edited by Tadeusz Trziszka and Maciej Oziembłowski

NEW CONCEPTS IN FOOD EVALUATION

Nutraceuticals – Analyses – Consumer

Wrocław 2009

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Nutraceuticals – Analyses – Consumer

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FOREWORD

The Polish food industry is one of the most important branches of economy taking into account both the size of production (over 20% of the sales value of all national industry), the number of production plants (ca. 28 thousand) and the level of employment (411 thousand persons, i.e. 8.4% of all employed in economy and ca. 16% of all employed in industry). The value of the Polish food industry is ca. 6% of GNP. Food processing plays a big and constantly increasing role in Polish export. Meat and meat products (15%), fruit and fruit products (11%) and dairy products (12.4%) have the biggest share in the commodity export structure.

The dynamics of the export of Polish products and high interest in Polish agri-food products in EU proves that Polish firms and their products are competitive on the markets characterised by high quality demands.

The high quality of Polish food as well as certificates and quality symbols confirm the unique features of such products. The production potential of agricultural processing plants creates optimum conditions for their development on the national and European market of food products.

The technologies used in food processing are based on the latest achievements and are harmonised with the food legal regulations. The knowledge about the biological principles of producing raw material is as important as the knowledge about the technological processes used in the production of food products. This means that monitoring of the processes "from field to table" should be applied. As food production issues are covered by three pillars of European law, i.e. food, safety and environment, food production requires multi-field expert knowledge. That is why No. simple rules of production management may be used in the technology and food processing and why numerous actions are taken to improve this area for the benefit of all Europeans.

A growing interest in high quality food products, including designed, regional and ecological products as well as products from integrated agriculture is observed in Poland. There is a growing demand for non-processed products which comply with high hygienic standards and for production of which innovative technologies are used. In the aspect of prevention of civilization diseases, new technologies of nutraceuticals and healthy food production are becoming an important and developing area in EU countries

Production of food is a complex and long chain of biological, chemical, physical, economic and psycho-sociological processes. It is, however, a key existential element of mankind and all economic and political activities must be subordinated to the idea of the quality of life, sustainable development and, first of all, to the basic existence needs of the humans.

The need of traceability in all links of food production chain (from field to table), so clearly indicated in European food legal regulations, is of high importance.

The development of Polish enterprises, growing expectations of the consumers as well as the demands of distribution networks have resulted in the use of novel systems of quality management.

The population of our continent is getting older and that is why a new style of life should be promoted in European countries. It is expected that in the year 2030, over 30% of the population will be older than 60 years. The value will be similar in the year 2050, when the persons aged 80 and more will constitute ca. 30% of the population.

This fact obliges us to make special efforts to create EU food policy that would take into account the perspective of future years and health protection so that the costs of treatment of metabolic disorders resulting from improper nutrition are lowered. The main objective of such policy is to promote healthy style of life, balanced diet and consumption of natural biologically active substances, including nutraceuticals, in diet.

It is also important to protect the consumer through monitoring of the food production chain and creation of a special pro-health education programme. These activities are part of the European programme of improving the quality of life realised by the European Technology Platform "Food for Life".

Taking the above data into account, a multiple-author monograph was written in 43 subchapters and the following 5 key chapters:

Chapter 1. Food improvement, nutraceuticals and organic food
Chapter 2. New trends in food sensory quality
Chapter 3. Selected analyses in food evaluation
Chapter 4. Quality management, marketing and consumer preferences
Chapter 5. Food quality in aspect of microbiology

The structure of chapters shows the high importance of food production processes, its improvement and search for food produced in natural conditions and of functional food (nutraceuticals) as well.

Evaluation of nutritional value and food safety, based on the latest analytical methods, is one of the most important elements of food production chain. Quality management, its influence of production processes and the role of marketing in distribution system, including the role of consumers in creating the quality of products, have been stressed.

The present publication does not exhaust the multitude of issues in this field but indicates the current problems which need to be solved.

Tadeusz Trziszka

Maciej Oziembłowski

CHAPTER 1

FOOD IMPROVEMENT, NUTRACEUTICALS AND ORGANIC FOOD

1

NANOTECHNOLOGY IN FOOD PROCESSING AND NUTRACEUTICALS/FUNCTIONAL INGREDIENTS DELIVERY

Introduction

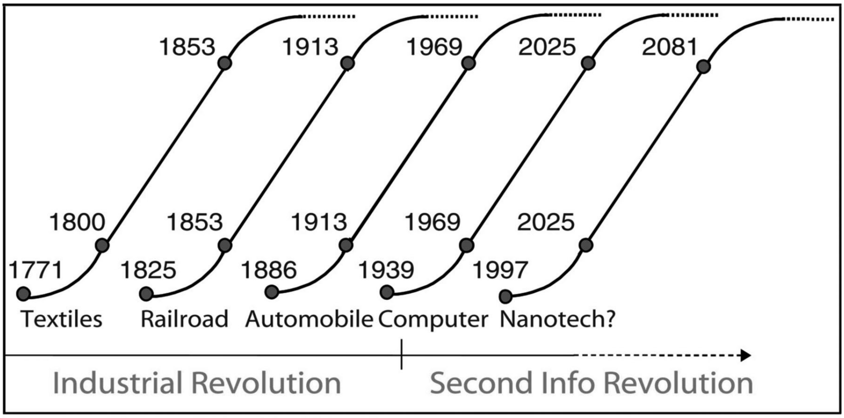
what is nanotechnology? Nanotechnology is the manufacture and use of materials and structures at the nanometre scale (a nanometre is one millionth of a millimetre). It offers a wide range of opportunities for the development of innovative products and applications in food system. Nanotechnology and nanomaterials are a natural part of food processing and conventional foods, because the characteristic properties of many foods rely on nanometer sized components, for example, nanoemulsions and foams. Recent technological developments lead the way for the manufacture of nanoparticles to be added to food. These could be finely divided forms of existing ingredients, or completely novel chemical structures. While many definitions for nanotechnology exist, the National Nanotechnology Initiative calls it "nanotechnology" only if it involves all of the following:

1. Research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1–100 nanometer range.
2. Creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size.
3. Ability to control or manipulate on the atomic scale.

Nanotechnology is predicted to rival the development of the automobile and the introduction of the personal computer. After industrial revolution of the last two centuries the nanotechnology initiated the second technical revolution as it is illustrated below as a function of growth innovations. We are only at the beginning of nanotechnology growth.

Nanotechnology applications in the food sector are on the increase worldwide and are expected to grow rapidly in the future. The types of material produced can be at the nanoscale in one dimension (very thin coatings), two dimensions (nanowires) or three dimensions (nanoparticles, such as a very fine powder preparations). Nanotechnologies are not new and scientists have been making polymers based on nanoscale subunits for many years. Due to new, previously unknown, properties attributed to engineered nanoparticles (NP) many new consumer products containing these NPs have been launched to the market recently. Application of NPs in electronics, medicine, textiles, defence, food, agriculture, cosmetics, and other areas are already a reality and applications are beginning to impact the food processing industries [Chen et al., 2006]. The potential benefits for consumers and producers of these new products are widely emphasized. In food and agricultural systems nanotechnologies cover many aspects, such as food security, packaging materials, disease treatment, delivery systems, bioavailability, new tools for molecular and cellular biology and new materials for pathogen detection [Maynard et al., 2006]. Nano-particles have tremendous potential for application in agro-food production/processing off:

- Pesticides, fertilizers
- Food additives
- Cosmetics
- Veterinary medicine, feed (e.g. vitamins)
- Packaging
- Textiles



Growth Innovations

Sources: Norman Poire, Merrill Lynch

Nano-technologies are key technologies of the 21st century, and huge research efforts are made in this field. New applications may become available in near future. Because applications with structural features on the nanoscale level have physical, chemical, and biological properties that are substantially different from their macroscopic counterparts, nanotechnology can be beneficial on various levels. Research in biology, chemistry, engineering, and physics drives the development and exploration of the nanotechnology field. Consequently, certain industries such as microelectronics, aerospace, and pharmaceuticals have already begun manufacturing commercial products of nanoscale size. Even though the food industry is just beginning to explore its applications, nanotechnology exhibits great potential. Food undergoes a variety of postharvest and processing-induced modifications that affect its biological and biochemical makeup, so nanotechnology developments in the fields of biology and biochemistry could also influence the food industry. Systems with structural features in the nanometer length range could affect aspects from food safety to molecular synthesis.

Nanotechnology focuses on the characterization, fabrication, and manipulation of biological and nonbiological structures smaller than 100 nm. Structures on this scale have been shown to have unique and novel functional properties. Interest and activities in this research area have greatly increased over the past few years. The use of the term "nano" does allow to highlight the fact that processes (for example, nanomanufacturing) or material structures (for example, nanomaterials) are designed and optimized to use specific properties and behaviours at lengths of 10^{-7} to 10^{-9} m.

Nanostructures/Nanomaterials. Nanostructures are materials that, in at least one dimension, measure approximately 1–100 nm. Nanostructures or nanomaterials exhibit properties different from their macroscale counterparts (their "big brothers") such as:

- Mechanical strength (how hard they are to break)
- Electrical conductivity (how fast electrons flow through them)
- Thermal conductivity (how fast heat flows through them)
- Chemical reactivity (how well/fast they react with other chemicals)
- Transparency (how well you can see through them)
- Magnetism (whether or not they are magnetic)

Microstructures, the cousin to nanostructures, typically measure between 100 nanometers and 100 micrometers in at least one dimension, but likely do not exhibit unique properties like nanostructures do. Discoveries in nanotechnology are beginning to impact the food industry and associated industries; this affects important aspects from food safety to the molecular synthesis of new food products and ingredients [Chen et al., 2006].

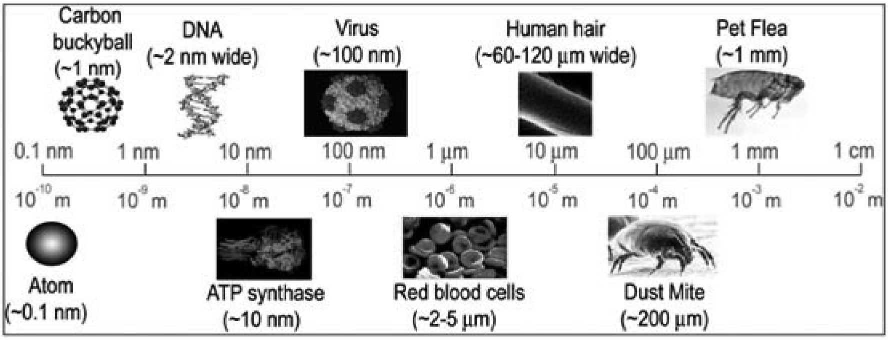
Types of Nanostructures/Nanomaterials

- Nanoparticles or nanospheres: nanoscale lengths are measured in all three dimensions
- Nanotubes or nanowires or nanorods: nanoscale lengths are measured in two dimensions only
- Nanoscale thin films or ultra-thin films: nanoscale lengths are measured in one dimension only
- Nanocomposites: a material comprised of many nanoscale inclusions (such as nanoparticles)
- Nanostructured materials: a material that exhibits a unique structure that can be measured at the nanoscale

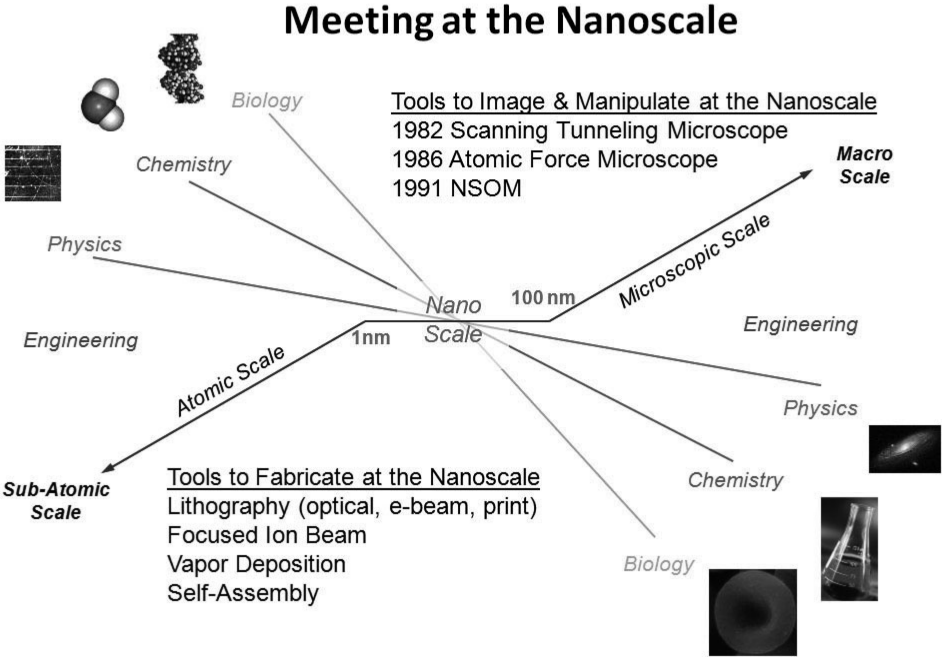
The fact that systems with structural features on the nanoscale have physical, chemical and biological properties substantially different from their macroscopic counterparts is changing the understanding of biological and physical phenomena in food systems. Since foods are complex biological systems that are governed by many of the same basic mechanisms and principles that biologists and biochemists study, expect that the discoveries made in nanotechnology may eventually also impact the food industry. However, foods undergo a variety of postharvest and processing-induced modifications that affect the biological and biochemical functionality of the system.

Meeting at the Nanoscale. Range of sizes of nanomaterials in the food sector and their relative position on nanoscale/microscopic scale are illustrated below.

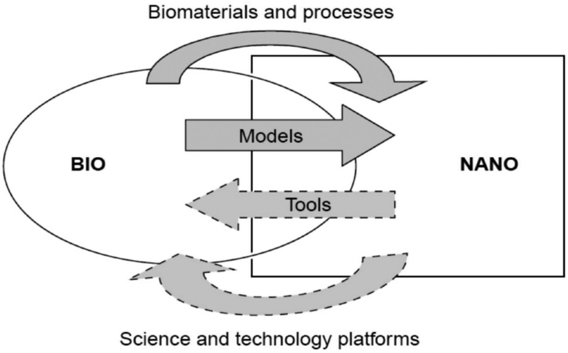
Structures	**Diameter or length (nm)**
DNA	12
Glucose	21–75
Liposome	30–10000
LDH	40–300
Amylopectin	44–200
Casein micelle	60–300
Zein	200
Nanosensors	<1000



Nanotechnology allows to measure, control, and manipulate matter at the nanoscale level to change those properties and functions in a beneficial way. Developments in nanotechnology are driven by fundamental and applied research in physics, chemistry, biology, engineering, and materials science.



Nanotechnology provides the tools to measure and understand biosystems in science and technology platform.



The potential benefits of nanofoods – foods produced using nanotechnology – are astonishing. Advocates of the technology promise improved food processing, packaging and safety; enhanced flavor and nutrition; ‘functional foods’ where everyday foods carry medicines and supplements, and increased production and cost-effectiveness. In a world where thousands of people starve each day, increased production alone is enough to warrant worldwide support. For the past few years, the food industry has been investing millions of dollars in nanotechnology research and development. Some of the world’s largest food manufacturers, including Nestle, Altria, H.J. Heinz and Unilever, are blazing the trail, while hundreds of smaller companies follow their lead. Yet, despite the potential benefits, compared with other nanotechnology arenas, nanofoods don’t get a lot of publicity. Applications of nanotechnology within the food industry are beginning to impact the important aspects of food and associated industries from food safety to the molecular synthesis of new food products and ingredients [Chen et al., 2006]. The fact that systems with structural features on the nanoscale have physical, chemical, and biological properties substantially different from their macroscopic counterparts is changing the understanding of biological and physical phenomena in food systems. Foods are complex biological systems that are governed by many of the same basic mechanisms and principles as in biology and biochemistry. Therefore, the discoveries made in nanotechnology will also impact the food industry. Nanotechnology allows to measure, control, and manipulate matter at the nanoscale level to change those properties and functions. Food is "nanofood" when nanoparticles, nanotechnology techniques or tools are used during cultivation, production, processing, or packaging of the food. It does not mean atomically modified food or food produced by nanomachines.

In the forefront of nanofood development is Kraft Foods, which took the industry’s lead when it established the Nanotek Consortium, a collaboration of 15 universities and national research labs, in 2000. Kraft’s focus is on "interactive" foods and beverages. These products will be customized to fit the tastes and needs of consumers at an individual level. Possible products include drinks that change colours and flavors to foods that can recognize and adjust to a consumer’s allergies or nutritional needs. Other large companies, such as Nestlé and Unilever, are exploring improved emulsifiers that will make food texture more uniform. In Australia for instance, nanocapsules are used to add Omega-3 fatty acids to one of the country’s most popular brands of white bread. According to the manufacturer, nanocapsules of tuna fish oil added to Tip Top Bread provide valuable nutrients, while the encapsulation prevents the bread from tasting fishy. These huge Western companies are responsible for the bulk of the food industry's research and development, however the nanofood industry is truly a global phenomenon.

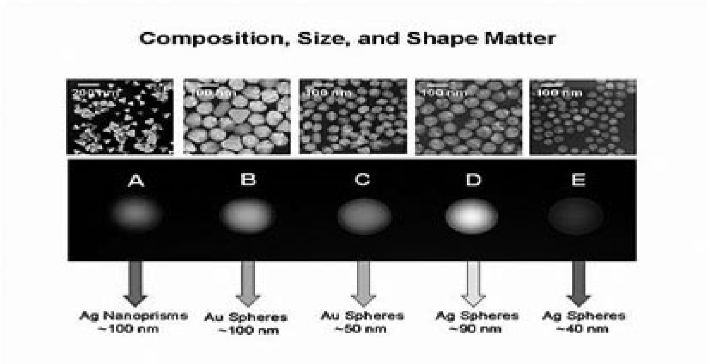
Summary of possible nanoscale size effect

- Realization of miniaturized devices and systems while providing more functionality
- Attainment of high surface area to volume ratio
- Manifestation of novel phenomena and properties, including changes in:
    - Physical Properties (e.g. melting point)
    - Chemical Properties (e.g. reactivity)
    - Electrical Properties (e.g. conductivity)
    - Mechanical Properties (e.g. strength)
    - Optical Properties (e.g. light emission)

Nanotechnology (NT) – Science and Technology

- NT = development and application of structures, devices, materials and systems with fundamentally new properties and functions
- Size and properties – at nanoscale, properties of materials – physical, chemical, optical, mechanical and biological – differ from those at larger scale or from individual atoms/molecules
- Size matters – especially surface area
- Shape matters – different properties even with same chemical comp
- Other properties matter – charge, surface coatings, structure
- Ability to understand and control the fundamental structure and function of matter at nanoscale at <100 nm (one nm = 1 billionth of a meter): the scale at which biological molecules and structures operate in cells
- Transformational set of enabling technologies with broad range of applications for the life sciences

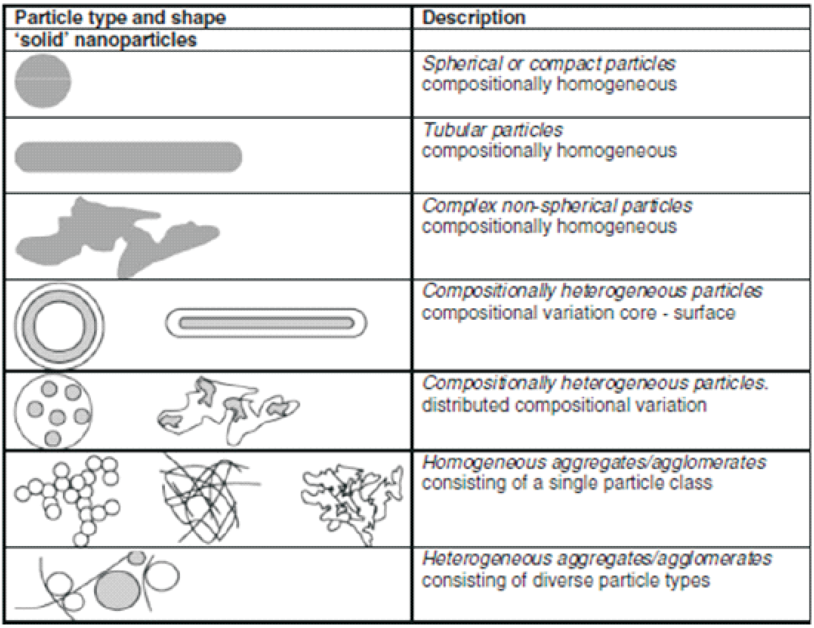
The composition, size and shape of nanoparticles (NP) produced or used affect their properties. For example, gold and silver NP are physically different at different sizes down the nano-scale in colour and shape – see picture below



Therefore, physicochemical properties of NP will depend upon:

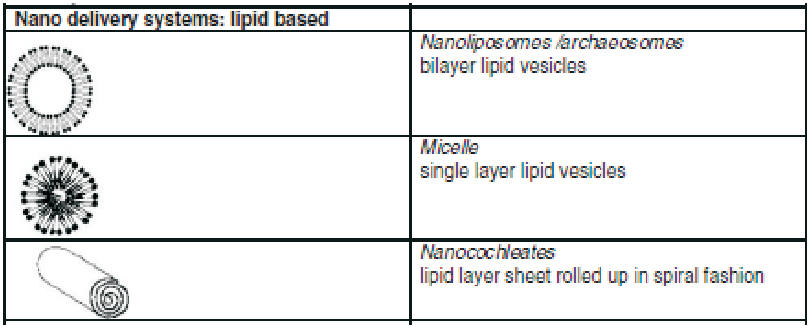
- Aggregation
- Shape
- Size
- Solubility
- Surface area
- Surface charge
- Surface coatings

Examples of forms and shapes of nano-structured particles



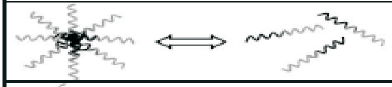
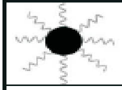
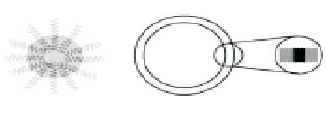
Particle type and shape	Description
'solid' nanoparticles	
	Spherical or compact particles compositionally homogeneous
	Tubular particles compositionally homogeneous
	Complex non-spherical particles compositionally homogeneous
	Compositionally heterogeneous particles compositional variation core - surface
	Compositionally heterogeneous particles. distributed compositional variation
	Homogeneous aggregates/agglomerates consisting of a single particle class
	Heterogeneous aggregates/agglomerates consisting of diverse particle types

Examples of forms and shapes of nano-structured particles



Nano delivery systems: lipid based	
	Nanoliposomes /archaeosomes bilayer lipid vesicles
	Micelle single layer lipid vesicles
	Nanocochleates lipid layer sheet rolled up in spiral fashion

Examples of forms and shapes of nano-structured particles

Nano delivery systems: polymer based	
	*Micelle* aggregated copolymers
	*Nanosphere* aggregated copolymers generating a solid central core
	*Nanocapsule / Polymersome* polymer membrane surrounding a central cavity: Nanocapsule: oily liquid cavity, single layer membrane Polymersome: aqueous cavity, bilayer membrane (similar to nanoliposome)

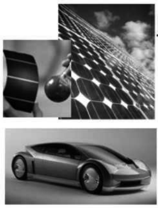
Nanotechnology applications. Most nanotechnological research focuses on the development of applications in:

- Information Technology
- Energy
- Medicine
- Consumer goods

Nanotechnology Applications

Information Technology

- Smaller, faster, more energy efficient and powerful computing and other IT-based systems



Energy

- More efficient and cost effective technologies for energy production
  - Solar cells
  - Fuel cells
  - Batteries
  - Bio fuels

Medicine

- Cancer treatment
- Bone treatment
- Drug delivery
- Appetite control
- Drug development
- Medical tools
- Diagnostic tests
- Imaging

Consumer Goods

- Foods and beverages
  - Advanced packaging materials, sensors, and lab-on-chips for food quality testing
- Appliances and textiles
  - Stain proof, water proof and wrinkle free textiles
- Household and cosmetics
  - Self-cleaning and scratch free products, paints, and better cosmetics

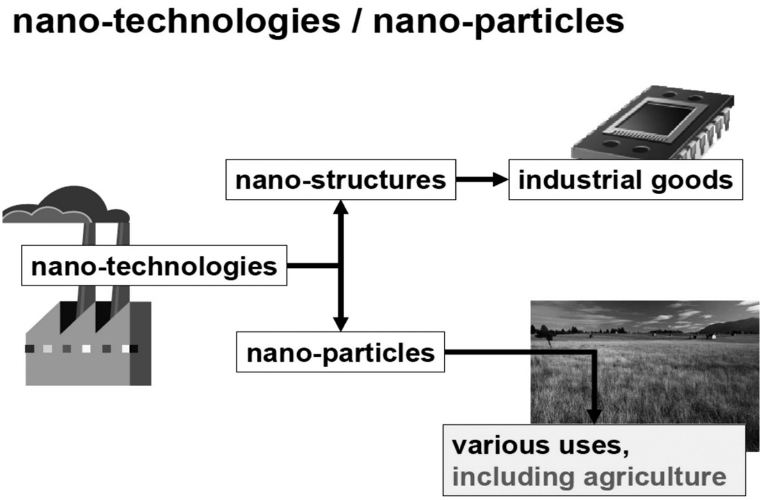
Nanotechnology has the potential to impact many aspects of food and agricultural systems. Food security, disease treatment delivery methods, new tools for molecular and cellular biology, new materials for pathogen detection, and protection of the environment are examples of the important links of nanotechnology to the science and engineering of agriculture and food systems.

Examples of nanotechnology as a tool for achieving further advancements in the food industry are as follows:

- Increased security of manufacturing, processing, and shipping of food products through sensors for pathogen and contaminant detection
- Devices to maintain historical environmental records of a particular product and tracking of individual shipments
- Systems that provide integration of sensing, localization, reporting, and remote control of food products (smart/intelligent systems) and that can increase efficacy and security of food processing and transportation
- Encapsulation and delivery systems that carry, protect, and deliver functional food ingredients to their specific site of action

Nanotechnologies and nanoparticles will affect food chain from primary production to consumer as is illustrated below.

Nanomaterials and nanostructures will provide specific function in targeted applications and some examples are listed below.



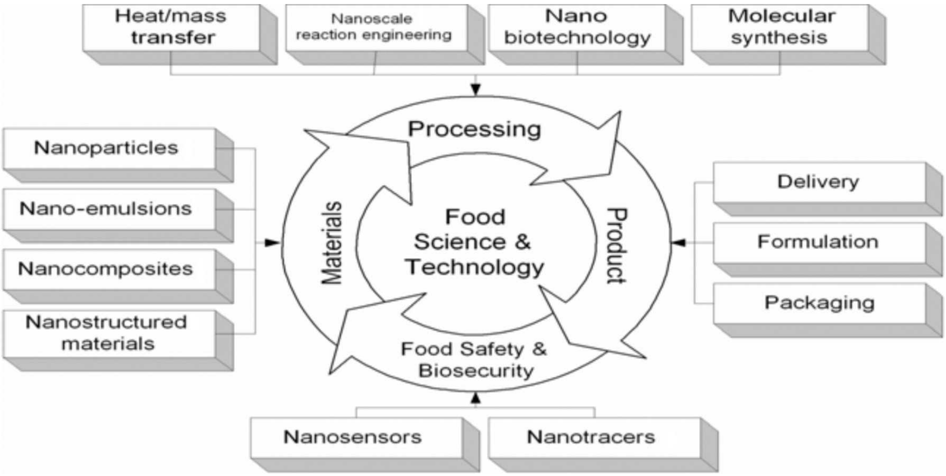
Type of NP	Application	Function
1	2	3
Metal nanoparticles Silver, ZnO)	Food additive/supplement	Claimed enhanced gastrointestinal uptake of metal
Packaging materials/storage	Increase barrier properties	
Food preparation devices	Clean surface	
Refrigerators, storage containers	Anti-bacterial coating	
Water purification/soil cleaning	Removal/catalysation/ oxidation of contaminants	
Sprays	Anti-bacterial	

1	2	3
Complex nanoscale structures	Nanosensors in packaging	Detection of food deterioration
Hand-held devices conditions detection of contaminants etc.	Monitoring storage	
Incorporated active nanoparticles	Migration out of packaging materials	Oxygen scavenging, prevention of growth of pathogens
Filters with nano-pores contaminants	Water purification	Removal pathogens
Equal sized emulsions	Product design (e.g., taste, texture)	
Nano-sized nutrients /foods	Food additives/supplements	Claimed enhanced uptake
Delivery systems (nano-encapsulates)	Food additives/supplements	Protecting and (targeted) delivery of content
	Pesticide	Increased efficacy

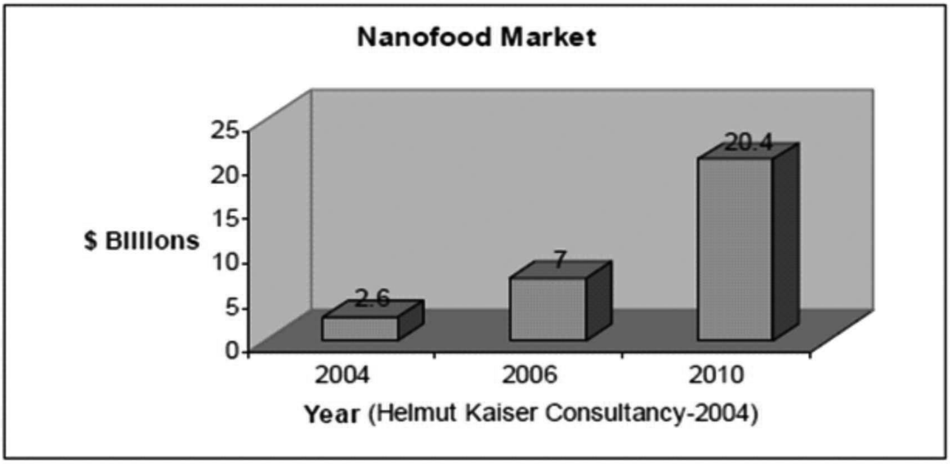
Strategies to apply nanoscience to the food industry are quite different from these more traditional applications of nanotechnology. Food processing is a multi-technological manufacturing industry involving a wide variety of raw materials, high biosafety requirements, and well-regulated technological processes. Four major areas in food production may benefit from nanotechnology:

- Development of new functional materials
- Microscale and nanoscale processing
- Product development
- and methods and instrumentation design for improved food safety and biosecurity

Application matrix of nanotechnology in food science and technology is schematically shown below.



It may be stated that there are a large number of potential applications of nanotechnology within the food industry. Applications in food packaging are considered highly promising because they can improve the safety and quality of food products. This includes intelligent packaging, which is reactive to the environment and active packaging, which is able to interact with the food product. The estimated world market for nanofoods is rapidly growing and is estimated at $ 20 billion in 2010.



Examples of nanotechnology as a tool for achieving further advancements in the food industry.

- Increased security of manufacturing, processing, and shipping of food products through sensors for pathogen and contaminant detection
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- Encapsulation and delivery systems that carry, protect, and deliver functional food ingredients to their specific site of action

Nanotechnology in Delivery of Nutraceuticals/Functional Ingredients. The nanosized self-assembled structured liquids (NSSL) technology allows for encapsulation of nutraceuticals, cosmeceuticals, and essential oils and drugs in food, pharmaceuticals, and cosmetics. Another advantage to the NSSL technology is that it allows the addition of insoluble compounds into food and cosmetics. One of the first products developed with this technology – a healthier version of canola oil – is already available to consumers in Israel.

Nanodispersions and Nanocapsules. The fundamental components of foods such as vitamins, antimicrobials, antioxidants, and preservatives come in various molecular and physical forms. They are rarely used in pure form, they are usually part of a delivery system. A delivery system has numerous functions, only one of which is to transport a functional ingredient to its desired site. Besides being compatible with food product attributes such as taste, texture, and shelf life, other functions of a delivery system include protecting an ingredient from chemical or biological degradation, such as oxidation, and controlling the functional ingredient's rate of release under specific environmental conditions. Because they can effectively perform all these tasks, nanodispersions and nanocapsules are ideal mechanisms for delivery of functional ingredients. These types of nanostructures include:

- association colloids
- nanoemulsions
- biopolymeric nanoparticles

A delivery system must perform a number of different roles:

- It serves as a vehicle for carrying the functional ingredient to the desired site of action
- It may have to protect the functional ingredient from chemical or biological degradation (for example, oxidation) during processing, storage, and utilization; this maintains the functional ingredient in its active state
- It may have to be capable of controlling the release of the functional ingredient, such as the release rate or the specific environmental conditions that trigger release (for example, pH, ionic strength, or temperature)
- The delivery system has to be compatible with the other components in the system, as well as being compatible with the physicochemical and qualitative attributes (that is, appearance, texture, taste, and shelf-life) of the final product

The characteristics of the delivery system are one of the most important factors influencing the efficacy of functional ingredients in many industrial products. A wide variety of delivery systems has been developed to encapsulate functional ingredients, including simple solutions, association colloids, emulsions, biopolymer matrices, and so on. Each type of delivery system has its own specific advantages and disadvantages for encapsulation, protection, and delivery of functional ingredients, as well as cost, regulatory status, ease of use, biodegradability, and biocompatibility.

Association Colloids. Association colloids–such as surfactant micelles, vesicles, bilayers, reverse micelles, and liquid crystals–have been used for many years to encapsulate and deliver polar, nonpolar, and/or amphiphilic functional ingredients [Garti et al., 2004, 2005; Golding and Sein, 2004]. Surfactant micelles, vesicles, bilayers, reverse micelles, and liquid crystals are all examples of association colloids.

A **colloid** is a stable system of a substance containing small particles dispersed throughout. An **association colloid** is a colloid whose particles are made up of even smaller molecules. Used for many years to deliver polar, nonpolar, and amphiphilic functional ingredients [Golding and Sein, 2004; Garti et al., 2004, 2005], association colloids range in size from 5 nm to 100 nm and are usually transparent solutions. The major disadvantages to association colloids are that they may compromise the flavor of the ingredients and can spontaneously dissociate if diluted. The major advantages of association colloid systems are that they form spontaneously, are thermodynamically favorable, and are typically transparent solutions. On the other hand, the major disadvantage is that a large quantity of surfactant (and in many cases, co-surfactant) is required to form them, which may lead to problems with flavor, cost, or legality. The formation of association colloids is concentration-driven, diluting the solutions containing the colloids can lead to their spontaneous dissociation.

Nano-emulsions. The use of high-pressure valve homogenizers or microfluidizers often causes emulsions with droplet diameters of less than 100 to 500 nm. In modern literature such emulsions are often referred to as "nano-emulsions". Nano-emulsions have been produced and studied for many years, so a large body of literature dealing with their preparation, characterization, and utilization exists [McClements, 2004]. Functional food components can be incorporated within the droplets, the interfacial region, or the continuous phase. Encapsulating functional components within the droplets often enables a slowdown of chemical degradation processes by engineering the properties of the interfacial layer surrounding them [McClements and Decker ,2000].

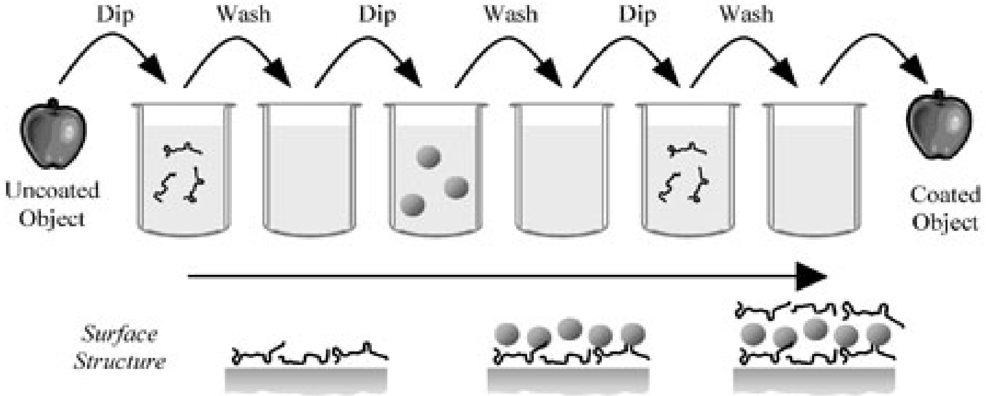
Nanostructured multiple emulsions. The use of multiple emulsions can create delivery systems with novel encapsulation and delivery properties. The most common examples of this are oil-in-water-in-oil (O/W/O) and water-in-oil in-water (W/O/W) emulsions [Garti and Benichou, 2001, 2004]. For example, a nanostructured (W/O/W) emulsion would consist of nanometer-sized water droplets or reverse micelles (W1) contained within larger oil droplets (O) that are dispersed within an aqueous continuous phase (W2). Functional food components could be encapsulated within the inner water phase, the oil phase, or the outer water phase, thereby making it possible to develop a single delivery system that contains multiple functional components [Flanagan and Singh, 2006].

Biopolymeric Nanoparticles. Food-grade biopolymers such as proteins or polysaccharides can be used to produce nanometer-sized particles [Chang and Chen, 2005; Gupta and Gupta, 2005; Ritzoulis et al., 2005]. Using aggregative (net attraction) or segregative (net repulsion) interactions, a single biopolymer separates into smaller nanoparticles. The nanoparticles can then be used to encapsulate functional ingredients and release them in response to distinct environmental triggers. One of the most common components of many biodegradable biopolymeric nanoparticles is polylactic acid (PLA). Widely available from a number of manufacturers, PLA is often used to encapsulate and deliver drugs, vaccines, and proteins, but it has limitations: it is quickly removed from the bloodstream, remaining isolated in the liver and kidneys. Because its purpose as a nanoparticle is to deliver active components to other areas of the body, PLA needs an associative compound such as polyethylene glycol to be successful in this regard [Riley et al., 1999].

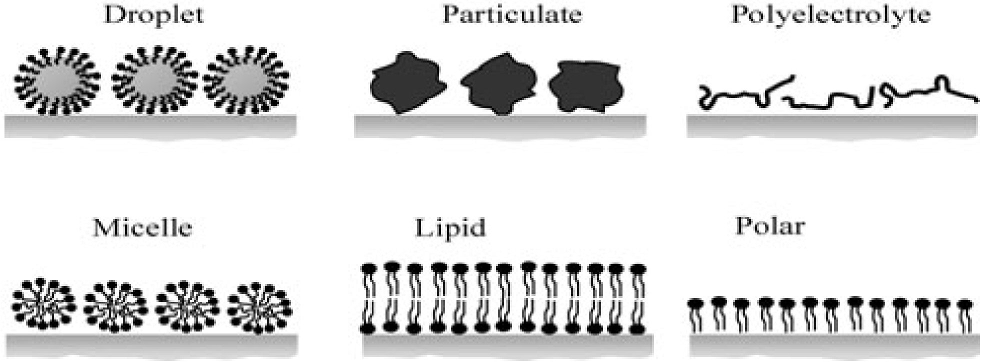
Nanolaminates. Nanotechnology provides food scientists with a number of ways to create novel laminate films suitable for use in the food industry. A nanolaminate consists of 2 or more layers of material with nanometer dimensions that are physically or chemically bonded to each other, see below.

Nanolaminates can give food scientists some advantages for the preparation of edible coatings and films over conventional technologies and may thus have a number of important applications within the food industry. Edible coatings and films are currently used on a wide variety of foods, including fruits, vegetables, meats, chocolate, candies, bakery products, and French fries [Morillon et al., 2002; Cagri et al., 2004]. These coatings or films could serve as moisture, lipid, and gas barriers. Alternatively, they could improve the textural properties of foods or serve as carriers of functional agents such as colours, flavors, antioxidants, nutrients, and antimicrobials. The basic functional properties of edible coatings and films depend on the characteristics of the film-forming materials used for their preparation. The composition, thickness, structure, and properties of the multilayered laminate formed around the object could be controlled in a number of ways, including:

- changing the type of adsorbing substances in the dipping solutions;
- changing the total number of dipping steps used;
- changing the order that the object is introduced into the various dipping solutions;
- changing the solution and environmental conditions used (pH, ionic strength, dielectric constant, temperature etc.)



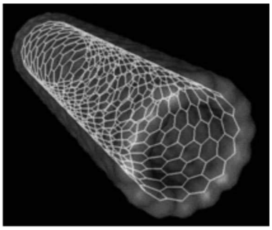
Coating an object with multilayers using a successive dipping and washing procedure



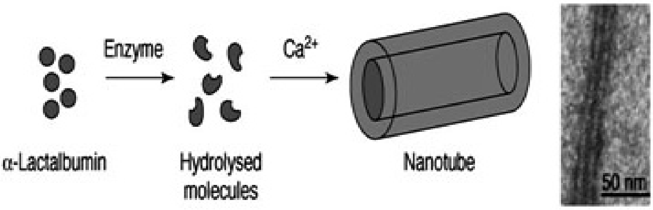
Possible components that could be used to assemble multilayered edible films or coatings.

The driving force for adsorption of a substance to a surface would depend on the nature of the surface and the nature of the adsorbing substance and it could be: electrostatic, hydrogen-bonding, hydrophobic interactive, thermodynamically incompatible, and so on.

Nanofibers and Nanotubes. Two applications of nanotechnology that are in the early stages of having an impact on the food industry are nanofibers and nanotubes. Because nanofibers are usually not composed of food-grade substances, nanofibers have only a few potential applications in the food industry. Produced by a manufacturing technique using electrostatic force, nanofibers have small diameters ranging in size from 10 nm to 1,000 nm. As advances continue in the area of producing nanofibers from food-grade materials, their use will likely increase. As with nanofibers, the use of nanotubes has predominantly been for non-food applications. Carbon nanotubes are popularly used as low resistance conductors and catalytic reaction vessels. Under appropriate environmental conditions, however, certain globular milk proteins can self-assemble into similarly structured nanotubes [Graveland--Bikker and de Kruif, 2005, 2006].

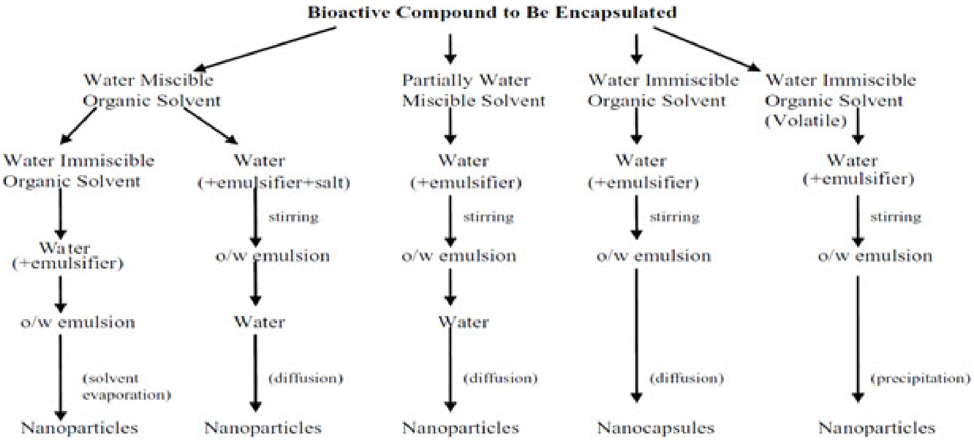


Carbon nanotubes (hollow fibers)

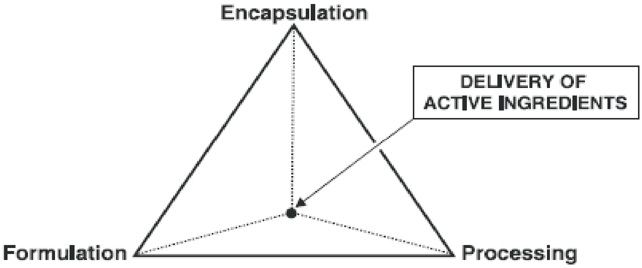


Unique milk protein based nanotubes

Technologies for the delivery of active nutraceutical/functional ingredients in foods. The goal is to improve the functionality of these ingredients in food systems, which may minimize the concentrations needed.



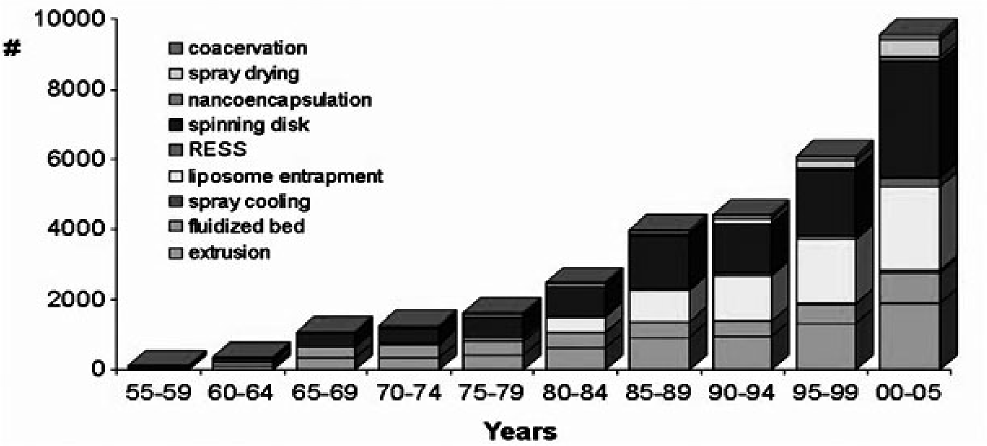
New functional ingredients are increasingly integrated into the food matrix development process [Haruyama 2003]. Food ingredients such as nanoparticulate lycopene and carotenoids are becoming commercially available. Bioavailability and the ability to disperse these compounds are typically higher than that of their traditionally manufactured counterparts.



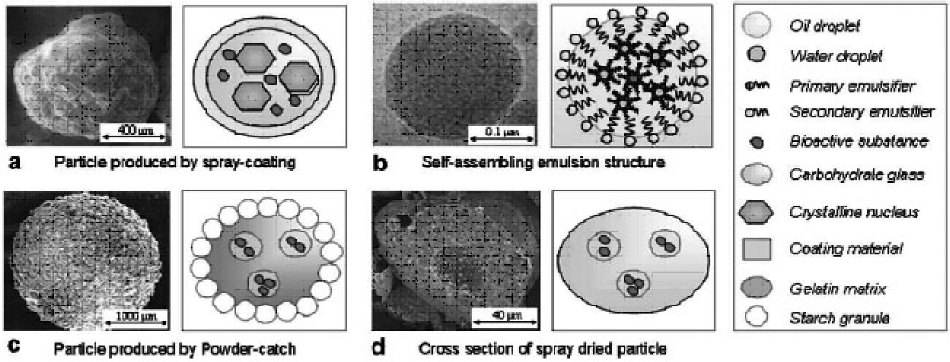
Example of technological approach for the delivery of active ingredients in foods.

Encapsulation is the isolation of the active ingredient within the food product using food-grade materials. Formulation encompasses the structuring of the active ingredient, often on molecular or nanoscale levels using food-grade ingredients interacting with the active ingredient.

Processing conditions will affect activity of ingredient in question.



Graph above, summarizes the major microencapsulation technologies used over the last 45 years. New microencapsulation technologies are relentlessly devised and invented by academics and industrial researchers: in 2002, over 1000 patents were filed concerning various microencapsulation processes and their applications and over 300 of these patents were directly related to food ingredient encapsulation.



a Particle produced by spray-coating

b Self-assembling emulsion structure

c Particle produced by Powder-catch

d Cross section of spray dried particle

Regulations/Safety of Nanoproducts. There are currently No. special regulations for the application or utilization of nanotechnology in foods in the United States. The U.S. Food and Drug Administration (FDA) states that it regulates "products, not technologies", and anticipates that many products of nanotechnology will fall under the jurisdiction of multiple centers within FDA and will therefore be regulated by the Office of Combination Products. FDA regulates on a product-by-product basis and stresses that many products that are currently regulated produce particles in the nano-size range. FDA says that "particle size is not the issue" and stresses that new materials, regardless of the technology used to create them, will be subject to the standard battery of safety tests (http://www.fda.gov/nanotechnology/regulation.html). In contrast to the FDA view on particle size, a recent report by the Institute of Food Science and Technology (IFST) - a United Kingdom-based independent professional qualifying body for food scientists and technologists states that size matters and recommends that nanoparticles be treated as new, potentially harmful materials until testing proves their safety.

Conclusion. As developments in nanotechnology continue to emerge, its applicability to the food industry is sure to increase. The success of these advancements will be dependent on consumer acceptance and the exploration of regulatory issues. Food producers and manufacturers could make great strides in food safety by using nanotechnology, and consumers would reap benefits as well. More than 200 companies are conducting research in nanotechnology and its application to food products [IFST, 2006], and as more of its functionalities become evident, the level of interest is certain to increase.

In addition to the scientific and technical advances needed to continue the application of nanotechnology to foods, regulatory considerations (including safety/toxicology and environmental impact), economics and consumer acceptance of nanotechnology will ultimately dictate its success in food applications. Agricultural producers and food manufacturers could gain a more competitive position through the application of nanotechnology, and in the long term, consumers may benefit from the advances in nanotechnology that contribute to a competitive and innovative domestic agricultural and food system and provide new methods to improve safety and nutritional value of food products.

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2

TAILORING EGGS FOR HEALTH BENEFITS: THE CHALLENGE OF EGGS MORE COMPATIBLE WITH RISKS FACTORS, INCLUDING FOR CVD AND DIABETES

Introduction

Egg vs. CVD Risks

The presumed association between egg intake and cardiovascular disease (CVD) risk is related to the perception of high plasma cholesterol being a major risk factor, and the recommendation of reduced dietary cholesterol for prevention. Such risk would be expected to be concentrated primarily in ‘cholesterol-responders,’ comprising approximately 30% of the general population. However hitherto available data on effects of egg consumption on CVD risk and mortality is limited and inconsistent.

Moreover, the effect of cholesterol intake on CVD risk was found to be less consistent than that of saturated fatty acids (SFA) and *trans* fatty acid (tFA) intakes, which typically raise concentrations of low-density lipoprotein (LDL) cholesterol more than do higher amounts of cholesterol in the diet [Gray & Griffin, 2009].

This is especially the case under conditions of restricted diet, in which increased cholesterol intake from two eggs/day did not increase total plasma or LDL cholesterol when accompanied by moderate weight loss. These findings further support the increasing consensus that cholesterol-rich foods should not be summarily excluded in general dietary advice, as for weight management, solely on account of assumed unfavorable influence on plasma LDL cholesterol [Harman et al., 2008]. A recent commentary from the American Egg Board [2008] reflects the current status, stating "These findings are now well established and, as a consequence, recommendations from major food and health bodies concerning dietary cholesterol have been relaxed in the UK and elsewhere in recent years. However, in the minds of the public, cholesterol in the diet, specifically from eggs, continues to be viewed with suspicion, and that view is still reflected in the advice of some professionals and for specific subpopulations".

Egg vs. glycemic and pre-/diabetes associated markers

Only limited and inconsistent findings have been reported for the relationship between dietary cholesterol or egg consumption and fasting glucose, and No. previous study has directly examined the association between egg consumption and risk for type 2 diabetes mellitus. A recent analysis using data from two completed randomized trials – 20,703 men from the Physicians’ Health Study I (1982–2007) and 36,295 women from the Women’s Health Study (1992–2007) – showed that during mean follow-up of 20.0 years in men and 11.7 years in women, adjusted hazard ratios for type 2 diabetes in men increased up to 1.46, and 1.58 for consumption of 5–6, and ≥7 eggs/week, respectively, and in women by 1.18 and 1.77 (p for trends <0.0001) [Djoussé at el., 2009].

Additional research in this cohort showed that even where egg consumption was not associated with incidence of myocardial infarction (MI) or stroke, adjusted hazard ratios for mortality increased from 0.94 to 1.23 for the consumption of <1 to ≥7 eggs/week, respectively (p<0.0001), and 2.0-fold among diabetic subjects [Djoussé & Gaziano, 2008]. Previous research evaluating 37,851 men and 80,082 women in the Health Professionals Follow-up Study (1986 to 1994) and the Nurses' Health Study (1980 to 1994) had also shown that only diabetics might have increased risk of developing coronary heart disease (CHD) or stroke if they ate ≥1 egg/day [Frank et al., 1999].

The questions raised address the relationship of egg consumption to all-cause mortality, beyond CVD, myocardial infarction, or stroke – and specifically the potential link with recently suggested diabetes-associated risk [Eckel, 2008]. The present paper discusses egg modifications that may potentially be more compatible with prevention of dyslipidemia and dysglycemia, and better management of risk factors for CVD, pre-diabetes, and diabetes.

Materials and methods

A. N-3 Polyunsaturated Fatty Acid (PUFA) Egg Fortification

Laying hens were fed for five weeks with either a standard corn-based feed, common to both the United States and Israel (control; Alonim, Israel), or the same feed supplemented with 5% extruded linseed [Shapira et al., 2008; Weill et al., 2002].

Egg n-3 PUFA contribution was calculated by comparing fatty acid (FA) contents to dietary intake of American [Ervin et al., 2004] and Israeli [ICDC, 2004] populations, and to calculated Dietary Reference Intakes (DRI; Institutes of Medicine, 2002) for adults aged ≥ 19 years (alpha-linolenic acid [ALA, 18:3] 0.6–1.2% daily kcal intake, and long-chain PUFA [LCPUFA] 0.06–0.12%). Egg cholesterol content was compared to DRI guidelines (≤300 mg/day) and to current consumption [Ervin et al., 2004; ICDC, 2004].

B. Antioxidative Egg Modification

Laying hens were fed either 'HPUFA-regular' (control) standard Israeli feed mixture based on corn (50.0%) and soy (31.0%); 'HPUFA-HAOX,' standard corn-soy mixture enhanced with a vitamin E- and carotenoid-fortified premix; or 'HMUFA-HAOX' feed based on milo (62.1%), with reduced n-6 PUFA and added antioxidant-rich premix. Study egg cholesterol ranged from 213–230 mg/egg in all types; vitamin E ranged from 1.0–2.0 mg in HPUFA-regular to 5–10 mg in modified HAOX-types, and carotenoids 350–800 µg/egg, respectively.

Human subjects added differing eggs to their regular diets for consecutive periods of three weeks: 'low-egg' 2–4 eggs/week of commercially-available HPUFA-regular, followed by two eggs/day of the same 'HPUFA-regular' composition, then of HUPFA composition fortified with antioxidants in 'HPUFA-HAOX, with reduced n-6 PUFA (displaced by n-9 monounsaturated fatty acids [MUFA]) and fortified with antioxidants in 'HMUFA-HAOX'. Dietary recalls and fasting blood samples were collected following each study regime.

Results

A. In n-3 PUFA-fortified eggs, concentrations of total n-3 PUFA increased to 3.8-fold that of control eggs (258.2 vs. 67.3 mg/egg), ALA to 6.4-fold (156.7 vs. 24.5 mg), and docosahexaenoic acid (DHA, 22:6) to 2.4-fold (101.6 vs. 42.8 mg). Correspondingly, total n-6:n-3 PUFA ratio was reduced 3.6-fold from control, LA:ALA ratio 5.7-fold, and LCPUFA AA:DHA ratio 3.0-fold (Table 1). Total n-3 PUFA increased 3.7- and 4.0-fold after the third and fifth weeks, respectively. SFA, MUFA, total PUFA, n-6 PUFA, and average cholesterol contents were maintained. The n-6 LCPUFA arachidonic acid (AA, 20:4) was reduced by 21% in fortified vs. control eggs.

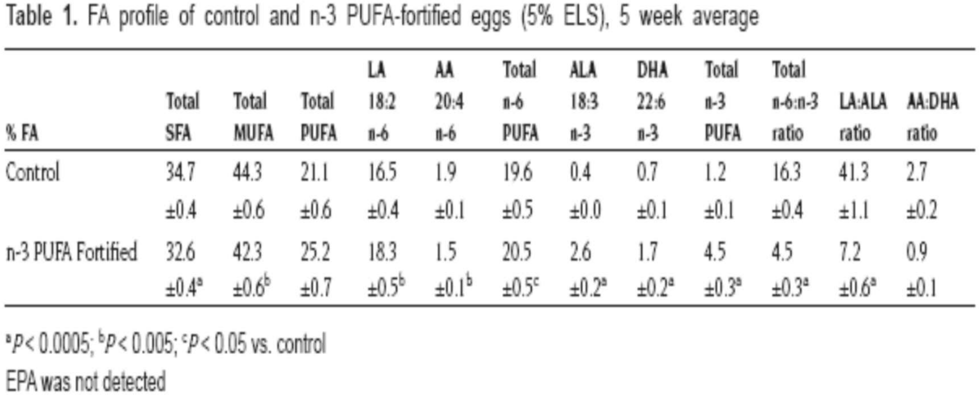

Table 1. FA profile of control and n-3 PUFA-fortified eggs (5% ELS), 5 week average

% FA	Total SFA	Total MUFA	Total PUFA	LA 18:2 n-6	AA 20:4 n-6	Total n-6 PUFA	ALA 18:3 n-3	DHA 22:6 n-3	Total n-3 PUFA	Total n-6:n-3 ratio	LA:ALA ratio	AA:DHA ratio
Control	34.7	44.3	21.1	16.5	1.9	19.6	0.4	0.7	1.2	16.3	41.3	2.7
	±0.4	±0.6	±0.6	±0.4	±0.1	±0.5	±0.0	±0.1	±0.1	±0.4	±1.1	±0.2
n-3 PUFA Fortified	32.6	42.3	25.2	18.3	1.5	20.5	2.6	1.7	4.5	4.5	7.2	0.9
	±0.4^a	±0.6^b	±0.7	±0.5^b	±0.1^b	±0.5^c	±0.2^a	±0.2^a	±0.3^a	±0.3^a	±0.6^a	±0.1

$^a P < 0.0005$; $^b P < 0.005$; $^c P < 0.05$ vs. control

EPA was not detected

B. Compared to the low-egg (2–4/week) regime, consumption of two eggs/day of all three types – HPUFA-regular, HPUFA-HAOX, and HMUFA-HAOX – was associated with increased total cholesterol by 12.5, 18.8 ($p<0.01$), and 8.3% (NS), respectively; LDL by 17.2, 27.6, and 17.2%; and LDL:HDL ratio by 33.3, 23.8, and 14.3%. Cholesterol increases were significant only with HPUFA-regular and HPUFA-HAOX but not with HMUFA-HAOX eggs**.** Lag-time to LDL oxidation was shortened with HPUFA-regular by 28.8%, $p<0.01$, HPUFA-HAOX by 27.2%, $p<0.01$, but with two HMUFA-HAOX eggs/day it was only 6.6% shorter (NS) (Table 2); LDL oxidation (as measured by optical density [OD] at 234 nm) (Fig. 3) was initiated faster following HPUFA-regular and HPUFA-HAOX regimes as compared to HMUFA-HAOX eggs, indicating that LDL oxidation was not significantly affected by the addition of antioxidants alone. The HPUFA-regular regime was the only one to reduce HDL (14%, $p<0.01$) and to increase fasting blood glucose and blood urea nitrogen (BUN) levels (14.9 and 32.5%, respectively, $p<0.01$). Other biochemical measures, i.e. liver function tests, very low-density lipoproteins (VLDL), triglycerides, and creatinine, were unchanged. Egg fortification with vitamin E and carotenoids increased plasma levels significantly ($p<0.01$).

Table 2

Plasma chemistry and FA following low-egg (2-4 weeks) and two egg/day HPUFA-regular, HPUFA-HAOX, or HMUFA-HAOX regimes (n=17)

Regime: Interval: **Measure** SI	HPUFA Week 0 2-4/d	HPUFA-Regular Week 3 2/d	HPUFA-HAOX Week 6 2/d	HMUFA-HAOX Week 9 2/d
Total Cholesterol mmol/L	4.8±0.6	5.4±1.0*	5.7±1.1*	5.2±1.1
HDL-Cholesterol mmol/L	1.4±0.2	1.2 ±0.2*	1.4±0.4***	1.4±0.2***
LDL-Cholesterol mmol/L	2.9±0.5	3.4±0.6*	3.7±0.5*	3.4±0.5
VLDL^{a}-Cholesterol mmol/L	0.6±0.3	0.5±0.3	0.6±0.3	0.6±0.2
Triacylglycerides mmol/L	1.2±0.5	1.1±0.5	1.3±0.4	1.3±0.4
Vitamin E µmol/L	23.2±0.2	23.7±4.4	33.9±9.1**	29.0±6.0**
Vitamin A µmol/L	1.9±0.4	2.0±0.5	2.7±0.6**	2.3±0.7**
Carotenoids µmol/L	2.1±0.4	2.0±0.5	3.0±1.1**	3.1±0.8**
Glucose mmol/L	4.7±0.4	5.4±0.4*	4.7±0.4***	4.7±0.4***
BUN mmol/L	4.3±1.4	5.7±0.7*	5.4±1.4	5.0±1.4
CK mg/dL	110±30	118±33*	111±45	107±32

Values are represented as mean±standard deviation

SD. * $p < 0.01$ (vs low-egg). ** $p < 0.01$ (vs HPUFA-regular). *** $p < 0.05$ (vs HPUFA-regular).

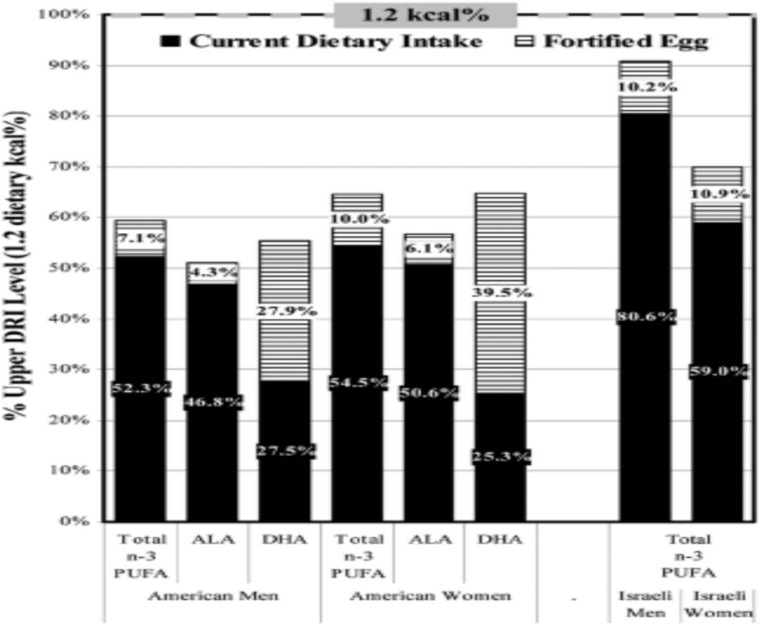

Fig. 1. n-3 PUFA in current diets and in fortified eggs as %upper DRI (kcal %) levels calculated for American (total, ALA DHA) and Israeli (total) men and women. DRI ranges: total n-3 PUFA and/or ALA = 0.6–1.2 kcal %. DHA = 0.06–0.12 kcal% of daily kcal intake for Americans [CDC, 2006] and Israelis [ICDC, 2003]. * No. information for current intake of individual n-3 PUFA among Israels is available

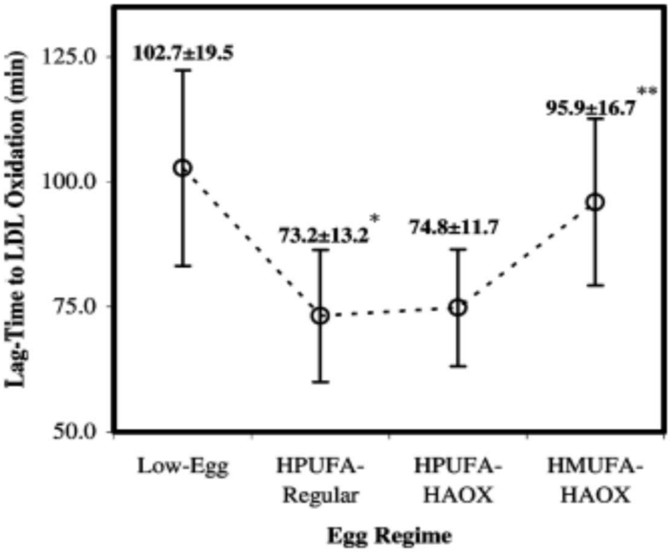

Fig. 2. Lag-time to plasma LDL oxidation following low-egg (2–4/week) and two eggs/day HPUFA--regular, HPUFA-HAOX, or HMUFA-HAOX regimes (*n* = 17).
* p < 0.01 (vs low-egg); ** p < 0.01(vs HPUFA-regular)

Discussion

Though eggs were repeatedly confirmed to be compatible with caloric restriction and CVD prevention, potential for improvement with relevant feed designed for modification may strongly suggest a composition-related approach. Moreover, a recently emerging suggested link between diabetes risk and egg intake may justify further exploration.

The substantial increase observed in egg n-3 LCPUFA with linseed fortification is in accordance with previous studies demonstrating the exceptional effectiveness of the egg for transforming ALA (18:3 n-3 PUFA) to DHA (22:6 n-3 LCPUFA), even in a high n-6 PUFA environment, and without an accompanying increase in n-6 LCPUFA, i.e. AA (Table 1). This is in contrast to low human conversion of ALA to DHA of approximately 0.05–4.0% [Burdge & Calder, 2005]. Consumption of one fortified egg (with increases in total n-3 PUFA by 3.8-fold and DHA by 2.4-fold) could make a significant dietary contribution, particularly of n-3 LCPUFA, as demonstrated by DHA content equaling 33.7 and 41.4% of the upper DRI for Americans and Israelis, respectively. Pre-formed n-3 LCPUFA is particularly important for people facing relative n-3 PUFA scarcity and enzymatic competition by high intake of n-6 PUFA LA, as n-6 and n-3 undergo transformation via the same enzyme.

The contribution of n-3 PUFA-fortified eggs can be understood following recently observed effects of moderate amounts of n-3 PUFA ALA, eicosapentaenoic acid (EPA, 20:5 n-3), and DHA [Egert et al 2009], which reduced fasting serum triacylglycerol concentrations significantly, and increased LDL EPA and DHA, as well as serum HDL cholesterol [Egert et al., 2009]. A high ALA diet was also associated with increased concentrations of the large, less atherogenic LDL1 and LDL2 subfractions [Harper et al., 2006]. High intake of n-3

PUFA, especially n-3 LCPUFA EPA and DHA, which compete with AA in meolic pathways, reducing its resultant pro-inflammatory icosanoids [Seo et al., 2005; Weisman et al., 2004], may thus be beneficial in reducing risk in a number of medical conditions – including type 2 diabetes, neurological disorders, and CVD [Hu & Willett, 2002; Lewis et al., 2000; Nettleton et al., 2004; Seo et al., 2005; Simopoulos, 2008; Weisman et al., 2004].

The consumption of n-3 PUFA-fortified eggs has recently been suggested to affect fasting glucose levels and inflammation markers as compared to standard eggs. This was shown in a double-blind cross-over study investigating addition of an extra egg/day of either standard or fortified composition for one month, where one/day of n-3 PUFA-fortified eggs was associated with higher levels of ApoA1, a lower ApoB/ApoA1 ratio, and lower plasma glucose. These effects were consistent with previous studies showing a reduced risk for cardiovascular mortality and diabetes [Ohman M et al., 2008; 18991244].

The finding that two eggs/day with reduced n-6 PUFA (LA) and increased n-9 MUFA (OA) and antioxidants (vitamin E and carotenoids) reduced LDL oxidizability – back to the low response shown with a low-egg regime (2-4 eggs/week) – shows the potential of egg modifications for affecting a known health risk (Table 2). The HMUFA-HAOX egg appears to represent a synergistic antioxidative composition with significant protective potential against plasma LDL oxidizability. The protective potential of a high-MUFA, high-vitamin E combination diet against small LDL particle oxidation [Reaven et al., 1994], had already been demonstrated.

The close relationship of LDL composition with dietary FA was previously shown following both high n-6 PUFA and high-MUFA diets [Montoya at el., 2002]. The larger proportional impact of minor FA changes (egg OA, LA ±1-3 g, ±15-25% of daily intake) on the LDL oxidation response (±30%), compared to relatively smaller changes in blood cholesterol (15-20%) following a major dietary increase (>200%), emphasizes the health significance of egg FA composition.

Interestingly, increased egg antioxidants appeared to also be associated with improved plasma glucose, BUN, and HDL, factors known to be associated with the metabolic syndrome and pre-diabetes, and thus highly relevant to the findings of increased CVD risk in diabetics and/or obese individuals. It may also be relevant to the recently suggested link between egg consumption and the increasing incidence of diabetes.

Recent findings that diabetics were exceptionally vulnerable to atherogenic processes following consumption of regular eggs (≥6/week), where non-diabetics remained unaffected [Qureshi at el. 2007], suggest the need for research regarding this potential link to metabolic risks, including dyslipidemia and conditions of increased LDL oxidation risk, such as abdominal obesity [Knopp at el., 2006] and impaired glucose tolerance [Schwab at el., 1998], and/or in diabetes mellitus [Dimitriadis at el., 1996]. The potential for modifications of egg composition targeting these specific health risks warrants research and development effort.

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3

LEGISLATION RELATING TO NUTRACEUTICALS, DIETARY SUPPLEMENTS AND FUNCTIONAL FOODS IN THE EUROPEAN UNION

Introduction

The concept of food which in addition to necessary nutrients would contain other substances with physiological effects on the human body, was first introduced in Japan in the early 1980s. These food products would not only provide ingredients required for normal development and functioning of the body, but also improve the health status of the consumers. The concept was subsequently promoted in the United States and Europe. The aim was ‘optimal nutrition’ to improve the physiological functions and enhance physical and mental well-being and at the same time reduce risk factors for disease [De Felice, 1992; Hardy, 2000; Kwak and Jukes, 2001a; Kwak and Jukes, 2001b, Andlauer and Furst, 2002; Jones, 2002; Ohama et al., 2006].

Such concept of food manufacturing was prompted by a vigorous increase in research into the physiological properties of nutrients and demonstration of their health-promoting properties on the one hand and alarming morbidity and mortality rates of so-called civilisation diseases due to improper life-style and nutrition. The arguments in favour of functional foods or nutraceuticals include their role in improving the health status and prevention of chronic disease [Hasler ,1998; Jones, 2002].

The terms ‘nutraceuticals’ and ‘functional food’ are now less commonly used in Japan, but they have been widely accepted in other industrialised countries, mainly the United States and Europe. However the very term ‘functional food’ was coined in Japan where in 1984 the Ministry of Education initiated a research project to develop its manufacture. Later the term ‘functional food’ was abandoned but the concept was abandoned but the concept was used in the system of "Foods for Specified Health Uses " or FOSHU which included regulations for control of their manufacture and safety of use [Ohama et al., 2006].

Nutraceuticals and functional foods: definitions

The term nutraceutical comes from the combination of two words "nutrition" and ‘pharmaceutical" and was introduced in 1889 by *S. DeFelice,* President of the Foundation for Innovation in Medicine, an American educational organisation encouraging discoveries in medicine [De Felice, 1992]. The name was soon accepted in biomedical research. The definition formulated by *DeFelice* [De Felice, 1992; De Felice, 1995; De Felice (http://www.fimdefelice.org/archives/arc.researchact.html) describes a nutraceutical as "any substance that may be considered a food or part of a food and provides medical or health benefits including the prevention and treatment of disease".

The category of nutraceuticals may include isolated nutrients, dietary supplements, herbal products or substances isolated from herbs (phytochemicals). Nutraceuticals may be used singly or in combinations. They are supplied in different pharmaceutical forms as tablets, capsules, syrups, powders etc and/or used as ingredients of food products. Nutraceuticals occupy a position between a food and a drug. Obviously they are not drugs and most consider them foods. They contain bioactive substances which may fortify, weaken or modulate the physiological and metabolic functions of the body with beneficial effects as they prevent chronic disease [De Felice, 1992; Hasler, 1998; Andlauer and Furst, 2002; Jones, 2002].

The concepts of functional food differ and it is difficult to formulate a good definition considering the diversity of food products which in the future may become functional foods used to improve health or prevent disease.

The International Food Information Council (IFIC) defines functional food as "providing health benefits over and above its normal nutritional values" [Barnes and Prasain, 2005].

The most comprehensive definition of functional food was described in 1998 in **the European Consensus of Scientific Concepts of Functional Foods** document produced by the European Commission's Concerted Action on Functional Food Science (FUFOSE) coordinated by ILSI Europe (International Life Science Institute). According to the definition "A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease". Additionally the claims must be "authorised and scientifically based" [Bellisle et al., 1998; Diplock et al., 1999; Roberfroid, 2000; Ashwell, 2002].

Still, 'functional food' is a concept rather than a well-defined food category. The term however has been accepted as differentiating functional food from other food categories such a nutraceuticals, pharmfood, medifood, designer food or vitafood. It does not include food supplements. It has been also more readily accepted by manufacturers and consumers than other terms proposed for foods having similar features. The definition implies that a food considered functional contains ingredients with known physiological effects, should reduce the risk of disease, but not treat it. According to the European Consensus of Scientific Concepts of Functional Foods, a functional food must have the following features:

1. it is a conventional food and thus the category includes food products for general, everyday consumption as part of a normal diet and it cannot be taken as tablets, pills, capsules, etc.;
2. apart from naturally occurring components, it should contain an active component in unnatural concentration or an additional active component it would not normally supply;
3. it should have a scientifically proven positive effect on health beyond nutritive value of consuming such food in normal amounts;
4. it should enhance well-being and health and/or reduce the risk of disease to improve the quality of life;
5. it should have authorised and scientifically based claims (evidence based on relevant biomarkers of a particular metabolic process or organ function) [Roberfroid, 2000; Ashwell, 2002].

Introduction of new terms and definitions has led to some confusion, especially as some regulations are lacking. Such terms as ‘enriched food’ or ‘fortified food’ partially fit the concept of functional food while ‘dietary supplements’ essentially differ from functional food by product form but to some extent correspond to the concept of nutraceuticals [Kwak and Jukes, 2001b]. This issue is however fairly complex and must be addressed separately.

Examples of nutraceuticals and functional foods

Nutraceuticals’ are biologically active substances with recognised health effects [Jones, 2002; Pennington, 2002; Stańczak and Ochocki, 2003; Kritchevsky and Chen, 2005]. These include dietary fibre, oligosaccharides, especially fructanes, lactic acid-producing bacteria, which are important for the digestive tract function; polyunsaturated fatty acids (fish oils), proteins, peptides, amiNo. acids, keto acids, antioxidants (including vitamins having this effect), conjugated linoleic acid (CLA), flavonoids and other phenols, carotenoids (β-carotene and lycopene), plant sterols and stanols, and stilbenes [Jones, 2002; Gosslau and Chen, 2004; Barnes and Prasain, 2005; Kritchevsky and Chen, 2005].

According to the definition given, raw fruits and vegetables are functional foods. These are e.g. broccoli and other cruciferous vegetables, carrots and tomatoes, spinach and red cabbage rich in such physiologically active substances as sulphopharane, β-carotene, lycopene or lutein. Black and green tea rich in polyphenols, soybean, oat products containing β-glucagon which reduce total and LDL cholesterol levels or cranberry juice which acts on the bacterial flora of the urinary tract may be also considered functional foods. The list of such products is fairly long [Pennington, 2002].

Production of functional foods consists of enriching food products with physiologically active substances and/or elimination and/or replacement of unwanted components (e.g. fat). The most common products of this kind are:

- fermented milk products or milk products with addition of probiotics (*lactobacillus, bifidobacterium*);
- spreads containing phytosterol and phytostanol esters which help to reduce LDL cholesterol levels and decrease the risk of cardiovascular disease (Benecol);
- beverages enriched with vitamins A, C and E or calcium and magnesium which reduce the risk of atherosclerosis and slow down ageing and osteoporosis;
- beef enriched with conjugated linoleic acid (CLA), which may prevent cancer; eggs enriched with omega-3 polyunsaturated fatty acids (EPA and DHA) which increase HDL-cholesterol levels and lower blood pressure and so decrease the risk of cardiovascular disease [Hasler, 2000; Stańczak and Ochocki, 2003; Kolanowski, 2003; Ferrari and Torres, 2003].

Functional food and nutraceuticals market

The development of functional food and nutraceuticals market varies depending on the country and local demand and has been the most dynamic in the United States and Japan. It was estimated that in 2000, the global market of functional food was 33 billion US$ with 50% of sales in the United States. Around 158 million of Americans, i.e. nearly half of the population, regularly take dietary supplements to maintain and/or improve health. The market volume of dietary supplements alone was 29.5 billion US$ in 2004 which meant that the demand had nearly doubled during the decade (from 1994). The value of nutraceuticals sold annually in the US is 30 billion US$ with a 5% increase [Adlauer W., Furst P.: 2002] which accounts for 2% of the total food market. In 2006, the estimated monetary market volume was 63.3 billion US$ and 71,9 -billion US$ for functional foods and nutraceuticals respectively [Menrad, 2003; Bagchi, 2006].

In the US, the most popular functional foods are products which reduce the risk of cardiovascular disease, such as products which contain dietary fibre of different origin, soybean proteins, sterol and stanol esters of plant origin and omega-3 fatty acids as well as products used in cancer prevention [Hilliam, 2000; Noonan and Noonan, 2006].

In Japan, in 2000, 174 products were approved under the FOSHU label and their estimated market value was around 2 billion US$. In total 1700 products were launched between 1988 and 1989. The dynamic increase of demand for the FOSHU products is demonstrated by the turnover of 5.7 billion US$ in 2005 (5). Over 90% of the FOSHU label products are used to modify function of the gastrointestinal tract followed by products lowering blood pressure and blood lipids [Hilliam, 2000].

In Europe, in 2000, the market share of functional food was around 1% of the total food and drinks market. Germany, France, The United Kingdom and The Netherlands are the leaders of the functional food market in Europe [Menrad, 2003].

The European functional food market is dominated by GI health products, especially probiotics. Another, commonly consumed group of products, e.g. in Germany, includes non-alcoholic beverages with the addition of vitamins A, C and E, and other functional ingredients. In recent years there has been an increasing demand for spreads containing phytosterol esters as well as for hypoallergic babyfood and pre- and pro-biotic products for young children [Menrad, 2003].

Legislation on health food in Japan

Such rapid development of the market of foods having health benefits required appropriate legislation to protect the consumer against various unpredictable effects on health. The earliest and fairly consistent initiative in this area was by the Japan Ministry of Health and Welfare. Several regulations concerning the manufacture and marketing of such products in 1991, 1999 and 2001 resulted in explicit legislation. In 2005, as a continuation of the FOSHU ("foods for specified health use) system, a new regulatory system was proposed – food with health claims (FNFO) [Ohama et al., 2006].

In 2005, another change was introduced by the Japan Ministry of Health, Labour and Welfare which subdivided the FOSHU category into: (1) standard GMP products, (2) qualified FOSHU products and 3. FOSHU products with claims to reduce disease risk.

European regulations on nutraceuticals, dietary supplements and functional foods

There is No. regulatory framework for nutraceuticals and functional foods in European Union Food Law. Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 (Regulation (EC) No.178/2002 of the European Parliament and the Council of 28 January 2002 –01/02/2002 str. 0001-0024). is applicable to these products. The Regulation lays down the general principles of food law within the framework of the European Food Safety, establishes the European Food Safety Authority and lays down procedures in matters of food safety. The aim of the Regulation is to "provide the basis for the assurance of a high level of protection of human health and consumer interest in relation to food.

It establishes common principles and responsibilities, the means to provide a strong science base, efficient organisational arrangements and procedures to underpin decision-making in matters of food and feed safety".

The Regulation is applicable to all foodstuffs and additionally it may be applicable to the functional properties of food (hence e.g. 'functional food', 'nutraceuticals', 'food supplements'). The application of appropriate legislation depends on the nature and properties of a foodstuff. The primary concern is food safety and that is why any potential risks for health should be evaluated including risk analysis, risk assessment, risk management and risk communication [Gulati and Ottaway, 2006; Coppens et al., 2006]

In his interpretation of the Regulation in question *Gulati* [Gulati and Ottaway, 2006] states that regulations on 'foods for particular nutritional uses' (PARNUTS) and regulations on 'novel foods' may apply to 'functional foods' depending on for how long they have been available (i.e. products authorised in the EU before 15 May 1997) [Coppens et al., 2006].

Directive 2002/46/EC (Directive 2002/46/EC of the European Parliament and of Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Official Journal L 183. 12/07/2002.str.0051–0057) on food supplements is applicable to nutraceuticals as well as the Act on Safety of Food and Nutrition of 25 August 2006 in Poland (Ustawa o bezpieczeństwie żywności i żywienia z dnia 25 sierpnia 2006 r. Dz. U. z 2006 r. Nr 171, poz. 1225). A food supplement is defined there as "a foodstuff the purpose of which is to supplement the normal diet, which is a concentrated source of vitamins and minerals or other substances with a nutritional or another physiologic effect, alone or in combination, marketed in dose form such as capsules, tablets, pastilles and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles and other similar forms of liquids and powders designed to be taken in measured small unit quantities with the exclusion of medicinal products as defined by pharmaceutical law".

Although, like food supplements nutraceuticals are considered foodstuffs, the questions remains of differentiating products used for medical indications from supplements indicated for nutritional uses. An important consideration when trying to establish similarities between nutraceuticals and food supplements is the requirement that both groups of products should contain substances with a nutritional or physiologic effect and according to food law supplements and nutraceuticals alike should be available as concentrates to be consumed in measured small unit quantities. The EU regulation controls the first stage of the harmonisation process which concerns vitamins and minerals. For other substances used as food supplements, the EU Committee is obliged to submit its conclusions to the European Parliament and the Council. At present, the European Union agencies are involved in work on determining the minimum and maximum amounts of active substances in dietary supplements (vitamins, minerals) and on the definition and information that can be included in the labeling of food products, also dietary supplements , nutrition and health claims and positive lists of such claims.

The report presents specialist opinions on the role of these substances and lists of substances for which approval should be sought for other categories of substances with physiological and nutritional effects [Coppens et al., 2006].

When a harmful effect on health of a functional food or nutraceutical has been identified, it should be banned. When there are suspicions of a harmful effect of any active substance, it should be placed on a list of dangerous compounds and re-authorised only when there is scientific evidence and experts' opinions confirming the safety of its use.

Although dietary supplementation is an effective way of dealing with deficiency of nutrients, it carries some potential risk resulting mainly from errors of supplements. Long- term supplementation without modifying the usual diet actually leads to decreased consumption of many substances naturally occurring in foodstuffs,including those whose role in the human body has not been yet fully understood and documented. The individual use several products in combination with the resulting risk of exceeding upper acceptable limits of intake.

In Europe, based on the data on current consumption of nutrients with non-fortified products, fortified products and water, it has been estimated that there is No. risk for exceeding the upper limits for intake of vitamins B_1, B_2, B_{12}, biotin, pantothenic acid and vitamin K. Nutrients, whose recommended intake may be exceeded by using supplements include vitamin A and β-carotene, calcium, copper, fluorine, iodine, iron, manganese and zinc. Such risk is due to different factors including excessive intake even in normal diet (vitamin A), the pro-oxidative effect in smokers (β-carotene), increased susceptibility in kidney disease (calcium) and a potential anatagonistic effect when the intake of other nutrients is low (calcium, zinc) [Rozporządzenie Ministra Zdrowia, 2007].

In 1998, a report was submitted on a study carried out on behalf of the European Commission by the European Association of the Self-Medication Industry (AESGP) which concluded that there was No. agreement between the EU member states concerning indications for use and identified medicinal effects of the same products of plant origin [Gulati and Ottaway, 2006].

Legislation on nutraceuticals of plant origin in the European Union

Another important issue is legislation on the use of nutraceuticals of plant origin (herbs). It is rather complicated because of the composition and effects of such products. There are problems related to obtaining the raw material and then the quantitative and qualitative identification of a physiologically active substance, its appropriate isolation, and conducting *in vitro* and *in vivo* studies to demonstrate its health effect and determine safe dosage range. Such product must be of good quality, safe and effective. Botanical materials are used in most nutraceuticals. Botanicals are whole or fragmented plants, algae, fungi, lichens and/or botanical preparations from these materials obtained by extraction, distillation, fractionation, purification, concentration and other processes. In the European Union countries, the regulatory status of botanical-sourced nutraceuticals is diversified. In some countries they are sold as foodstuffs or included in the category of functional/fortified food or are considered food supplements and thus have No. health claims. In other countries, these products are considered medicinal herbs and the marketing authorisation may be obtained after a rigorous registration process [Gulati and Ottaway, 2006].

According to the regulations now in force botanical-sourced nutraceuticals may added to e.g. functional food based on the Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients (Ustawa o bezpieczeństwie żywności i żywienia z dnia 25 sierpnia 2006 r., Dz. U. z 2006 r. Nr 171, poz. 1225), ratified by the EU Standing Committee in 2005. The regulation is applicable to such foods used in the EU before 15 May 1997. They may continue to be sold but only in the same food category as up to 1997. If the manufacturer wants to change the category of food and add the active substance to a food belonging to another category, e.g. a beverage, they should seek a new authorisation based on toxicological studies. If a herb-sourced substance was used in a food supplement or a nutraceutical before 1997, then the dossier would probably need to be revised and completed (additional data submitted) before the addition of such substance to food is authorised. The amount and type of an active substance in a nutraceutical which is used as a food supplement must correspond to its amount in a natural diet and cannot be classified as 'medicinal by function' since such categorising precludes its use in foodstuffs [Coppens et al., 2006].

The ongoing work on legislation concerning medicinal plants, in particular herbs, used as food supplements is expected to be completed by 2012. The establishment of positive lists of herbs which may added to food and of negative lists of herbs banning their use in foodstuffs has been proposed.

Problems related to the use of ingredients obtained from plants, especially those containing active substances, are diverse. Their potential interactions with drugs or supplements must be taken into consideration. In general, doctors and patients alike do not have sufficient knowledge of the activities of particular ingredients in food supplements or nutraceuticals and are not aware of potential adverse effects related to their use. There are also substances which are safe at low doses but may be toxic when taken in larger quantities. Such was the case of *Ephedra* used in traditional Chinese medicine in low and therefore nontoxic doses. In the United States, *Ephedra* was authorised under the DSHEA as a food supplement used to reduce weight. However young athletes took the supplement in excess of the prescribed doses, sometimes with fatal effects. As a result the product was banned in 2004 [Barnes and Prasain, 2005].

When considering nutraceuticals of botanical origin, it is important to remember that often they contain a single ingredient isolated from plant material. Not infrequently studies of their efficacy in which a purified botanical is used are disappointing. A well-known example are clinical trials of β-carotene which was expected to prevent lung cancer. In such studies potential interactions between components of the diet should be taken into consideration [Barnes and Prasain, 2005].

Studies of safety of use of nutraceuticals and functional foods containing botanical ingredients

Safe use of such foods requires both *in vitro* (isolated cells, microorganisms, enzymes, receptors and DNA) and *in vivo* studies (studies of acute and repeated dose toxicity, genotoxicity and toxicity to reproduction) which may disclose potential hazards to humans. Also of importance are clinical studies focusing on adverse effects of substances. Epidemiological studies are required, in particular regarding the use of functional food [Kruger and Mann, 2003; Coppens et al., 2006]. Efficacy studies are also conducted, to verify the manufacturers' claims in the labelling and advertisements. Effective consumer protection requires careful scrutiny pf nutritional and health claims in advertising and such claims must be based on generally accepted scientific evidence [Ashwell, 2002; Coppens et al., 2006]. The final data on the effects of functional foods should be verified by thorough analyses of the study methods and selection of study groups, reliability and repeatability of studies, and critical assessment of conclusions [Ashwell, 2002; Kruger and Mann, 2003].

Three groups of biomarkers are used in the assessment of functional ingredients: indices of the systemic exposure to the ingredient, markers of the body's biological response to the ingredient, and markers of the health effect of the ingredient, which are the most important.

The Indices of the systemic expose give information about the substance levels in the body. Markers of the body's biological response to active ingredient provide information about its target effect, e.g bone mineral density as an indicator of consumption of calcium-containing products. Markers of the health effect show changes in the physiological process related to the expected effect, e.g. decreased BMI as evidence of the reduced risk of obesity as a result of a diet which contains such ingredient [Ashwell, 2002].

The future of nutraceuticals and functional foods

The present volume of nutraceuticals and functional food production and demand for such products indicate further vigorous development in this area should be expected. There is great interest from both consumers and manufactures but there is No. consistent and uniform legislation on these products. Scientific research is expected to play a significant role in the further development of nutraceuticals and functional foods.

Many ingredients of functional foods have recognised health benefits, but little is known about the physiological health-promoting effects of even more compounds or they have not been fully understood. It is necessary to develop methods to identify these substances and to find biomarkers to determine their effects in the human body. The future of functional foods and nutraceuticals depends on quality control and good manufacturing processes and safety of use relying on a strong science base, establishing recommended allowances for nutraceuticals to prevent adverse affects and continuous improvement of food safety control [Noonan and Noonan, 2004].

There are also some concerns about the widespread use of functional foods and nutraceuticals, which according to some authors contains to many and too much nutritive and functional ingredients. That is why more studies are needed to find out which food supplement contain biologically active substances, what amounts of these ingredients are beneficial and what amounts may be harmful to people of different ages, healthy individuals and patients suffering from different diseases. This is especially important in the case of ingredients which should be taken regularly and for longer periods of time [Erbersdobler, 2002].

While functional food will be manufactured mostly by the food industry, manufacturing nutraceuticals will be essentially the responsibility of the pharmaceutical industry, with regulation by pharmaceutical rather than food law. Further development of such products will result in new legislation to protect the consumer.

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4

ENRICHMENT OF FISH OIL IN BIOACTIVE FATTY ACIDS EPA AND DHA AND ITS APPLICATION IN ORDER TO DECREASE LIPID INDICES OF BLOOD

Introduction

The number of people hospitalized and dying from circulatory system diseases like atherosclerosis, coronary diseases, heart failure or stroke has increased rapidly in last years. According to medicine doctors one of the reasons of that phenomenon is an improper diet that results in an increase in a level of triglycerides and cholesterol in blood [Temple, 1996].

Cholesterol plays very important role in human's organism. However it appears that its excess that is accumulated in walls of vessels may be dangerous and even harmful. Since cholesterol is not soluble in watery blood plasma, its transporting function is played by proteins that are connected to it forming so called VLDL, LDL, HDL lipoproteins [Ziemiański and Budzyńska-Topolowska, 1991]. A strong atherogenic activity is currently attributed to LDL fraction since its high level in blood serum favours a deposition of cholesterol in cells of blood vessels creating thus conditions for atherosclerosis development [Halpern, 1995]. LDL lipoproteins influence also thrombocytes increasing their aggregation abilities, that leads to the formation of microclots that atherosclerotically block contracted parts of arterial vessels [Halpern, 1995]. LDL fraction is also the main carrier of lipid peroxides that inhibit a synthesis of a prostacyclin – the most active inhibitor of thrombocytes aggregation [Ziemiański and Budzyńska-Topolowska, 1991].

The study conducted mainly in developed countries of Western Europe, USA and Japan proved that the food, except the nutritional function, i.e. supply of basic building and energetic components, may also play a role in health status improvement [Diplock et al., 1999]. Certainly, fish oils may be included to functional food due to very beneficial profile of fatty acids, i.e. uncommon in other kinds of fats content of omega-3 acids, especially EPA and DHA ones [Kolanowski, 2007].

An increased interest in fish oils as a source of omega-3 acids started from the observation that indigenous people from Greenland rarely suffer from cardiovascular disease (CVD), and their diet consist mainly of fat fish and sea animals of high content of eicosapentaneoic (EPA) and docosahexanoic (DHA) acids [Connor, 2000]. The results of epidemiological and experimental studies provided some further evidence of profitable activity of omega-3 acids in CVD prevention [Arens, 1997; De Lorgeril and Salen, 2006; Jacobson, 2006; Psota et al., 2006; Robinson and Stone, 2006; Schmidt et al., 2006; Colhoun, 2007; Cundiff et al., 2007; Von Schacky and Harris, 2007; Yokoyama et al., 2007; Zatsick and Mayket P, 2007; Harris et al., 2008; Saito et al., 2008]. In 2002 the American Heart Association (AHA) published a scientific statement approving an application of omega-3 acids in primary and also secondary CVD prevention [Kris-Etherton et al., 2002].

An increase of the interest in food as one of ways of well health status maintaining is currently observed all over the world. Functional food is a subject of a growing interest among consumers in Poland as well. The aim of the present paper was an enrichment of fish oil obtained from muscular tissue in biologically active omega-3 fatty acids (including EPA and DHA ones) and its application in order to reduce atherogenic lipid indices of blood in rats.

Material and methods

Part I. Obtaining of fish oils of elevated content of omega-3 acids (EPA + DHA).

Research material was oil obtained from muscular tissue of fat Atlantic mackerel (*Scomber scombrus*) (Table 1).

After filleted, skinned and mixed, the mackerel was subjected to lyophilization. Fat extraction from lyophilized samples was done using a mixture of solvents hexane/acetone (4:1 *v/v*) in Soxtek Avanti apparatus. After careful removing of solvent, the oil was protected against oxidation processes by ascorbic acid addition, and then soaped under reflux condenser in nitrogen stream using ethanol solution of potassium hydroxide. Oil in amount of 100 g was weighted to a flask, and 0.8 g of ascorbic acid, 264 ml of 96% ethanol and 23 g of potassium hydroxide dissolved in 44 ml of deionizated water was added. Saponification process was conducted for 1 hour in a temperature of solution boiling. The flask was cooled, and 200 ml of distilled water was added. The content of the flask was moved to the two dividers (about 270 ml to each) and 200 ml of hexane was added to them. Upper layers containing unsaponified residues were removed, and lower ones containing salts of fatty acids were turned back to the divider and extracted with 200 ml of hexane. Next, upper layers were removed again, and lower ones were acidified to pH 1 using 6 M HCl (water solution) and were transferred to dividers. Free fatty acids were obtained in a result of three-fold extraction with hexane (100 ml for three times). Free fatty acids obtained were then subjected to complexing with urea process. The method of fish oil enrichment in omega-3 acids was chosen basing on literature data [Wanasundara and Shahidi, 1999], however it was modified on a basis of the own laboratory tests. The ratio of an amount of urea added to amount of free fatty acids (from 4.5 to 3) and also time and temperature of (from 24 hours in temperature of -10°C to 5 hours in room temperature, and 18 hours in temperature of 4°C) were modified.

A determination of fatty acids profile in raw mackerel oil and after its enrichment was done on gas chromatograph Agilent Technologies 6890N equipped with FID detector and 100 m long capillary column Restek RT-2560 in the presence of standard mixture of 37 fatty acids. In order to conduct the analysis, 2 μl of the sample was proportioned in split mode 100:1. The temperature of a dosimeter was 250ºC, while of a detector 260ºC. Helium was used as a carrier gas, and nitrogen as screening one. The flow of carrier gas through the column was 1.1 ml/min. Temperature schedule of the furnace: initial temperature 140ºC (2 min.), next an increase of temperature in a rate of 2ºC/min to 225ºC, and 10 min isotherm, increase of 40ºC/min to 240ºC, and 10 min isotherm.

In order to protect polyunsaturated fatty acids (PUFA), mainly omega-3 ones (EPA and DHA) against unprofitable oxidation processes, 200 mg of α-tocopherol was added to 100 g of enriched mackerel oil.

The process of fish oil enrichment in omega-3 acids, its protection against oxidative changes and chromatographic analysis were done in an accredited laboratory of Sea Fisheries Institute in Gdynia.

Part II. The study on laboratory animals concerning an influence of an application of fish oil enriched in omega-3 acids on decrease of blood lipid indices.

The research material were 80 rats of Wistar strain, coming from homozygous breeding in Medical University of Warsaw. Animals were randomly divided into 4 groups, 20 animals in each, and were kept individually in cages in Vivarium of Department of Veterinary Medicine, Wrocław University of Environmental and Life Sciences. All rats were fed with Labofeed B fodder, and animals from experimental groups were additionally given pork lard (mixed in loose fodder and given in a form of balls) and/or enriched fish oil (given to a muzzle using a syringe), according to following scheme:

- **I** – control (only Labofeed loose fodder) ;
- **II** – loose fodder + addition of pork lard in amount of 7.5 g/head/day;
- **III** – loose fodder + an addition of pork lard (7.5 g/head/day) for 3 weeks, and at the same time (preventively) fish oil enriched in omega-3 acids in amount of 1 ml/head/day;
- **IV** – loose fodder + an addition of pork lard (7.5 g/head/day). After lard discontinuance, 1 ml/head/day of fish oil enriched in omega-3 acids for 3 weeks (therapeutically).

According to methodological assumptions, the blood from *vena saphena lateralis* (*vena saphena lateralis*) was collected from all rats at the beginning and at the end of the experiment in each experimental group, i.e. after 3 weeks in the case of group I, II and III, and after 6 weeks i.e. the case of group IV. Blood analysis covered the content of triglycerides, total cholesterol and its LDL and HDL fractions. Above analysis were done in Vetlab laboratory using an enzymatic colourimetric test on Cobas Mira S apparatus using following reagents: LT-sys (triglycerides), Ellitech (cholesterol) and Bio-Systems (HDL and LDL).

Results and discussion

Since the mackerel comes from an Atlantic reservoir, its fat is characterised by a low content of stable organic compounds, especially dioxins and dioxin-like polychlorinated biphenyles (dl-PCB). Moreover, muscular tissue of that fish contains relatively high amount, i.e. about 22% of fat (Table 1), and oil obtained from that fish is characterised by less intense smell comparing to oil obtained from Baltic fish during fishmeal production [Usydus, 2005].

Table 1

Basic composition of muscular tissue of Atlantic mackerel (X±SD)

Fat [%]	Protein [%]	Dry matter [%]	Ash [%]
22.2±0.4	17.3±0.2	40.4±0.3	1.1±0.1

The process of complexing with urea that mackerel oil was subjected to (3 trials in modified conditions) cause a decrease in percentage content of saturated fatty acids (SFA) from 28.9 to 0.3%, and an increase in the content of polyunsaturated ones (PUFA) from 40.8 to 97.7% in a lipid concentrate obtained (Table 2). In a group of polyunsaturated fatty acids, the content of omega-3 fatty acids increased from 35.5 to 90.4%, and EPA + DHA acids from 23.9 to 70.7% (Table 2).

Table 2

Fatty acids profile of fish oil before and after the process of complexing with urea (X±SD)

Fatty acids	Raw oil [%]	After complexing with urea process [%]
Σ Saturated (SFA)	28.9±2.2	0.3±0.0
Σ Unsaturated (UFA) including	71.1 ± 2.6	99.7 ± 2.4
Σ Monounsaturated (MUFA)	30.3±2.9	2.5±0.4
Σ Polyunsaturated (PUFA)	**40.8±2.5**	**97.2±3.55**
n-3	35.5±2.1	90.4±3.2
n-6	5.3±1.2	6.8±1.21
n-3/n-6	6.7	13.3
EPA+DHA	23.9	70.7±2.3

Analysing in turn the changes in the content of particular fatty acids it was observed that the majority of saturated fatty acids (SFA) was removed from mackerel oil during the process of complexing with urea, only miristic, palmitic and stearic acids remained there in trace amounts (Table 2a). Moreover, the content of all monounsaturated fatty acids (MUFA), mainly oleic and eicosanoic ones, decreased significantly (Table 2a). An increase in the concentration of polyunsaturated fatty acids (PUFA) mainly biologically active ones - EPA (over 2.5-fold) and DHA (3-fold) (Table 2a) was noted.

The lipid preparation obtained as a result of the modified complexing with urea process is characterized by higher concentration of EPA and DHA acids as compared to capsulated diet supplements avaliable on the market.

About 42% of EPA+DHA was in turn obtained in the process of mackerel oil freezing out (Final Report). Lower concentration of omega-3 acids was obtained also in the case on an enzymatic method (to about 45.%), chromatographic adsorption, molecular distillation or an extraction in a supercritical state [Shahidi and Wanasundara, 1998].

As a result of the complexing with urea, a considerable increase in a ratio of omega-3/omega-6 acids was observed in the preparation obtained (from 6.7 to 13.3) that is very profitably from health point of view (Table 2).

Table 2a

Fatty acids profile of fish oil before and after the process of complexing with urea (X±SD)

Fatty acids	Raw oil [%]	After complexing with urea process [%]
SFA		
Lauric C12:0	0.05 ± 0.01	–
Myristic C14:0	9.90 ± 1.10	0.1 ± 0.00
Pentadecanoic C15:0	0.60 ± 0.03	
Palmitic C16:0	15.05 ± 1.50	0.1 ± 0.00
Heptadecanoic C17:0	0.40 ± 0.05	–
Stearic C18:0	2.50 ± 0.40	0.1 ± 0.00
Arachidic C20:0	0.2 ± 0.02	–
Behenic C22:0	0.10 ± 0.02	–
Tricosanic C23:0	0.10 ± 0.01	–
MUFA		
Myristoleic C14:1	0.06 ± 0.02	0.01 ± 0.01
Palmitoleic C16:1 n7	4.10 ± 1.50	1.60 ± 0.0
Oleic C18:1 n9	9.84 ± 0.90	0.60 ± 0.1
Eicosanoic C20:1 n9	12.80 ± 1.10	0.24 ± 0.05
Erucic C22:1 n9	2.30 ± 0.6	0.05±0.01
Nervonic C24:1	1.20 ± 0.04	–
PUFA		
Linolelaidic C18:2 n3	0.10 ± 0.00	0.20 ± 0.02
Linoleic C18:2 n6 (LA)	3.30 ± 0.30	4.00 ± 0.30
γ- linolenic C18:3 n6	0.20 ± 0.00	0.30 ± 0.03
α- linolenic C18:3 n3 (ALA)	4.00 ± 0.90	2.70 ± 0.30
Stearidonic C18:4 n3	4.60 ± 0.70	13.90 ± 1.10
Eicosadienic C20:2 n6	1.10 ± 0.20	1.20 ± 0.05
Eicosatrienic C20:3 n6 c8,c11,c14	0.10 ± 0.02	–
Eicosatrienic C20:3 n3 c11,c14,c17	0.60 ± 0.01	–
Arachidonic C20:4 n6	0.60 ± 0.07	1.30 ± 0.30
Eicosapantaenoic (EPA) C20:5 n3	7.60 ± 1.60	19.20 ± 1.80
Docosapantaenoic (DPA) C22:5 n3	2.10 ± 0.40	2.90 ± 0.80
Docosahexaenoic (DHA) C22:6 n3	16.50 ± 1.50	51.50 ± 2.20

According to scientists, a profitable activity of unsaturated fatty acids is first of all connected to a proper a ratio of consumed acids from omega-6 and omega-3 family that should be 6–4:1, with an average daily consumption of essential unsaturated fatty acids (EUFA) on a level of about 8 g [Hu, 2001; Simopoulos, 2002; Harris et al., 2006]. A maintaining of such a ratio profitably influences immunological system, conduces to an improvement of an overall health status, decreases a risk of cardiovascular system diseases, and tumours.

Some food components, besides cholesterol and LDL lipoproteins described earlier, also take part in a development of atherogenic changes in arteries and incidence of circulation system diseases. The high content of saturated acids in a diet, that come mainly from animals fat, is a factor causing an increase in all lipid indices of blood, and thus exhibits a strong atherogenic activity [Weintraub, 2002]. That observation was also confirmed in the present study. Rats fed with a high-fat diet rich in saturated fatty acids were characterised by considerably higher level of all atherogenic lipid indices of blood comparing to rats from the control group (Table 3). The level of triglycerides, total cholesterol and LDL cholesterol in a group receiving an addition of lard only (group II) was higher of 77, 46 and 120%, respectively (all differences significant statistically), in a group receiving lard and at the same time, preventively, fish oil enriched in ω-3 acids (group III) of 31%, 18% and 68%, respectively, (all differences highly significant statistically), while in the group where the level of those indices was first elevated on a nutritional way (lard addition) and then an addition of fish oil enriched in ω-3 acids was applied therapeutically (group IV), of 41, 22 and 81%, respectively (all differences highly significant statistically).

Table 3

The results concerning lipid indices of blood of rats (nmol/l)

Groups	Lipid indices [nmol/l]			
	Triglycerides	Total cholesterol	LDL cholesterol	HDL cholesterol
I	1.199^{Aa}	2.231^{Aa}	0.325^{A}	1.043^{a}
II	2.119^{Bb}	3.258^{Ba}	0.715^{Ba}	1.174^{b}
III	1.568^{Cc}	2.641^{C}	0.546^{Cb}	1.158
IV	1.692^{Cd}	2.730^{Ca}	0.588^{Cc}	1.094

However, the results concerning differences between the group fed with high-fat diet (pork lard) (group II) and groups receiving the fish oil enriched on omega-3 acids (groups III – IV) (Table 3) were more important in the present experiment. An application of fish oil enriched in omega-3 fatty acids in a preventive form (group III), caused a decrease in the content of triglycerides, total cholesterol and LDL cholesterol of about 26, 19 and 24%, respectively ($P \leq 0.01$), comparing to the group II. In turn, and application of an addition of enriched fish oil in a therapeutic form (group IV), caused a decrease in the content of triglycerides, total cholesterol and LDL cholesterol of about 20, 16 and 18%, respectively ($P \leq 0.01$), comparing to the group II (an addition of lard only). Any statistical differences between these groups were however noted in a range of the content of HDL cholesterol fraction (Table 3).

The profitable influence of fish oils or biologically active EPA and DHA fatty acids contained in them is also confirmed by the results of other authors studies [Davidson et al., 1991; Silva et al., 1996; Clandinin et al., 1997; Harris, 2001; Balk et al., 2006; Davidson 2006; Castro et al., 2007; Henderson et al., 2008 Chang et al., 2009; Wergedahl et al., 2009].

The results of the present study prove moreover, that preventive, i.e. together with high-fat diet rich in saturated fatty acids, application of fish oil enriched in omega-3 acids is more profitable in respect of the decrease in all atherogenic lipid indices of blood, comparing to its application in therapeutic form, i.e. in animals that have already their high level.

Conclusions

The supplementation of a diet with oils rich in omega-3 acids is currently more and more often used as an alternative for a diet rich in fish. Oils from fish livers (e.g. cod or halibut) containing about 30% of EPA + DHA acids and about 30% of saturated fatty acids are usually available in pharmacies. An application of such kinds of supplements may be connected with a risk of vitamin A and D overdosing, and unprofitable influence of increased consumption of cholesterol and saturated fatty acids and also the presence of undesirable toxic compounds in the supplements. Thus, more and more often oils from muscular tissues of sea animals containing concentrated from of omega-3 PUFA instead of oil from fish livers are used. Such a preparations are mostly free of saturated and monounsaturated fatty acids, thus are much better supplements that oils from sea organisms since they allow to maintain a daily overall fats consumption on as low level as possible. An application of concentrated forms of omega-3 fatty acids is also of a big significance in the processes of food enrichment (e.g. yogurts and margarines). The application of oils of an increased concentration of EPA and DHA acids allows to obtain a product of considerably higher content of biologically active fatty acids beneficial for health, with the same oil dose. That is important since with higher dose of fish oil, some unprofitable sensoric changes may be observed in the enriched product [Kolanowski, 2007].

Due to an application of modified method of complexing with urea, the authors of the present paper managed to remove saturated fatty acids and most of monounsaturated ones almost entirely from oil obtained from muscular tissue of mackerel. That resulted in almost 3-fold increase of omega-3 acids concentration, including mainly biologically active EPA an DHA ones. Moreover, also all contaminations classified as xenobiotics (such as polychlorinated biphenyls or dioxins) and other undesirable substances were removed with unsoaped fraction from fish oil during the process of complexing with urea.

An application of those supplement in rats fed with high-fat diet rich in saturated acids, resulted in a reduction of all atherogenic lipid indices of blood. That points the possibility of that supplement application in a prevention and treatment of atherosclerosis and some circulatory system diseases. Since an obtained fraction of fatty acids is thoroughly safe as regards the content of toxic organic substances, the supplement may be also applied in humans.

The study concerning an influence of bioactive lipid complex (BLC) worked out by the authors of the paper on reduction of blood lipid indices, adipose tissue and inhibition of neoplastic cells proliferation, are currently being conducted. The complex is formulated just on a base of fish oil enriched in EPA and DHA acids, grape oil enriched in isomers of linoleic acid 9c,11t and 10t,c12, and sheep milk fat enriched in isomer of linoleic acid c9t11 and oleic acid t11.

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5

THE EFFECT OF FEEDING OF THE LAYERS ON THE FATTY ACIDS AND PHOSPHOLIPIDS CONTENT IN THE YOLK

Introduction

Besides genetic and environmental factors, the nutritional habits and pro-health quality of food influence the health of human beings. Improper style of life and bad feeding habits result in numerous diseases, such as diabetes, diseases of the immune and circulatory systems, obesity, various diseases of alimentary tract, some tumours or osteoporosis [Linko and Hayakawa, 1996; Horrocks and Yeo, 2000; McNamara, 2000; Kris-Etherton et al., 2002; Calder, 2004; Simopoulos, 2006;].

This results in an increased interest of consumers in food products enriched with bioactive substances characterised by the desired, directed effect on human body, increasing the effectiveness of head and manual work, improving the state of mind, good health, slowing down the process of ageing and protecting from numerous civilisation diseases [Daniewski et al, 1997; Balas et al., 2004].

Functional food is an example of food bringing about specific physiological effects of the human body. The increased pro-health quality of functional food results from the presence of one or several bioactive substances in it and from the proper proportions of its components [Horrocks and Yeo, 2000; Świderski and Waszkiewicz-Robak, 2005].

The present knowledge and practice makes it possible to enrich eggs in a natural way. Polyunsaturated fatty acids (PUFA), vitamins A, E, D and such microelements as selenium, iodine or silicone are introduced into eggs as a result of specific feeding of layer hens. It is also possible to produce eggs containing antibodies (IgY immunoglobulins) which help fight microorganisms [Trziszka, 2002a; Trziszka, 2002b; Świątkiewicz and Koreleski, 2007].

In the above context, apart from their traditional nutritional function, eggs take the role of nutraceuticals, i.e. pro-health food products and material for the production of diet supplements. The possibility of modification of the content of fatty acids, esp. increasing the content of n-3 polyenic fatty acids and introducing them into the structure of phospholipids is very interesting for scientists [Van Elswyk et al., 2000; Anton et al., 2006; Froning, 2006; Sparks, 2006]. Research studies in this direction are extremely challenging for science.

Triacylglycerols (63.1%) and phospholipids (29.7%) dominate among the lipids contained in egg yolk. The content of free and estrified cholesterol in egg yolk fat is ca. 6.2% [Pisulewski, 2000]. Contemporary knowledge about lipids obtained from plants and animals make it possible to identify fats not only as compounds providing energy, but also as substances with numerous biological functions important for proper functioning of human body.

From the physiological point of view, phospholipids and fatty acids play a special role. Lecithin, constituting as much as 75% of phospholipids in egg yolk, is the main source of choline which is important in many metabolic processes. It takes part in transformations of methyl groups and is a substrate for building neurotransmitters [Ishinaga et al., 2005; Fischer et al., 2005]. Human body is able to synthesise small amounts of choline which are, however, not sufficient to cover the daily demand. Insufficient amounts of choline may lead to health disorders in such organs as, e.g. liver. The cholesterol-phobia so visible a few years ago led to lowered consumption of meat and eggs, which resulted in deficiency of this compound in persons who limited the consumption of products containing fats of animal origin.

Polyenic fatty acids are characterised by protective functions, including anti-atherogenic and anticancer functions [Simopoulos, 2006]. The average content of fatty acids in an egg is 27 g/100 g egg yolk, monounsaturated acids (MUFA) – ca. 47% and polyunsaturated acids (PUFA) – 22% are dominant, whereas the remaining 31% are constituted by saturated fatty acids (SFA) [Rudnicka, Dobrzański, 2002].

Hen eggs belong to low-calorie and low-fat group of food products. The content of two eggs (ca. 100 g) covers most of the daily demand of an adult for basic nutritional compounds, esp. amino-acids and some vitamins. Due to the content of desirable full-value substances, hen eggs are especially recommended for young persons, who are growing up and whose organs are developing. The material, a source of essential biological substances, is also recommended for elderly persons as it helps maintain good body condition [Pisulewski, 2000].

The present study is an attempt at the assessment of the influence of various feed additives used with standard feed mixture for layer hens and of their influence on the profile of fatty acids and phospholipids contained in egg yolk.

Materials and methods

Eggs obtained from Lohmann Brown layer hens in 40–50 weeks of life, kept in bedding system, were the experiment material. The layers were fed with standard feed (control group) and with feed additives, including plant fats and fish oil (Experiment group I) and enriched with herbs and algae (Experiment group II).

Eggs, 60 pcs/group, were collected in three age intervals – 40th, 45th and 50th week of hens' life. A total of 540 eggs was used.

Determination of fatty acids profile

Extraction of fat from 2 g egg yolk was conducted according to a modified Folch method (1957) using a 2:1 solution of methylene and methanol. 50 mg fat was weighed and added to 0.5M of NaOH in methanol. The mixture was then heated in a reflux condenser at boiling temperature, then 14% of BF_3 solution in methanol were added and heating was performed again. After the process of heating, hexane and saturated NaCl solution were added. After drying with ahydrous magnesium sulphate, the organic phase was analysed using a gas chromatographer.

The profile of fatty acids was performed using a gas chromatographer coupled with a mass spectroscope (GC/MS Agilent 6890N, 5973 MS detector).

Conditions of chomatographic analysis
Chromatography column – DB 225MS
Carrier gas – He, flow – 1.0 ml/min
Feeder temperature – 230^{o}C
Detector temperature – 240^{o}C.

Isolation of phospholipids from hen egg yolk

Extraction of phospholipids from homogenous yolk was performed using 96% EtOH. 30 cm^3 (m/v) of alcohol were poured over 10 g of yolk and shaken for 1h. The process was repeated three times, extraction was preceded by centrifuging and collection of supernatants in a tared round-bottom flask. The supernatant was evaporated to ca. 5 cm^3 using a vacuum evaporator (Büchi Rotavapor 461) at the negative pressure of 77 mbar and bath water temperature of 45^{o}C. The condensed mixture of phospholipids was quantitatively transferred to polypropynele tubes placed in a vacuum Labconco concentrator to steam dry the solvent. The phospholipids were then used to analyse the purity (expressed as a substance not soluble in acetone), the composition of phospholipid fraction (phosphatidylcholine and phosfatydyloetanoloamin) and the profile of fatty acids.

Determination of the content of phospholipid groups using HPLC equipped with a crown detector

The distribution of phospholipids in high-pressure liquid chromatography (HPLC) was conducted using a modified Shefiq-ur-Rehman method (1991).

Elution solution of acetonitrile/methanol/phosphoric acid (100:10:1.8 v/v/v) was prepared, and it was then out-gassed with a vacuum pump. In the later part of the experiment, the column was balanced by pumping the elution solution through it for 30 min. at the flow of 1.5 ml/min, the detector was set for 203 nm.

The extract was then transferred to a test tube and the solvent was out-gassed in a stream of nitrogen. The dry fraction was then dissolved in a hexane-isopropanol mixture, so that the lipid concentration equalled their concentration in the original extract. The process was conducted for ca. 20 min.

Results and discussion

Specialists in nutrition, medicine and egg production technology emphasise the culinary and biological value of eggs which are products of high nutritional value. [Trziszka, 2002]. They have always been a source of protein, vitamins, mineral components and unsaturated fatty acids. Egg protein is characterised by favourable content of amino-acids and is highly digestible [Świątkiewicz and Koreleski, 2007].

From the technological point of view, eggs are an important and often indispensable ingredient of numerous dishes, which is conditioned not only by their chemical composition, but functional qualities as well [Trziszka, 2002a,b].

The physical properties and chemical composition of hen eggs depend on many factors. The origin of birds, type of feeding, age and the system of breeding are the key factors conditioning the nutritional value of eggs [Lewko i in., 2007].

The results of the analysis of the profile of fatty acids and phospholipids are presented in Tables 1–4.

Table 1

The content of saturated and monoenic fatty acids in egg yolks from various feeding groups [in %], n = 3

Fatty acid	Feeding group		
	Control (C)	Experiment plant oils and fish oil (I)	Experiment herbs and algae (II)
C14:0	0.18	0.29	0.27
C15:0	0.05	0.09	0.09
C16:0	25.40	23.60	24.41
C17:0	0.16	0.23	0.22
C18:0	7.88	8.14	8.01
C20:0	0.04	0.04	0.02
C22:0	0.05	0.05	0.00
∑ saturated	**33.75**	**32.42**	**33.02**
C16:1	2.49	2.43	2.16
C17:1	0.06	0.19	0.06
C18:1	40.68	41.58	42.58
C20:1	0.20	0.32	0.23
∑monoenic	**43.42**	**44.56**	**45.03**

Table 2

The content of polyenic fatty acids in egg yolks from various feeding groups [in %], n = 3

Fatty acid	Feeding group		
	Control (C)	Experiment plant oils and fish oil (I)	Experiment herbs and algae (II)
C18:2 LA	19.52	16.58	16.47
C20:2	0.16	0.09	0.07
C20:3	0.10	0.09	0.06
C20:4 AA	1.52	0.73	0.69
∑ omega 6	**21.30**	**17.48**	**17.29**
C18:3 ALA	0.75	3.12	2.49
C20:5 EPA	0.03	0.21	0.07
C22:6 DHA	0.75	2.20	2.01
∑ omega 3	**1.53**	**5.54**	**4.57**
n6/n3	14.1	3.2	4.0

Table 3

The content of fatty acids in extracted phospholipid fraction [in %]

Fatty acids	Symbol	Feeding group		
		Control (C)	Experiment plant oils and fish oil (I)	Experiment herbs and algae (II)
Saturated: Miristinic	14:0	0.17	0.25	0.17
Palmitic	16:0	23.18	22.83	22.85
Stearinic	18:0	17.84	17.86	18.44
		∑ =41.19	**∑ = 40.95**	**∑ =41.48**
Monounsaturated: Palmitooleinic	16:1	1.34	1.50	1.28
Oleinic	18:1	28.32	27.24	25.79
		∑= 29.66	**∑= 28.73**	**∑= 27.07**
Polyunsaturated: Linoleic	18:2	18.05	17.35	19.90
α-Linolenic	18:3	0.38	1.17	0.43
Arachidonic (AA)	20:4	6.83	2.70	6.93
Docosapentaenoic	20:5 (EPA)	0.09	0.76	0.24
Docosahexaenoic (DHA)	22:6 (DHA)	3.80	8.34	3.95
		∑=29.15	**∑=30.32**	**∑= 31.45**
n6/n3		**5.8**	**1.9**	**5.8**
AA/DHA		1.8	0.32	1.75

Table 4

Percentage content of lecithin and cephalin in eggs obtained from three feeding groups (n = 3)

Feeding group	Lecithin (PC)		Cephalin (PE)	
	$\overline{X}$	SD	$\overline{X}$	SD
Control	75.44%a	± 0.63	24.56%b	± 0.63
Experiment Plant oils and fish oil (I)	79.16%b	± 0.49	20.84%a	± 0.49
Experiment Herbs and algae (II)	75.49%a	± 1.13	24.51%b	± 1.13

The content of saturated and monoenic fatty acids in three experiment groups is presented in Table 1. The percentage content of acids was similar in all groups, the highest value was observed in Group II, in which the feed was supplemented with herbs and algae.

Polyenic fatty acids, esp. from n-3 family and the n-6 and n-3 ratio are very important from the nutritional point of view.

The highest content of n-6 polyenic fatty acids (21.30%) and the lowest content of n-3 acids (1.53%) was observed in the control group, where the n-6/n-3 ratio was less favourable (14.1) than in experiment groups.

It was showed that enrichment of feed significantly reduces the content of n-6 acids and increases the content of n-3 acids. The n-6/n-3 ratio is also very favourable, esp. when feed additives of fish origin are used.

The results of the analysis of percentage content of fatty acids in phospholipid fraction of egg yolk are presented in Table 3. The level of saturated acids was ca. 41% both in the control and experiment groups. The level of monoenic acids was the highest in the control group (29.66%) and, in the control groups, was 28.73 and 27.03% in Group I and Group II, respectively. The lowest level of polyenic fatty acids was observed in the control group (29.15%). In the experiment groups the level was 30.32% in Group I and 31.45% in Group II. The results clearly indicate the effect of using feed enrichment and show the differences between the effect of various feed additives on the profile of fatty acids in phospholipids.

The phospholipid fraction of egg yolk is one of the most valuable groups of nutritional substances, characterised by high nutraceutical value which determines its fatty acids profile.

In the experiment group (I), where feed was supplemented with plant oils and fish oil, the values, esp. for polyenic fatty acids, were especially favourable. The n-6 acids constituted 20.05% and the acids from n-3 family constituted 10.27%, which gave a very desirable value of n6/n3 ratio, i.e. 1.9. As clearly visible in Table 3, an addition of fish oil results in a lowered content of arachidonic acid (AA) and an increased content of docosahexaenoic acid (DHA). From the medical point of view, the low AA/DHA ratio is very favourable. The AA/DHA value was the highest in the control group (1.8) and the lowest in the experiment group I (0.32), which shows the effect of fish oil.

It should be noticed, that in the experiment group II, supplemented with herbs and algae, a significant increase of the content of polyenic acids was observed. This, however, concerned mainly linoleic acid (n-6).

In the above context, it may be suggested that feed additives with fish oils should be used in feeding layer hens to obtain eggs for the production of phospholipid preparations from the yolks. This problem requires further study.

The percentage composition of egg yolk phospholipids, taking into account two fractions, i.e. lecithin and cephalin, is presented in Table 4. Significant differences between the experiment group I and the control group and experiment group II were observed. In the group supplemented with plant oils and fish oil, the content of lecithin was 79.49%, whereas in the other groups the content of lecithin was ca. 75%. This fact is important if pure lecithin is to be isolated. If phospholipids are to be isolated as a multi-compound fraction, it will be less important.

The results obtained in the study clearly indicated the influence of plant and fish oils on the improvement of the composition of egg yolk phospholipids, which is of practical importance in production of phospholipid preparations, esp. lecithin.

Phospholipid preparations, mainly with complex composition and containing DHA and EPA, may play an important role in preventing liver, heart, vascular and nerve system disorders [Hosokawa 2000; Szuhaj 2003]. Lecithin, a diet supplement, positively influences the functioning of nerve system and on the process of memorising. Moreover, it has a positive effect on cholesterol management, stimulation of digestion and prevention of cardio-vascular diseases [Na, 1990]. These qualities are related to n-3 and n-6 long-chain unsaturated acids (PUFA) which may be carried by phosphatidylcholine (lecithin). When compared to eggs, the commonly used soya preparations are not a rich source of high quality and pro-health lecithin. Moreover, soya lecithin preparations cannot be used by a group of persons allergic to soya protein that may be present in such preparations.

Lecithin obtained from egg yolks, especially from yolks enriched with n-3 polyenic fatty acids (esp. DHA, EPA and ALA), is an attractive alternative. That is why intensive studies on natural methods of enriching eggs with beneficial fatty acids are conducted. In the above context, egg yolks enriched with PUFA become a source of not only perfect food, but also become a key material for the production of nutraceuticals and biomedical preparations.

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6

THE EFFECT OF BREEDING AND FEEDING OF LAYERS ON FATTY ACIDS IN EGG YOLK

Introduction

In recent years, intensive search for natural materials from which bioactive substances that may be used in the production of diet supplements can be obtained has been observed. Eggs seem to be the best material for this purpose and long-chain fatty acids as well as phospholipids, esp. lecithin and kephalin, are extremely important and life-giving substances contained in egg yolk [Kidd, 2007].

The methods of natural, through specially designed feeding of layer hens, enrichment of egg yolks with n-3 polyenic fatty acids, esp. EPA and DHA, are constantly improved [Farell, 2000; Sim, 2000; Van Elswyk et al., 2000; De Meester, 2006; Simopoulus, 2006; Yannakopoulos, 2007]. These valuable fatty acids are probably located in particles of yolk phospholipids, which may be of big importance for the use of eggs, or lecithin isolated from eggs, as nutraceuticals.

The research conducted by our team prove high transformation of n-3 fatty acids from feed to egg yolk [Kaźmierska, 2007].

The process of transformation is a very complex phenomenon an it requires wide ranging and specialist research. Presently patent applications are submitted in this field.

Phospholipids contained in hen egg yolk are an important source of choline, relevant for the development of brain, functioning of liver and in prophylaxis of cancer [Gutierrez et al., 1997]. Docosahexaenoic *acid* (DHA) and arachidonic *acid* (AA) are necessary for proper functioning of the nervous system, they reduce the level of triglycerides in blood, are characterised by anticoagulant, anti-inflammatory and vasodilatory activity [Sheppard, 2002]. Thus, daily ingestion of high doses of n-6 PUFA, n-3 PUFA and MUFA fatty acids, at low n-6/n-3 value, is highly beneficial for health. Modifications of the composition of feed mixtures for layer hens made it possible to obtain eggs enriched (up to 10-fold) with omega-3 fatty acids [Sim and Sunwoo, 2002]. Research on enrichment of hen eggs has been conducted for over 20 years and enrichment components and their proportions have been improved. Flax seed, flax seed oil, rapeseed oil, and products of marine origin are used most frequently. Moreover, antioxidants (vitamin E, carotenoids, polyphenols) and detoxicants, e.g. humine compounds, are important additives as well. Recently, interest has been paid to the use of algae in feeding layer hens, which results in an increased content of DHA in yolk lipids.

The currently used natural methods of enriching the egg matter are more and more sophisticated and, due to high competition on the new generation food market, secret. New bioactive components of feed mixtures as well as layer hens with the most desired genotype are sought for.

The aim of the study was to find the best method of enriching eggs with PUFA and also to demonstrate the effect of adding plant oils and humine preparation on the one hand, and the influence of layer hens with various genotypes, i.e. Lohmann Brown (LB) and Polish native breed Zielononóżka kuropatwiana (ZN) on the profile of fatty acids contained in the yolk, on the other.

Material and methods

The experiment material was obtained from experimental farm of Tronina Co. where the experiment was conducted in specially designed rooms for layer hens in floor system (with stable zoohygienic conditions) in 4 experiment groups, 40 hens in each:

a) two genetic groups (i.e. Lohmann Brown [LB] and Polish native breed Zielononóżka kuropatwiana [ZN]), and

b) two feeding groups, i.e. one fed with standard feed DJ and one fed with feed enriched with PUFAs, vitamins as well as humine and mineral compounds [Humokarbowit (HKW) and Humobentofet (HBF)] used as carriers.

The composition of feed mixtures is presented in Table 1.

Table 1

The profile of fatty acids in the feeds (average values given in %, n = 3)

	Standard feed	**Enriched feed**
C14:0	0.23	0.43
C15:0	0.07	0.11
C16:0	30.47	29.91
C17:0	0.18	0.22
C18:0	7.56	7.83
C20:0	0.09	0.05
C22:0	0.03	0.03
∑	38.64	38.58
C16:1	1.72	2.06
C17:1	0.08	0.07
C18:1	39.23	38.94
C20:1	0.22	0.37
∑	41.25	41.43
C18:2	16.80	14.76
C20:2	0.18	0.11
C20:3	0.13	0.12
C20:4	1.48	0.68
∑	18.59	15.67
C18:3	0.83	2.57
C20:5	0.00	0.23
C22:6	0.69	1.52
∑	1.52	4.32
n6/n3	12.2	3.6

Humokarbowit (HKW), although not yet very popular, should be mentioned as an important feed component. It is derived from humine and carbonate materials enriched with calcium and magnesium compounds. Besides, humine acids and their salts, carboxyl groups, bitumins, hemicellulose, lignin, waxes, resins, phytohormons, proteins, aminoacids as well as macroelements and trace elements are also present [Dobrzański and Tronina, 1999].

The experiment started when the layer hens were 22 weeks old and was conducted for 40 weeks, with 6 experiment intervals – study periods (every 8 weeks). Material was collected in each interval and the following were performed:

- egg mass, percentage of yolk and the basic chemical composition were determined (protein content was determined by Kjeldahl method according to PN-ISO 75/A-04018 norm using Foss Kjeltec 2300 equipment; fat content was determined according to PN-ISO 1444:2000 norm, by Soxhlet method, using Büchi equipment (Extraction System B-811);
- the profile of fatty acids was analysed using a gas chromatographer combined with mass spectroscope (GC/MS by Agilent 6890N, 5973 MS detector, using a chromatography column - DB-225MS (0.25 mm*30 m*0.25 µm).

Results

The results of the study, taking into account the effect of feeding type and genotype of layer hens on the analysed parameters, are presented in Tables 2–5.

Table 2

The mass of egg and the yolk, its basic chemical composition with respect to the age of layer hens and types of feeding (Lohmann Brown layer hens)

LAYERS	PERIOD	STATISTICAL PARAMETER	WEIGHT OF EGG [g]		WEIGHT OF YOLK [g]		PROTEIN OF YOLK [%]		FAT OF YOLK [%]	
			Contr.	Exp.	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.
LOHMANN BROWN	1	Mean	56.12	56.43	13.77	13.91	15.94	15.62	31.32	31.13
		Std Dev.	2.36	3.03	1.02	1.01	0.58	0.55	0.28	0.39
	2	Mean	60.61	60.96	16.11	16.27	16.85	16.36	30.74	31.76
		Std Dev.	4.23	3.66	1.16	1.01	0.93	0.12	0.27	0.26
	3	Mean	63.80	63.46	17.56	17.01	15.87	15.83	30.28	30.29
		Std Dev.	5.07	4.96	1.45	2.12	0.16	0.40	0.86	1.04
	4	Mean	64.79	65.50	17.64	18.07	16.31	16.30	29.78	30.09
		Std Dev.	3.57	3.62	0.99	1.50	0.17	0.14	1.37	1.23
	5	Mean	64.59	67.30	18.68	19.07	16.36	16.32	29.51	30.10
		Std Dev.	4.24	4.00	3.12	1.38	0.11	0.16	1.06	0.90
	6	Mean	65.18	67.11	18.76	18.65	15.88	15.64	30.74	30.29
		Std Dev.	5.15	5.76	1.66	1.40	0.19	0.33	0.34	0.11

Std Dev. = standard deviation
Contr. = control group
Exp. = experimental group
Control group = N3+N7
Experimental group = N1+N5

Table 3

The mass of egg and the yolk, its basic chemical composition with respect to the age of layer hens and types of feeding (Zielononóżka layer hens)

LAYERS	PERIOD	STATISTICAL PARAMETER	WEIGHT OF EGG [g]		WEIGHT OF YOLK [g]		PROTEIN OF YOLK [g]		FAT OF YOLK [g]	
			Contr.	Exp.	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.
ZIELONO-NÓŻKA	1	Mean	45.13	45.51	11.63	11.68	16.07	16.02	31.54	31.12
		Std Dev.	4.11	3.34	1.22	1.11	0.27	0.20	0.22	0.26
	2	Mean	51.04	48.00	14.66	13.92	16.25	16.37	31.15	31.02
		Std Dev.	4.64	3.80	1.17	0.98	0.20	0.11	0.11	0.20
	3	Mean	55.01	54.45	16.91	16.14	16.20	16.17	31.25	29.17
		Std Dev.	3.54	5.53	1.61	2.12	0.39	0.14	0.61	0.37
	4	Mean	56.15	55.73	17.70	17.70	16.34	16.43	31.25	29.17
		Std Dev.	3.17	3.60	2.61	1.40	0.23	0.18	0.61	0.31
	5	Mean	61.48	60.26	19.02	18.90	16.30	16.43	31.07	29.53
		Std Dev.	3.99	4.07	2.09	1.92	0.13	0.09	0.51	0.20
	6	Mean	58.85	59.25	19.19	18.96	15.90	15.91	31.42	31.31
		Std Dev.	5.52	3.33	1.75	1.40	0.29	0.25	0.23	0.06

Std Dev. = standard deviation
Contr. = control group
Exp. = experimental group
Control group = N4+N8
Experimental group = N2+N6

Table 4

The average mass of egg and yolk, its basic chemical composition with respect to two genetic groups of layer hens (Lohmann Brown and Zielononóżka) and the feeding methods

LAYERS	STATISTICAL PARAMETER	WEIGHT OF EGG (g)		WEIGHT OF YOLK (g)		PROTEIN OF YOLK (%)		FAT OF YOLK (%)	
		Contr.	Exp.	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.
Lohmann Brown	Mean	62.51	63.46	17.08	17.16	16.20	16.01	30.39	30.61
	Std Dev.	3.54	4.19	1.89	1.90	0.38	0.35	0.67	0.68
Zielononóżka	Mean	54.61	53.86	16.52	16.21	16.18	16.22	31.28	30.22
	Std Dev.	5.84	5.96	2.91	2.92	0.17	0.22	0.17	1.03

Std Dev. = standard deviation
Contr. = control group
Exp. = experimental group

Table 5

The characteristics of fatty acids contained in egg yolk (average values in the laying period from 22nd to 62nd week of life) depending on the genetic group (Lohmann Brown and Zielononóżka) and feeding type

LAYERS	GROUP	STATISTICAL PARAMETER	FATTY ACIDS											
			UNSATURATED		MUFA		n-6 PUFA			n-3 PUFA			n6/n3	AA/DHA
			Σ	16:00	Σ	18:01	Σ	18:02	20:04	Σ	18:03	22:06		
Lohmann Brown	Contr.	Mean	34.28	25.02	43.43	40.57	21.36	19.00	1.51	1.25	0.63	0.59	17.57	2.58
		Std Dev.	0.83	1.15	3.60	3.12	3.98	3.91	0.16	0.31	0.20	0.13	2.28	0.53
	Exp.	Mean	32.43	23.18	43.50	40.58	21.04	19.01	1.08	3.18	2.21	1.24	7.85	1.05
		Std Dev.	1.35	1.32	2.55	2.60	3.43	3.15	0.28	0.93	0.95	0.36	5.23	0.79
Zielononóżka	Contr.	Mean	33.11	24.55	43.61	40.68	21.99	19.83	1.45	1.26	0.70	0.57	17.71	2.58
		Std Dev.	1.65	1.53	3.12	2.90	3.69	3.73	0.12	0.27	0.20	0.11	2.01	0.31
	Exp.	Mean	32.67	23.49	44.08	41.14	20.38	18.25	1.12	2.85	1.67	1.16	8.05	1.05
		Std Dev.	1.10	1.08	2.19	2.37	2.98	2.76	0.20	0.74	0.55	0.25	4.38	0.50

Std Dev. = standard deviation
Contr. = control group
Exp. = experimental group

As presented in Table 2 and Table 3, egg mass was increasing with the age of Lohmann Brown layer hens, from 56.1 g in the first period, to 65.2 g in the sixth period of the experiment. It should be noted that, after the 46th week of layer's age, the mass of experiment eggs was ca. 2 g higher and, in the 6th study period, was 67.1 g. Similarly, in Zielononóżka layer hens, an increase of mass from 45.1 g to 58.8 g in the control group and from 45.5 g to 59.2 g in the experiment group, was observed. Similar changes, depending on the yield period and the breed of layer hens, were observed in the content of yolk. However, its percentage was significantly higher in the group with native Zielononóżka hens. Generally, the percentage content of proteins and fat in yolk did not differ depending on the age of layer hens or on the research groups. The values presented in Table 2 and Table 3 reflect only the deviations typical for the biological material.

Table 4 presents the results with average values observed within the whole experiment, i.e. until 62nd week of the layer hens' life. The results present the characteristics of eggs as material that, taking into account the breed of hens and the method of feeding (including enrichment), may be widely used. The results also show that the mass of eggs obtained from Lohmann Brown layer hens was 12–15 % higher, whereas the participation of yolk in egg mass was ca. 3% higher in case of eggs obtained from Zielononożka layer hens.

Table 5 presents the results of the analysis of fatty acids profile and indicates the differences in groups. Graph 1 and Graph 2 present the changes in selected fatty acids taking place within the whole experiment period.

Generally, the level of saturated fatty acids (SFA) decreased as a result of the use of feed additives in both genotype groups of layer hens, whereas the content of n-3 PUFAs increased. No. changes in the family of monoeic acids (MUFA) were observed in the experiment groups. The 2–4 fold increase of n-3 PUFAs, esp. ALA (18:3) and DHA (22:6) acids should be listed as important changes resulting from the modification of feed in both groups of layer hens. The improvement of n-6/n-3 was over twofold. The level of arachidonic acid (AA -20:4) decreases significantly as a result of the use of feed additives in both groups of layer hens (LB and ZN). At the same time, the ARA/DHA value decreases 2.5 times, which indicates a beneficial change of the proportion between arachidonic and docosahexaenoic acids from 2.6 to 1.05.

The results presented in the graph 1 and 2 clearly indicate the increase of the content of n-3 fatty acids in experimental groups both the LB and ZN layer hens, and there were No. significant differentiating tendencies in the group of n-6 acids.

It should be noted that, as a result of the use of experimental feed additives, Zielononóżka (ZN) layer hens are characterised by absorption of polyenic n-3 acids which is worse than in Lohman Brown (LB) layer hens. Moreover, the content of n-6 PUFAs is also lowered. This proves that the LB layers, with improved genotype, are more efficient in digesting the feed additives. Taking into account the higher egg yield of LB layers, enrichment of eggs using these hens is highly justified. In the context of the data presented in the tables, the ZN layer hens should not be used for the production of eggs enriched with PUFAs which is also influenced by their lower yield

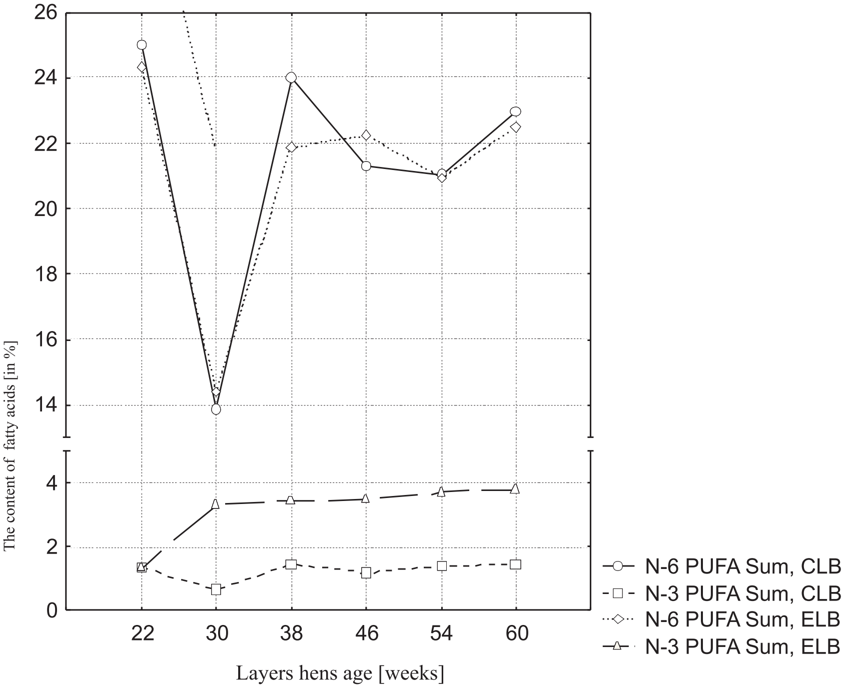

Fig. 1. The content of fatty acids (n-6, n-3) in egg yolks from Lohman Brown group [in %]

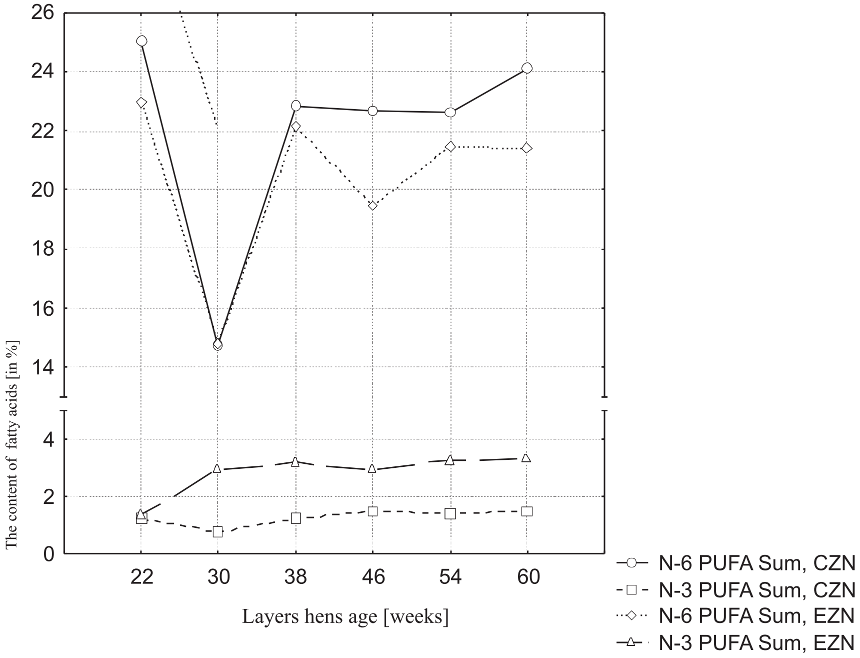

Fig. 2. The content of fatty acids (n-6, n-3) in egg yolks from Zielononóżka Kuropatwiana group [in %]

Discussion

Until recently, the consumption of eggs in all types of diet was very limited due to cholesterol. At the same time, intensive research on the characteristics of the nutritional value of eggs and the possibility of their biological and nutraceutical application was conducted. As a result, high nutritional value of eggs was confirmed and the opinion that cholesterol plays a negative role in our diet was ignored [Kamiński, 2000; Sim, 2000]. Eggs enriched with bioactive substances, esp. with PUFAs and vitamins, are especially valuable.

The research results clearly indicate that the use of properly prepared feed mixture may result in a multiple increase of the content of bioactive substances, incl. PUFAs, which confirms that it is possible to improve the chemical composition of egg yolk, especially its lipid fraction. The present study proves that the effects of enrichment vary depending on the breed of layer hens. Zielononóżka (ZN), the Polish native breed so frequently used in ecological breeding, gives lower yield and accumulates less n-3 PUFAs than LB layer hens. Thus, from the economical point of view, ZN layers should not be used for enriching eggs with PUFAs on an industrial scale. On the other hand, it is known that the level of bioactivity of eggs obtained from ZN layers is higher than in case of other genotypically improved layer hens (including LB). The activity of lysozyme and cystatin obtained from ZN layer hens is 20–50% higher than from LB hens. The results of the study prove that the level of lipids and vitamins in the yolk depends not only on the composition of feed mixture, but on the genotype of the layer hens as well.

Research on the enrichment of eggs with n-3 polyenic fatty acids should be continued and embedding of DHA particles into selected phospholipid fractions of the yolk, especially lecithin, should be developed. As far as their biomedical value is concerned, phospholipids are the most valuable components used in the therapy and prevention of civilisation diseases. Phospholipid preparations of plant (mainly soy) origin are widely available on the market.

It is commonly known that phosphatidylcholine or phosphatidylethanolamine are not homogenous compounds, but mixtures of particles including saturated acylic chains, mainly in *sn*-1 position and unsaturated chains in *sn*-2 position. [D'Arrigo and Servi, 1997]. The composition of fatty acids in lecithin and kephalin of soy origin is different than the composition of these phospholipids obtained from egg yolk [Hammond, 2005]. There are more saturated fatty acids in lecithin isolated from egg yolk than in soy lecithin. Soy lecithin is a rich source of linolic acids, whereas lecithin obtained from egg yolk contains arachidonic and docosahexaenoic acids, used in treatment of numerous diseases and not present in soy lecithin.

Extensive studies of phospholipids have showed that the compounds play various functions, are ubiquitous and fundamental for many life processes.

Due to the amiphilic character of phospholipid particles, they are surface active agents, which facilitates emulgation and digestion of fats in the alimentary tract [Ziemlański, 1991]. Phosphatidylserine is highly concentrated in the membranes of nerve cells where it is used for intercellular identification and communication. Moreover, the phospholipids obtained from egg yolk may be used in prophylaxis of some neuropsychological disorders, esp. depression and dementia, including Alzheimer disease [Bourre et al., 2004].

It has been observed that many groups of phospholipids play a specific role in protection against degeneration of liver, heart, blood vessels and nerve system [Hosokawa, 2000; Szuhaj, 2003]. Phosphatidylcholine (lecithin) is especially important for the functioning of these systems. Lecithin, which contains polyunsaturated fatty acids, regulates the concentration of LDL cholesterol and the management of triacyloglycerols which prevents their accumulation on the walls of blood vessels, thus reducing the risk of heart diseases and arteriosclerosis. Lecithin is, first of all, a source of choline which is necessary for the creation of acetylcholine, an important transmitter of nerve impulses.

Despite numerous applications in food industry, lecithin (esp. soy lecithin) is mainly used as diet supplement which is beneficial for the functioning of nerve system and the processes of memorising. Lecithin has a positive influence on the management of cholesterol, stimulates digestion and prevents cardiovascular diseases [Na, 1990]. These qualities, however, should be attributed to fatty acids, esp. long-chain unsaturated acids from the PUFA family, which may be transmitted by phosphatidylcholine. Soy, when compared to eggs, is not a rich source of high quality lecithin. Moreover, the commercially available preparations based on soy lecithin cannot be used by a group of people who are allergic to soy protein which may contaminate the preparations. Lecithin obtained from egg yolk is an attractive alternative, especially if the yolks are enriched with n-3 polyenic fatty acids, mainly with DHA, EPA and ALA. Egg yolk, naturally rich in lecithin and enriched with long-chain fatty acids, mainly DHA, is valuable not only for culinary, but also for nutraceutical and biomedical applications.

Conclusions

1. There is a significant difference between the morphology and the percentage content of yolk between eggs from ZN and LB layer hens. However, the content of proteins and lipids is at a similar level in both breed groups.
2. The enrichment of eggs through designed feeding of layer hens resulted in 2–4 fold increase of n-3 PUFA and, at the same time, lowered (2.5 times) content of arachidonic acid, which led to a 2-fold decrease on the n-6/n-3 value in both genotype groups of the layer hens.
3. It has been showed that the LB line of layer hens is better for the enrichment of eggs with n-3 polyenic acids than the ZN line. Moreover, the yield of eggs from LB layers is two times higher than from ZN layers, which indicates that production of Lohman Brown eggs is more economical.
4. The results show that it is possible to improve the yolk lipids, both as the n-3 PUFAs content, esp. DHA, and the lowered n-6/n-3 value are concerned.

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7

FUNCTIONAL FOODS TAILORED TO GENES: CURRENT RESEARCH AND FUTURE HYPOTHESIS

Introduction

Functional foods have been suggested to play an important role in combating cardiovascular diseases (CVD) and other chronic illnesses. The aim of this review is to examine recent studies, which have used genomics techniques to look at the action of functional foods on CVD. In particular, n-3 polyunsaturated fatty acids (n-3 PUFAs) and plant sterols (PSs) will be looked at to provide insights on nutrient-gene interaction effects that may modulate CVD risk factors. First, it is well known that consumption of n-3 FA decreases the risk of CVD by decreasing triglycerides (TG) levels and pro-inflammatory parameters. Yet, studies have observed an inter-individual variability in TG-lowering and inflammatory response. Current research has investigated the impact of n-3 PUFA supplementation on their impact on gene expression rates and the influence of a variety of polymorphisms; yet, there are No. definitive conclusions and more research is needed. Secondly, PS consumption decreases low-density lipoprotein cholesterol (LDL-C) levels; however, high variability of responsiveness of lipid levels to PS intervention has been observed. Still, No. polymorphisms have been demonstrated to consistently explain the non-responsiveness to PS. In addition, PSs have not shown clear effects on gene expression rates. Therefore, studies are needed to clarify the genetic basis related to the variability to PS consumption and to determine the precise molecular mechanism of PS action. Overall, genomic techniques have the potential to assist in the development of functional foods that are able to: (WHO Statistical Information System, 2009] alter favourably gene expression in individuals and [Kersten, 2008] develop a "personalized nutrition" approach, where nutrient intake is optimized based on an individual's genetic profile to reduce the disease risk and/or improve the effectiveness of dietary guidelines in general.

Functional Foods and Nutrigenomics

Cardiovascular diseases (CVD) are the number one cause of death worldwide and are projected to remain so. An estimated 17.5 million people died from CVD in 2005, representing 30% of all global deaths (WHO Statistical Information System, 2009). There exist many lipid lowering functional foods that can aid in preventing and treating CVD. Functional foods are defined as similar in appearance to, or may be, conventional foods that are consumed as part of a usual diet, and are demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. Common examples of functional foods on the market include: n-3 polyunsaturated fatty acids (n-3 PUFAs), plant sterols/stanols (PSs), nuts, soluble fiber, garlic and many more. However, the data demonstrates a large inter-study variability in response to functional foods that is possibly due to the dose or ratio of bioactive compound, the duration of study, the health status, diet and other confounding factors. Yet, the large heterogeneity within studies is likely to be attributable to genetic variability within the study population.

The study of nutrigenomics describes both the effects of diet on gene expression and the effects of genetic variation on response to diet. First, the diet effects on gene expression describe changes in transcriptional rates of various genes due to specific bioactive components. Secondly, genetic variations, such as single nucleotide polymorphisms (SNPs), occur within the promoter region of a large number of genes. Certain of these SNPs influence the transcriptional activity of genes, resulting in intra-individual differences in the amount of protein produced by the gene. Other SNPs can influence the gene function, for example, different structural and thus functional properties of the gene. The aim of this review is to examine recent studies, which have used genomics techniques, either gene expression or genetic variations, to reveal the mechanism of action of functional foods on CVD risk factors. In particular, n-3 PUFAs and PSs will be examined to determine the nutrigenomic mechanisms on metabolic factors.

Omega-3 Fatty Acids

Long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in relatively high concentrations in oily fish. Numerous epidemiological studies suggest that consumption of n-3 PUFA and/or fish oil decrease the risk of CVD. The effects of n-3 PUFA are likely mediated by changes in gene expression, which precede changes in membrane composition, by directly governing the activity of nuclear transcription factors. First, peroxisome proliferator-activated receptor alpha (PPAR-alpha) is a nuclear transcription factor regulating multiple genes involved in energy, glucose and lipid metabolism. However, studies have shown that PPAR-alpha is not the single transcription factor involved in mediating fatty acid effects on gene transcription. Additionally, several other transcription factors have been shown to be targets for FAs regulation, including: PPAR-gamma, hepatic nuclear factor-4alpha (HNF-4alpha), sterol regulator element-binding protein (SREBP), liver X receptors (LXR-alpha and beta), retinoid X receptor (RXR-alpha), and nuclear factor-kappaB (NF-kappaB). Overall, n-3 PUFA supplementation influence gene expression by acting on a variety of key nuclear transcription factors.

Omega-3 Fatty Acids and Gene Expression

The effects of n-3 PUFAs on lipid metabolism are likely mediated by changes in gene expression. More specifically, FAs and their derivatives are natural ligands of the nuclear receptor PPAR-alpha, which heterodimerize with the RXR before triggering the expression of target genes [Kersten, 2008]. Particular target genes include lipoprotein lipase (LPL)

[Michaud and Renier, 2001], a central enzyme in triglyceride metabolism, and apolipoprotein AI (apo-AI) [Vu-Dac et al., 1994], a key structural element in high-density lipoproteins (HDL). Overall, omega-3 FAs bind to PPAR-alpha and consequently have the potential to lower plasma triglyceride (TG) levels and increase HDL-cholesterol (HDL-C) levels.

Secondly, n-3 PUFAs have strong anti-inflammatory effects: suppress plasma interleukin 1 beta (IL-1beta), tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6). The anti-inflammatory effects of n-3 PUFAs appear also to be exerted at the level of altered gene expression [Calder, 2005]. It is thought that n-3 PUFAs might exert their effects on inflammatory gene expression through direct actions on the intracellular signalling pathways that lead to activation of one or more transcription factors such as PPAR-alpha and NF-kappaB. However, these effects have been demonstrated only a small number of *in vitro* studies and the extent of these effects *in vivo* are not yet clear. Nevertheless, studies indicate potentially very potent effects of n-3 PUFA on the expression of a range of lipid and inflammatory genes. Further, a more detailed comparison of the gene expression rates after different dietary interventions, such as n-3 PUFAs, offers the possibility to reveal gene networks and signal transduction pathways.

Omega-3 Fatty Acids and Polymorphisms

Lipid Response to Omega-3 Fatty Acids

Numerous studies have suggested that variation in the genes for PPAR-alpha, PPAR-gamma, apolipoprotein (apo) AI, apo AIV, apo B, apo E, apo CIII, LPL, hepatic lipase, endothelial lipase, liver fatty acid-binding protein, $[\text{beta}]_3$-adrenergic receptor, and adipsin contributes to the heterogeneity in the lipid response to dietary intervention [Masson et al., 2003; Masson and McNeill. 2005]. However, relatively few studies in the literature have focused on dietary changes with n-3 PUFAs and genetic variations.

One of the most common genetic variations studied with n-3 PUFA is the gene encoding the PPAR-alpha. Several polymorphisms exist within the PPAR-alpha gene including L162V. PPAR-alpha L162V polymorphism has been associated with obesity and with changes in specific metabolic parameters in numerous studies [Flavell et al., 2000; Sparso et al., 2007; Tanaka et al., 2007]. Recent, *in vitro* results also indicate that both allelic variants of the PPAR-alpha L162V are activated by n-3 PUFAs; however, the mutated form displays a lower transcriptional activity than the wildtype variant after incubation with n-3 PUFAs [Rudkowska et al., 2009]. Additionally, an epidemiological study in a sample of 260 French-Canadians found that the interaction between the PPAR-alpha L162V polymorphism and fat intake estimated from a food frequency questionnaire explains differences observed in waist circumference [Robitaille et al., 2004]. Further, Tai et al. [2005] also established that the effect of the L162V polymorphism on plasma TG and apo CIII concentrations depends on the PUFA, with a high intake triggering lower TG in carriers of the V162-PPAR-alpha allele. Further, to confirm these epidemiological study, an interventional study by Paradis et al. [2002] demonstrated that the inter-individual variations in total cholesterol (TC), apo A-I and cholesterol concentrations in small low-density lipoprotein (LDL) particles observed after modification of the PUFA/saturated FA ratio of the diet is partly attributable to the PPAR-alpha L162V polymorphism [Paradis et al., 2005]. Since n-3 PUFAs are the strongest ligands for PPAR-alpha, a recent study investigated the impact of n-3 PUFAs supplementation on PPAR-alpha L162V polymorphism [Caron-Dorval et al., 2008]. The n-3 PUFA supplementation decreased TG concentrations in both genotype groups [Caron-Dorval et al., 2008]. Yet, there was nutrient-gene interaction for plasma C-reactive protein (CRP) concentrations [Caron-Dorval et al., 2008]. Overall, these studies show that PPAR-alpha L162V polymorphism contributes to the inter-individual variability in the CVD risk factor response to n-3 PUFAs.

Similarly, Volcik et al. [2008] suggested that PPAR-alpha 3'UTR SNP can also modulate the association between TC and LDL-C and n-3 PUFAs intake in African Americans. Clearly, the above studies reveal a relationship between the PPAR-alpha polymorphisms, lipid parameters and PUFAs intake.

Other studies have demonstrated the relation between different SNPs and n-3 PUFA supplementation. First, the apo A1 is highly polymorphic and the -75G/A SNP, a common variant of this gene, has been extensively studied in relation to variation in the concentration of serum apo A1 and HDL-C. Ordovas et al. [2002] found a nutrient-gene interaction associated with the apo-A1 G-A polymorphism and PUFA intake. Secondly, SNPs have been identified in the promotor region of the apo CIII gene. In particular, the T-455C polymorphism in the insulin-responsive element of the apo CIII gene was found to influence TG and apo CIII concentrations [Olivieri et al., 2005]. Thirdly, apo E polymorphisms and n-3 PUFA and their effect on lipid metabolism has not been extensively studied, despite the fact that apo E is one of the most-widely investigated genotypes in relation to lipid metabolism. A clinical trial demonstrated that the apo E genotype may in part determine the blood response to fish oil intervention, and that the LDL-C increases may be largely evident in the apoE4 carriers [Minihane et al., 2000]. Taken as a whole, the effects of these genetic variations on lipid metabolism are not consistently reported and results are sometimes conflicting. Future studies need to have much larger sample sizes and carefully controlled dietary intervention, which should investigate the effects of polymorphisms in multiple genes instead of the effects of polymorphisms in single genes. In sum, the lipid response to dietary change, such as n-3 PUFA supplementation, is highly complex that involves many polymorphisms in genes.

Inflammatory Response to Omega-3 Fatty Acids

Since inflammation is at the base of many chronic diseases, including CVD, dietary intake of n-3 PUFAs plays an important role in the symptom of disease, particularly in persons with genetic variation. Therefore, the possession of genotypes associated with raised inflammatory stress may support the association between lipid metabolisms. However, the influence of n-3 PUFAs on cytokine production is inconsistent. For example, only 6 out of 12 studies, investigating the effect of fish oil on TNF-alpha production by peripheral blood mononuclear cell from healthy subjects, report a suppressive effect [Grimble et al., 2002]. These discrepancies in findings can be explained by the effects of inherent TNF-alpha production and by polymorphisms in the TNF-alpha and lymphotoxins genes [Grimble et al., 2002]. For instance, a study by Markovic et al., [2004] concluded that the ability of n-3 PUFA to exert a lipid-lowering, anti-inflammatory is influenced by the possession of the lymphotoxin-alpha (TNF-beta) +252 A allele and body mass index (BMI). Further, IL 1beta is an important cytokine that has a multiple of actions, including a pronounced pro-inflammatory effect and increasing the expression of adhesion molecules. Shen et al., [2007] suggested that IL 1beta genetic variants were associated with measures of chronic inflammation and the metabolic syndrome risk. Further, a greater understanding of the genomic determinants of the ability of n-3 PUFAs to act as an anti-inflammatory agent will enable n-3 PUFA supplementation to be used more effectively in suppressing inflammation as well as potentially lipid parameters.

Plant sterols

Plant sterols (PS) have been shown to reduce LDL- cholesterol (C) by 10% [AbuMweis and Jones 2008]. Thus, CVD risk can be lowered by introduction of PS in the diet. PSs are widely distributed in human diet but are poorly absorbed. However, when fed in large amounts, they lower plasma cholesterol levels by interfering with cholesterol absorption. The cholesterol-lowering action of PS is thought to occur, at least in part, through competition with dietary and biliary cholesterol for intestinal absorption in mixed micelles. However, mechanisms by which PSs lower plasma cholesterol levels have not been clearly elucidated. It was suggested that these beneficial effects are attributable in part to the increased activity of ATP-binding cassette (ABC) transporters ABCG5 and ABCG8, or a decrease in Niemann-Pick C1 Like 1 (NPC1L1) could mediate the reduction in intestinal cholesterol absorption caused by PSs. The section below describes the progress on the effect of PS and nutrigenomics.

Plant Sterols and Gene Expression

Studies have suggested that cholesterol-lowering effect of PS is unrelated to changes in gene expression levels of intestinal ABC sterol transporters or NPC1L1 [Calpe-Berdiel et al., 2008; Madden et al., 2008]. Investigators have also suggested PS action on hepatic sterol regulatory element binding protein 2 (SREBP2), cholesterol esterification and lipoprotein assembly (ACAT, apo B), cholesterol internalisation (ANXA2), cholesterol synthesis (HMG-CoA reductase, C-24-reductase) and removal of apoB100-containing lipoproteins (LDLr) [Calpe-Berdiel et al., 2008; Madden et al., 2008]. Yet, the impact of PSs intake on these physiological processes *in vivo* remains unclear. PSs may influence levels of a variety of gene expressions for lipid metabolism. It would be interesting to use large-scale gene expression profiling- measuring simultaneously the expression of thousands of genes- to determine molecular mechanisms of action and other potential beneficial or negative effects. This type of research would be valuable since targeted approaches may favour the systematic focus on a few genes or proteins that are already known to be implicated but may not be the only or the most important roles.

Plant sterols and Polymorphisms

Lipid Response to Plant Sterols

Plant sterol (PS) consumption decreases LDL-C levels; however, high variability of responsiveness of lipid levels to PS intervention has been observed. Recently published data indicates that PS intake did not decrease cholesterol absorption rates to the same degree in certain subjects, possibly clarifying the inter-individual variability in the cholesterol-lowering effect; yet, No. single genotype was identified [Rudkowska et al., 2008].

Various polymorphisms in the ABCG8 and ABCG5 gene have been found to be associated with several components of cholesterol metabolism [Rudkowska and Jones, 2008]. Further, NPC1L1 gene is an intestinal cholesterol transporter has been found to have a key role in PSs metabolism [Simon et al., 2005]. Therefore, recent studies hypothesized common SNPs in the genes encoding the ABCG5 and G8, NPC1L1 or other proteins of the cholesterol pathway, would underline inter-individual variations in response to PS. First, Plat et al., [2005] determined that genetic variations in ABCG8 1289C>A explain differences in serum PS concentrations and a subject's responsiveness to changes in serum PS during PS interventions. Similarly, Zhao et al. [2008] determined that A allele carriers of the ABCG8 1289C> A polymorphism with high basal plasma PS concentrations demonstrated a greater reduction in serum LDL-C than their low basal plasma PS counterparts. Further, individuals carrying mutant alleles in the NPC1L1 haplotype of 872 C>G and 3929 G>A, showed a greater reduction in LDL-C levels, compared to wildtype counterparts. Thirdly, Gylling et al. [2008] determined that the serum cholesterol lowering with absorption inhibition was not associated with SNPs of ABCG5 and ABCG8. As a whole, these studies do not show a consistent pattern of polymorphisms in the ABCG8 or NPC1L1 genes.

Since the apo E polymorphism is the most extensively studied polymorphism in gene-diet interactions and lipid levels [Bennet et al., 2007], Sanchez-Muniz et al., [2008] examined the responses to PSs vary by apo E genotype and concluded that PS treatment may be of little value in apo E4 carriers, who did not reduce TC, LDL-C, or apo B levels. Another study determined that the decrease of TC and LDL-C by a proportional decrease in cholesterol absorption are most consistent in subjects with apo E4 allele after PS treatment [Miettinen and Vanhanen, 1994]. Yet, other research found No. differences between the polymorphisms in apo A-IV, scavenger receptor class-B, type I (SR-BI), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), cholesterol ester transfer protein (CETP), or apo E genotype and dietary responsiveness to PS consumption [Plat and Mensink, 2002]. In sum, No. clear and consistent polymorphism has been associated with the responsiveness to PS consumption.

Future controlled dietary intervention studies with large sample sizes are required. Additionally, the variability in lipid response to PSs is probably under polygenic control. Therefore, a slight effect of one polymorphism may be overshadowed by other polymorphisms. Construction of haplotypes, a combination of SNPs, may reveal effects that are not obvious by analysis of single SNPs. Therefore, the use of genetic and phenotypic biomarkers together may predict inter-individual lipid level responsiveness to PS-intervention, and thus could be useful in devising individualized cholesterol lowering strategies.

Conclusion

Functional foods, including n-3 PUFAs and PSs, offer an interesting alternative through which optimal dietary advice can be tailored to a population's needs. Further, genomic techniques can assist in the development of functional foods that are able to: [WHO Statistical Information System, 2009] alter favourably gene expression in individuals and [Kersten, 2008] develop a "personalized nutrition" approach, where nutrient intake is optimized based on an individual's genetic profile to reduce the disease risk and/or improve the effectiveness of dietary guidelines in general.

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8

THE CONTENT OF BIOACTIVE COMPOUNDS IN PICKLED SWEET PEPPER FROM ORGANIC AND CONVENTIONAL CULTIVATION

Introduction

Consumers consider organic produce safe due to the absence of pesticide residues and of excess nitrates; synthetic fertilizers and plant protection products are inadmissible in organic farming. At the same time, many studies indicate that organic vegetables and fruit have high nutritional value [Rembiałkowska et al. 2005, Pérez-López et al. 2007]. Only natural, farm fertilizers are used in organic crops (manure, compost and green manure), and only natural biological products are used in plant protection (plant extracts, predatory insects and pheromone traps). Such systems result in plants producing a specific system of resistance against disease and pests. Phenolic compounds are thought to be "natural pesticides" [Young et al. 2005]. These are bioactive compounds which protect plants against pest infestation or fungal infection. That is why organic vegetables may contain more of these antioxidant constituents [Rembiałkowska et al. 2003, Young et al. 2005]. Many studies have confirmed that organic vegetables contain more bioactive compounds as compared with conventional vegetables [Caris-Veynard et al. 2004, Hallmann and Rembiałkowska 2007, Juroszek et al. 2009]. One important vegetable in our diet is sweet pepper, otherwise known as bell pepper, which is a very rich source of vitamin C, flavonols, phenolic acids and carotenoids (beta-carotene, capsorubin and capsantin) [Marin et al. 2004]. If fresh vegetables are richer in bioactive compounds, then it may be assumed that processed vegetables (e.g. pickles) can, despite the loss arising from processing, be still richer in antioxidants. Unfortunately the available literature provides little information about the nutritional value of organic preserves, including pickled sweet pepper. Processing of vegetables has had a long tradition in Poland, and is a very good method to prolong their keeping quality and diversify the diet. Therefore, it has been found useful to study nutritional value of preserves made from organically grown sweet pepper.

In addition to agricultural practice, one of the most significant factors to determine nutritional value of vegetables is the variety. For instance, studied carried out so far indicate that from two unprocessed sweet pepper varieties studied, Ożarowska was richer in rutin, a flavonoid, as compared with the other variety, Roberta [Hallmann and Rembiałkowska, 2007]. According to the quoted studies, the cultivation system had No. significant effect on the accumulation of rutin in sweet pepper fruits [Hallmann and Rembiałkowska, 2007]. These results differ from those presented by Asami et al. [2003] and Rembiałkowska et al. [2003], Rembiałkowska et al. [2005]. They found a higher content of flavonol compounds in the organic fruit and vegetable species studied. The content of bioactive compounds in sweet pepper fruits from the organic system may be also strongly influenced by variety. From the two fresh sweet pepper varieties studied only one (California Wonder) contained more vitamin C than the fruits of the same variety in the conventional system, while the other, Excalibur, was characterized by higher content of vitamin C in the conventional system [Chassy et al., 2006]. Also the content of flavonols in sweet pepper fruits was higher in the conventional system, except for quercetin, which was found to be richer in the fruits of organic sweet pepper of the California Wonder variety. Obviously, a large year-to-year variability was also observed [Chassy et al., 2006]. The studies carried out by Pérez-López et al. [2006] found more vitamin C, carotenoids and total polyphenols in the fruits of organic sweet pepper. Similar results were obtained by del Amor et al. [2008], who demonstrated that organic sweet pepper of the Almuden variety was characterized by significantly higher content of total polyphenols, and demonstrated statistically significantly higher activity of polyphenol peroxidase in the fruits.

This literature review shows that the results obtained so far are not unequivocal – most of the studies confirm better parameters of nutritional value of organically-managed sweet pepper, yet some results run counter to it. The studies presented have been aimed at broadening the knowledge in this regard.

Material and Methods

The experiment used two varieties of sweet pepper [Roberta and Berceo] from organic and conventional production. These plants were grown in two pairs of certified organic and conventional farms, located in Mazowieckie Province of Poland.

The organic farm no. 1 was located at Kaszewska Wola (Przytyk commune, Radom District, Mazowieckie Province). Coordinates 51°30’ N 20°55’ E. The farm used green fertilizers based on phacelias (500 kg), and a mineral fertilizer called Patentkami over the previous year. The total fertilizer balance was as follows: nitrogen /N/ 90 kg; phosphorus /P/ 15 kg; potassium /K/ 250 kg; calcium /Ca/ 80 kg, magnesium /Mg/ 61 kg per one hectare of area, with the addition of all the necessary micronutrients. The following products were used for protection: Bioczos BR, Grevit 200 SL, for the protection against potato blight, Biohumus EKO, nettle extract.

The organic farm no. 2 was located at Radzanów (Radzanów commune, Białobrzegi District, Mazowieckie Province). Coordinates 51°33’ N 20°51’ E. The following was used as fertilizer: compost from agricultural waste in the quantity of 150 kg per tunnel, Biohumus EKO produced at the farm in the quantity of 47 l for the whole duration and area of cultivation. The total fertilizer balance was as follows: nitrogen /N/ 230 kg; phosphorus /P/ 58,5 kg; potassium /K/ 143 kg; calcium /Ca/ 823 kg, magnesium /Mg/ 111 kg per hectare, with the addition of all the necessary micronutrients. The following product were used for plant protection: Grevit 200 SL, for the protection against potato blight.

The conventional farm no. 1 was located at Kaszewska Wola (Przytyk commune, Radom District, Mazowieckie Province), very close to the organic farm; coordinates 51°31’ N 20°56’ E. The following was used as fertilizers: cattle dung 10 t per ha (in the year preceding the experiment, i.e. 2007), chalk, calcium-magnesium nitrate 5 kg per 125m^2. The detailed NPK balance was as follows: nitrogen /N/ 104 kg; phosphorus /P/ 110 kg; potassium /K/

123 kg; calcium /Ca/ 651 kg, magnesium /Mg/ 35 kg per hectare. Mospilan 20 SP was used as protection against greenhouse whitefly.

The conventional farm no. 2 was located at a distance of 7 km from the organic farm, at Sewerynów (Przytyk commune, Radom District, Mazowieckie Province). Coordinates 51°28' N 20°54' E. The following was used as fertilizers: cattle dung 0,25 t per are and mineral fertilizers: Polidap (10 kg per are), Grantar (10 kg per are) and calcium nitrate (8 kg per are). Together with Polidap fertilizer, all necessary micronutrients were used in the cultivation. The total fertilizer balance was as follows: nitrogen /N/ 409 kg; phosphorus /P/ 389 kg; potassium /K/ 512 kg; calcium /Ca/ 362 kg, magnesium /Mg/ 55 kg per hectare. The following products were used for plant protection: Sumilex 500 SC against grey mould, Previcur Energy 840 SL against brown spot and root rot, Topsin M 500 SC for the protection against brown and septoria leaf spot, Mospilan 20 SP for the protection against greenhouse whitefly.

Ripe sweet-pepper fruits were harvested (at the same ripening stage from all farms) and transferred for pickling. Sweet-pepper fruits were washed and cut into stripes. They were inserted to jars and spices were added: 2 bay leafs, 2 grains of allspice and 5 grains of black pepper, and then filled with pickle. The pickle was composed of: 10% vinegar, 1% cooking salt, 0.5% sugar. The pickle was heated up to 90°C and one spoon of Florinol edible oil was added. Such pickle was poured into the jars filled with sweet pepper. Subsequently, some samples, in line with the experimental assumptions, were subjected to the pasteurization process at a temperature of 85°C for 18 minutes. After the pickles were made, sweet-pepper samples were freeze-dried directly after the pickling, and subjected to chemical analysis, covering the estimation of dry matter by gravimetric method (PN-A-75101-03:1990), vitamin C by the Tillmans method (PN-A-75101-11:1990), flavonols expressed as rutin by the Christ-Müller's method, and carotenoids by column chromatography [Saniawski and Czapski 1983]. Because the selected analytical method allows to determine only the flavonol compounds, and rutin (quercetin-3-rutinoside)was used for the preparation of the standard, in the following, the term rutin is used interchangeably with the term flavonols [Strzelecka et al., 1978].

Results

The results obtained indicate that the content of dry matter in the pickled sweet pepper fruits significantly depended on the cultivation system applied. Organic sweet pepper contained significantly more dry matter than the fruits of conventional sweet pepper (Fig. 1). The variety was not a significant factor in the accumulation of dry matter in the samples of pickled sweet peppers, but in both cultivation systems Roberta variety was characterized by slightly increased content of dry matter as compared with Berceo variety (Fig. 1).

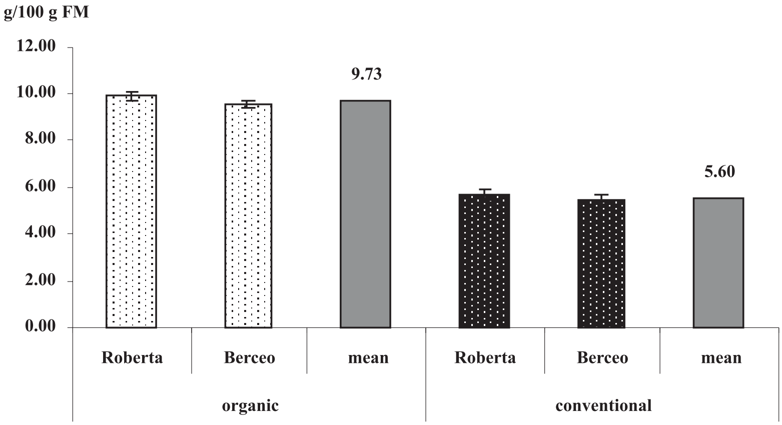

Fig. 1. The content of dry matter in pickled red pepper fruits from organic and conventional production

p-value	**cultivation**	**variety**	**interaction**
	<0.00001	n.s.	n.s.

Pickled organic sweet pepper was characterized by significantly higher content of vitamin C in fruits as compared with conventional sweet pepper (Fig. 2). From the varieties studied in the organic system, more vitamin C was found in Roberta variety fruits, and in conventional system – this was Berceo variety (Fig. 2).

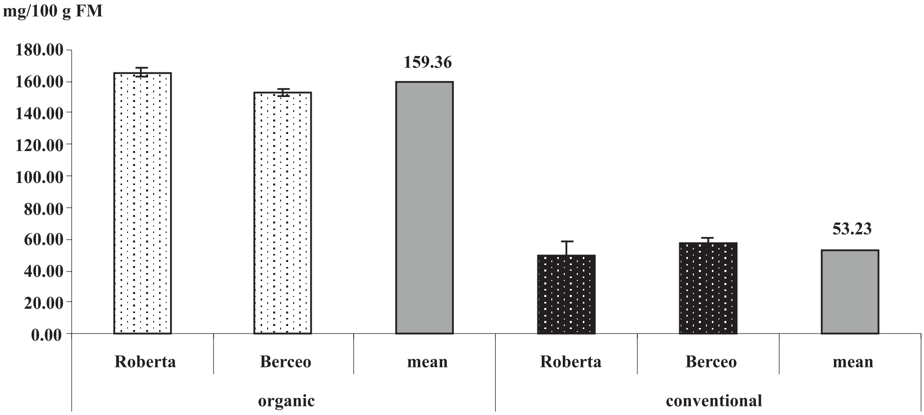

Fig. 2. The content of vitamin C in pickled red pepper fruits from organic and conventional production

p-value	**cultivation**	**variety**	**interaction**
	<0.00001	n.s.	n.s.

The content of flavonols in pickled sweet pepper fruits was significantly affected by cultivation method and variety (Fig. 3). Pickled organic sweet peppers were characterized by significantly higher content of flavonols than conventional sweet peppers (Fig. 3). In the varieties studied in the organic system, more flavonols were found in Roberta variety fruits, and in conventional system Berceo was the richer variety (Fig. 3).

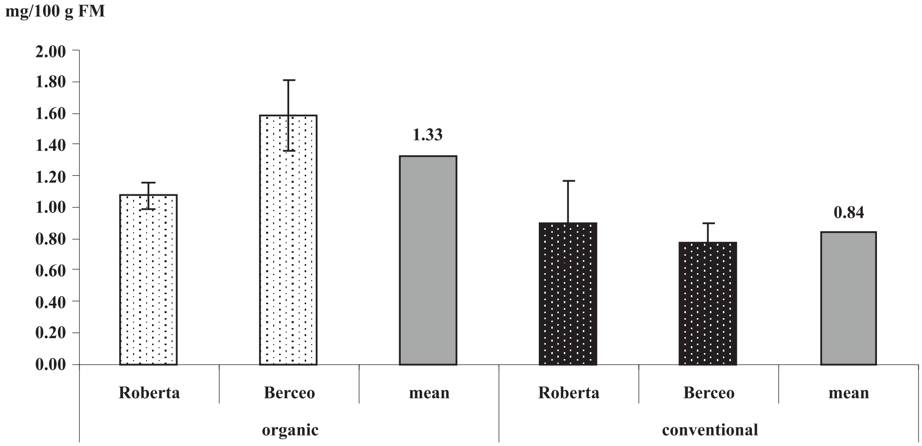

Fig. 3. The content of flavonols in pickled red pepper fruits from organic and conventional production

p-value	**cultivation**	**variety**	**interaction**
	<0.00001	0.0027	0.003

The content of phenolic acids in pickled sweet pepper fruits was significantly affected by cultivation system. The samples of pickled organic sweet peppers contained 86.3% more phenolic compounds than conventional samples (Fig. 4). In the varieties studied in both cultivation systems, the samples of Roberta variety were characterized by a higher content of phenolic acids as compared with the samples of the other variety under study (Fig. 4).

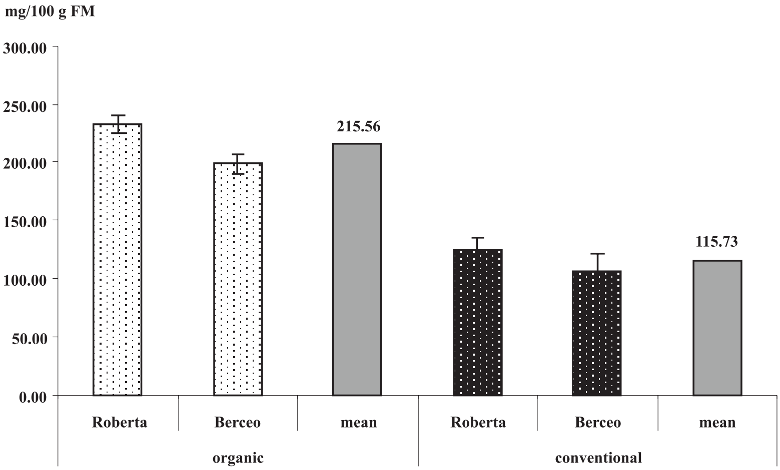

Fig. 4. The content of phenolic acids in pickled red pepper fruits from organic and conventional production

p-value	**cultivation**	**variety**	**interaction**
	0.0017	n.s.	n.s.

The content of beta-carotene was significantly higher in the samples of pickled organic sweet pepper (Fig. 5). The varieties under study differed significantly from each other in beta-carotene content. In the organic system, more of this compound was found in Berceo variety fruits, and in the conventional system - it was the Roberta variety (Fig. 5).

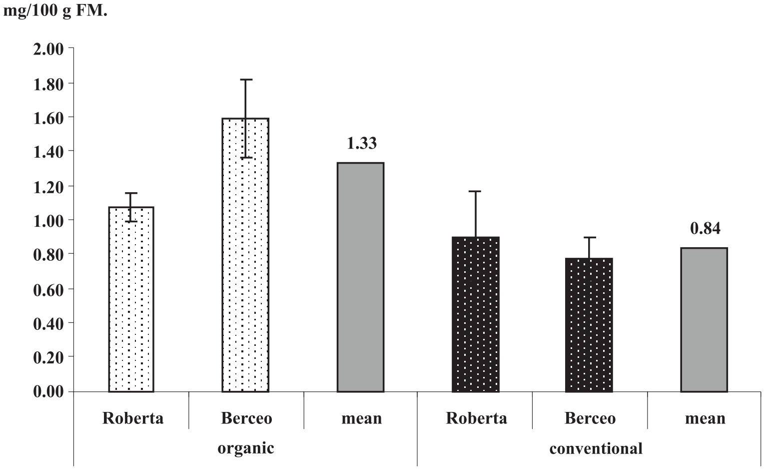

Fig. 5. The content of beta-carotene in pickled red pepper fruits from organic and conventional production

p-value	**cultivation**	**variety**	**interaction**
	0.0001	0.0211	0.0006

The content of lutein in pickled sweet pepper fruits was significantly affected only by the cultivation system. The organic sweet peppers contained 45.9% more of this compound relative to the conventional samples. In the varieties under study in both cultivation systems, more lutein was found in the Roberta variety fruits (Fig. 6).

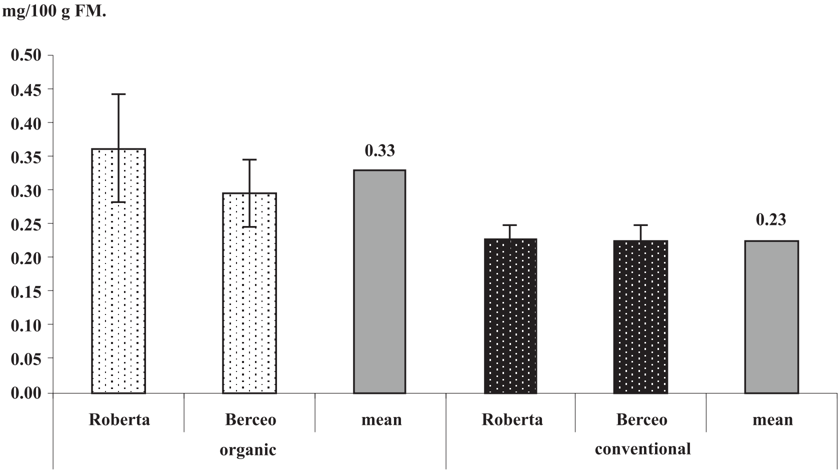

Fig. 6. The content of lutein in pickled red pepper fruits from organic and conventional production

p-value	**cultivation**	**variety**	**interaction**
	0.0006	n.s.	n.s.

Discussion

In the experiment presented here, pickled sweet peppers from the organic production were characterized by significantly higher content of dry matter in the fruits. Since there is deficiency of information in the available literature about nutritional value of pickled sweet peppers from different cultivation systems, the results obtained could only be compared with the previous study by authors in this field [Rembiałkowska and Hallmann, 2008] and in some cases with the results for fresh organic and conventional sweet pepper. The sweet peppers grown in the organic system contained 6.15–6.83 g dry matter per 100 g FM [Rembiałkowska and Hallmann, 2008]. The results presented here are slightly higher in the case of pickled organic sweet peppers, in which the dry matter content was found to be within the range of 9.53–9.93 g per 100 g FM (fig.1). For fresh conventional sweet peppers Deepa et al. [2007] obtained the dry matter content within a much wider range of 6.80–11.60 g per 100 g FM. This may suggest that the variety of sweet pepper has a strong effect on the dry matter content in fresh and pickled fruits. Vitamin C content in sweet peppers depends not only on the variety, but also on cultivation conditions. In this experiment, the vitamin C content was found to depend only on the cultivation system studied. The fruits of organic pickled sweet peppers contained significantly more vitamin C (Fig. 2). The results presented here are much more distinctive than those obtained by Rembiałkowska and Hallmann [2008] obtained the vitamin C content of 57.24 mg per 100 g FM for pickled organic sweet peppers. For fresh organic sweet peppers Pérez-López et al. [2007] obtained vitamin C content of 148.85 mg per 100 g FM, and only 120.65 mg per 100 g FM for the conventional sweet peppers. In this experiment, the flavonols content in pickled organic sweet peppers was found to be significantly higher than in conventional fruits, reaching 5.92 mg per 100 g FM and 3.00 mg per 100 g FM, respectively (Fig. 3). Rembialkowska and Hallmann [2008] recorded previously the opposite results – pickled organic sweet peppers contained 16.4% less flavonols as compared with conventional samples. Chassy et al. [2006], however, found a similar flavonols content expressed as quercetin – 3.08 mg per 100 g FM for organic fruits, and 2.41 mg per 100 g FM for conventional fruits. The content of phenolic acids was found higher in a freshly prepared organic pickle (215.56 mg per 100 g FM) than in conventional samples – only 115.73 mg per 100 g FM. However, Chassy et al. [2006] found significantly smaller quantities for fresh organic sweet peppers (36,4 mg per 100 g FM), and 34.4 mg per 100 g FM. On the other hand, Deepa et al. [2007], who studied only fresh conventional fruits, found the content of phenolic compounds to be 93.6 mg per 100 g FM. This is consistent with these authors' studies, which found smaller concent of phenolic compounds in sweet peppers following pickling [Rembiałkowska and Hallmann, 2008]. In this research, the beta-carotene content of pickled organic sweet peppers was higher (1.33 mg per 100 g FM) as compared with the fruits of pickled conventional sweet peppers (0.54 mg per 100 g FM) (Fig. 5). In contrast, earlier studies conducted by Rembiałkowska and Hallmann [2008] found the opposite result, i.e., it was pickled conventional sweet peppers that was richer in this compound (by 54%) as compared with organic samples. Significantly higher proportions, but only for beta-carotene and in fresh conventional sweet peppers are reported by Deepa et al. [2007]. Next, the content of total carotenoids in fresh organic sweet peppers reported by Pérez-López et al. [2007] was 3.2 mg per 100 g FM, and in fresh conventional fruits only 1.8 mg per 100g FM. Earlier research by Rembiałkowska and Hallmann [2008] found that the cultivation system had No. significant impact on the content of lutein in the pickled sweet peppers: the content of lutein in the pickled organic sweet peppers was contained within the range of between 0.67 and 0.73 mg per 100g FM, while for the conventional samples it was between 0.65 and 0.69 mg per 100 g FM. However, this study found slightly lower values for lutein in both cultivation systems, but pickled organic sweet peppers contained significantly more lutein than conventional samples (Fig. 6).

Conclusions

Pickled organic sweet peppers were characterized by a significantly higher content of all bioactive compounds under study, i.e. vitamin C, flavonols, phenolic acids, beta-carotene and lutein as compared with conventional sweet peppers. Organic sweet peppers are very good material for preparing pickles. Pickles from organic sweet peppers should be recommended as an important element of healthy diet in the winter season.

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9

THE EFFECT OF PASTEURIZATION ON THE NUTRITIONAL VALUE OF CARROT JUICE FROM ORGANIC AND CONVENTIONAL PRODUCTION

Introduction

The research of recent years has demonstrated that organic vegetables have better nutritional value and higher content of bioactive compounds than conventional vegetables. This is related to a different fertilization and plant protection practices used in organic farms. Chemical fertilizers and synthetic plant protection products are inadmissible in the organic system. Natural fertilizers are widely used (manure, compost, green manure), as are natural protection methods (natural enemies, pheromone traps, board traps). Due to a different management system, plants in the organic production develop their own defences in form of intensified synthesis of bioactive compounds (e.g. phenolic compounds). These are called "natural pesticides" [Young et al., 2005]. Many research studies find that these compounds have a positive effect on human health [Brandt and Mølgaard, 2001].

Carrots contain phenolic acids (chlorogenic and isochlorogenic acids), a group of phenolic compounds [Alasalvar et al., 2001], and are also rich in fibre and simple sugars (glucose and fructose). They are also a rich source of carotenoids: beta-carotene and lutein. Carrots from organic production can be a valuable material for juice production. Abele [1987] found that organic carrots were characterized by a lower content of dry matter, but at the same time produced more total and reducing sugars, as well as more beta-carotene as compared with conventional carrots [Abele, 1987]. Similar results in terms of the content of dry matter, sugars and beta-carotene are reported by Rembiałkowska and Hallmann [2007]. The content of bioactive compounds in carrot roots and their suitability for juice production depends on many external factors (primarily agricultural practice and weather conditions in the particular growing season) and on the internal factor, i.e. the variety. This is why research has been undertaken to study nutritional value of puree juices obtained from carrot varieties cultivated in the organic and conventional systems.

Material and Methods

Two carrot varieties, Perfekcja and Flacoro, were grown in organic (certified) farms and in conventional farms located in the same region. Below is presented a system of fertilization and plant protection used in these organic farms.

The organic farm no. 1 was located at Orońsko (Orońsko commune, Szydłowiec District, Mazowieckie Province). Coordinates 51°18' N 20°59' E. Manure was used as fertilizer in the quantity of 20t per ha. Mineral fertilizer called Patentkali (allowed for use in organic system), ground phosphate rock. The total fertilizer balance was as follows: nitrogen /N/ 107 kg; phosphorus /P/ 74 kg; potassium /K/ 210 kg; calcium /Ca/ 200 kg, magnesium /Mg/ 44 kg per hectare. No. protection against disease and pests was used.

The organic farm no. 2 was located at Kaszewska Wola (Przytyk commune, Radom District, Mazowieckie Province). Coordinates 51°30' N 20°55' E. Green manure based on mustard, Humobak – a product activating soil fauna and flora, and ground basalt. The total fertilizer balance was as follows: nitrogen /N/ 374 kg; phosphorus /P/ 54 kg; potassium /K/ 400 kg; calcium /Ca/ 29 kg, magnesium /Mg/ 25 kg per hectare. The following products were used for protection: Bioczos BR, Biohumus EKO and nettle extract to protect against fungal diseases.

In conventional farms, a different fertilization and protection system was applied, as described below.

The conventional farm no. 1 was located 34 km away from the organic farm no. 1 at Sewerynów (Przytyk commune, Radom District, Mazowieckie Province). Coordinates 51°28' N 20°54' E. Cattle dung an mineral fertilizer Polidap were used for fertilization. The total fertilizer balance was as follows: nitrogen /N/ 450 kg; phosphorus /P/ 255 kg; potassium /K/ 278 kg; calcium /Ca/ 215 kg, magnesium /Mg/ 50 kg per hectare. Afalon 450 S.C. was used as protection against weeds.

The conventional farm no. 2 was located 2 km away from the organic farm no. 2 at Suków (Przytyk commune, Radom District, Mazowieckie Province). Coordinates 50°48' N 20°41' E. Cattle dung and chalk were used as fertilizers. The total fertilizer balance was as follows: nitrogen /N/ 107 kg; phosphorus /P/ 22 kg; potassium /K/ 110 kg; calcium /Ca/ 1843 kg, magnesium /Mg/ 86 kg per hectare. No. protection against disease and pests was used.

The carrots were harvested at the same time from all farms, and used for the production of puree juices. The roots were manually washed and sorted, broken up into smaller pieces and scalded with water in proportion 1:1. Then, water was evaporated (at 90°C). The carrot puree thus obtained was rubbed through sieves, 1.2 mm mesh. Next step was juice condensation and mixing. 1 dm^3 of carrot juice contained 540 g carrot puree, 46 g sugar and 2 g citric acid, and was filled up to 1 liter with water. In line with the experiment guidelines, the selected samples were pasteurized at 92–93°C for 20 min.

The following measurements were carried out in juice samples thus prepared: dry matter (gravimetric analysis) (PN –R-04013:1988), total and reducing sugars [Fortuna et al., 2003], organic acids (PN-A- 75101-04:1990), vitamin C (pn-a-75101-11:1990), carotenoids (by HPLC) [Helsper et al. 2003], phenolic acids (by colourimetric method) [Strzelecka et al., 1978]. The results were subjected to bi-factor statistical analysis using Tukey's test, at the significance level of $\alpha = 0.05$; in addition, standard deviation and p-value (probability) were generated for the samples.

Results

The results in terms of the content of dry matter and total and reducing sugars in fresh carrot juice are presented in table 1. The statistical analysis carried out has demonstrated that the content of dry matter in the organic carrot juice tends to be higher as compared with the conventional juice. In both cultivation systems, the product derived from the roots of the Perfekcja variety was characterized by a higher content of dry matter than the other juice that was derived from the roots of the Flacoro variety. After pasteurization, dry matter was found to have increased (water loss) in all tested samples, although the increase in dry matter content was greater in the samples of conventional juices. Following pasteurization, the samples of the organic juice contained, on average, more dry matter than conventional samples (Table 2). In the organic system, juice derived from the roots of the Perfekcja variety contained more dry matter, and in the conventional system - it was the juice derived from the Flacoro variety. The content of total sugars in the carrot juices was significantly related to the cultivation system applied. The organic juice contained significantly less total sugars than the conventional juice (Table 1). Only in conventional system, the juice made from the roots of the Perfekcja variety was characterized by a higher content of total sugars. The pasteurization process contributed to a loss of total sugars in all tested samples of carrot juices, although a greater loss was observed for conventional samples. Following pasteurization, the samples of the organic juice were more abundant in total sugars than conventional samples (Table 2). The samples of the organic juice tended to contain more reducing sugars than the conventional samples (Table 1). The pasteurization process contributed to increased reducing sugars in all tested samples, although the content of these compounds was similar in organic and conventional samples. Following pasteurization, both samples contained similar level of reducing sugars. Variety was the factor that significantly influenced the content of reducing sugars in the pasteurized carrot juice. In both cultivation systems, juices derived from the roots of the Perfekcja variety contained more reducing sugars than the other product under study. The content of organic acids in carrot juices was significantly related to the cultivation system. The samples of organic juices contained more organic acids than conventional juices (Table 1). The pasteurization process contributed to a rise in the content of these compounds in all tested samples, although this increase was observed to be greater in conventional samples. Following pasteurization, the conventional samples contained slightly more organic acids than organic samples. In both cultivation systems, the juice derived from the roots of the Perfekcja variety was characterized by a higher content of organic acids than the other juice, derived from the roots of the Flacoro variety (Table 2).

Table 1

The content of dry matter, total and reducing sugars, and total acidity in fresh carrot juices from organic and conventional production [g/ 100 g FM]

		dry matter	**sugars**		**organic acids**
			total	**reducing**	
Cultivation method	**variety**				
organic	Perfekcja	8.77	6.53	3.81	0.42
	Flacoro	8.68	6.59	3.79	0.41
	mean	**8.73a**	**6.56a**	**3.80a**	**0.41b**
conventional	Perfekcja	8.95	7.43	3.84	0.37
	Flacoro	8.19	6.57	2.62	0.33
	mean	**8.57a**	**7.00a**	**3.23a**	**0.35a**
p-value					
Cultivation method		n.s.	n.s.	n.s.	0.03
variety		n.s.	n.s.	n.s.	n.s.
interaction		n.s.	n.s.	n.s.	n.s.
n.s. = not significant					

Table 2

The content of dry matter, total and reducing sugars, and total acidity in pasteurized carrot juices from organic and conventional production [g/ 100 g FM]

		dry matter	**sugars**		**organic acids**
			total	**reducing**	
Cultivation method	**variety**				
organic	Perfekcja	8.95	6.28	5.97	0.58
	Flacoro	9.13	6.49	5.94	0.56
	mean	**9.04a**	**6.38a**	**5.95a**	**0.57a**
conventional	Perfekcja	9.16	6.52	6.22	0.60
	Flacoro	8.83	5.83	5.58	0.55
	mean	**8.99a**	**6.18a**	**5.90a**	**0.58a**
p-value					
Cultivation method		n.s.	n.s.	n.s.	n.s.
variety		n.s.	0.015	0.046	0.008
interaction		n.s.	n.s.	n.s.	n.s.
n.s. = not significant					

The content of phenolic acids in carrot juices was closely related to the cultivation method. Juices from organic production were characterized by significantly lower content of these compounds than conventional juices (Fig. 1). The pasteurization process contributed to a loss of phenolic acids in all tested samples of carrot juices, although a greater loss was observed for conventional samples. Following pasteurization, slightly more phenolic acids were found in organic juices than in conventional ones, however, this difference was statistically insignificant (Fig. 1).

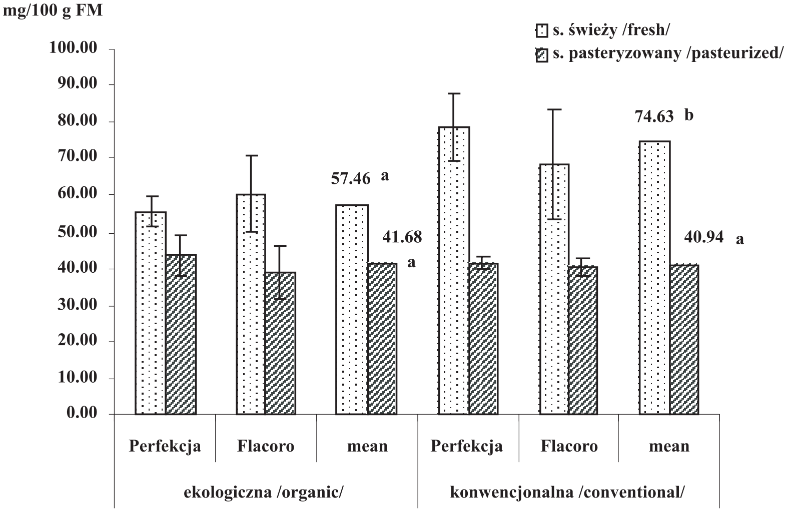

Fig. 1. The content of phenolic acids in fresh and pasteurized carrot juice from organic and conventional production

p-value	**fresh**	**pasteurized**
cultivation	0.0015	n.s.
variety	n.s.	n.s.
cultivation x variety	n.s.	n.s.

The content of vitamin C in fresh carrot juices was significantly higher in conventional samples than in organic ones (Fig. 2). The pasteurization process contributed to a loss of vitamin C in all products tested, with a greater loss found in conventional samples. Following pasteurization, the conventional samples were still richer in vitamin C than organic samples, but this difference was statistically insignificant (Fig. 2).

The content of beta-carotene was slightly higher in conventional samples than in organic ones, but this difference was statistically insignificant (Fig. 3). In the organic system, the juice made from the roots of the Flacoro carrot variety was characterized by a higher content of beta-carotene, and in the conventional system the Perfekcja variety proved to be better (Fig. 3). The pasteurization process contributed to a loss of beta-carotene in all tested samples of carrot juices, although a greater loss was observed for conventional samples. The pasteurized carrot juice contained the same proportion of beta-carotene as the organic one (Fig. 3).

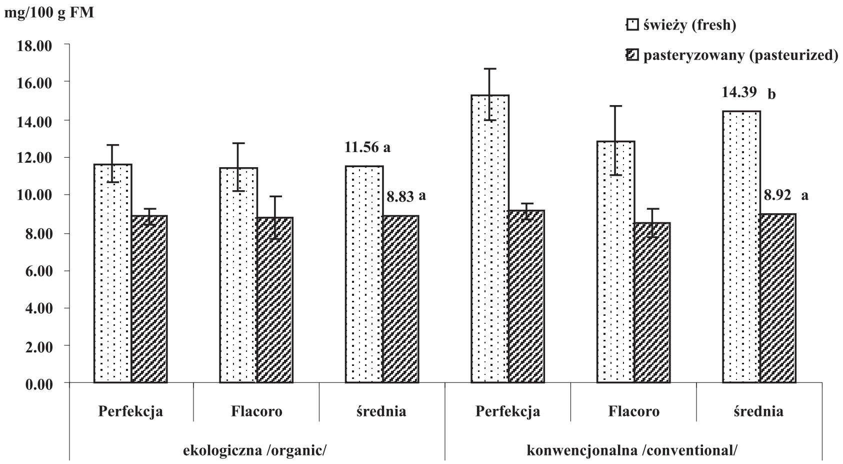

Fig. 2. The content of vitamin C in fresh and pasteurized carrot juice from organic and conventional production

p-value	**fresh**	**pasteurized**
cultivation	0.0002	n.s.
variety	0.0375	n.s.
cultivation x variety	n.s.	n.s.

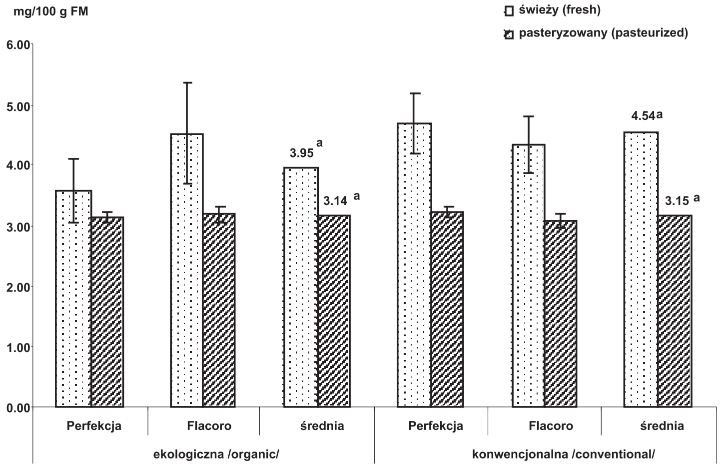

Fig. 3. The content of beta-carotene in fresh and pasteurized carrot juice from organic and conventional production

p-value	**fresh**	**pasteurized**
cultivation	n.s.	n.s.
variety	n.s.	n.s.
cultivation x variety	0.0014	0.0026

The content of lutein in fresh carrot juice was significantly higher in conventional samples, but this difference was statistically insignificant (Fig. 4). The pasteurization process contributed to a growth of the content of total sugars in all tested samples of carrot juices, both organic and conventional ones, although a greater increase was observed for conventional samples. However, following pasteurization, the conventional samples were still slightly richer in lutein than organic samples, but this difference was statistically insignificant (Fig. 4).

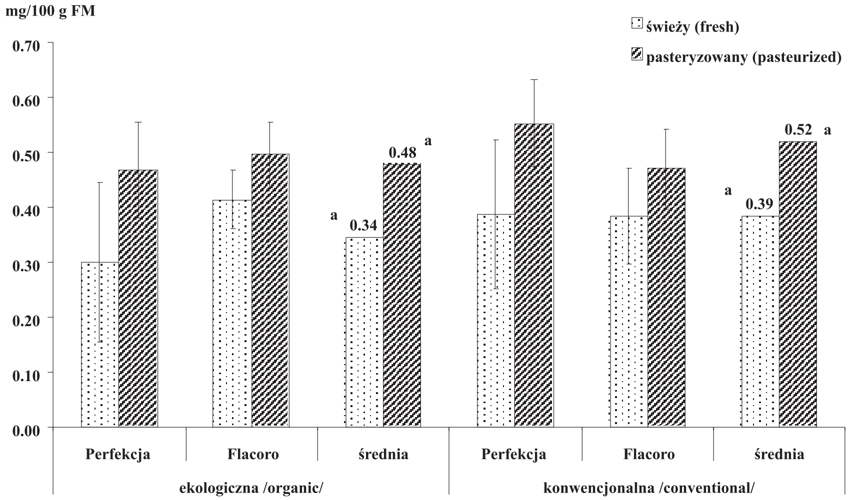

Fig. 4. The content of lutein in fresh and pasteurized carrot juice from organic and conventional production

p-value	**fresh**	**pasteurized**
cultivation	n.s.	n.s.
variety	n.s.	n.s.
cultivation x variety	n.s.	0.026

Discussion

Carrot juice is an excellent source of fibre and simple sugars. At the same time, it provides the body with valuable bioactive compounds, such as carotenoids and phenolic compounds. The carrot varieties recommended for juice production should be characterized by a high content of dry matter, simple sugars and carotenoids. Since there is No. information in the available literature about the chemical composition of organic and conventional carrot juices, the presented results were compared with publications about conventional carrot juice, as well as publication about other kinds of organic and conventional juices, and in sporadic cases, with nutritive value of fresh carrots from two cultivation systems. Rembiałkowska and Hallmann [2007] found that carrots from organic production contained slightly less dry matter, but at the same time more total and reducing sugars than conventional carrots. Similar result were obtained by Abele [1987], who reported that organic carrots was characterized by a higher content of dry matter, and total and directly reducing sugars. This study reports a higher content of dry matter in organic juice, and these juices, while fresh (directly after production), were characterized by higher content of reducing sugars (Tab. 1). Following pasteurization, a change in the sugar balance occurred in the products under study, because organic juices contained slightly more total sugars, while conventional juices were characterized by higher content of reducing sugars (Tab. 2). Heat-treatment of carrot juice significantly influenced the content and proportions of carotenoids. Rising temperatures and storage times contributes to degradation of pigments [Chen et al., 1996]. In their experiment, Marx et al. [Marx et al., 2003] report that increasing temperature of juice (they only studied the conventional juice) prior to pasteurization proper (10 min) by 10^{o}C made beta-carotene slightly increase (by 17%). On the other hand, the process of blanching (10 min) after pre-heating did not change the content of beta-carotene in juice samples. At the same time, extending the pasteurization time to 30 min at 80^{o}C made beta-carotene content increase by 17.8%. In this paper, the opposite process was found to occur: the content of beta-carotene following juice pasteurization fell in all products, regardless of the cultivation system.

The content of beta-carotene in the source material, i.e. carrot roots, depends on agricultural practice applied and genetic factors. In the presented studies, the content of beta-carotene in fresh carrot juice from organic and conventional production was 3.14 mg per 100 g FM and 3.15 mg per 100 g FM, and lutein 0.48 mg per 100 g FM and 0.52 mg per 100 g FM, respectively. Carrot varieties with dark-orange root pigment (high-*β*C orange) contain on average even up to 18.5 mg per 100 g FM beta-carotene and 0.44 mg per 100 g FM lutein, while typical orange roots 12.8 mg per 100 g FM and 0.26 mg per 100 g FM beta-carotene and lutein, respectively [Surles et al., 2004] Similar results are reported by Strackea et al. [2008] who found that organic carrot contained 12.1 mg per 100 g FM beta-carotene, and conventional one – 11.6 mg per 100 g FM. According to the research carried out by other authors, the levels of beta-carotene in carrot juice in the presented studies was much lower than in raw carrot roots. This is probably the result of the negative impact of processing and pasteurization on the levels of this vitamin in carrots.

Conclusions

Freshly-made carrot juices from organic production had significantly higher total acidity; they also contained slightly more dry matter and reducing sugars than conventional juices, which, however, were characterized by significantly higher content of phenolic acids and vitamin C, and contained slightly more beta-carotene and lutein. Pasteurization of carrot juices, both organic and conventional ones, contributed to increased content of dry matter, of reducing sugars, acidity and lutein, and decreased total sugars, phenolic acids, beta-carotene and vitamin C. In all cases, more profound changes (both increase and decrease) were observed for conventional samples. Following pasteurization, organic juice contained slightly more total sugars and phenolic acids than conventional juice, and conventional juice tended to have higher proportion of dry matter, reducing sugars, organic acids, carotenoids and vitamin C. Further research is needed to study the factors influencing the content of bioactive compounds in preserves from organic and conventional vegetables.

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10

NUTRITIONAL VALUE AND QUALITY OF ORGANIC FOOD

Introduction

Advance in industry, intensification of agricultural production as well as excessive mineral fertilization and the concomitant use of pesticides greatly contribute to the contamination of natural environment. The pollution of environment exerts an immediate effect on the quality of foods supplied by agriculture. Food products of plant and animal origin produced with conventional methods are not devoid of ample chemical compounds being detrimental to man [Pilarski et al., 2003; Żakowska-Biemans et al., 2003]. An alternative to conventional agriculture is organic farming aimed at producing high-quality food, while maintaining traditional methods of plant cultivation and animal breeding, without the use of artificial fertilizers, pesticides, growth regulators nor feed additives [Wieczorek, 2006; Łuczka-Bakuła, 2007].

A considerable interest in foods produced with the ecological methods observed in the Member States of the EU, made that over the last 12 years the area of arable lands exploited by ecological farms has increased nearly ten times, i.e. from ca. 0.7 mln ha in 1993 to 6.3 mln ha in 2005, which constitutes ca. 4% of the total arable lands [Stalenga and Kuś, 2007]. In Poland, in the year 2008 there were 14.970 ecological farms and 236 processing plants of raw materials originating from organic farming. In spite of the fact that their number is increasing successively each year, they are still sparse as compared to the other countries of the EU [www.ijhars.gov.pl, 2008].

The production of organic food is subject to legal regulations referring to agricultural crops, animal breeding as well as processing and market of organic food. Legal regulations referring to the principles of organic food production, the labeling of organic food products, granting certificates and the control system are stipulated in the Directive of the Council (EC) No. 834/2007 of the 28th of June 2007 and in the Directive of the Commission (EC) No. 889/2008 of the 5th of September 2008. In Poland, those regulations have been in force since the 1st of January 2009 [Dziennik Urzędowy Unii Europejskiej L.250, 2008].

In Poland, the customers who buy organic food are mainly inhabitants of big cities, young, educated persons, vegetarians, mothers of young children, and persons suffering from chronic diseases. 87% of consumers buying this type of food substantiate their choice with a care over their health [Żakowska-Biemans et al., 2003; Rembiałkowska, 2000]. Organic food is commonly perceived as having a higher nutritive value and a lower level of contaminants as compared to the foods produced with conventional methods. Still, a question arises whether the nutritive value and quality of foods produced with ecological methods are indeed higher than those of the foods produced with conventional methods [Bartnik, 1996].

The objective of the study was to compare the nutritive value and quality of food produced with methods of organic farming and conventional agriculture based on an overview of literature published over the last 18 years.

Content of nutrients in organic and conventional food

Protein

The content of total protein in various potato cultivars originating from conventional crops and organic farming was estimated by Rembiałkowska [2000]. Protein content of potato, garden beet and carrot from both these crops was also determined by Wawrzyniak et al. [1997]. Those investigations demonstrated that the content of total protein in potatoes and garden beets cultivated with ecological methods was lower by ca. 9% on average than that in the respective products from conventional crops, whereas in carrot its content was alike in both types of crops.

Carbohydrates

In tomatoes and paprika from ecological farming, the content of carbohydrates was found to be significantly higher than in the respective conventional crops, i.e. by ca. 40% and 8–80%, respectively [Hallmann and Rembiałkowska, 2007; Hallmann et al., 2007]. In such fruits as: apples [Rembiałkowska et al., 2004], plums [Lombardi-Boccia et al., 2004] and grapefruits [Lester, 2007], however, their content was found not to be highly significant different. In vegetables and fruits originating from both types of crops, contents of carbohydrates were differentiated, irrespective of cultivation method [Rembiałkowska et al., 2004; Lombardi-Boccia et al., 2004; Hamouz et al., 2004]. In turn, potatoes from organic farming were characterized by a significantly higher content of starch than those from conventional crops [Rembiałkowska, 2000; Rutkowska, 2005; Hajšlová et al., 2005].

Fatty acids

Cow's and buffalo's milk and dairy products from organic farms were demonstrated to have a significantly higher content of polyunsaturated fatty acids, conjugated dienes of linoleic acid, α-linolenic acid and vaccenic acid, i.e. on average by 70–80% [Bergamo, 2003]. The percentage contribution of n-3 fatty acids in those products was higher by ca. 1.7 times than in the products originating from conventional farms [Ellis, 2006]. Meat of calves bred with ecological methods contained ca. 2 times more, whereas meat of chickens – ca. 2 times less fat in respect of the meat of animals bred with conventional methods [Walshe et al., 2006; Castellini et al., 2002]. In veal, chicken meat and lamb meat, the concentration of polyunsaturated n-3 fatty acids was higher by 20–40% than in the meat of animals from conventional breeding [Castellini et al., 2002; Walshe et al., 2006; Angood et al., 2008].

Vitamin C

60% of investigations addressing the content of vitamin C in potatoes and selected species of vegetables and fruits demonstrated that the highest concentrations of that vitamin occurred in crops from ecological farming as compared to those produced with conventional methods. Apart from potatoes [Rembiałkowska, 2000; Hajšlová et al., 2005], the higher content of vitamin C was also reported in cabbage [Rembiałkowska, 2000; Rembiałkowska, 1998], tomatoes [Caris – Veyrat at al., 2004; Chassy et al., 2006; Hallmann and Rembiałkowska, 2007], paprika [Chassy et al., 2006; Hallmann et al., 2007], as well as in strawberries [Leskinen et al., 2002] and grapefruit juice [Lester et al., 2007]. 25% of the cited references pointed to a lower content of vitamin C in potatoes and other vegetables from organic farms [Rutkowska, 2005; Warman and Havard, 1998; Rembiałkowska et al., 2003; Owsikowski et al., 2008]. In the other investigations, No. difference was demonstrated in the content of that vitamin between the crops obtained with both cultivation methods compared [Kunachowicz et al., 1993; Warman and Havard, 1997; Lombardi – Boccia et al., 2004].

Carotenoids and tocopherols

The overview of literature does not provide any explicit answer as to the difference in the content of β- carotene between vegetables from various types of farming, since half the investigations indicated a higher, whereas the other half pointed to a lower content of that component in products from organic farming as compared to those from conventional cultivation. In organic paprika and tomatoes, the content of β-carotene was higher [Rembiałkowska et al., 2003; Caris – Veyrat et al., 2004; Hallmann and Rembiałkowska, 2007]. In turn, organic carrot was characterized by its lower content [Warman and Havard, 1997; Rembiałkowska, 1998; Rembiałkowska, 2000; Rutkowska, 2005; Owsikowski et al., 2008]. The concentration of lycopene in tomatoes [Rembiałkowska et al., 2003; Hallmann and Rembiałkowska, 2007], paprika [Hallmann, et al., 2007] and grapefruits [Lester et al., 2007] from organic farming was lower than in the respective products obtained with conventional methods. Milk and dairy products from eco farms were characterized by a higher content of β-carotene than those from conventional farms [Bergamo et al., 2003].

The content of α-tocopherol in plums, peaches and cabbage from both types of farming was differentiated and independent of the cultivation method [Warman and Havard 1997; Carbonaro et al., 2002; Lombardi-Boccia et al., 2004]. In turn, organic milk and dairy products were found to contain 1.5 times more α-tocopherol than the conventionally-obtained products [Bergamo et al., 2003].

Minerals

Results of most of the domestic and international studies referring to contents of micro- and macroelements in vegetables, fruits and cereals from organic and conventional farming were diversified and inconsistent [Rembiałkowska, 2000; Hajšlová et al., 2005; Warman and Havard, 1997; Mazurkiewicz, 2005]. No. significant differences were determined in contents of such minerals as: calcium, magnesium, iron, zinc, phosphorus or potassium, that would indicate an explicit dependency between concentrations of those elements on the type of farming system [Warman and Havard, 1998; Wiśniowska – Kielian and Klima, 2006; Wiśniowska – Kielian and Klima, 2007].

Polyphenols

Contents of polyphenolic compounds – flavonols and anthocyanins – in potatoes as well as selected vegetables and fruits originating from different types of farming were diversified [Rembiałkowska, 1999; Hamouz et al., 2004; Lester et al., 2007]. In peaches and pears from organic farms, the content of total polyphenolics was found to be significantly higher as compared to the respective fruits produced with conventional methods [Carbonaro et al., 2002]. A significantly higher concentration was also demonstrated for quercetin in tomatoes and apples originating from ecological crops [Rembiałkowska et al., 2003; Rembiałkowska et al., 2004; Chassy et al., 2006], whereas a significantly higher content of total polyphenolic compounds and quercetin – for plums from conventional crops [Lombardi-Boccia et al., 2004].

The occurrence of contaminants in organic and conventional food

Nitrates(V) and (III)

Results of assays conducted for nitrates(V) and (III), presented in ample research works, point to diversified contamination of crops from ecological and conventional farming with those compounds, irrespective of cultivation methods applied. In most cases, concentrations of those compounds in vegetables and potatoes from both types of farming did not exceed the admissible levels [Rutkowska, 2005]. Most of the domestic and international research (80%) demonstrated a significantly lower content of nitrates(V) in organic potatoes and other vegetables, including: carrot, cabbage, garden beet, tomatoes and lettuce, as compared to the respective crops obtained with conventional methods of cultivation [Leszczyńska, 1996; Rembiałkowska, 1998; Woźniak and Pokorska-Lis, 1999; Rembiałkowska 2000; Rembiałkowska et al., 2001; Rutkowska, 2001; Wawrzyniak et al., 2004; Bożym and Jagiełło 2005; Guadagnin. et al., 2005; Hajšlová et al., 2005]. The crops from organic farming were characterized by from 2 to 10 times lower concentrations of those compounds than those from conventional farming [Rembiałkowska, 1998; Woźniak and Pokorska-Lis, 1999; Rembiałkowska, 2000a; Rembiałkowska, 2000b; Bożym and Jagiełło, 2005]. 10% of investigations demonstrated a higher content of nitrates(V) in organic crops as compared to the conventional ones, whereas the others did not indicate any differences between concentrations of those compounds in potatoes and other vegetables originating from both types of farming [Kunachowicz et al., 1993; Wawrzyniak et al., 1997; Woźniak and Pokorska-Lis 1999]. The content of nitrates(V) in potatoes and selected species of vegetables from both types of farming was presented in Table 1.

In 40% of the analyzed investigations, the concentration of nitrates(III) in potatoes and vegetables from organic farming was demonstrated to be 2–3 times lower than in the respective conventional products [Leszczyńska, 1996,. Leszczyńska, 1999; Woźniak and Pokorska-Lis, 1999; Wawrzyniak et al., 2004]. In turn, 20% of studies showed a higher content, whereas the others indicated either No. differences or great diversity in the content of those compounds between both types of farming [Wawrzyniak, et al., 1997; Rembiałkowska, 2000b; Rutkowska, 2005].

Table. 1

Contents of nitrates (V) [mg/kg fresh mass] in organic and conventional crops
(average ± standard deviation)

Foods	Organic	Conventional	Reference
Potatoes	110.5 ± 46.4	145.8 ± 98	[45]
	30.4 ± 9.9*	53.8 ± 27.5	[45]
	133.72 ± 198.20*	229.14± 145.13	[44]
	157.5 ± 146.7*	250.3 ± 185.7	[18]
	175.2 ± 5.8*	114.3 ± 6.2	[40]
	77	151	[10]
	167.8 ± 86.7*	201.1 ± 81.0	[39]
	145	203	[38]
	52.7 ± 48.2*	120.8 ± 84.06	[43]
	358.9 ± 335.3*	1290.8 ± 1080.9	[8]
	224.2 ± 273.3*	18705 ± 955.6	[8]
Carrots	52.2 ± 36.8*	209.7 ± 175	[24]
	154.5 ± 224.4	266.2 ± 226.7	[45]
	49.1 ± 37*	126.2 ± 61.8	[45]
	131.58 ± 143.01*	261.43 ± 185.18	[44]
	40.3 ± 20.3	323.8 ± 283.3	[42]
	24.7 ± 2.3*	238.7 ± 5.1	[40]
	197.7 ± 107.2	191.8 ± 103.8	[39]
	154	293	[38]
	52.2 ± 36.8*	209.7 ± 175.0	[8]
White cabbage	344.3 ±193.9*	907.8 ± 479.7	[8]
	303	433	[38]
	788.7 ± 362.8*	1061.6 ± 220.0	[39]
	594.4 ± 12*	31.9 ± 0.9	[40]
	344.3 ± 193.9*	907.8 ± 479.7	[24]
Beetroots	1342. 83 ± 892.49	2216.50 ± 1427.87	[8]
	350	834	[38]
	1986. 1 ± 1004.7	1963. 3 ± 606.4	[39]
	714	1412	[10]
	926.5 ± 9.4*	1861. 1 ± 17.0	[40]
	1342.8 ± 892.5	2216.5 ± 1427.9	[45]
	265.0 ± 76.9*	891.2 ± 672.4	[45]
Lettuce	1306.4 ± 822.6	1357.3 ± 931.4	[39]
	818 ± 489*	1303 ± 430	[41]
Parsley	164.94 ± 299.45*	351.19 ± 314	[44]
	132.9 ± 163.8	456.2 ± 257.7	[42]
	166.3 ± 0.5	159.1 ± 4.6	[40]
	469.0 ± 270.0	477.4 ± 124.9	[39]
	234	383	[38]

* statistically significant differences according to references

Heavy metals

A higher content of lead in the crops from ecological farming was demonstrated in ca. 50% of the works analyzed. Potatoes and other vegetables, including: carrot, cabbage or garden beets, as well as cereal grains from that type of farming contained, on average, 1.3–2 times more Pb than the respective vegetables cultivated with conventional methods [Rembiałkowska et al., 2001; Wiśniowska-Kielian and Klima 2007]. In turn, over 30% of works demonstrated a lower concentration of that element in organic potatoes, vegetables and cereals. In the other investigations, No. differences were shown in lead content between the crops originating from both types of faming compared [Leszczyńska, 1996; Leszczyńska, 1999; Rembiałkowska, 1999; Rembiałkowska, 2000a].

In ca. 30% of the works analyzed, the concentration of cadmium was found to be lower in potatoes, vegetables and cereals from eco farms, whereas ca. 30% of studies demonstrated a higher content of that element in ecological products [Leszczyńska, 1999; Rembiałkowska et al., 2001; Hajšlová et al., 2005]. The other cited references did not indicate any differences in the level of cadmium in the crops produced with both types of farming compared in this study [Kunachowicz et al., 1993; Leszczyńska, 1999; Wiśniowska-Kielian and Klima 2007].

In milk originating from both types of farming, No. statistically significant differences were confirmed in the level of both these heavy metals, i.e. Pb and Cd [Ghidini et al., 2005].

In beef from organic farms the concentration of lead and cadmium was ca. 3-fold lower than in the meat of animals from conventional breeding [Ghidini et al., 2005].

Pesticides

Crops originating from organic farms not applying pesticides over the entire vegetative seasons of plants were demonstrated to contain trace amounts of those compounds [Gonzaoles et al., 2003]. Cow's milk obtained from ecological as well as conventional farms also contained trace amounts of pesticides. In contrast, beef originating from ecological farms was shown to contain higher concentrations of pesticides, ranging from 3 to 6.23 μg/kg fat, than the meat of animals bred with conventional methods in the case of which the concentration of pesticides accounted for < 3μg/kg fat. Still, both these values did not exceed the admissible levels [Ghidini et al., 2005].

Mycotoxins

The level of contamination of cereal grains from organic farming with ochratoxin A was very diversified. According to a study by Czerwiecki et al. [2000a, 2000b] conducted in the years 1997–1998, the frequency of occurrence of ochratoxin A in all domestic types of cereals, i.e. wheat, rye and barley, in the year 1997 was significantly higher in grains originating from ecological farms, and in the year 1998 – in grains from conventional farms. In turn, Biffi et al. [2004] did not observe any significant differences in the content of ochratoxin A in Italian cereal products produced from raw materials obtained with ecological and conventional methods.

Baturo [2005], having analyzed the quality of barley kernels of domestic cultivars, demonstrated that ca. 60% of the kernels harvested from ecological crops and ca. 8% of the kernels from conventional crops were infested by pathogenic fungi *Bipolaris sorokiniana*. Ca.

48% of the grains from conventional farming were infested by fungi of the genus *Fusarium spp*., which is 2 times more frequently than in the case of organic grain.

The incidence of potato tuber diseases induced by the fungi *Rhizoctoma solani, Streptomyces scabies* and *Fusarium species* was similar both in the ecological and conventional system of farming [Sadowski et al., 2002; Sadowski et al., 2004].

Results reported in the cited works for the content of mycotoxins in milk were divergent. A study by Skaug [1999] demonstrated a higher, whereas that by Ghidini et al. [2005] – a lower concentration of those compounds in milk from ecological farms as compared to the milk produced with conventional methods.

Glycoalkaloids

In most of articles addressing the content of solanine in potatoes, higher concentrations of that component were determined in tubers cultivated with ecological methods. Results of solanine content determinations in potatoes conducted by Hamouz et al. [2004] showed a higher concentration of that compound in organic tubers (97.9 mg/kg on average) as compared to the potato tubers from conventional crops (77.0 mg/kg on average). According to Hajslova et al. [2005], the mean content of glycoalkaloids in potato tubers cultivated with ecological methods accounted for 80.8 mg/kg and was significantly higher than in the potatoes cultivated with conventional methods (58.5 mg/kg). A research by Abreu et al. [2007] demonstrated that the total content of solanine was 2 times higher in potatoes from conventional crops than in the organic ones, i.e. 79.5 mg/kg and 44.6 mg/kg, respectively.

Conclusions

The conducted overview of domestic and foreign literature addressing the nutritive value and quality of organic food enables concluding that:

1. Organic food did not contain significantly higher levels of nutrients as compared to the conventional food, except for contents of total carbohydrates in such vegetables as tomatoes and paprika, for vitamin C in most of vegetables and fruits, for starch in potatoes as well as for concentrations of α-linolenic acid, conjugates dienes of linoleic acid and the sum of polyunsaturated fatty acids in milk and dairy products.

2. Most of the works analyzed demonstrated a significantly lower content of nitrates (V) in food of plant origin from ecological farming.

3**.** Due to inconsistent results reported for concentrations of such contaminants as: heavy metals, pesticides and mycotoxins, it may not be stated explicitly that the organic food is more safer than the conventional one.

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CHAPTER 2

NEW TRENDS IN FOOD SENSORY QUALITY

1

COMPARATIVE EXAMINATION OF MEAT SENSORY TRAITS IN FIVE CONSERVATIVE STRAINS OF DUCKS

Introduction

The local poultry breeds are an excellent material for studies on the history of farm animal evolution as reported in many papers on poultry genetics [Książkiewicz, 2002]. The characteristics of certain quality traits of duck meat has not been investigated to such extent as it is in the case of land fowl.

Chartrin et. al. [2006] examined the effect of the intramuscular fat (IMF) content in the breast muscles on colour, taste and tenderness by using sensory evaluation and shear-force measurements. Their study revealed that the muscles in ducks subjected to force-feeding system demonstrated a higher fat content, lower water content, lighter colour, lower juiciness and more intense flavor in comparison with the breast muscles of ducks fed *ad libitum.*

Baéza et.al. [2002] in earlier studies reported that selection of ducks for increased body weight resulted in pH decrease in the breast muscles postmortem, increased protein and minerals content and decreased moisture content but had No. effect on colour, fat content and water loss.

Studies on carcase meat yield and chemical composition in the ducks from conservative flocks in Poland were conducted by Książkiewicz and Kontecka [1993], Mazanowski et. al. [2003] and Mazanowski and Gornowicz [2003]. However, the investigations on certain meat quality traits in ducks from conservative flocks were carried out for the first time by Kisiel [2003] and comprised: pH_{15} value, colour and muscle fibre thickness αW and βR in the pectoralis superficialis muscle and biceps thigh muscle. The author demonstrated that the examined parameters were found to have the desired values. He paid particular attention to the observed fact that the thickness of muscle fibres of the pectoral superficial muscle and femoral biceps muscle in the K-2, P-8 and P-33 female ducks exhibited the required fine-fibre structure.

The results of comparative studies facilitate selection of optimum qualitative and quantitative traits of meat with particular attention to the fleshing and fatness of the carcase and nutritional value of meat [Pingel et al., 1997; Kisiel, 2001].

The purpose of this study conducted in the year 2008 was the evaluation the sensory traits of meat in the selected population of conservative strains of ducks (P-8, P-9, P-33, synthetic LsA and K-2).

Material and methods

The experimental material comprised five populations of conservative ducks: K-2 mini-ducks, P-8, P-9 and P-33 Pekin type and LsA birds. In each group of ducks there were 15 males and 15 females kept in the same environmental conditions and feed ad libitum on the same diet. The birds were reared in 2008 for eight weeks in the Waterfowl Genetic Resources Station in Dworzyska within the National Research Institute of Animal Production in Cracow.

Sixteen birds of each group (eight males and eight females) taken at random were used for the evaluation of slaughter yield and meat quality. All those birds were slaughtered and subjected to postmortem processing under the same technological procedure. After 24 h period the eviscerated carcases were dissected according to the method described by Ziołecki and Doruchowski [1989].

Left pectoral superficial and profound muscles and left leg muscles from 16 carcases (8♂ and 8♀) of each conservative flock of ducks were taken for sensory analysis. All muscles were without adjacent skin with subcutaneous fat. The sensory evaluation was conducted on chilled muscles and the following quality attributes were taken into consideration: external appearance, fatness, colour, odour and the total quality of product. A four point hedonic scale from 2 to 5 was used in the sensory assessment where score 2 denoted the undesirable quality whereas score 5 the most desirable quality. For each examined attribute the scores in the sensory quality scale were given with the accuracy to 0.5. The final (total) quality was calculated as the arithmetic mean from the individual scores with the accuracy of 0.1 point. The sensory assessment panel consisted of five judges [Ziołecki, 1988].

All data from the experiments on the sensory assessment of raw meet of ducks from five conservative flocks were subjected to statistical analysis. Moreover, the correlation between the sensory attributes and the body weight prior to slaughter, eviscerated carcase weight and carcase fleshing was also analysed. The significance of differences was determined with the use of Duncan multiple range test in the orthogonal system.

Results and discussion

The general appearance of breast muscles (Table 1) was differentiated among female ducks from the examined strains and ranged from 4.28 (K-2) scores to 4.89 scores (P-33, LsA) and was statistically significant ($p \leq 0.05$). That trait was in male ducks assessed by 0.17 lower than in females, except the LsA strain where the breast muscles of males obtained the maximum score (5.0). The odour of the raw muscles was evaluated on the same level in all examined strains and reached 4.67 score. Breast muscles in female ducks of P-9 strain were given the highest scores for fatness (4.94), colour (4.94) and in the case of total score (4.82). The total score for breast muscles in both males and females was on the highest level (4.79) in the LsA strain, while the score in P-8 strain was significantly ($p \leq 0.05$) lower, i.e. 4.60. The evaluation of odour and fatness revealed No. statistically significant differences. However, in the case of those two attributes a high variation coefficient from 15.8 to 19.6% was noted.

In the total assessment of leg muscles (Table 2) of both sexes , LsA strain was given the highest scores for all examined sensory traits, i.e. appearance (4.64); odour (4.67), fatness

(4.42) as well as colour (4.75) and the total score (4.62). Leg muscles of LsA females obtained the highest scores for the general appearance (4.67), fatness (4.56) and total the score attained (4.64). Fatness of leg muscles in males was scored from 3.78 in P-9 to 4.44 in K-2. As far as muscle colour and odour are concerned the same score level (4.67) was noted in all groups of ducks.

No. statistically significant difference was noted in the colour of duck breast and leg muscles from both sexes, even in the comparison of Pekin type strains (P-8, P-9 and P-33) with the K-2 miniduck strain. On the other hand, Larzul et. al. [2006] reported significantly lighter muscle colour in Muscovy ducks in comparison with muscle colour in Pekin ducks, while in the hybrid ducks the effect of hetherosis was observed in that muscle trait. Similar results were reported by Alexieva et. al. [1999]. They noted that the darker muscle colour in Pekin type ducks is associated with the higher concentration of haem pigments (3.26 mg/g) in that type of duck.

Table 1

Sensory traits of raw breast muscles in male and female ducks from selected conservative flocks (evaluation in points*)

Trait\Group		K-2		P-8		P-9		P-33		LsA	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
General appearance	$\bar{x}$	4.28^{a}	4.00^{a}	4.61^{ab}	4.50^{b}	4.72^{ab}	4.39^{ab}	4.89^{b}	4.67^{bc}	4.89^{b}	5.00^{c}
	SEM	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Odour	$\bar{x}$	4.67	4.67	4.67	4.78	4.67	4.67	4.67	4.67	4.67	4.67
	SEM	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Fatness	$\bar{x}$	4.89^{a}	4.94	4.28^{b}	4.61	4.94	4.56	4.78	4.89	4.67	5.00
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Colour	$\bar{x}$	4.78^{ab}	4.83^{a}	4.67^{ab}	4.50^{b}	4.94^{a}	4.83^{a}	4.72^{ab}	5.00^{a}	4.50^{b}	4.94^{a}
	SEM	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Total score	$\bar{x}$	4.65^{ab}	4.61^{a}	4.56^{a}	4.60^{a}	4.82^{b}	4.61^{a}	4.77^{ab}	4.81^{ab}	4.68^{ab}	4.90^{b}
	SEM	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09

Explanations:
* – scale from 2.0 to 5.0 where score 2.0 denotes negative result and 5.0 most desired result
^{a,b}– different superscripts in the rows denote statistically significant differences at $p \leq 0.05$

Table 2

Sensory traits of raw leg muscles in male and female ducks from selected conservative flocks (scores in points*)

Trait\Group		K-2		P-8		P-9		P-33		LsA	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
General appearance	$\bar{x}$	3.56^{a}	3.89^{ac}	4.39^{b}	4.44^{b}	4.39^{b}	3.94^{c}	4.50^{b}	4.39^{bc}	4.67^{b}	4.61^{b}
	SEM	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Odour	$\bar{x}$	4.67	4.67	4.67	4.72	4.67	4.67	4.67	4.67	4.67	4.67
	SEM	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Fatness	$\bar{x}$	3.89^{a}	4.44^{a}	3.83^{a}	4.28^{ab}	4.22^{ab}	3.78^{b}	3.94^{a}	4.33^{ab}	4.56^{b}	4.28^{a}
	SEM	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Colour	$\bar{x}$	4.28^{a}	4.89	4.56^{ab}	4.50	4.83^{b}	4.61	4.67^{ab}	4.78	4.67^{ab}	4.83
	SEM	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Total score	$\bar{x}$	4.67^{ab}	4.47^{ab}	4.36^{ab}	4.49^{ab}	4.53^{b}	4.25	4.45^{b}	4.54^{ab}	4.64^{b}	4.60^{b}
	SEM	0.14	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11

Explanations:

* – scale from 2.0 to 5.0 where score 2.0 denotes negative result and 5.0 most desired result

^{a,b} – different superscripts in the rows denote statistically significant differences at $p \leq 0.05$

In total the sensory traits in male ducks were scored higher than in females by 0.02 for odour; 0.09 for fatness; 0.1 for colour and 0.01 for the total score; on average. Those differences were statistically not confirmed. Only females in the P-9 strain were scored higher that males in all attributes mentioned above. Omojola [2007] investigated the effect of sex on the sensory quality traits in ducks and found that meat in females from Pekin and Muscovy ducks was significantly better. On the other hand, in the Rouen ducks that author observed an opposite situation since here the sensory attributes of meat were scored higher.

The observed slight differences of the sensory traits in the examined constructive flocks of ducks are in line with the results of study by Baeza et. al. [1998]. They reported that the differentiation of the sensory attributes of breast muscles in ducks were observed with the increase of fat and protein content in the muscles noted between the 8th and 12th week of bird age. The quality assessment of duck muscles in this study was conducted on birds slaughtered at 8 weeks of age.

The summarized evaluation of sensory attributes of breast and leg muscles presented on Figure 1 revealed clearly that the ducks of LsA strain demonstrate meat quality mostly desired by consumers.

Significant correlations were noted between the muscle general appearance (Table 3) and the total score; as well as muscle odour and its fatness. Only few correlations between body weight (Table 4) as well as meat yield (Table 5) and sensory attributes of raw muscles in ducks from selected conservative strains appeared to be significant. That fact indicates the sensory traits of duck meat can also be influenced by other factors, for example of environmental and nutritional nature.

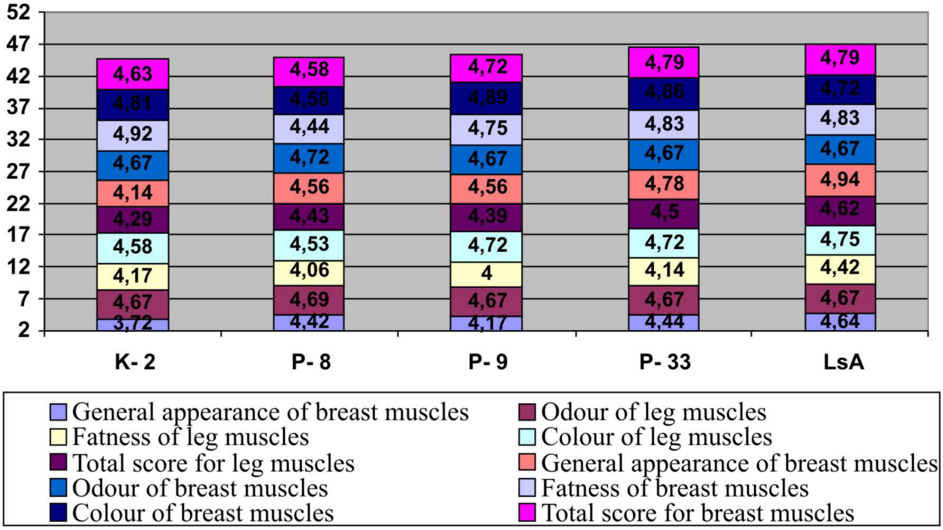

Fig. 1. Summarized sensory evaluation of raw breast and leg muscles from selected conservative flocks (scores in points*).

Table 3

Correlations between general appearance and the other sensory traits of muscles in ducks from selected conservative flocks

	K-2	P-8	P-9	P-33	LsA
General appearance of breast muscle					
x breast muscles traits					
– colour	0.418	0.617*	0.373	0.113	0.662*
– odour	-0.480*	0.365	-0.449	-0.115	0.343
– fatness	0.367	0.444	0.582*	0.187	0.700*
– total score	0.815*	0.846*	0.632*	0.472*	0.845*
General appearance of leg muscle					
x leg muscles traits					
– colour	0.394	0.596*	0.286	0.583*	0.679*
– odour	-0.369	0.081	0.000	0.053	0.245
– fatness	0.330	0.561*	0.745*	0.531*	0.638*
– total score	0.579*	0.797*	0.776*	0.685*	0.843*
total score for breast and leg muscles	0.713*	0.730*	0.775*	0.606*	0.796*

Explanations:
* – statistically significant correlation at $p \leq 0.05$

The noted differences in the breast and leg muscles in the ducks from various conservative flocks indicate that those populations can avail an opportunity to use them in the agro-touristic farms as an element of a mini-zoo (K-2 flock) or an element for preparation of attractive meals, *e.g.* (LsA flock).

Table 4

Correlation between final body weight and sensory traits of raw muscles in duck from selected conservative strains

	K-2	**P-8**	**P-9**	**P-33**	LsA
Body weight in 56 day of life					
x breast muscles traits					
– general appearance	-0.211	-0.335	-0.331	-0.295	0.015
– colour	0.134	-0.477*	-0.196	0.458	0.285
– odour	0.000	0.079	0.000	0.000	0.000
– fatness	-0.183	0.268	-0.461	0.191	0.529*
– total score	-0.157	-0.132	-0.436	0.100	0.265
x leg muscles traits					
- general appearance	0.196	-0.132	-0.453	-0.040	-0.073
– colour	0.445	-0.343	-0.284	0.205	0.163
– odour	0.000	0.041	0.000	0.000	0.000
– fatness	0.283	0.379	-0.496*	0.507*	-0.196
– total score	0.378	-0.027	-0.452	0.237	-0.047
total score for breast and leg muscles	0.179	-0.084	-0.477*	0.206	0.103

Explanations:
* – statistically significant correlation at p≤0.05

Table 5

Correlation between carcase meat yield and sensory traits of raw muscles in ducks from selected conservative flocks

	K-2	**P-8**	**P-9**	**P-33**	**LsA**
The percentage of muscles in eviscerated carcase with neck without giblets					
x breast muscles traits					
- general appearance	0.167	0.349	-0.156	0.147	-0.045
– colour	-0.042	-0.070	-0.165	0.547*	-0.035
– odour	0.000	-0.034	0.000	-0.000	0.000
– fatness	0.540*	0.048	-0.253	0.403	0.310
– total score	0.228	0.111	-0.239	0.399	0.056
x leg muscles traits					
- general appearance	-0.006	-0.143	0.284	0.116	0.098
– colour	-0.020	-0.044	0.108	0.046	0.168
– odour	0.000	-0.092	0.000	-0.000	0.000
– fatness	0.182	-0.216	0.355	0.171	0.432
– total score	0.064	-0.175	0.282	0.111	0.238
total score for breast and leg muscles	0.174	-0.025	0.118	0.243	0.170

Explanations:
* – statistically significant correlation at p≤0.05

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2

ANALYSIS OF VARIOUS BREEDS OF PIGS AND WILD BOARS HEALTH STATUS AND MEAT NUTRITIONAL VALUE

Introduction

21.6 thousands tons of pork meat has been imported into the Lithuanian Republic during the year 2004, 41.3% from Estonia, 17.83% from Germany, 5.83% from Denmark and 5.13% from Finland. Due to the fact, that prices of pork meat are considered to be among the highest in the EU (ES), during the previous year only 870 tons of this meat was exported [Ziziute and Kleinaite, 2005]. 304 thousands of pigs were bought during January – May in 2004 for the inner market. Therefore, supply of pig carcasses from Poland and Estonia made 90% from all the pork meat imported in 2004 [Radzevicius et al, 2004].

In 2003 pork meat from Lithuania was mainly exported to Latvia and imported meat tended to be cheaper when it was fresh or chilled as well as frozen carcasses or halves [Guseinova and Zilaitis, 2004].

On the basis of the data of the Lithuanian Ministry of Environment, collected according to the statistics of the Ministry of environment it was calculated that during the period of 2003–2004 years in the forests of Lithuania were 32059 boars, from which 10919 were hunted [Gudas, 2004]. During the period mentioned 532 boars were hunted in Alytus region, 2732 – in Kaunas region, 670 – in Klaipeda region, 778 – in Marijampole region, 1919 in Panevezys region, 2015 in Siauliai region, 1185 – in Utena region and 1097 – in Vilnius region [State forest survey service, 2004].

Pork meat has been traditionally widely used not only on the inner market, but exported as well. Especially popular hunting of wild boars is among hunters from Germany. Pork meat is traditionally more popular in Lithuania, but it seems to be worth pointing out that using of wild animals meat for food helps to decrease development of such diseases as aterosclerosis, heart diseases as this meat doesn‘t contain hormones, medicines, amount of cholesterol tends to be lower. Besides, pork meat independently on the species contains on average 22.3% of protein, 4.9% of fat while in boar meat protein and fat make respectively 28.3% and 4.8% [Etling, 1992]. Nevertheless, boar meat is distinguished by other good nutritional and biological properties [Korzeniowski et al, 1991]. Carcasses of boars are of good quality, the output fluctuates within the range of 59.9–74.3% [Zmijewski and Korzeniowski, 2000].

Recently "game" meat has been becoming more and more popular among people in various countries, especially wild boar meat.

A great number of research on hunted boars, their meat quality and muscle physicochemical properties, citogenetic studies when pigs were crossbred with wild boars has been carried out in Poland [Sysa et al., 1984; Żmijewski and Korzeniowski, 2001].

In France a great attention is paid not only to the quality of commercial pork meat, but comparative experiments are carried out in order to define the effect of various factors on boar meat colour L*, a*, b* [Marchiori and Felicio, 2003].

Meat quality parameters of commercial pigs and analysis of genetic markers is being carried out not only in the countries of the European Union [Thomsen et al., 2004].

The peculiarities of new stress-resistant breeds of pigs are widely studied all over the world, genome of wild boars is analysed and new methods for meat quality investigation are being searched for [Geldermann et al., 2003].

Generally, scientists in different countries study health status of pigs and wild boars only in their geographic territories, carry out investigations on biological changes in boars, study the effect of environment and plants on wild boars population, evaluate morphological changes of their spermatozoids, defects of meat quality and protein structural analysis in meat [Leaper et al., 1999, Nixdorf et al., 2001, Pospiech et al., 2003, Kozdrowski et al., 2004].

Three years ago scientists from Croatia and Slovakia investigated health status of wild boars according to pathological changes in the tusks of shot animals [Konjevic et. al, 2004]. Nowadays various breeds of pigs are grown in Lithuania. However, data in literature about the most suitable breeds of pigs according to the health state, hematological parameters and nutritional value of the meat in Lithuanian conditions are rather limited. Besides, we also failed to find any data about the comparison of different breeds of pigs and boars, concerning their health status, hematological parameters and meat physico-chemical analysis as well as the effect of breed on these parameters. The interaction of these parameters and inner relationship among them undoubtedly is of great importance on public health when carcasses of these animals are used for human consumption. Furthermore, there are No. data about composition of intestinal microflora in pigs and boars, microbiological processes which evidently effect not only animal health, but production quality as well.

The aim of this study was to evaluate health status of various breeds of pigs and hunted in Lithuania wild boars, to examine bacteriological state of the digestive system and carry out analysis of meat nutritional value.

Materials and methods

Research was carried out on 80 pigs of four different breeds and 10 wild boars. Morphological and biochemical parameters of animal's blood, meat physico-chemical properties and microbiological composition in the digestive system were studied. The experiments were carried out in Lithuania during 2005 year, at the State of pig breeding station, the Department of Animal Husbandry, Laboratory of meat characteristics and quality assessment, Department of Non-infection diseases and the Department of Anatomy and Physiology of the Lithuanian Veterinary Academy. The wild boars were hunted in five different according to geographical location regions of Lithuania, the weight fluctuated within the ranges of 105±0.48 kg. All experimental samples from pigs were taken during the control slaughtering, meat samples from wild boars - immediately after hunting, studying *m. longissimus dorsi.* Breeds of pigs were distributed into four groups, each of them contained twenty animals. The experimental pigs were of four different genotypes: Lithuanian White, Landrace, Yorkshire and Big White.

The pigs were fed concentrates KRET-KOM58-157GR/05. The main quality parameters of the concentrates were the following: metabolizable energy – 13.4 MJ/kg DM, protein – 16%, fat – 3.28%, fiber – 2.99%, lysine – 1%, metionin+cistin – 0.6%. The control slaughterings were made at the slaughtering house of the State pigs husbandry station when pigs reached 95–100 kg weight.

Amount of glucose (GL), triglycerides (TG), cholesterol (CH) in blood of pigs and wild boars was measured by a reflexive fotometer Accutrend GCT 2001 (Germany), hemoglobin (Hb) – by a colourimetric method, number of erythrocytes (ER) – in a Goriajev chamber, blood oxygen volume (BOV) was calculated according to Hüfner, protein in blood serum (BP) – by a refractometer RL3 (Poland).

The amount of dry matter was measured by the automatic scale for humidity assessment Scaltec SMO – 01, drying samples at 105ºC, pH – by a pH-meter Inolab 3, by a contact electrode (pH ISO 2917:1999 Meat and meat products Measurement of pH). Meat colour by a Minolta Chroma Meter 410, measuring L* values for lightness, a^* values for redness and b^* values for yellowness, fat – by an authomatic system for fat extraction Soxterm SE 416 macro (ISO 1443;1973 Meat and meat products determination of total fat content), ash – by organic matter inceneration at 700ºC (ISO 936:1998 Meat and meat products determination of total ash), protein according to Kjeldal method.

Drip loss was measured by a bag method, the meat was kept in special bags for 24 hours at + 4 C temperature [Honikel, 1987], water holding capacity was defined according to Grau and Hamm [Offer and Knight, 1988], cooking loss packaged under vacuum – in a circulating water bath at 75°C temperature for 30 min., meat tenderness – according to Warner-Bratzler.

Faecal samples from the colon of pigs and wild boars were collected with an aseptic manipulation into a special box filled with some ice and taken back to the laboratory. Dilution of the samples and inoculation were completed according to the method described by Oberauskas et al., [2004]. Selective culture media, MRS agar, McConkey agar, Bile Aesculin agar and Sabouraud agar (Liofilchem, Italy) were used. The final result was expressed by logN/g.

Statistical analysis. Data obtained were submitted to the analysis of variance (ANOVA) and the differences between the groups under study were analyzed using the Student *t-test* for comparison of means ($P< 0.05; 0.01; 0.001$) by using the R 2.2.0 statistical computering package [Juozaitiene and Kerziene 2001; Venables and Smith, 2005].

Results

The morphological and biochemical parameters of blood of different breeds of pigs and wild boars are demonstrated in Table 1.

The results of this Table evidently demonstrate, that amount of GL in blood from various breeds of pigs tended to be twice as higher if to compare to the analogous parameter in wild boar pigs. The highest amount of GL was found in the blood of Large white pigs. Fluctation of this parameter may be caused by nutritional factors, stress and functional activity of pancreas as well as by the intensity of carbohydrates metabolism and some other factors [Zymantiene et al., 2005].

Table 1

Haematological and biochemical studies of blood in adult pigs and wild boars

Parameters	Lithuanian white	Landrace	Yorkshire	Large White	Wild boars
GL [mmol/l]	5.6 ±0.86	5.7 ±1.05	7.0 ±0.58	7.4 ±0.87	3.1 ±1.52
TG [mmol/l]	0.87 ±0.22	1.52 ±0.52	0.94 ±0.53	0.88 ±0.00	6.87 ±0.37
CH [mmol/l]	3.88 ±0.00	3.88 ±0.03	3.88 ±0,04	3.88 ±0.00	3.88 ±0.39
BP [g/l]	54.7 ±11.8	56.8 ±5.88	59.7 ±12.52	52.5 ±1.61	54.7 ±3.14
ER [X*10^{12}/l]	5.8 ±0.33	4.7 ±0.85	6.2 ±0.48	4.4 ±0.51	5.7 ±0.63
Hb [g/l]	120 ±12.84	120 ±12.32	131 ±3.94	142 ±8.98	130 ±10.92
BOV [0_2/ml]	160.8 ±17.14	160.8 ±16.50	175.0 ±5.28	190.3 ±12.04	174.0 ±14.64

The highest amount of TG was found in the blood of boar pigs if to compare to the blood from all breeds of pigs studied. The highest amount of TG was defined in the blood of Landrace and it made 77.87% ($P<0.001$), in Lithuanian white – 87.34% ($P<0.001$), Yorkshire – 86.32% ($P<0.001$), and in Lithuanian large white – by 87.19% ($P<0.001$) lower in comparison with boars blood.

The amount of CH in blood among different breeds of pigs and boars tended to be similar.

The amount of BP in the blood serum obtained from Landrace was less by 4.86% ($P<0.05$) if to compare to the blood of Yorkshire, and in Lithuanian white and wild boars blood it was found to be similar. Nevertheless, the amount of BP was by 8.37% ($P<0.01$) higher compared with boars, and in the blood of Lithuanian white pigs – by 4.02% ($P<0.05$) higher if to compared to Large white pigs. The amount of protein in Yorkshires blood tended to be by 12.06% ($P<0.01$) higher in compare with Large white pigs.

It was also defined during the experiments that the highest number of erythrocytes was typical for the Yorkshire blood. This number of red blood cells was by 8.06% ($P<0.01$) higher if to compare to the boars blood. However, the number of erythrocytes in the blood from Landrace was by 17.55% ($P<0.05$) lower, and in Lithuanian white pigs – by 1.75% ($P<0.05$) higher, while in Lithuanian large white pigs the number of erythrocytes was by 22.80% ($P<0.05$) lower if to compare to the blood from boars.

The highest concentration of hemoglobin was found in the blood samples from Yorkshire if to compare to the Lithuanian white and Landrace.

The amount of Hb and BOV value in the blood of Yorkshire was by 0.76% higher in comparison with the blood from boars. Among different breeds of pigs these parameters were statistically unreliable.

According to the data of one-way analysis of variance (ANOVA) the effect of pig's breed and group of boars on blood parameters was analyzed. Comparison of the data about changes of protein amount in the blood serum from Landrace and Yorkshire breeds led to the conclusion that the effect of breed made 57.3% (P<0.05) on the amount of BP in blood serum. In case of changes in the amount of triglycerides between Landrace and boars the effect of breed made 79.8% (P<0.001), and on the changes of the erythrocytes – 39.2% (P<0.05).

The effect of breed on the changes of triglycerides between Lithuanian white pigs and boars is equal to 86.0% (P<0.001) and on the fluctuation of protein amount – to 45.2% (P<0.05).

Comparative analysis of Yorkshire and boars led to the conclusion that the effect of breed was measured by 77.9% (P<0.001), and on the number of erythrocytes – by 36.2% (P<0.05).

Between Lithuanian large white pigs and Lithuanian white pigs the effect of breed was 49.0% (P<0.05) on the changes in the amount of protein in blood serum.

Between Lithuanian large white pigs and Yorkshire the effect of breed on the changes in the amount of protein in blood serum was even to 71.5% (P<0.01).

The breed of Large white pigs and boars effected changes in the amount of GL in blood serum by 28.8% (P<0.05), changes of triglycerides – by 87.7% (P<0.001) and fluctuations in the number of erythrocytes – by 33.1% (P<0.05).

Analysis of physico-chemical composition of meat of different breeds of pigs and wild boars are demonstrated in Table 2.

During ANOVA analysis the effect of various breeds of pigs and wild boars on meat physico – chemical properties was studied. Breed of pigs effected meat colour L* by 46.8% (P<0.05), colour a* – by 88.0% (P<0.001), colour b* – by 57.6% (P<001), tenderness – by 40.1% (P<0.05), the amount of intramuscular fat – by 61.1% (P<0.001) and variety of ash – by 45.3 % (P<0.05). The analysis of all pigs and boars studied without paying attention to the breed demonstrated that the effect of sex on meat tenderness was 21.8% (P<0.05).

As it becomes evident from the data presented in Table 2, the amount of fat in the intramuscular meat of Lithuanian white pigs was by 0.5% (P<0.05) higher than in Landrace. However, the amount of fat in the intramuscular meat of Landrace was by 0.2% (P<0.05) higher than in the meat from Yorkshire.

Besides, tenderness of Yorkshire meat was by 92.85% (P<0.05) higher if to compare to the meat of Landrace. Cooking loss of Landrace meat were by 4.40% (P<0.05) higher than of analogous parameter of wild boars meat.

It was also defined, that colour value L* of Landrace meat was by 16.98% (P<0.05) higher, colour a* value – by 28.94% (P<0.05) lower and colour b* value – by 4.30% (P<0.05) lower than in wild boars meat. Meat tenderness of Lithuanian white pigs was by 40.75% (P<0.05) lower than in Yorkshire meat. Colour values a* and b* in Lithuanian white pigs were respectively by 29.45% and 43.00% (P<0.05) lower than in wild boars meat.

The amount of fat in the intramuscular meat of Yorkshire was by 0.60% (P<0.05) lower compared with Lithuanian white pigs. However, colour value L* in Yorkshire was by 18.04% (P<0.05) higher, but a* – by 32.49% (P<0.001) and b* values – by 47.00% (P<0.05) lower compared to the analogous parameters of wild boar meat.

Meat tenderness of Yorkshire was by 92.85% (P<0.05) higher, the amount of intramuscular fat – by 1.20% (P<0.01) lower, but the amount of ash – by 0.10% (P<005) higher if compared to the wild boar meat.

Cooking loss in Lithuanian large white pigs was by 4.00% (P<0.05) higher, meat colour value L* – by 16.98% (P<0.05) higher, colour a*value 28.43% (P<0.001) and colour b* value by 40.00% (P<0.05) higher if compared to the boars meat.

Table 2

Analysis of meat chemical composition and physical properties in different breeds of pigs and wild boars

Parameters	Lithuanian white	Landrace	Yorkshire	Large white	Wild boars
Dry matter [%]	26.1 ±0.64	25.1 ±0.32	25.0 ±0.52	25.8 ±0.56	26.1 ±0.65
Protein [%]	23.0 ±0.53	22.6 ±0.30	22.7 ±0.49	22.9 ±0.71	22.3 ±0.46
Intramuscular fat content [%]	1.8 ±0.21	1.3 ±0.07	1.1 ±0.08	1.7 ±0.23	2.3 ±0.27
Ash [%]	1.2 ±0.02	1.2 ±0.02	1.2 ±0.01	1.2 ±0.02	1.1 ±0.02
pH	5.6 ±0.13	5.4 ±0.04	5.5 ±0.06	5.4 ±0.02	5.5 ±0.05
Colour value L*	54.9 ±1.89	55.1 ±0.81	55.6 ±0.75	55.1 ±1.25	47.1 ±3.15
a*	13.9 ±0.50	14.0 ±0.26	13.3 ±0.60	14.1 ±0.54	19.7 ±0.56
b*	5.7 ±0.55	5.7 ±0.32	5.3 ±0.48	6.0 ±0.32	10.0 ±1.47
Drip loss [%]	5.8 ±1.17	6.9 ±1.28	6.6 ±1.55	8.9 ±2.34	7.5 ±2.02
Water holding capacity [%]	57.9 ±2.51	55.3 ±1.46	53.7 ±2.30	57.7 ±1.89	56.5 ±1.23
Cooking loss [%]	28.1 ±2.71	30.1 ±1.48	28.1 ±0.53	29.7 ±0.68	25.7 ±1.35
Tenderness [kg/cm^2]	1.6 ±0.36	1.4 ±0.32	2.0 ±0.29	2.2 ±0.66	1.4 ±0 29

The content of different bacteria in the colon of pigs and wild boar pigs is demonstrated in Figure 1.

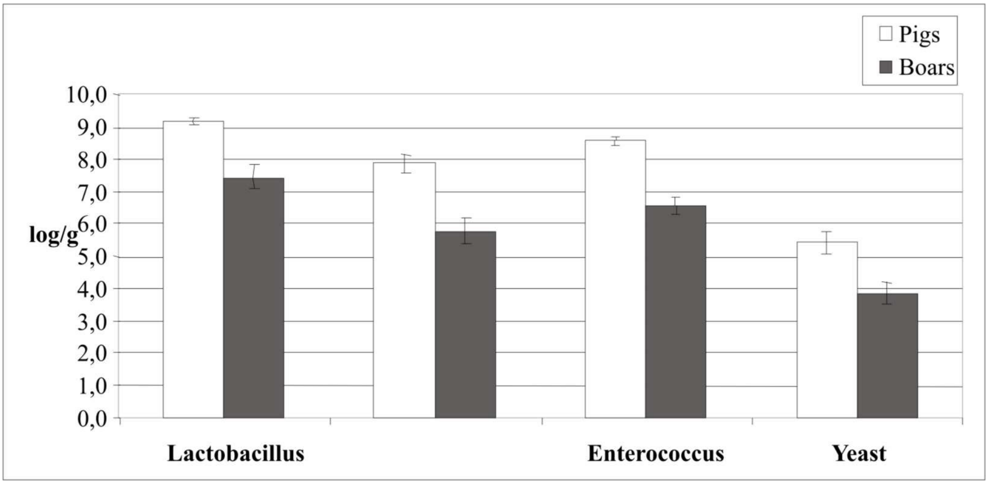

Fig. 1. The content of different bacteria in the colon of pigs and wild boars

The correlation among different species of bacteria in the colon of pigs and wild boars is presented in Table 3 and 4. It becomes evident that total count of lactobacillus in the colon of pigs positively correlated with the count of enterobacteria and enterococcus ($P<0.05$). Negative correlation was stated for enterococcus and yeast ($P<0.05$).

In the colon of wild boars count of lactobacillus positively correlated with enterobacteria, but it was not statistically reliable. However, negative correlation was found between enterobacteria and enterococcus with yeast.

Table 3

The correlation between different species of bacteria in the colon of pigs

Pearson correlation	Lactobacillus	Enterobacteria	Enterococcus	Yeast
Lactobacillus	1	0.401	0.507*	-0.043
Enterobacteria	0.401	1	-0.244	0.213
Enterococcus	0.507*	-0.244	1	-0.464*
Yeast	-0.043	0.213	-0.464*	1

Note: * $P<0.05$

Table 4

The correlation between different species of bacteria in the colon of wild boars

Pearson correlation	Lactobacillus	Enterobacteria	Enterococcus	Yeast
Lactobacillus	1	0.615	0.210	-0.028
Enterobacteria	0.615	1	-0.012	-0.517
Enterococcus	0.210	-0.012	1	-0.428
Yeast	-0.028	-0.517	-0.428	1

Discussion

The data found in literature state that amount of GL in blood of pigs of various breeds fluctuated within the ranges of 6.61± 0.96 mmol/l [Sederevicius, 2004]. The average GL amount in the investigated pigs tended to be similar to those stated in literature, however it was the highest in the blood from Yorkshire and Lithuanian large white pigs. Such an increase of GL maybe caused by stress. In our opinion these breeds are the most susceptible to stress during slaughtering. This fact was observed by other authors as well, especially concerning Yorkshires [Jakubka and Miceikiene, 2001].

It can be concluded from our experiments that significantly lower level of GL in the blood from wild boar pigs was caused during shooting and prevailing ingredients of their diet were natural components of plant and animal origin, only rarely they were given supplemented feeding by hunters.

The amount of TG in the blood of Lithuanian white, Yorkshire and Lithuanian large white pigs remained very similar, so we dare to conclude that the organism of these animals assimilated forage lipids and carbohydrates better than Landrace and wild boars.

CH is component elements of cell membranes, quite considerable amount of which is found in nervous tissue, suprarenal cortex, genital glands, it participates in the metabolism of bile acids, processes of detoxication, help to improve resistance of membranes of erythrocytes. Increased amount of CH is observed in case of disturbed metabolism of carbohydrates and functional disturbances of pancreas [Zymantiene et al., 2005; Egbe-Nwiyi and Erivo, 2001]. During our experiments No. differences among the amount of CH in the blood of different breeds of pigs as well as wild boar pigs were found, so we can conclude that in the organisms of pigs and wild boar pigs the metabolism of lipids and carbohydrates independently on the type of feeding fluctuated within the limits of physiological norm and processes of aterosclerosis in blood vessel system, related to CH metabolism were not observed.

The protein metabolism in Lithuanian white pigs and Yorkshire tended to be more intensive as in Lithuanian large white. According to the data of the experiments carried out the effect of breed on the amount of protein in blood serum may reach 49.0–71.5%.

The intensity of the hemopoiesis in the organisms of pigs and wild boars is characterized by the number of erythrocytes, hemoglobin concentration and value of oxygen volume. The most intensive blood production and processes of oxidation were defined in Lithuanian white pigs and Yorkshire. Lithuanian white pigs are considered to be of local origin. Blood parameters in their organisms were the best and it can be confirmed by the best adaptation to environmental conditions. However, the highest amount of hemoglobin was defined in the breed of Yorkshire compared to the Lithuanian white, Landrace and wild boar pigs. The increased amount of hemoglobin in blood evidently demonstrates better assimilation of oxygen and nutritive substances in the organism.

Particular parameters of meat in pig breeds widespread in Lithuania differed. Similar results were stated by other authors about the meat of the most popular breeds and their combinations [Dziaugys et al., 1992; Klimas et al., 1999; Jukna et al., 2005; Jukna et al., 2007]. The amount of dry matter and protein is very similar in wild boars and pigs. However, the amount of intramuscular fat was higher in boars, what characterizes better gustatory quality. Meat chemical composition in Lithuanian population of boars is very similar to that in Poland [Żmijevski et al., 2001]. Nevertheless, our data contradict to the data of Etling [1992]. According to his data, meat of wild boars contained 6.0% of protein, but the amount of fat was by 2.5 % higher than in our experiments. Such a difference can be caused by the differences in age and other factors. Colour of boar meat was more intensive and darker. The colour of meat depends on the amount of protein myoglobine sarcoplazma of muscles or on the chemical changes in protein part of the molecules [Brown-Brandl et al., 2001]. The colour of wild boar pigs meat was caused by a greater variety of natural forage and more intensive movement. Water holding capacity of meat and water coherence in wild boars were very similar to these parameters in pigs. They are considered to be rather important technological characteristics. As some authors indicate [Lundstrom et al., 1995; Van-Lack et al., 1999], problems related to water holding capacity may become primary important in improvement breeding characteristics of animals and regulation of the process of glycogenolysis after slaughtering. Cooking loss of meat in wild boar pigs is lower than in pigs. According to Zajas [1981] cooking loss during thermal processing of meat is effected by pH and proteins of microfibriles. As pH of wild boars and pigs are quite similar, lower boiling loss in boars may be explained by the peculiarities of microfibriles proteins. Tenderness of boar meat is higher than of Yorkshires, Large white and Lithuanian white pigs. Increase of pigs muscularity causes increased area of muscular fibers cross-section. The increased area of muscular fibers cross-section strenghtens resistance to cutting energy. It explains the fact that meat with higher muscularity has higher tenderness [Candek-Patokar et al., 1999; Fiedler et al., 2001].

Data about normal composition of microflora in boars are rather poor. It was defined during our experiments that the ratio of lactobacillus, enterococcus and yeast in the colon of various breeds of pigs and wild boars was rather similar. Count of these bacteria in boars was from 18.9 to 29.1% lower than in the colon of pigs. In our opinion, such a difference may be caused by different nutrition. Lactobacillus had weak superiority to enterobacteria and enterococcus in quantities as in the colon of pigs so in wild boars. The changing tendency of intestinal microflora of pigs and wild boars obtained in this experiment was very similar as in the colon of calves [Cong Peiqing et al., 2001].

The study also statistically analyzed the correlationship of the quantities of pigs and boars colon microflora. It becomes evident from the results that positive correlation of lactobacillus was observed as in pigs so in boars with enterobacteria and enterococcus and it was negative with yeast. It indicated that the quantities of lactobacillus and enterococcus with positive and negative corelationship increased or decreased simultaneously to some extent. We suppose to have something to do with synergism among different species of bacteria.

Conclusions

The amount of GL in different genotypes of pigs was twice as higher as in the blood of wild boars. The amount of TG in all breeds of pigs was by 17.3% higher than in the blood of wild boars. The most intensive protein metabolism and erythropoiesis was defined in Yorkshire. The most intensive process of oxygenization was in Lithuanian large white pigs. The breed of pigs effected the variety of proteins, erythrocytes and triglycerides in blood.

Meat quality parameters of all the breeds of pigs investigated (Lithuanian white, Landrace, Yorkshire, Large white) and wild boar pigs were different. The meat of wild boars contained by 0.5–1.3% higher amount of intramuscular fat, it was darker, cooking loss was lower and except from Landrace, tended to be softer. The breed of pigs effected meat colour L^* – by 46.8%, redness a* – by 88,0%, yellowness b^* – by 57.6%, rigidity – by 40.1%, the amount of intramuscular fat – by 61,1% and the amount of ash – by 45.3%.

The ratio of lactobacillus, enterobacteria, enterococcus and yeast tended to remain similar in the colon of pigs and wild boars. However, the bacterial count in wild boars was from 18.9 to 29.1% lower than in the colon of pigs. Count of lactobacillus positively correlated with enterobacteria and enterococcus and negatively – with yeast as in pigs so in wild boars, negative correlation was defined between enterococcus and yeast. Nienaberg

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3

SENSORY EVALUATION OF SOUS VIDE – PROCESSED SALAD WITH MEAT IN MAYONNAISE DURING THE STORAGE TIME

Introduction

Lifestyles of European families are changing rapidly. Urban families have witnessed tremendous pressure on time and this has resulted in visible change in their eating habits as well. Changing lifestyles have led to an increased demand for convenient, ready-to-eat products [Jang and Lee, 2005, Galimpin-Johan et al., 2007]. In the recent years ready-to-eat products like salads in mayonnaise have become popular in the Baltic States day by day. Statistical data show that the tendency to eat salads with mayonnaise is increasing in the local market [http://www.lango.lv]. Salad business of Latvian food producers is stabilizing, and focusing on innovations. The most popular innovations of packaging technologies for salad with mayonnaise in the local market are vacuum and modified atmosphere packaging, which give better quality, taste, and shelf life for salads with mayonnaise compare with conventional packaging. New products are being introduced and new uses promoted. The same time consumers expect and demand tasty, safe, high quality salad products [http://www.lango.lv].

An innovative approach that could give all good sensory properties, and quality for salad products and satisfy consumers is using of "Sous vide" technology. The "Sous vide" technologies for ready-to-eat meals developed over the last 20 years as food service industry's need to become more efficient while simultaneously satisfying the consumer's growing demand for higher quality in food and food services [Nissen et al., 2002]. The "Sous vide" technology has been used a long time as a method for cooking catering products and enhancing quality and sensory attributes compared with conventional cooking methods [Lassent et al., 2002, Stea et al., 2006]. Very recently, "Sous vide" products are being increasingly steered towards the retail market in consumer sized packs as ready-to-eat meals [Gracia-Linares et al., 2004; Baldwin, 2008].

"Sous vide", is French for "under vacuum" and describes a method of cooking in vacuum sealed in individual packages and then pasteurized at low temperatures for long times to destroy vegetative pathogens but mild enough to maximize the sensory characteristics of the product [Jang and Lee, 2005; Galimpin-Johan et al., 2007; Baldwin, 2008].Vacuum packaging prevents evaporative losses of flavour volatiles and moisture during cooking and inhibits off-flavours from oxidation [Church and Parsons, 2000]. This results in especially flavourful and nutritious food [Lassen et al., 2002; Gracia-Linares et al., 2004; Stea et al., 2006].

The literature studies point the effects of "Sous vide" packaging upon the sensory characteristics of chicken breast and of sliced potatoes in cream both immediately after cooking (70–80°C) and following subsequent chilling, chilled storage and reheating – higher hedonic scores were associated with higher flavours and juiciness scores for chicken and with higher flavour and moisture scores for potato [Church and Parsons, 2000].

Some of the key global trends include health, convenience, and speciality. Consumers trust a food product to be safe, which means that it should not produce any negative health effects when eaten [Meng and Genigeorgis, 1994; Church and Parsons, 2000]. The industries reflect this by developing low-sodium and organic products [Meng and Genigeorgis, 1994; Obotolu and Veronica, 2007]. Fresh and semi-processed products need a preservative or mild pasteurization to give them commercially acceptable shelf life. For example, meat products, prepared salads, dressings, mayonnaise and sauces need cooling and/ or preservatives to ensure safety and shelf life [Ahvendinen, 2003]. Natural lactic acid, lactates and products based on lactic acid, produced and marketed by Purac, have proven their ability as "natural" preservatives in meat and fish products, dressings, pickles, beer etc. In salads, which are not heated after production, preservatives are primarily used to extend the shelf life. Using PURASAL in salads with mayonnaise, it has mild acid taste and functions primarily as a preservative, flavouring and pH regulator. The flavour of a dressing or salad containing lactic acid and acetic acid is less sour and sweeter than one with only salt and vinegar in it [http://www.purac.com/]. When PURASAL was added the cooked beef/brothy flavour was enhanced at zero days of storage. During storage, this flavour declined, but the products containing PURASAL tended to maintain higher lever of cooked beef/brothy aromatics compared with control roasts. In addition, lactate tends to limit the development of aromatics associated Warmed-over Flavour. PURASAL in low-salt products offers the opportunity to reduce the sodium content of the meat product while maintaining and optimal flavour. Trained panel evaluations relevealed positive flavour notes. Consumer panellists described treated roasts as flavourful, with a stronger beefy, meaty flavour than control samples 3% PURASAL is the optimum level for use as bacteriostatic agent and flavour enhancer in cooked uncured beef [Devlieghere et al., 2000]. The new generation of potassium lactate has very neutral flavour, which allows meat processors to produce good-tasting reduced-sodium products with extended shelf life [Devlieghere et al., 2000]. PURASAL Powder Opti Form gave to salad with mayonnaise, soft taste treated by "Sous vide" technology [Levkane et al., 2008; Muizniece-Brasava et al., 2008]. The "Sous vide" technology prolongs the shelf life of salads with meat in mayonnaise till 52 days [Muizniece-Brasava et al., 2008].

Material and methods

Experiments have been carried out at the Latvia University of Agriculture Department of Food Technology in 2009. The object of research is – salad with meat in mayonnaise. The salads with meat in mayonnaise used in the research have been made in LLC "VSL Food group" for the local market. The ingredients in the salads were potatoes, cooked beef, boiled eggs, pickled cucumbers, spicery, and mayonnaise Provansa purchased on the local market. The aim of the study was to identify the influence of salad with meat in mayonnaise on the intensity of the main sensory properties (aroma, flavour, sourness, aftertaste, consistency and colour) and on the degree of liking (the ranking test) using "Sous vide" technology and natural preservative PURASAL Powder Opti Form addition at the storage time.

The three salads with meat in mayonnaise samples submitted for sensory evaluation are aggregated in Figure 1.

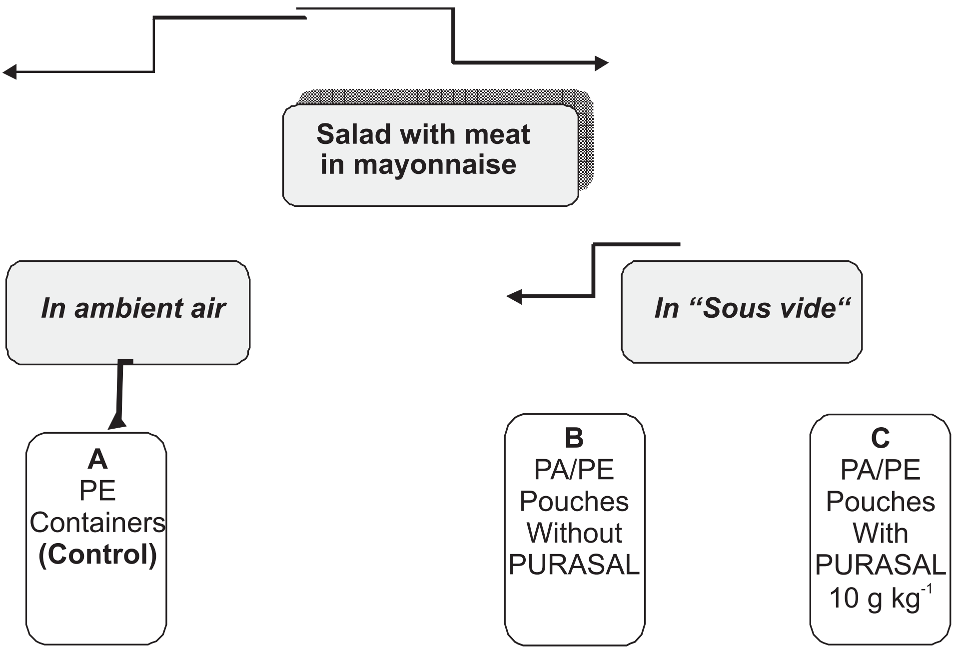

Fig. 1. Structure of performed experiments

The salad samples with PURASAL addition 0.1% and without PURASAL were placed in pouches polyamide/polyethylene ((PA/PE) thickness 20/45 µm) with barrier properties size of 200 x 300 mm, mass of each sample 200±1 g from witch substantially all air was removed prior to final sealing of the containers on MULTIVAC A 300/16, following-up by thermal treatment – "Sous vide" [Levkane et al., 2008]. A control sample without preservatives was packed in traditionally used polyethylene (PE) containers covered with non hermetical lids. All samples were stored in Commercial Freezer/Colder ELCOLD at +4±0.5°C temperature. In the experiments on days 1, 15, 30, 42, and 52, the sensory properties of salad with meat in mayonnaise were characterised by the line scale to evaluate the intensity of sensory properties and the degree of liking (the ranking test) was evaluated in sensory laboratory. The evaluation took place in the laboratory of food sensory evolution, Faculty of Food Technology, at the Latvia University of Agriculture, in an individual evaluation booth.

The line scale was used to evaluate the intensity of sensory properties (aroma, flavour, sourness, aftertaste, consistency and colour) of the salads with meat in mayonnaise. The ranking was used to carry out the sensory estimation of the samples; it helps to evaluate the salad with meat and mayonnaise degree of liking. Altogether 25 panellists – 19 females, 6 males (mean age 23) took part in this evaluation. Three samples of salad with meat in mayonnaise submitted for sensory evaluation are aggregated in Table 1.

Table 1

Salad with meat and mayonnaise samples prepared for sensory evaluation

Salad with meat in mayonnaise samples	Deciphering of samples	Used salt	Amount of salt added, % (per kg of salad mass)
A	Salad with meat in mayonnaise (control)	NaCl	0.05
B	Salad with meat in mayonnaise ("Sous vide" technology)	NaCl	0.05
C	Salad with meat in mayonnaise ("Sous vide" technology)	PURASAL Powder Opti Form	0.1

Salad samples each of 28 g was served to panellists for testing. The samples were arranged in small plates that were coded with three figure numbers, and were ranked on a tray. The positions of the salad samples on the tray were randomized. The panellists got a questionnaire, were they needed range samples most liked to least liked for row, and the line scale were they needed evaluate the intensity of sensory properties (aroma, flavour, sourness, aftertaste, consistency and colour). The sensory data were analyzed using Fizz Forms – Sensory Analysis and Consumer Test Management Software. Statistical analysis of ranking test was established with software SPSS 16.0 for Windows, the analysis of variance ANOVA. Use of nonparametric ranked ANOVA by Friedman and ANOVA Tukey's test [Castura and Findaly, 2006] were used to analyze the results of sensory evaluation significance was defined at $p< 0.05$.

Results and discussion

The results of line scale evaluation showing the intensity of the sensory properties of the salad with meat in mayonnaise at storage time are demonstrated in Table 2 and 3.

In accordance with the results of the analysis of variance of aroma at the storage time after 1, 15, 30, 42 and 52 days F(calculated) < F (critical) (Tab. 3), the conclusion is that there are not significant differences in aroma intensity among three estimated salad with meat in mayonnaise samples.

The results of the analysis of variance compiled of flavour F(calculated) < F (critical) (Tab. 3) show that there are not significant differences in flavour intensity among of all three samples at the storage time in 52 days.

The results of the analysis of variance of sourness F(calculated) < F (critical) (Tab. 3) show that there is not significant differences at the storage time after 1, 15 and 30 days. The results of the analysis of variance of sourness after 42 days of storage F(calculated) = 15.67 > F (critical) = 3.19 ($p\leq5$), and after 52 days of storage F(calculated) = 15.54 > F (critical) = 3.19 ($p\leq5$), it means there exist significant differences in sourness intensity among the three estimated salad with meat in mayonnaise samples. PURASAL 10 g kg-1 addition influences the intensity of sourness at 42 and 52 days of storage, the salad with meat in mayonnaise become sourer.

Table 2

Results of sensory evaluation by line scale of the salad with meat in mayonnaise mean values at the storage time

Samples	Aroma	Flavour	Sourness	Aftertaste	Consistency	Colour
	after 1 day of storage					
A	7.3	7.2	2.7	6.0	6.9	5.6
B	6.3	6.9	2.4	5.4	7.6	6.4
C	5.9	6.1	2.4	4.8	7.0	6.5
	after 15 days of storage					
A	5.8	6.6	4.3	5.2	6.6	6.0
B	6.5	6.8	4.6	6.4	7.2	6.5
C	4.9	6.7	3.3	5.2	6.8	6.1
	after 30 days of storage					
A	6.9	6.7	2.8	6.0	6.8	6.3
B	6.5	6.3	3.6	5.5	6.9	6.1
C	6.4	6.6	3.3	5.7	6.9	6.3
	after 42 days of storage					
A	6.6	6.9	2.8	5.5	5.9	6.2
B	5.2	7.4	4.2	5.9	6.8	6.7
C	6.0	5.2	2.9	5.2	6.3	6.0
	after 52 days of storage					
A	6.0	5.2	2.9	5.2	6.3	6.0
B	6.4	6.1	4.1	6.4	6.8	6.5
C	6.6	5.9	5.9	6.6	7.0	7.1

The results of the analysis of variance of aftertaste F(calculated) < F (critical) (Tab. 3) show that there is not significant differences at the storage time after 1, 15, 30 and 42 days. The results of the analysis of variance of aftertaste after 52 days of storage F(calculated) =3.33 > F (critical) = 3.19 ($p \leq 5$) show that significant differences in aftertaste intensity exist among the three evaluated salad with meat in mayonnaise samples.

The results of the analysis of variance compiled of consistency F(calculated) < F (critical) (Tab. 3) show that there are not significant differences in consistency intensity among of all three samples at the storage time in 52 days. The consistency of the salad with meat in mayonnaise is not influenced by using "Sous vide" technology.

In accordance with the results of the analysis of variance of colour F (calculated) < F (critical) (Tab. 3), the conclusion is that there are not significant differences in colour intensity among three estimated salad with meat in mayonnaise samples at the 52 days of storage. Using the "Sous vide" technology for the salad with meat in mayonnaise, it is not affecting colour at the storage time.

The sensory properties intensity of salad with meat in mayonnaise results are shown in Table 2. The control sample (A) salad with meat in mayonnaise has a distinctly marked difference in aroma, because control sample (A) was taken in every experiment day as a fresh salads compare with other samples which were stored 52 days.

The most intensive flavour has control sample (A), then sample B ("Sous vide" without PURASAL) has intensive flavour at the storage time. Intensity of flavour on sample C ("Sous vide" with PURASAL 10 g kg^{-1}) change at storage days 42 and 52, it decrease and stay less intensive flavour.

Table 3

Results of analysis of variance of main sensory properties at the storage time

Sensory properties	Variance ratio $F_{calculated}$	Variance ratio $F_{critical}$
after 1 day of storage		
Aroma	2.70	3.19
Flavour	2.57	3.19
Sourness	0.17	3.19
Aftertaste	1.35	3.19
Consistency	0.65	3.19
Colour	0.84	3.19
after 15 days of storage		
Aroma	1.91	3.19
Flavour	0.04	3.19
Sourness	2.15	3.19
Aftertaste	1.27	3.19
Consistency	0.79	3.19
Colour	0.38	3.19
after 30 days of storage		
Aroma	0.52	3.19
Flavour	0.80	3.19
Sourness	1.32	3.19
Aftertaste	0.36	3.19
Consistency	0.03	3.19
Colour	0.16	3.19
after 42 days of storage		
Aroma	1.68	3.19
Flavour	1.29	3.19
Sourness	15.67	3.19
Aftertaste	0.73	3.19
Consistency	0.93	3.19
Colour	0.97	3.19
after 52 days of storage		
Aroma	0.52	3.19
Flavour	2.42	3.19
Sourness	15.54	3.19
Aftertaste	3.33	3.19
Consistency	0.84	3.19
Colour	1.73	3.19

$\alpha \leq 0.05$

Sample C ("Sous vide" with PURASAL 10 g kg^{-1}) has the highest degree of sourness at the storage time. The addition of PURASAL 10 g kg^{-1} affects taste of salad with meat in mayonnaise. Then it is followed by sample B ("Sous vide" without PURASAL), it come sourer at end of storage days. The control sample (A) has the least sourness.

The samples B ("Sous vide" without PURASAL) and C ("Sous vide" with PURASAL 10 g kg^{-1}) aftertaste intensity are less compare with control sample (A). The "Sous vide" technology gives mild taste for salad with meat in mayonnaise and soft aftertaste for a long time after eating. The sample C ("Sous vide" with PURASAL 10 g kg^{-1}) aftertaste is increasing at the storage time. Addition of PURASAL 10 g kg^{-1} is affecting aftertaste of salad with meat in mayonnaise and after 52 days of storage it comes more intensive.

The consistency of the salads with meat in mayonnaise for samples B ("Sous vide" without PURASAL) and C ("Sous vide" with PURASAL 10 g kg^{-1}) are influenced by using "Sous vide". It came more intensive (dryer) after "Sous vide" treatment, potatoes of the salads absorb mayonnaise. The sample B ("Sous vide" without PURASAL) and C ("Sous vide" with PURASAL 10 g kg^{-1}) did not became watery after 52 days of storage.

The colour intensity of salad with meat in mayonnaise was not influenced by using "Sous vide" and addition of PURASAL 10 g kg^{-1} at the 52 days of storage. The sample C ("Sous vide" with PURASAL 10 g kg^{-1}) colour intensity come more intensive at 52 days of storage.

One of the aims of this research was liking degree's appreciation of meat salad in mayonnaise using "Sous vide" technology modes of salads. The results of sensory evaluation of the salad with meat in mayonnaise after the first experiment day are demonstrated in following results. In accordance with the results of the analysis of variance F (calculation) = 0.27 < F (critical) = 3.19 ($p \leq 5$), the conclusion is that there are not significant differences between the degrees of liking estimated salad with meat in mayonnaise control sample and "Sous vide" packaged without PURASAL and with PURASAL 10 g kg^{-1} at the first storage day. The results of Tukey's test show that there is No. significant difference between all three samples A – control sample, B – "Sous vide" without PURASAL, and C – "Sous vide" with PURASAL 10 g kg^{-1}, it shows that the panellists most liked samples A (control sample), C ("Sous vide" with PURASAL 10 g kg^{-1}), and B ("Sous vide" without PURASAL) samples. The "Sous vide" treatment with PURASAL Powder Opti Form 10 g kg^{-1} gave less sour but mild taste for salad with meat in mayonnaise, which were positive accepted from panellists.

The results of the analysis of variance of salad with meat in mayonnaise at the 15 days of storage F (calculation) = 8.48 > F (critical) = 3.19 ($p \leq 5$). There are significant differences between the degrees of liking of salad with meat in mayonnaise samples. The results of Tukey's test show that there are a significant difference between C ("Sous vide" with PURASAL 10 g kg^{-1}) sample and the sample B ("Sous vide" without PURASAL) and A (control sample). Panellists the most liked samples B ("Sous vide" without PURASAL) and A (control sample), and the least liked sample is C ("Sous vide" with PURASAL 10 g kg^{-1}) in which is significant difference in taste among the three estimated salad with meat in mayonnaise samples.

The results of the analysis of variance complied of salad with meat in mayonnaise at the 30 days of storage F (calculation) = 3.47 > F (critical) = 3.19 ($p \leq 5$). There are significant differences between the degrees of liking of salad with meat in mayonnaise samples. The results of Tukey's tests show that the panellists the most liked sample A (control sample) and B ("Sous vide" without PURASAL), there are not significant differences between those samples. The less liked is sample C ("Sous vide" with PURASAL 10 g kg^{-1}), there is significant difference between other two samples. The flavour intensity of salad sample "Sous vide" with PURASAL Powder Opti Forn 10 g kg^{-1} is influenced by the amount of the added PURASAL 0.1 % of salad mass, what decrease the intensity of tenacity flavour, also consistency in the salad with meat in mayonnaise sample to gave to it tasteless flavour, which reduces the degree of liking of the estimated samples. The PURASAL Powder Opti Forn in amount 10 g kg^{-1} is changing flavour of salads with meat in mayonnaise packaged in "Sous vide" technology at the 30 days of storage time. The flavour comes sourer not like it was in the first days of storage.

The results of sensory evaluation of the salad with meat after 42 days of the storage are demonstrated in following results. In accordance with the results of the analysis of dispersion analysis F (calculation) = 8.89 > F (critical) = 3.19 ($p \leq 5$). The conclusion is that there are significant differences between the degrees of liking estimated salad with meat in mayonnaise of all samples. The results of Tukey's test show that the panellists the most liked sample A (control sample), which one is significant different between B ("Sous vide" without PURASAL) and C ("Sous vide" with PURASAL 10 g kg^{-1}) samples. At the same time there are not significant differences between samples B ("Sous vide" without PURASAL) and C ("Sous vide" with PURASAL 10 g kg^{-1}). The panellists the less liked sample C ("Sous vide" with PURASAL 10 g kg^{-1}). The control sample is more likeable with fresh flavour for the salad with meat in mayonnaise samples, which increase the degree of liking of the estimated samples. Addition of PURASAL Powder Opti Forn in amount 10 g kg^{-1} at the 42 days of the storage is decreasing mild taste and comes sourer.

The results of the analysis of variance compiled of salad with meat in mayonnaise at the 52 days of storage, F (calculation) = 22.30 > F (critical) = 3.19 ($p \leq 5$) show that there exist significant difference in salad with meat in mayonnaise samples. The results of Tukey's test show that the panellists the most liked sample A (control sample), which one is significant different between B ("Sous vide" without PURASAL) and C ("Sous vide" with PURASAL 10 g kg^{-1}) samples. The control sample (A) has the least sourness and mild flavour.

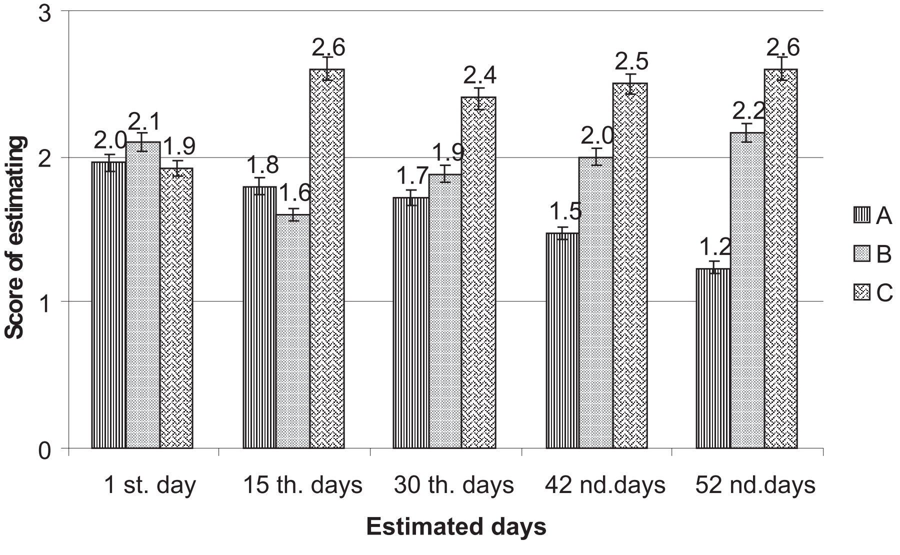

Fig. 3. Results of ranking test evaluation of salad with meat in mayonnaise used "Sous vide" technology A – control sample with NaCl; B – "Sous vide" without PURASAL; C – "Sous vide" with PURASAL10 g kg^{-1}

In order to determine the degree of liking for salad with meat in mayonnaise packaged in "Sous vide" with and without addition of PURASAL, as well as control with NaCl the ranking test scaling used at storage time on 1, 15, 30, 42, and 52 days.

The results are shown in Figure 3. Control sample was taken in every experiment day as a fresh salad with meat in mayonnaise. The results show that the average estimation in raking test of salad with meat in mayonnaise at the storage time the most liked control sample, then "Sous vide" without PURASAL, and "Sous vide" with PURASAL 10 g kg^{-1}. The technology of "Sous vide" without PURASAL can be used for 52 days of storage without significant changing in quality and safety of salad with meat in mayonnaise.

Conclusion

The results of the sensory analysis showed that the use of "Sous vide" technology and addition of PURASAL 10 g kg^{-1} to salad with meat in mayonnaise have various influence on aroma, flavour, sourness, aftertaste, consistency and colour.

The PURASAL 10 g kg^{-1} addition influences the salad with meat in mayonnaise. PURASAL affects more sourness and aftertaste of salad with meat in mayonnaise, salad with meat in mayonnaise become sourer and aftertaste become more intensive at the storage time.

Evaluation of sensory properties demonstrates that the best sample, compared with the control sample, is the salad with meat in mayonnaise treated by "Sous vide" technology without PURASAL.

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4

EFFECT OF RAW MATERIAL COMPOSITION AND TECHNOLOGICAL FACTORS ON GLUTEN-FREE PASTA QUALITY

Introduction

Celiac disease, is an autoimmune disorder of the small intestine caused by a reaction to gluten. An estimated prevalence of the disease is 1% of population. It develops mainly in early childhood or in people over 40–50 years, more often in women [Ciclitira et al., 2005]. Factors involved in celiac disease development are: genetic, metabolic, immunologic, environmental and probably infectious. Celiac disease is connected with particular genetic make-up (HLA type) with the genes DQ8 and DQ2 being identified as the "celiac genes" present in over 95% of patients [Catassi and Fasano, 2008]. The disease is triggered by a contact of gluten with small intestine mucosa. Exposure to gliadin causes a disruption of structure and function of the small bowel's mucosal lining. As a result of villous atrophy the surface area, which enables the absorption of nutrients and minerals, is seriously reduced which leads to malabsorbtion and nutritional deficiencies [Koning et al., 2005].

The only effective treatment of celiac disease is an eliminatory diet excluding foods containing gluten proteins with a specific amiNo. acid sequence in peptide chains. Especially toxic are prolamins (wheat gliadins, rye secalins, barley hordeins) but it was found that glutenin fractions can exert a harmful effect too [Ciclitira et al., 2005; Catassi and Fasano, 2008].

Nutritional value of gluten proteins is not high but in case of bread, confectionery or pasta gluten plays a very important structure-forming role [Obuchowski, 1997; Day et al., 2006]. Manufacturing of such gluten-free products with good sensory properties is still a challenge. Different raw materials are used to obtain a product of good quality and high nutritional value. Starches of various origin: corn, potato, rice or even wheat and also corn, rice, buckwheat, soy and tapioca flours are used most often. Pasta quality can be influenced by such factors as: kind of starch, size of starch granules or amylose, fat and protein content [Chen et al. 2003;Tan et al., 2009].

Since in pasta dough without gluten it is difficult to bind all the ingredients sufficiently, different innovations in traditional technologies are introduced into production process [Miedwiediew, 1999; Wang et al., 1999; Gallagher et al., 2004]. In gluten-free pasta the role of binding and structure-forming factor takes over gelatinized starch. However properties of such a product during and after cooking are much worse. Moreover because of complicated technology gluten-free pasta is more expensive than traditional product.

Using hot extrusion method, in conditions of high pressure, homogenization, even gelatinization and binding of all the particles occurs. While using cold extrusion part of the starch should be previously subjected to gelatinization and than combined with the rest of the ingredients. In this case binding of all the ingredients is usually worse. That is why components which can substitute for gluten such as polysaccharides (guar gum, xanthan gum, modified starches, locust bean) or egg white are added [Gallagher et al., 2004; Alamprese1 et al., 2007; BeMiller, 2008]. Hydrocolloids increase viscosity of starch systems but in the presence of salt the synergic interaction of starch and xanthan gum increasing and the viscosity is less effective. It was also found that gelatinization temperature of starch decreased with the addition of xanthan gum. [Sudhakar et al., 1995]. Studying the effect of guar gum on starch properties using microscope methods it was shown that the starch components tended to be inhibited from leaching out of the starch granules as the molecular weight of added guar gum increased [NagaNo. et al., 2008].

Apart from hydrocolloids emulsifiers addition is also applied. Some of the most often used include sodium and calcium stearoyl–2-lactylate, lecithin, monoglycerides and fatty acids esters [Kovacs et al., 1992]. Monoglycerides significantly decrease surface stickiness of cooked spaghetti and slightly decrease its hardness. In the extrusion process, emulsifiers can provide some lubrication and that is why the addition of monoglycerides reduces torque, specific mechanical energy and dough temperature [Shiau, 2004; Tao et al. 2009]. Besides emulsifiers react with amylose during gelatinization [Zobel, 1988]. The helical inclusion complexes formed between glycerol monostearate and the amylose affect the cooking time and reduce cooking loss of the noodles [Kaur et al. 2005]. An increase in cooking time of pasta with the emulsifier addition was also reported by Schreurs et al. [1986]. Eliasson [1986] reports that the addition of emulsifiers increases initial pasting temperature, hot paste viscosity and temperature of peak viscosity.

In this work an attempt was made to make gluten-free pasta from traditional gluten-free raw materials mixed in different proportions basing on two technologies. The first of them consisted in gelatinization of starch before pasta dough mixing and the second one was based on preparing pasta dough in a twin-screw cooking extruder where hydrotermic treatment of the ingredients took place.

Materials and methods

Corn starch, corn flour, gluten-free wheat starch, potato starch, defatted soy flour, guar gum, xanthan gum, and fresh hen's egg white were used in the study for making pasta samples. The selection of raw materials was made on the basis of the ingredients in gluten-free pasta available on the market.

The raw materials were characterized for moisture (PN-90/A-74009), protein content by Kjeldahl method (protein conversion factor – 6,25 except wheat starch – 5,7), minerals content (PN-ISO 2171:1994) and yellow pigments content expressed as beta-carotene (AACC 14–50).

The raw material composition of all pasta samples is shown in table 1.

Two methods were used to produce pasta: a traditional method with pasta-extruder GOLTZ POLO 5, with partially gelatinized starch in recipe and an extrusion method with the use of twin-screw cooking extruder ZSK-25 (made by Krupp Werner & Pfleiderer) at the following parameters of the process: L:D = 20; a temperature of the 1 zone 110°C; a temperature of the 2 zone 120°C; a temperature of the head 95°C; corotating screw revolutions = 50/min. Pasta samples were dried at 60°C for 6 hours.

Table 1

Raw material composition [percent with reference to amount of starches and flours]

Symbol	Corn flour [%]	Corn starch [%]	Wheat starch [%]	Potato starch [%]	Soy flour [%]	Egg white [%]	Guat gum [%]	Xanthan gum [%]	Emulsifier [%]
A_G	70	10	10	–	10	–	0.5	–	1
A_{GW}	70	10	10	–	10	5	0.5	–	1
A_X	70	10	10	–	10	–	–	0.5	1
A_{XW}	70	10	10	–	10	5	–	0.5	1
B_G	55	15	15	–	15	–	0.5	–	1
B_{GW}	55	15	15	–	15	5	0.5	–	1
B_X	55	15	15	–	15	–	–	0.5	1
B_{XW}	55	15	15	–	15	5	–	0.5	1
C_G	30	50	15	–	5	–	0.5	–	1
C_{GW}	30	50	15	–	5	5	0.5	–	1
C_X	30	50	15	–	5	–	–	0.5	1
C_{XW}	30	50	15	–	5	5	–	0.5	1
D_G	30	–	15	50	5	–	0.5	–	1
D_{GW}	30	–	15	50	5	5	0.5	–	1
D_X	30	–	15	50	5	–	–	0.5	1
D_{XW}	30	–	15	50	5	5	–	0.5	1

Cooking quality of obtained samples was evaluated by determining cooking loss and degree of pasta hydration – weight increase (AACC 66–50). Pasta colour was evaluated by measuring the L*, a* and b* parameters by means of a reflectance colourimeter (CR 400 Chroma-Metter, Minolta, Japan) (AACC 14–22).

Pasta samples were also subjected to sensory evaluation test for colour, taste, smell, firmness and adhesiveness on a 5-point scale (1- unsatisfactory, 5- very good), using a 15 member panel. Additionally commercial gluten-free pasta produced by ZPC "Glutenex" and pasta prepared from semolina were subjected to evaluation.

In the next stage of the experiment the effect of hydrocolloid on pasta firmness was determined. Guar or xanthan gum was added to corn flour or corn starch at the levels of 0.5% and 1%. Pasta was produced with the use of twin-screw cooking extruder, samples were dried and then cooked for 10 minutes. For comparison pasta prepared in the same conditions from semolina was also tested. Cooked firmness of pasta was measured using texture analyzer TA-XT2i fitted with razor blade shear attachment A/LKB. A cutting tests were conducted according to AACC 66–50 method at the following parameters of the process: crosshead speed 10 mm/min, crosshead upper position 3 mm, crosshead lower position 0.5 mm from bottom plate. Measurements were repeated eight times, using fresh subsamples. Maximum cutting stress was calculated by dividing maximum force value by tooth-sample contact area and work of cutting was represented by the area under curve.

Sensory evaluation of sample texture was conducted by fifteen panelists, using a 1 to 5 scale, with 1 representing unacceptable texture (too firm or too soft) and 5 the optimum, most desirable texture. In addition cooking loss and cooked weight, as a measure of the degree of pasta hydration, was determined.

Results and discussion

when a recipe formulation for gluten-free pasta is developed the aim is to obtain a product with properties similar to traditional product. It is possible to produce pasta with desirable colour and nutritional value by composing specific mixtures. Gluten-free pasta available on the market contains wheat starch. Its use for gluten-free food production arouses controversy. In some opinions all the wheat, rye, oat or barley products should be eliminated from this branch of food industry for the safety reasons. In USA and Canada products naturally containing gluten are forbidden to use for gluten-free food production whereas in Scandinavia and Great Britain the use of wheat starch is acceptable [Thompson, 2001].

All the starches used in the experiment had very low protein and mineral content so their nutritional value was rather small. Corn flour was characterized by protein content at the level of 8% while defatted soy flour contained over 50% of protein and moreover it had high mineral content. The addition of this component to the other ingredients could improve nutritional value of pasta.

An important pasta characteristic which affects its attractiveness is colour. Pasta colour is a result of yellow pigments content in raw material or additives used. Pigments contained in wheat, which determine the colour of traditional pasta, are mainly lutein and its esters, while in corn the main pigment apart from lutein is zeaxantin [Gąsiorowski, 2006]. In case of traditional pasta turmeric is often added. In all the starches used in the experiment yellow pigments content, expressed as beta-carotene, was low whereas in corn flour high content of yellow pigments (over 15 ppm) was found. In good quality traditional raw material, which is semolina, their content amounts to about 6–8 ppm.

Pasta quality depends not only on quality of raw material but also on the production process used. Manufacturing of good quality gluten-free pasta is a difficult task. In case of traditional pasta products an essential structure-forming role plays gluten. Firmness, elasticity and strength of pasta depend on quantity and quality of gluten. In gluten-free products different additives or technologies should be applied to enable maintaining of appropriate structure.

In the present work two kinds of hydrocolloids: guar gum (nonionic hydrocolloid) and xanthan gum (anionic hydrocolloid) were used. It is found that they can influence rheological properties of starch systems in different way depending on a charge [Chaisawang et al., 2005; Chaisawang et al., 2006].

The results of sensory evaluation of obtained pasta samples are presented in Table 3.

The highest score among evaluated samples was obtained by pasta from corn flour with the addition of xanthan gum and hen's egg white made with traditional method (A_{XW}). While the lowest score was obtained by pasta with the addition of soy flour at the level of 15% made with cooking-extrusion process (B). In case of these samples an adverse change of colour during production process was noted. Pasta was intensively brown and after cooking the colour changed into gray. The addition also adversely affected smell of the product. The effect of colour and smell change was not noted while manufacturing pasta of the same composition with traditional method and these pasta samples were evaluated higher. Samples of the same composition but with the addition of hen's egg white got a little better score. When the amount of soy flour in pasta composition was decreased the colour and smell improved.

Table 2

Characteristics of raw materials used in the experiment

Raw material	Moisture [%]	Protein content [% d. m.]	Minerals content [% d.m.]	Fat content [% d.m.]	Yellow pigments content [ppm]
Corn starch	12	0.3	0.06	–	0.49
Wheat starch	12	0.2	0.06	–	0.71
Potato starch	10	0.6	0.43	–	–
Corn flour	12	8.3	1.20	1.8	15.55
Soy flour	7	54.0	3.71	2.6	–

Table 3

Sensory evaluation of pasta samples

Sample	Colour	Smell	Taste	Firmness	Stickiness	Overall Score
Pasta made with traditional extruder						
A_G	4.0	3.0	3.6	3.4	3.4	3.5
A_{GW}	4.0	3.5	3.7	3.6	3.8	3.7
A_X	3.0	4.0	3.7	3.5	3.6	3.6
A_{XW}	4.0	4.0	3.7	3.7	3.7	3.8
B_G	4.2	3.0	3.7	3.3	3.5	3.5
B_{GW}	4.3	3.0	3.9	3.8	3.6	3.7
B_X	4.1	3.3	3.8	3.8	3.6	3.7
B_{XW}	4.0	3.5	3.8	3.9	3.2	3.7
C_G	2.6	2.7	2.9	2.6	2.5	2.7
C_{GW}	2.8	3.1	3.4	3.3	2.8	3.1
C_X	3.0	3.0	3.0	3.4	3.0	3.1
C_{XW}	2.8	3.0	3.2	3.1	3.5	3.1
D_G	3.0	1.7	2.3	2.9	2.9	2.6
D_{GW}	2.9	2.0	2.5	3.3	2.7	2.7
D_X	3.0	1.9	2.8	3.1	2.6	2.7
D_{XW}	3.1	2.0	2.3	3.0	3.0	2.7
Pasta made with twin-screw cooking extruder						
A_G	2.4	2.7	2.6	3.2	3.0	2.8
A_{GW}	2.2	3.0	2.9	3.3	3.3	2.9
A_X	2.1	2.9	2.8	2.7	2.8	2.6

A_{XW}	2.2	3.2	2.9	3.0	3.0	2.9
B_{G}	1.5	2.0	2.6	2.5	2.8	2.3
B_{GW}	1.0	2.2	2.5	2.7	3.0	2.3
B_{X}	1.4	2.4	2.3	2.4	3.0	2.3
B_{XW}	1.3	2.3	2.7	2.7	3.5	2.5
C_{G}	2.8	3.3	3.3	3.3	3.4	3.2
C_{GW}	3.0	3.5	3.3	3.6	3.8	3.4
C_{X}	3.2	3.3	3.0	3.7	3.5	3.3
C_{XW}	3.0	3.6	3.1	3.5	3.8	3.4
D_{G}	2.6	2.8	2.6	2.9	2.7	2.7
D_{GW}	2.8	3.0	2.9	3.2	3.2	3.0
D_{X}	2.7	3.0	2.5	2.8	3.0	2.8
D_{XW}	2.4	3.1	2.7	3.4	3.3	3.0
Commercial gluten-free pasta	3.9	3.7	3.9	3.9	3.9	3.9
Pasta from semolina	4.5	4.2	4.0	4.6	4.4	4.4

Pasta with 50% of corn starch (C) was evaluated higher than samples in which this component was replaced with potato starch (D). The addition of potato starch worsened taste and smell of pasta. The differences were more pronounced in pasta produced with traditional method.

Pasta manufactured in the experiment was characterized by lower quality than commercial pasta. In comparison with pasta made from semolina all the gluten-free pasta samples were evaluated definitely lower. It was found that pasta made with traditional method is of better quality than that manufactured with the use of cooking-extrusion process. A major influence on overall evaluation of pasta samples had the colour of product. Extruded pasta (cooking extrusion) before cooking were characterized by unattractive brown colour. After cooking the colour changed – cooked samples turned gray. The effect was the most evident in case of pasta with the highest addition of soy flour.

It was also shown that hen's egg white positively affects gluten-free pasta quality. The addition had a favorable influence on firmness, stickiness and also taste of pasta. Unfortunately people suffering from celiac disease are often allergic to egg white too, what limits the possibility to use it for manufacturing of gluten-free products. A positive effect of egg white addition on properties such as texture and shortening of hydration time of pasta made with cooking extrusion technique was reported by Wójtowicz [2007].

On the basis of sensory evaluation it was also found that pasta with the addition of xanthan gum had a little bit better quality than pasta with guar gum. The addition of xanthan gum in gluten-free pasta production was also applied by Hung et al. [2001]. Using sensory evaluation they found that xanthan addition at the level of 0.8% enables to obtain better quality (hardness, cohesiveness, adhesiveness) pasta in comparison with the pasta containing 0.4% of this polysaccharide.

On the basis of cooked pasta colour measurements (brightness L* and yellowness b*) it was found that pasta manufactured with the extrusion process was characterized by lower L and b indexes than pasta of the same composition produced with traditional method.

Higher yellowness index was noted for pasta containing higher ratio of corn flour (A and B). The highest brightness had C samples whereas the lowest value of this characteristic was noted for D samples in which corn starch was replaced with potato starch. It was shown that in case of pasta manufactured from traditional raw materials applying extrusion method causes a decrease in brightness index and an increase in yellow colour saturation [Makowska, 2008]. In case of gluten-free pasta evaluated after cooking a decrease in both brightness and yellowness of samples was noted. While evaluating pasta colour after extrusion it was shown that extruded pasta made with cooking extruder was darker and glossier than that extruded in low temperature.

Pasta cooking properties are determined on basis of such characteristics as weight increase and cooking loss. Weight increase determined for obtained samples ranged from 1.98 to 3.13. It is assumed that for good quality pasta made from semolina this index ranges from 2.5 to 3.5 while cooking loss should not exceed 8–10%. It was found that in pasta manufactured with traditional method cooking loss was definitely higher and ranged up to 20%. Considerably lower values were obtained while analyzing cooking-extruded pasta samples. The values were even threefold lower than in case of pasta made with traditional method. On the basis of pasta cooking properties evaluation it was found that cooking-extruded pasta had better quality than products obtained with traditional technology. High cooking loss is the result of poor binding of pasta dough components together before extrusion. Products for people suffering from celiac disease lack gluten proteins which could bind all the components together through forming protein network in which starch granules are trapped. It is starch what in gluten-free products becomes a binding agent.

In the next stage of the experiment an attempt was made to determine the effect of kind and amount of hydrocolloid added on cooking properties and texture of model pasta samples. As raw materials in this part of the experiment corn flour, corn starch and wheat starch were used. Pasta made on the basis of corn starch demonstrated higher values of maximum cutting stress and work of cutting than pasta made of corn flour. Corn starch pasta with the addition of xanthan gum obtained higher values of these texture indexes than pasta with guar gum. Differences in cutting stress between pasta manufactured from corn starch with guar gum and xanthan gum were smaller than differences in work of cutting.

On the other hand pasta made from corn flour showed the opposite tendency i.e. samples of pasta made from corn flour with the addition of guar gum showed higher values of maximum cutting stress and work of cutting than the same pasta with xanthan gum.

Wheat starch pasta was characterized by even higher values of these texture indexes.

In sensory texture evaluation test the highest scores among all the gluten-free samples were obtained by samples of corn pasta with the addition of xanthan gum at the level of 0.5%.

However the results were still lower than the score obtained by traditional pasta made from semolina.

It was also found that in pasta with higher addition of hydrocolloid the cooking loss was lower while weight increase was higher. No. clear effect of kind of flour (starch) on the above characteristics was found.

Table 4

Cooking properties and colour measurements of cooked pasta samples

Samples	Colour				Weight increase [g/g]		Cooking losses [g/100g d.m.]	
	Brightness L		Yellowness b					
	T. E.*	C. E.**	T. E.*	C. E.**	T. E.*	C. E.**	T. E.*	C. E.**
A_{G}	63.5 ±0.78	59.2 ±0.61	25.3 1.18	18.1 ±0.51	2.90 ±0.02	2.44 ±0.04	20.57 ±2.28	5.63 ±0.69
A_{GW}	63.8 ±1.33	57.6 ±0.55	24.7 ±0.24	19.1 ±0.62	3.02 ±0.04	2.61 ±0.05	18.25 ±1.52	5.11 ±0.39
A_{X}	62.1 ±1.07	58.7 ±0.87	27.2 ±0.71	20.0 ±0.37	3.10 ±0.05	2.66 ±0.05	14.47 ±0.57	6.92 ±0.87
A_{XB}	63.0 ±0.74	56.9 ±0.45	24.1 ±1.12	19.1 ±0.66	3.15 ±0.04	2.80 ±0.06	12.73 ±0.94	4.86 ±0.03
B_{G}	57.9 ±0.94	53.1 ±1.03	27.3 ±0.94	21.6 ±0.58	2.53 ±0.02	2.48 ±0.04	18.37 ±1.43	8.62 ±0.90
B_{GW}	61.3 ±0.66	55.6 ±0.33	24.2 ±1.21	20.4 ±0.67	2.72 ±0.05	2.44 ±0.03	14.23 ±1.04	7.22 ±0.88
B_{X}	59.9 ±1.08	55.7 ±0.58	25.7 ±1.03	20.5 ±0.93	2.63 ±0.03	2.50 ±0.04	16.58 ±1.33	8.19 ±0.81
B_{XB}	60.8 ±1.16	56.2 ±0.37	24.2 ±0.69	21.0 ±0.47	2.68 ±0.06	2.55 ±0.05	15.11 ±0.91	7.34 ±0.71
C_{G}	66.1 ±0.57	61.0 ±1.22	24.7 ±2.07	19.1 ±0.68	2.79 ±0.03	2.68 ±0.05	13.16 ±0.64	4.42 ±0.10
C_{GW}	65.0 ±1.32	59.6 ±0.98	21.9 ±1.06	18.9 ±0.73	2.77 ±0.05	2.71 ±0.03	12.06 ±0.59	4.15 ±0.35
C_{X}	65.0 ±1.97	59.8 ±0.73	22.4 ±0.97	19.9 ±0.61	2.94 ±0.04	2.51 ±0.04	14.30 ±1.65	5.80 ±0.47
C_{XB}	66.9 ±0.75	62.0 ±1.13	21.6 ±0.41	18.7 ±0.91	3.11 ±0.04	2.54 ±0.03	12.63 ±1.12	4.88 ±0.27
D_{G}	61.0 ±1.33	57.1 ±0.48	23.4 ±0.17	18.4 ±0.45	1.98 ±0.02	2.65 ±0.06	17.60 ±1.24	4.02 ±0.61
D_{GW}	60.4 ±0.98	56.5 ±0.55	22.6 ±0.66	18.5 ±0.51	2.06 ±0.02	2.56 ±0.05	15.44 ±0.88	4.22 ±0.52
D_{X}	60.6 ±1.06	55.4 ±0.37	21.5 ±0.27	19.1 ±0.61	2.17 ±0.04	2.50 ±0.04	18.24 ±2.02	5.81 ±0.46
D_{XB}	61.4 ±1.27	55.7 ±0.62	22.3 ±1.34	19.4 ±0.84	2.25 ±0.01	2.73 ±0.05	16.52 ±0.97	4.17 ±0.33
Commercial gluten-free pasta	80.1±0.50		19.3±0.78		2.9±0.05		17.5±1.62	
Pasta from semolina	73.3±0.63		27.3±0.62		3.1±0.02		6.5±0.52	

.*T. E – pasta made with traditional extruder

**C. E. – pasta made with twin-screw cooking extruder

Table 5

Texture evaluation and cooking properties of model pasta samples

Samples		Maximum cutting stress [g/cm^2]	Work of cutting [g*s]	Sensory evaluated texture	Cooking loss [g/100g d.m.]	Weight increase [g/g]
Corn starch	Guar gum 0.25	1072±37	654±52	3.2	6.8±0.1	2.23±0.37
Corn starch	Guar gum 0.5	1082±50	694±31	3.8	3.7±0.3	3.73±0.01
Corn starch	Xanthan 0.25	1078±72	710±88	3.4	4.2±0.2	1.86±0.03
Corn starch	Xanthan 0.5	1096±30	721±44	4.4	3.3±0.4	2.56±0.01
Corn fluor	Guar gum 0.25	821±81	601±100	3.2	6.8±0.6	2.20±0.01
Corn fluor	Guar gum 0.5	995±17	619±14	3.6	5.0±0.3	2.30±0.02
Corn fluor	Ksantan 0.25	572±3,6	372±46	3.0	7.9±0.6	2.14±0.03
Corn fluor	Ksantan 0.5	706±68	507±56	4.4	5.8±0.7	2.37±0.04
Semolina	–	761± 25	612±34	4.6	6.4±0.4	2.80±0.03
Wheat starch	Guar gum 0.25	914±32	655±47	3.0	5.9±0.6	1.99±0.01
Wheat starch	Guar gum 0.5	1155±57	802±57	3.0	4.5± 0.7	2.54±0.01
Wheat starch	Xanthan 0.25	1277±43	871±65	4.2	5.9±0.2	2.37±0.01
Wheat starch	Xanthan 0.5	1903±118	1283±68	4.2	3.9±0.2	2.30±0.04

Conclusion

It still presents a challenge to manufacture gluten-free pasta of the quality resembling durum wheat pasta. Gluten-free pasta obtained in the present work was significantly worse than pasta from semolina.

It was found however that the addition of xanthan gum better affected sensory evaluated texture of gluten-free pasta than guar gum and the best were samples with 0.5% of this hydrocolloid added.

A positive effect of egg white on pasta quality was also determined but the addition of defatted soy flour (a good source of protein) had a negative influence on colour of pasta manufactured in hot extrusion conditions used in the experiment.

Since the quality of gluten-free products is still significantly worse than that of traditional pasta further studies have to be conducted to obtain product of high nutritional and sensory quality.

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5

POLYPHENOLS IN EMULSION AND LIPOSOMES AS EFFECTIVE PROTECTORS OF MEAT PRODUCTS AGAINST OXIDATION

Introduction

Technical and technological development as well as still growing consumer demands towards high quality foods force food producers to either update used technologies or to design new methods of food preservation and processing. Safe foodstuffs should be characterized by high quality standard and desired sensory traits [Li and Zang, 2001]. Oxidation processes commonly occurring in foods initiate by free radicals ($O_2^{-\cdot}$, $^{\cdot}OH$) and/or reactive oxygen species (singlet oxygen $^1O_2$, hydroxyl peroxide H_2O_2, lipid peroxides LOOH) are one of the most important factors changing character of safety foods [Gray et al., 1996]. Results collected in biological systems studies revealed that oxidation processes disrupt structure and functions of many biological molecules such as: nucleic acids, enzymes dependent on SH groups and lipids including cholesterol [Bartosz, 2003]. Biological molecules breakages can cause some dangerous diseases such as tumors, cardiovascular system diseases or neurodegenerative changes in older people [Rice-Evans et al., 1994; McNamara, 2000]. Autooxidative processes occuring *in vitro* in animals and birds muscle tissues play a significant role in the formation of final products characteristic properties [Gardner, 1979]. Lipids oxidation is one of the best recognized process leads to e.g. formation of toxic products either *in vivo* or in foodstuffs. Lipids oxidation effected in decreasing of fats nutritive value as a result of n = 3 and n = 6 polyunsaturatted fatty acids breakdown as well as cis-trans isomerisation processes. Cholesterol, constituent of membrane cells of all animal organisms, is also prone to oxidation processes. Oxysterols, oxidized forms of cholesterol, occurring in app. 70 different forms, can be formed during lipids autooxidation as well as during smoking, heating, sterilizing of food by ionizing radiation [Ahn et al., 2001] or in fried products. Oxidised forms of cholesterol present *in vivo* also in protein-lipid associated forms, especially as low density lipoprotein (LDL) contribute in heart and blood vessels diseases (atherosclerosis), they also express mutagenic and carcinogenic activities [Smith and Jahnson, 1989]. Moreover, on the cell level they can effect calcium channel, which impair ionic transport through biological membranes and express inhibitory activity on specific enzymes (Na- and K-ATP-ase), which has indirect influence on initiation of different inflammations in living organisms. Lipid oxidation processes in meat products decrease aminoacids digestability and bioavailability as well as deteriorate taste, flavour and colour of the products known as WOF (warmed-over flavour) [McMillin, 1996].

The use of antioxidants is a simple and effective method of foodstuffs durability prolongation. Many natural and synthetic substances express antioxidative properties [Heim et al., 2002; Sokół-Łętowska et al., 2007; Bąkowska-Barczak and Kołodziejczyk, 2008]. Literature data, including last few years toxicological research pointed out disease aspects of some synthetic antioxidants [Kupfer et al., 2002]. Moreover, growing consumer awareness and many law restrictions towards limitation of synthetic antioxidant use lead to intensive interest in raising and applying of natural antioxidative substances. Polyphenols form a large group of natural antioxidants, which are mainly extracted from fruits, flowers, seeds, barks and roots. Numerous studies on screening effective antioxidants, either in form of polyphenol's extract or highly purified components, such as flavonoids, are very intensively carried out. Some of the studies concern the evaluation of antioxidative substances activity e.g. *in vitro* towards model biological membranes [Gabrielska et al., 1997; Gabrielska and Oszmiański, 2005; Gabrielska et al., 2007; Chaudhurii et al., 2007; Bonarska-Kujawa et al., 2008a, 2008b], emulsions and oils [Frankel et al., 1996; Wojdyło et al., 2007a] and model systems containing stable free radicals DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ [Bąkowska-Barczak et. al., 2008; Wojdyło et al., 2007b]. However, there is still lack of knowledge about polyphenols application in food of animal origin. Main interest in this area is focused on utilization of spices (rosemary, oregano, sage) [Botsoglou et al., 2002; Cornardo et al., 2002; Waszkowiak et al., 2007], as well as green tea catechins [Mitsumoto et al., 2005] in meat technology. Anyway, there is No. evidence on research on antioxidants application methods, which can improve their effectiveness especially in fresh foods containing fats e.g. meat. Our previous study conducted on polyphenols acted in phospholipids liposome membranes enabled the selection of natural polyphenols (hawthorn, buckwheat and rosemary (used as a reference sample) extracts and rutin, catechin and quercetin) used in further research on inhibition of peroxidation reaction occur in poultry and beef meat. The objective of the study was the evaluation of antioxidation effectiveness of selected polyphenols applied to frozen and fresh poultry and beef meat in forms of emulsion and liposomes, in relation to the activity of the same substances applied in powder state.

Materials and methods

Materials. Phosphatidylcholine from eggs was purchased from Lipids Product [South Nutfield, U.K.). The lipid stock solution in pure ethanol was maintained at -20°C in glass vials under nitrogen. Rutin hydrate, quercetin dihydrate, catechin hydrate, Tween 80 and sorbitol were obtained from Sigma [Deisenhoffen, Germany]. The extract of rosemary containing 56.20% of carnosic acid was purchased from TKI PINUS Rače d.d. (Czech Republic). Soy oil was purchased in local market. The remaining chemicals of highest analytical grades were bought from Sigma (Deisenhoffen, Germany). Turkey and beef meat were purchased in local markets, mechanically deboned turkey meat was obtained from turkey industry plant in Slupia Kapitulna (Gizewski).

Preparation of plant extracts. Buckwheat seeds was purchased in local market. Isolation methods of phenolic constituents from buckwheat hulls (*Fagopyrum esculentum* Moench) was compiled on the basis on procedure reported by Kim et al. [2005] and Kreft et al. [1999]. The bark of hawthorn (*Crataegus oxyacantha)* was collected from Botanical Garden of the Medical Academy of Wrocław. Extract from those plant was prepared according to the method proposed by Oszmiański and Bourzeix [Oszmiański and Bourzeix, 1995] and determined by HPLC. Procyanidins from the bark of hawthorn contained (-)-epicatechin (10.1%) and their polymers: B2 (24.2%), B4 (14.0%) and other compounds B4+B5 (10.5%).

Liposome preparation. An ethanol solution of egg yolk phosphatidylcholine with appropriate amount of studied plant extracts or flavonoids was dried in vacuum under nitrogen [Gabrielska and Oszmiański, 2005]. 50 mmol/L Tris:HCl [(hydroksymethyl) aminomethane] buffer of pH 7.4 was added and the sample was vortexed to obtain a milky suspension of multilamellar vesicles. The final concentration of lipids in the vesicle suspension was 1.5 g/L Prepared suspension was then sonicated for 10 min with a 20 kHz sonicator.

Preparation of submicronic emulsions o/w types. Preparation of the emulsions was based on ultrasounds desintegration methods according to procedure reported by Jaromin [2005] and Meller and Hildebrand [1998] with some modifications. On the basis on the initial results quercetin was considered as standard antioxidant, thus in further stages of the experiment rutin and catechin concentrations were calculated in relation to molecular mass of the quercetin. Appropriate amount of the polyphenols extract (5.2 mg) or pure flavonoid (quercetin – 5.2 mg, catechin – 4.5 mg, rutin – 9.4 mg) were dissolved in 0.5 ml of ethanol, then 20 mg of phosphatidylcholine (lipid) was added and evaporated in nitrogen atmosphere under vacuum. Following, 15 mg of Tween'u 80 (emulgator), 50 mg of soy oil and 2 ml of 4.5% of water solution of sorbitol were addend to dry phosphatidylcholine films. The mixture was then mixed for app. 5 min and homogenized using ultrasound (3–4 W) for app. 2 min.

Analyses of lipids oxidation. The amount of lipids oxidation products in meat was analysed spectrophotometrically using TBARS method based on the quantity of lipids oxidation products reacting with 2-thiobarbituric acid [Buege and Aust, 1978; Rey et al., 2005]. Control sample as well as samples containing antioxidants in forms of: powder, emulsion or liposomes, were joined with 10 g of meat (turkey breast muscles, turkey meat mechanically recovered, beef rib steak) and thoroughly mixed. Prepared samples were incubated at the temperature 4°C for 3 days. The amount of oxidized lipids was evaluated after 3 days of incubation. For this analyses 10 g of meat samples was mixed with 54 ml of perchloric acid water solution (1:1), homogenized and filtered. Then, 2 ml of filtrate was added to 2 ml of thiobarbituric acid and trichloroacetic acid, incubated for 15 minutes at the temperature 98°C –100°C. Finally, solutions were centrifuged (2000 x g, 10 minutes) and absorbance was measured at 535 nm. Percent of lipid oxidation inhibition was calculated according to:

$$\%\ \mathrm{INHIBITION} = \{(1 - \Delta A_A) / \Delta A_0\} \times 100\%$$

where ΔA_A – increase of absorbance (at $\lambda = 535$ nm) at day 3 of incubation the samples with antioxidant, ΔA_0 – increase of absorbance (at $\lambda = 535$ nm) at day 3 of incubation the control samples (without antioxidant).

Statistical analysis. The standard deviation was calculated on the basis of collected results of two or three independent experiments (n = 6). All results were statistically analysed by using STATISTICA ver. 7.0 with the Duncan test with probability level of $p \leq 0.05$.

Results and discussion

The results of our previous studies on antioxidative properties of selected plant polyphenols and flavonoids showed a high protective activity of buckwheat, hawthorn and rosemary (as a standard due to its wide use in food) extracts, as well as quercetin, rutin and catechin against lipids oxidation analysed in model systems of liposome and erythrocyte membranes [Gabrielska et al., 2007]. In present study previously checked plant polyphenols extracts and flavonoids were applied to meat in order to inhibit lipid oxidation. Prepared polyphenols preparations were applied to meat as o/w dispersion or as phosphatydilcholine's liposome associates in order to enhance their distribution within lipid tissue. This kind of application was done for better antioxidants molecules transfer together with lipids droplets into fat. Figure 1 shows the percentage of inhibition of lipid oxidation analysed in comminuted turkey muscles (Fig. 1 A) and mechanically deboned turkey meat (MDTM, Fig. 1 B) in the presence of quercetin, rutin and catechin. Antioxidants were applied to those systems as dispersion, liposome and powder (Fig. 1). Statistical analysis of the results (Fig. 1 A i B) revealed that rutin was the most effective antioxidant when analysed inhibition of lipids oxidation in turkey comminuted and mechanically deboned meat (excluding MDTM with liposome). The antioxidative activity of analysed substances was as follows: rutin (75–90%) > quercetin (38–63%) ≥ catechin (37–56%). Inhibitory activity of analysed flavonoids in relation to application form was arranged as follows: liposome (37–90%) ≥ emulsions (44–75%) ≥ powders (21–78%). Analysis of variance showed that there are No. statistically significant differences between application methods for rutin, which expressed the highest inhibitory activity among analysed flavonoids either in comminuted or mechanically deboned turkey meat (Fig. 1 B). Lower inhibitory activity of quercetin in relation to rutin is in opposite to the results reported by Bors et al. [1990] and Rey et al. [2005], as well as our previous study conducted on liposome membranes [Gabrielska et al., 2007]. Cited authors pointed out higher activity of quercetin than quercetin diglucoside (rutin) *in vitro* in lipids protection against oxidation. Those contradictory results can effect from the analytical system specifity (comminuted meat) as well as different experimental conditions (pH, buffer types, oxidizing agents) [Sun and Ho, 2005].

Analysis of inhibition of oxidation processes initiated in turkey mechanically deboned meat by selected polyphenol's extracts (Fig. 2 A) revealed that the inhibitory activity of extracts applied in emulsion and liposome forms decreased as follows: buckwheat (78%, a)≥ rosemary (74%, a) >> hawthorn (57%, b) for emulsions and hawthorn (73%, a) > rosemary (62%, b) ≈ buckwheat (57%, b) for liposomes. Polyphenol extracts applied in powder form were characterised by significantly lower inhibitory activity (hawthorn – 41% c, > buchwheat – 31% c, ≈ rosemary – 37% c), however, they was not significant differences within analysed group. Polyphenols extracts protective ability against lipid oxidation occurs in comminuted beef muscles (Fig. 2B) expressed a similar to MDTM tendency in relation to application method (emulsion ≥ liposome > powder). The levels of oxidation process inhibition by polyphenols extracts applied in forms of emulsion and liposomes were as follows: buckwheat (83%, a) ≥ hawthorn (73%, a) >> rosemary (33%, b) and hawthorn (74%, a) ≈ rosemary (72%, a) ≈ buckwheat (67%, b). However, when powder form of polyphenols extracts were used the inhibition of lipid oxidation was much lower (buckwheat (42%, c) > hawthorn (39%, b) > rosemary (29%, b)). Statistical analysis of collected results revealed that there was a positive correlation between antioxidant activity of buckwheat extract (78%, a) and rutin (72%, a), as well between hawthorn extract (57%, b) and catechin (44%, b) incorporated into MDTM in form of emulsion and between hawthorn extract (73%, a) and catechin (79%, a) applied into the same material in form of liposome. This kind of correlation was expected due to the fact that rutin is the main constituent of buckwheat extract. Moreover, buckwheat is commonly used for rutin extraction for further pharmaceutical purposes [Kreft et al., 1999, 2006; Quettier-Deleu et al., 2000; Jiang et al., 2007]. Hawthorn extract is rich in catechins, present also as polymers, which are responsible for its antioxidant and pharmacological properties [Ammon and Handel, 1981; Hobbs and Foster, 1999; Joseph et al., 1995; Zang et al., 2001; Rigelski and Sweet, 2002].

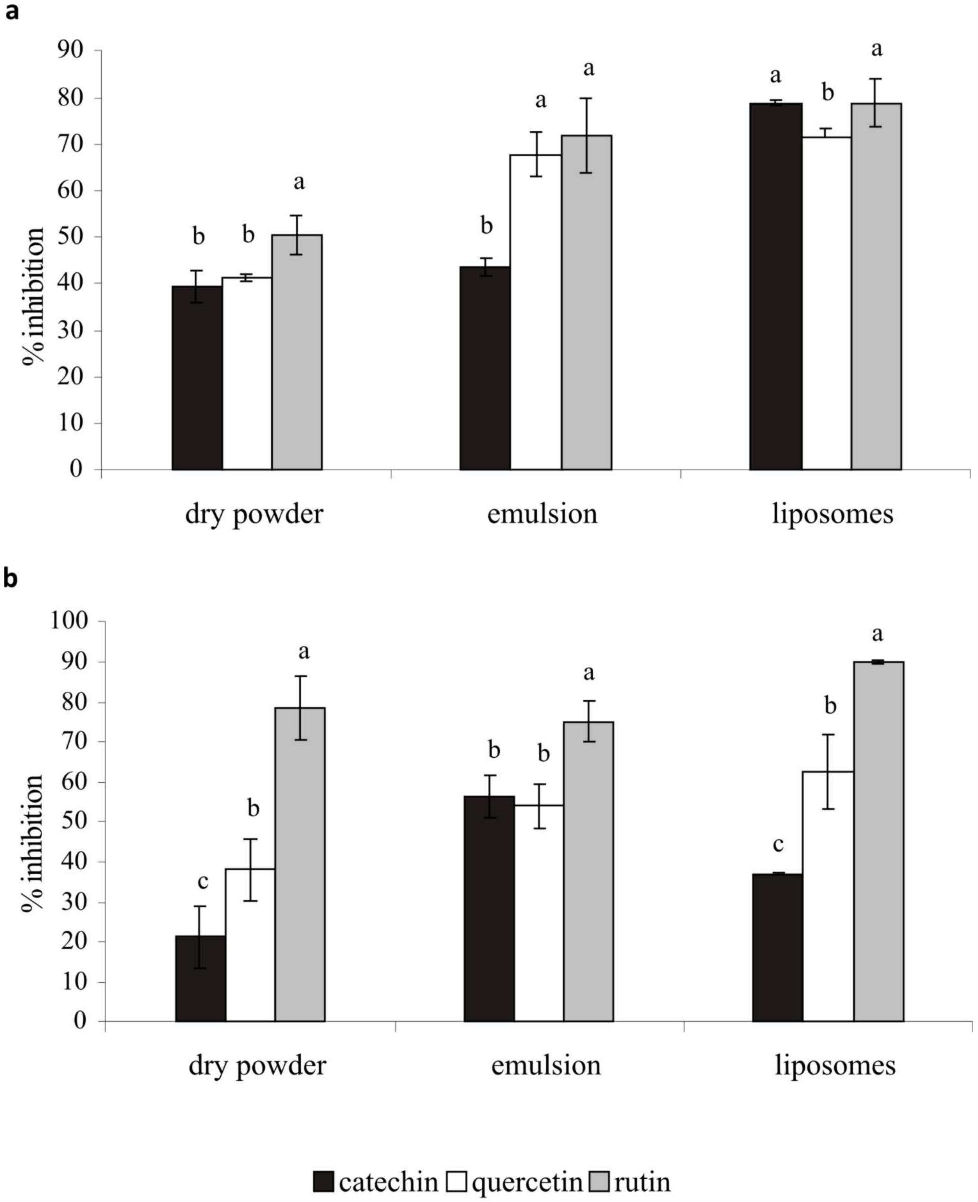

Fig. 1. Percentage of lipid oxidation inhibition by flavonoids (catechins, quercetin, rutin) in relation to application method (powder, emulsion, liposome) in:
a) mechanically deboned turkey meat MDTM
b) comminuted turkey meat

a,b,c,…, – the same letters determine not statistically significant differences at p>0.05 n = 6

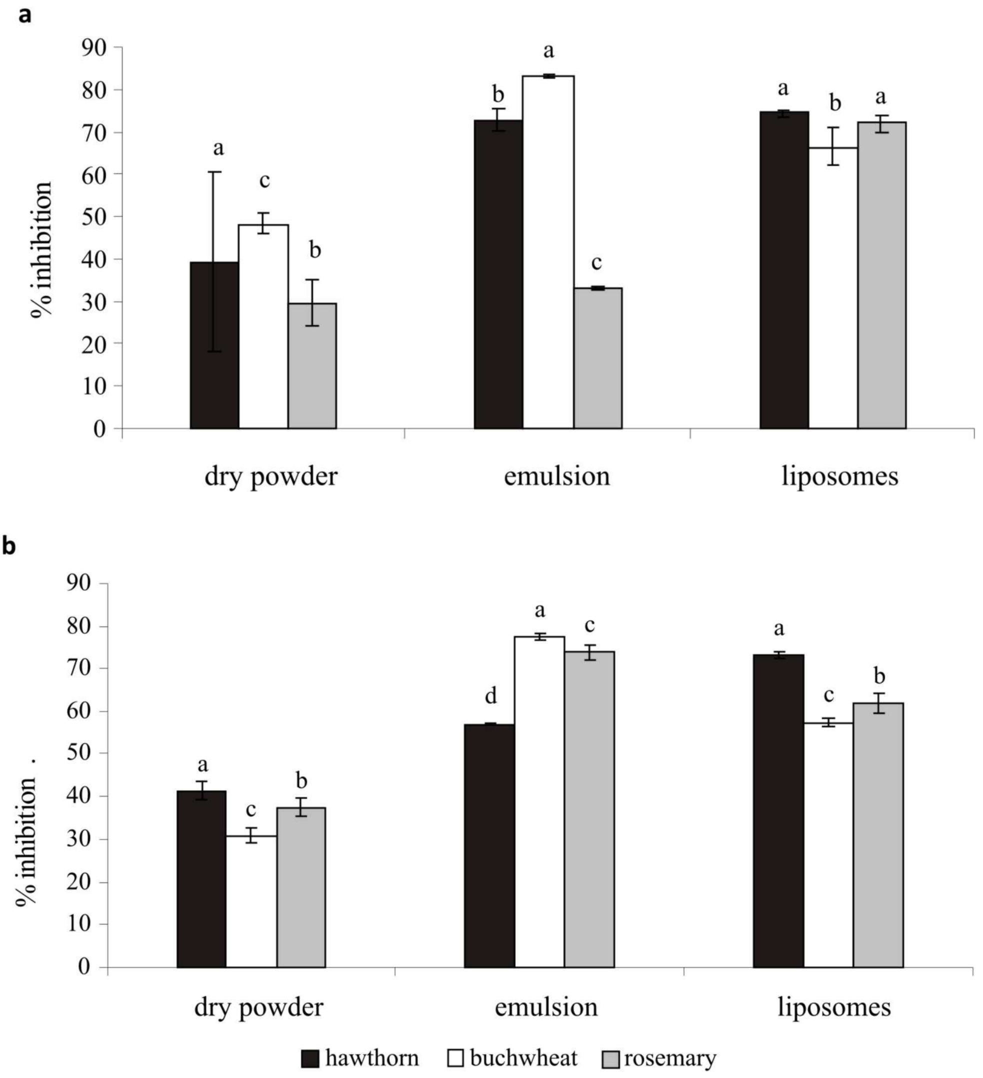

Fig. 2. Percentage of lipid oxidation inhibition by polyphenols extracts from hawthorn bark, buckwheat hulls and rosemary in relation to application method (powder, emulsion, liposome) in:
a) mechanically deboned turkey meat MDTM
b) comminuted beef meat

a,b,c,…, – the same letters determine not statistically significant differences at $p>0.05$ n = 6

Quite high differences between antioxidant activity of buckwheat preparation (57%, b) and rutin (79%, a) analysed in MDTM can be explained by lower association ability of molecules present in plant extract towards liposome membranes in comparison to pure rutin. Asociation constants designated by DPH probe using Stern-Volmer method [Verkam, 1980] were 799 ± 35 $l \cdot g^{-1}$ and 1050 ± 120 $l \cdot g^{-1}$ for buckwheat extract and rutin, respectively. Buckwheat extract constituents were less bound by bilayer of liposome membrane and smaller amount of them were transported to muscle lipids, thus they were much less protected against oxidation. Oteiza et al. [2005] suggested that flavonoids moleculeas are bound by liposome membrane by the formation of hydrogen bonds with polar side of lipid's bilayer, which can protect the membrane against free radicals.

The study carried out on two different meat systems (MDTM and beef) showed that buckwehat preparation was more effective in inhibition of lipid oxidation when applied into comminuted beef muscles, which were characterized by higher fat content in comparison to mechanically deboned turkey meat (Fig. 1b and Fig. 2b). The antioxidative activity of buckwheat extract was similar to those expressed by commercial rosemary preparation. Moreover, extract application form had a significant influence on the level of lipid oxidation inhibition. The highest level of oxidation inhibition was analysed when polyphenol extract was incorporated into meat system in form of liposome or emulsion (Fig. 1b and 2b). There are many literature data on rosemary antioxidative avtivity analysed in different model systems [Foti et al., 1996; Frankel et al*.,* 1996; Basaga et al., 1997; Perez-Fons et al*.,* 2006]. However, there is still lack of any comparative study about rosemary and other polyphenolic substances in relation to application method. Our study showed that antioxidative activity of rosemary is lower than buckwheat and hawthorn preparation when applied in form of emulsion and powder, and it is comparable to studied plant polyphenols extract when incorporated into meat systems as liposome associates. Perez-Fons et al. [2006] suggested that effectivity of rosemary towards liposome membrane resulted from the activity of diterpenes and genkwain, which are able to tighten the membrane. It can hinder free radicals diffusion in such modified membrane.

The effectiveness of inhibition of lipid oxidation by rutin (the most effective flavonoid among all used in the study) was also analysed in comminuted beef and turkey muscles. The percentage of oxidation inhibition by rutin measured in beef muscles was in frame from 25% for powder up to 74% for emulsion and 76% for liposome form of application. Regarding to turkey muscles the oxidation inhibition was as follows: 78, 75% and 90% for powder, emulsion and liposome forms, respectively. These results indicated that rutin is a very effective antioxidant in meat systems (with the exception of beef muscles when applied in powder form), especially in turkey meat where its protective activity was very high even when incorporated in powder form. Higher protective ability against lipid oxidation in turkey meat can be partially explained by higher concentration of other antioxidants in this type of food products, especially with the level of carnosine, natural occurs quite strong antioxidant. Lower level of oxidatively induced changes in pure turkey meat (control) comparing to beef, was probably connected with the interaction between rutin and other antioxidants.

Results collected in the study showed higher antioxidant activity of polyphenols preparations and flavonoids against lipid oxidation when they were applied into meat systems in forms of emulsion and liposomy in comparison to powder form, which can be connected with better availability of active compounds in plant extracts. This resulted probably from the opportunity for binding (dissolving) the extract or flavonoid molecules by bilayer of liposome membrane or emulsion particles, which led to easier mixing and bounding with muscles lipids. Similar explanation of this phenomenen was reported by Barclay et al. [1997], when the effectivity of selected phenolic hydroksychromanic antioxidants (including α-tocopherol) applied in form of liposome build from saturated lipids were analysed in systems containing azine compounds. Cited authors revealed that antioxidant properties of analysed substances were much higher when applied in form of liposome than directly (as solution) to the model system. It is also probable that lecithin (either in emulsion or liposome) enhanced the inhibition of lipid oxidation through the interaction with incorporated substances, due to lecithin (lecithin compounds) expresses a good antioxidative activity [Sikorski, 2001].

In conclusion it can be stated that rutin, as well as buckwheat hulls and hawthorn bark extracts can be used as effective antioxidants in turkey and beef comminuted muscles, especially when applied in form of emulsion (o/w type) or liposome.

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6

THE INFLUENCE OF THE FREEZING PROCESS AND STORAGE PERIOD ON THE SENSORY QUALITY OF COOKED CARROTS OF SELECTED VARIETIES

Introduction

The sensory analysis plays a significant role in food industry and gastronomic production, because it ensures the production on the appropriate level and constitutes an indispensable factor in the continuous improvement of the quality of the goods produced. The sensory characteristics are: taste, smell, appearance including colour, and consistency of the food that can be distinguished and evaluated with the help of human senses [Baryłko-Pikielna, 1975]. The sense organs in people allow identifying the quality of the impression and determining its intensity as well as stating to what degree this impression is desirable or undesirable [Baryłko-Pikielna, 1998].

Human sensory apparatus allows the identification of quality experience, the determination of its intensity, as well as determining desirable or undesirable of the impression extent [Baryłko-Pikielna, 1998]. The characteristics of sensory impressions are presented in Figure 1. The sensory assessment combines the interaction of the product chemical composition and physicochemical characteristics with human's psychophysical features, his expectations and beliefs, including religious [Borowski, 2004].

Sensory analysis plays an important role in the food industry and catering as prerequisite the production maintaining at an appropriate level and is an indispensable factor in the continuous quality improvement of manufactured products [Baryłko-Pikielna, 1975; Ewan, 1992; Sidel, Stone, 1993]. In general, the quality evaluation of vegetables is defined by the senses relative to the product and their sensory attraction. Cooking processes aim at giving vegetables the right consistency, appearance, smell and taste [Zalewski, 1988; Zalewski, 1992]. The research on nutritional behaviours [Czarnocińska and Wądołowska, 2003; Czarnocińska and Babicz-Zielińska, 2004] showed that the most important factors in choosing vegetables and vegetables products are sensory features independent of the socio-demographic profile presented by Polish respondents. Among the factors influencing the choice of vegetables are their freshness and taste [Babicz-Zielińska and Zagórska, 1998].

The carrot is one of the most popular vegetables cultivated and consumed in Poland. About her nutrition value decide the chemical constitution of granary root. This vegetable is a rich and valuable source of such compounds as: β-carotene, saccharides, mineral components and vitamins, among which the most present are vitamins: A, E, from group B and the ascorbic acid [Sady et al., 2000; Adamiecki, 2001; Grudzień and Sikora, 2002]. The content of saccharose in the carrot carries out about 8.7 g per 100 g of edible part whereof

4.4 g determines content of saccharine and 2.4 g the alimentary fiber. Saccharides structure in the carrot shape on gustatory and reological properties of products. The content of these components in the carrot is first of all variety features [Borowska et al., 2003; Kowalska et al., 2003].Carotenoids perform the essential part in the creation of carrot root colour. In the human body carotenoids keep such chemical reactivity as in plants by catching free radicals and active atomic oxygen. This is especially important for heavily working peoples, record-seeking sportsmen and people being in the situation of the prolonged stress. The diet rich into carotenoids diminishes of coronary disease, tumours of the lungs, urinary diseases and skin problems [Godlewska, 1991a; Kleszkowska, 2001; Mosiewicz , 2002]. About the nutritive value of the carrot decide also contracted in roots mineral components and alkaline salts regulating the water economics of the human body. Mineral components delivered along with the food realize important matter in the behavior of the human-body acid-alkaline equilibrium [Godlewska, 1991b; Godlewska, 1992].

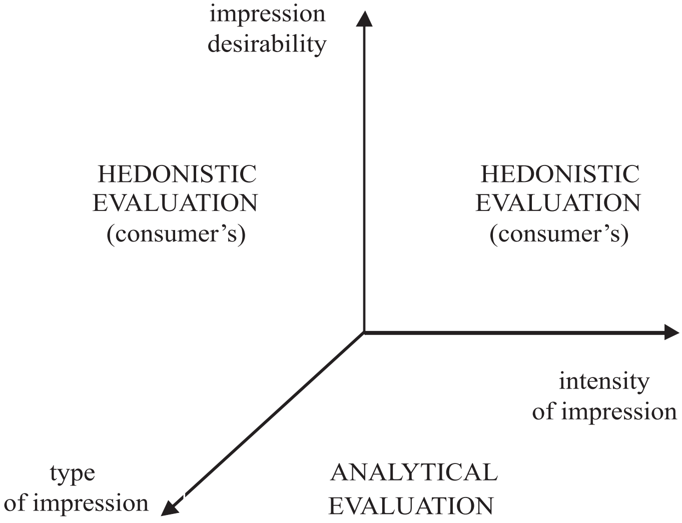

Fig. 1. Three-dimensional characteristics of sensory impressions and related types of the sensory evaluation [Baryłko-Pikielna, 1998].

In the winter-spring season the carrot is present in the consumer meal not only as the raw product but also in the preserved form. Frozen vegetables gain from year to year the greater acknowledgment among consumers [Górecka and Flaczek, 2000; Górska-Warsewicz, 2003]. To the most important advantages of frozen food belongs: all around the year accessibility, the comfort in preparation, economic, the improvement of the wholesome safety by the elimination of secondary microbiological contagious, the high nutritive value, the good product information the many months persistence without perceptible quality changes the lack of waste material [Bąkowski, 2003]. The carrot belongs to vegetable assorts especially useful to the consolidation in the frozen form. For growing requirements both on the part of the industry as and consumers turns the attention not only on the profitable surface of the finished product as frozen mixed vegetables but also on his high nutritive value [Bąkowski, 2002; Górska-Warsewicz, 2002].

The carrot is consumed all around the year as fresh product, in the form of salads, however in the greatest degree is used after thermal treatment to determine the perfect material in the culinary art. The heat treatment has principle meaning in the meals production and leads to changes of appearance, consistency, colour, taste and chemical constitution of semimanufactured products and grants their characteristic sensory properties [Grzesińska, 1997; Gołaszewska and Czarniecka-Skubina, 2000; Platta et al., 2003]. During the heat treatment nutritive components as vitamins and mineral components decay or leaching away to the solution [Gołaszewska and Czarniecka-Skubina, 2000].

The aim of this work was to define the sensory quality of selected carrot varieties after cooking them with various methods. In the research the influence of carrot varieties, the form of material, the time of storage and the cooking processes on the sensory feature of the cooked carrots was studied. The scope of the research included: 1. the estimation of the sensory feature of six carrot varieties after the traditional cooking process, in a pressure cooker and in a steam cooker; 2. the estimation of the sensory feature of raw and frozen carrot varieties cooked in the traditional way taking into account the tested material storage period.

Material and methods

The research took place after collecting selected carrot varieties from the field and preparing the frozen carrots and after the third and the sixth month of storage of the raw and frozen carrots. The tested material used in the experiment consisted of ten carrot varieties: Nerac F_1, Narbonne F_1, Nandrin F_1, Napa F_1, Nagadir F_1, Bristol F_1, Nigel F_1, News F_1, Nipomo F_1, Niagara F_1. The examined carrot samples were cooked in the traditional way – starting from boiling water, in the pressure cooker and in the steam cooker. The examined vegetables were blanched and deep frozen at -22°C. After the freezing process carrot samples were cooked only in the traditional way.

The sensory estimation of the cooked carrots was analysed with the use of the points method in the range from 1 for the minimum value to 5 for the maximum value. Four quality distinctive factors were taken into consideration: general appearance, smell, consistency and taste (Table 1). The scope of the research included the sensory evaluation of the raw and frozen carrot varieties cooked in the traditional way taking into account the tested material storage period.

Table 1

Standard card of the boiled carrot's sensoric rating – 5 -points method (own's elaboration)

Quality Feature		5 pts	4 pts	3 pts	2 pts	1 pts
General appearance	Colour	intense, orange-red, clear, uniform, for typical for boiled carrot	typical for boiled carrot, a bit clearer or darker than original	a bit changed colour, clearer or darker than original	changed colour, seal, darker or faded than original	highly changed colour, seal or pale
	Shape	original shape, No. damages	few damages, shape a bit changed if ever	shapeless, partially crambled, a bit cracked	crambled significant part	crambled, shapeless, many bits distracted in boiling process
Scent		intense, fresh scent of boiling carrot, aromatic and sweet	a bit less intense	slight scent	slight scent, soft strange scent present	No. typical scent for boiled carrot, No. scent at all, abnormal scent (bitter, acid, etc.)
Consistency		soft, succulent	soft, a bit less succulent, or a bit watery	too soft or slightly hard, nonuniform, a bit succulent or a bit watery	too hard or overboiled, dry or watery, nonuniform	very soft (pulp) or very hard, very dry or very watery, nonuniform
Taste		slightly sweet, soft taste typical for fresh boiled carrot	typical for boiled carrot	a bit different than for typical of boiled carrot, too sweet, slightly watery	soft strange taste present, No. taste at all	acid, bitter, strange aftertaste present

Results and discussion

The examination results obtained from the evaluated sensory features have shown that the transformations occurring during the coking process influenced the quality. The size and the character of transformations depended on the used method of cooking. The type of the applied cooking method had the influence on the four quality distinctive factors which were taken into consideration in the experiment.

The general quality evaluation of the carrot samples cooked in the traditional way was defined in the range between 3.93 points (Nandrin F_1 carrot variety) and 4.85 points (Nigiel F_1 carrot variety). The carrot samples cooked in the pressure cooker were estimated in the range between 3.86 points (Nagadir F_1 carrot variety) and 4.65 points (Nipomo F_1 carrot variety). The carrot samples cooked in the steam cooker were estimated in the range between 3.81 points (News F_1 carrot variety) and 4.88 points (Nipomo F_1 carrot variety). The Nigiel F_1 carrot variety and the Nipomo F_1 carrot variety gained the highest sensory evaluation by the points method with regard to the cooking methods used. The Nipomo F_1 carrot variety obtained the lowest sensory evaluation by the points method with regard to the cooking methods used (Table 2, Figure 2).

It was presumed in the work that the storage period has an influence on the quality distinctive factors in the cooked vegetables. The general sensory evaluation of the selected raw carrot varieties cooked in the traditional way after three months of storage was defined in the range between 3.33 points (Nerac F_1 carrot variety) and 4.48 points (Nigel F_1 carrot variety), and after 6 months of storage it was defined in the range between 2.74 points (Narbonne F_1 carrot variety) and 3.65 points (Napa F_1 carrot variety) (Table 3, Figure 3).

The general sensory evaluation of the frozen carrot samples cooked in the traditional way was defined in the range between 3.21 points (Nandrin F_1 carrot variety) and 3.88 points (Nigiel F_1 carrot variety). The frozen carrot samples cooked after three months of storage were defined in the range between 2.63 points (Niagara F_1 carrot variety) and 4.23 points (Napa F_1 carrot variety), and after six months of storage they were defined in the range between 2.44 points (Niagara F_1 carrot variety) and 4.40 points (Narbonne F_1 carrot variety). Among the examined frozen carrot varieties, the Bristol F_1, Nagadir F_1, Napa F_1, Narbonne F_1 and Nigiel F_1 carrot varieties gained the highest sensory quality by the points method with regard to the storage period (Table 4, Figure 4).

The statistical analysis of the obtained sensory results have shown the essential influence of the cooking methods on the general appearance, the consistency and the taste of the cooked carrot samples (Table 5). It also has shown the essential influence of the storage time and the carrot variety on the general appearance, the smell, the consistency and the taste of the cooked fresh and frozen samples of the selected carrot varieties (Table 6, 7).

The sensory estimation of the selected carrot varieties after cooking: in the traditional way, in the pressure cooker and in the steam cooker has shown that the traditional cooking way gives the most valuable product as far as the sensory feature is concerned. The cooking process in the pressure cooker gives the least valuable product as far as the sensory feature is concerned (Table 2, Figure 2).

Table 2

Sensory assessments of the fresh carrots after selected cooking processes (points)

Carrot varieties	Cooking methods	Sensory assessment (1–5 points)				General quality evaluation x ± δ
		General appearance x ± δ	Smell x ± δ	Consistency x ± δ	Taste x ± δ	
Bristol F_1	in the traditional way	5.0±0.00	4.5±0.55	3.0±0.89	3.8±0.75	3.93±0.41
	in the pressure cooker	4.5±0.58	4.7±0.52	4.3±0.83	4.3±0.82	4.43±0.64
	in the steam cooker	4.2±0.14	4.5±0.55	3.8±0.82	3.7±0.55	3.99±0.49
Nagadir F_1	in the traditional way	4.3±0.89	4.7±0.52	4.7±0.82	4.7±0.82	4.65±0.68
	in the pressure cooker	3.8±0.47	4.0±0.63	4.0±0.89	3.7±0.82	3.86±0.61
	in the steam cooker	4.3±0.56	4.3±0.52	4.0±0.63	4.0±0.89	4.11±0.38
Napa F_1	in the traditional way	4.8±0.14	4.1±0.41	4.3±0.52	4.3±0.52	4.31±0.38
	in the pressure cooker	4.3±0.22	3.8±0.75	4.0±0.52	4.1±0.75	4.03±0.50
	in the steam cooker	3.9±0.33	3.9±0.89	3.0±0.89	3.4±0.55	3.49±0.47
Narbonne F_1	in the traditional way	4.4±0.14	4.3±0.52	4.1±0.41	4.4±0.84	4.30±0.29
	in the pressure cooker	4.1±0.14	3.8±0.41	4.0±0.89	3.8±0.41	3.89±0.25
	in the steam cooker	4.3±0.47	4.0±0.63	3.5±0.84	4.1±0.98	3.95±0.37
Nigel F_1	in the traditional way	5.0±0.00	4.8±0.41	4.8±0.41	4.8±0.41	4.85±0.31
	in the pressure cooker	4.3±0.22	4.5±0.55	3.5±0.55	4.0±0.63	4.04±0.26
	in the steam cooker	5.0±0.00	4.5±0.55	4.2±0.75	4.7±0.52	4.54±0.26
News F_1	in the traditional way	4.8±0.14	4.3±0.82	4.3±0.52	4.5±0.55	4.46±0.42
	in the pressure cooker	4.2±0.14	3.8±0.41	4.0±0.63	3.8±0.41	3.92±0.37
	in the steam cooker	4.3±0.22	3.8±0.41	4.0±0.89	3.5±0.55	3.81±0.26
Nandrin F_1	in the traditional way	5.0±0.00	4.3±0.52	3.8±0.75	3.7±0.52	4.04±0.33
	in the pressure cooker	4.5±0.25	4.0±0.00	4.0±0.63	3.8±0.41	4.00±0.34
	in the steam cooker	4.8±0.14	4.8±0.41	4.7±0.52	4.2±0.75	4.54±0.44
Nipomo F_1	in the traditional way	4.8±0.14	3.8±0.75	4.5±0.55	4.3±0.52	4.31±0.38
	in the pressure cooker	4.8±0.14	4.5±0.55	4.2±0.41	5.0±0.00	4.65±0.26
	in the steam cooker	5.0±0.00	4.5±0.52	5.0±0.00	5.0±0.00	4.88±0.13
Nerac F_1	in the traditional way	4.7±0.22	4.5±0.55	4.2±0.75	4.3±0.52	4.38±0.30
	in the pressure cooker	4.2±0.47	4.3±0.52	4.2±0.41	3.8±0.41	4.08±0.14
	in the steam cooker	4.5±0.25	4.2±0.75	4.2±0.41	4.3±0.52	4.27±0.33
Niagara F_1	in the traditional way	4.8±0.14	4.5±0.55	4.7±0.52	4.5±0.55	4.58±0.44
	in the pressure cooker	4.7±0.22	4.0±0.89	4.2±0.41	4.0±0.63	4.13±0.46
	in the steam cooker	4.5±0.25	4.2±0.75	4.0±0.63	4.3±0.52	4.23±0.27

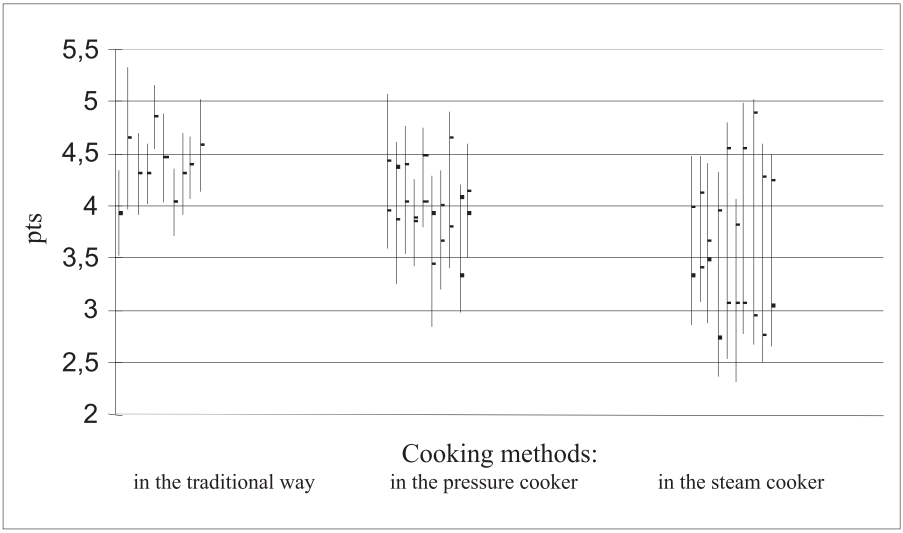

Fig. 2. The general quality evaluation of the fresh carrots after selected cooking processes (points)

Table 3

Sensory assessments of the fresh carrots cooked in the traditional way (points)

Carrot varieties	Time of storage period (months)	Sensory assessment (1–5 points)				General quality evaluation x ± δ
		General appearance x ± δ	Smell x ± δ	Consistency x ± δ	Taste x ± δ	
1	2	3	4	5	6	7
Bristol F_1	0	5.0±0.00	4.5±0.55	3.0±0.89	3.8±0.75	3.93±0.41
	3	4.2±0.47	4.0±0.63	4.0±0.00	3.8±0.41	3.95±0.35
	6	2.8±0.14	3.7±0.52	3.7±0.82	3.0±0.89	3.33±0.46
Nagadir F_1	0	4.3±0.89	4.7±0.52	4.7±0.82	4.7±0.82	4.65±0.68
	3	4.5±0.25	4.7±0.52	4.2±0.75	4.2±0.75	4.36±0.26
	6	4.0±0.10	3.5±0.55	3.3±0.52	3.2±0.75	3.4±0.32
Napa F_1	0	4.8±0.14	4.1±0.41	4.3±0.52	4.3±0.52	4.31±0.38
	3	4.5±0.25	4.5±0.84	4.3±0.82	4.3±0.52	4.38±0.38
	6	4.3±0.22	3.5±1.05	3.7±1.03	3.5±0.84	3.65±0.76
Narbonne F_1	0	4.4±0.14	4.3±0.52	4.1±0.41	4.4±0.84	4.30±0.29
	3	4.3±0.56	3.7±0.52	3.8±0.41	3.8±0.75	3.84±0.41
	6	3.0±0.33	3.0±0.89	2.7±0.82	2.5±0.55	2.74±0.37
Nigel F_1	0	5.0±0.00	4.8±0.41	4.8±0.41	4.8±0.41	4.85±0.31
	3	4.3±0.22	4.8±0.41	4.7±0.52	4.2±0.41	4.48±0.27
	6	4.2±0.47	3.2±0.75	2.7±0.52	2.7±0.82	3.06±0.52
News F_1	0	4.8±0.14	4.3±0.82	4.3±0.52	4.5±0.55	4.46±0.42
	3	3.3±0.22	3.3±0.52	3.0±0.89	3.8±0.75	3.44±0.59
	6	3.8±0.47	3.1±0.75	3.0±0.89	2.8±0.75	3.05±0.75

Table 3 cont.

1	2	3	4	5	6	7
Nandrin F_1	0	5.0±0.00	4.3±0.52	3.8±0.75	3.7±0.52	4.04±0.33
	3	4.2±0.14	3.5±0.55	4.0±0.89	3.3±0.52	3.65±0.45
	6	4.0±0.10	2.5±0.55	2.5±0.52	3.5±0.55	3.06±0.30
Nipomo F_1	0	4.8±0.14	3.8±0.75	4.5±0.55	4.3±0.52	4.31±0.38
	3	4.2±0.14	4.0±0.89	3.8±0.41	3.5±0.84	3.79±0.38
	6	4.2±0.14	3.0±0.10	2.5±0.52	2.7±0.55	2.94±0.26
Nerac F_1	0	4.7±0.22	4.5±0.55	4.2±0.75	4.3±0.52	4.38±0.30
	3	3.5±0.25	3.3±0.52	3.0±0.63	3.5±0.55	3.33±0.34
	6	4.0±0.10	3.0±0.63	3.7±0.52	1.5±0.55	2.75±0.25
Niagara F_1	0	4.8±0.14	4.5±0.55	4.7±0.52	4.5±0.55	4.58±0.44
	3	4.0±0.10	4.0±0.63	4.2±0.82	3.7±0.52	3.92±0.42
	6	4.2±0.56	3.2±0.82	3.0±0.10	2.5±0.55	3.03±0.37

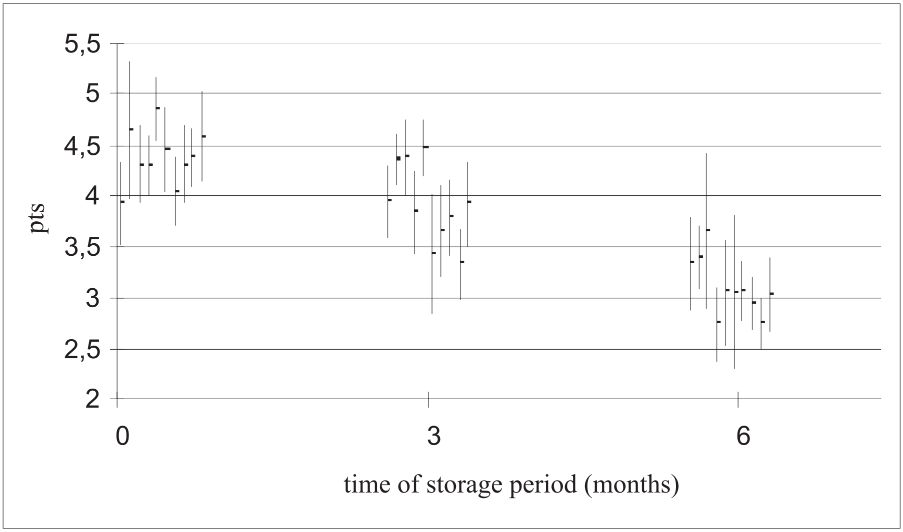

Fig. 3. The general quality evaluation of the fresh carrots cooked in the traditional way (points)

Table 4

Sensory assessment of the frozen carrots cooked in the traditional way (points)

Carrot varieties	Time of storage period (months)	Sensory assessment (1–5 points)				General quality evaluation x ± δ
		General appearance x ± δ	Smell x ± δ	Consistency x ± δ	Taste x ± δ	
Bristol F_1	0	4.5±0.58	3.7±0.82	3.5±0.84	3.5±0.84	3.68±0.64
	3	4.0±0.33	4.3±0.52	3.5±0.55	3.7±0.52	3.84±0.31
	6	4.7±0.22	4.3±0.82	3.5±0.55	4.2±0.75	4.11±0.41
Nagadir F_1	0	4.5±0.58	3.7±0.82	3.5±0.84	3.5±0.84	3.68±0.64
	3	4.5±0.25	3.7±0.52	3.8±0.41	3.5±0.55	3.75±0.26
	6	4.5±0.25	3.7±0.52	4.0±0.10	3.8±0.75	3.91±0.35
Napa F_1	0	4.7±0.14	3.5±0.55	3.5±0.84	3.2±0.41	3.54±0.35
	3	4.8±0.14	4.2±0.41	4.0±0.63	4.2±0.75	4.23±0.46
	6	4.8±0.14	4.3±0.52	3.8±0.75	3.7±0.82	4.01±0.34
Narbonne F_1	0	4.2±0.47	3.8±0.75	3.8±0.98	3.6±0.80	3.78±0.79
	3	4.2±0.14	4.5±0.55	4.0±0.89	3.3±0.52	3.89±0.35
	6	4.7±0.22	4.3±0.52	4.2±0.98	4.5±0.55	4.40±0.49
Nigel F_1	0	3.8±0.14	4.2±0.41	3.2±0.75	4.2±0.75	3.88±0.41
	3	4.7±0.22	3.3±0.52	4.3±0.52	3.8±0.41	3.94±0.10
	6	3.8±0.14	2.7±0.52	3.0±0.10	2.8±0.41	2.96±0.30
News F_1	0	3.2±0.14	4.3±0.52	3.3±0.52	3.7±0.52	3.69±0.31
	3	3.5±0.25	3.5±0.55	3.3±0.52	3.0±0.10	3.27±0.38
	6	3.3±0.56	2.7±0.52	3.2±0.75	2.5±0.55	2.81±0.54
Nandrin F_1	0	3.3±0.22	3.3±0.52	3.3±0.84	3.0±0.00	3.21±0.39
	3	4.3±0.22	3.2±0.41	2.5±0.55	2.8±0.41	3.02±0.25
	6	3.0±0.10	2.8±0.41	3.2±0.41	2.0±0.90	2.63±0.32
Nipomo F_1	0	3.5±0.25	3.7±0.52	3.8±0.41	3.3±0.82	3.56±0.49
	3	3.3±0.22	3.0±0.63	2.8±0.41	2.3±0.52	2.75±0.44
	6	3.3±0.22	2.3±0.52	2.8±0.41	2.2±0.41	2.52±0.38
Nerac F_1	0	3.3±0.22	4.5±0.55	3.0±0.63	2.5±0.84	3.23±0.34
	3	3.3±0.22	3.2±0.41	3.0±0.10	3.0±0.89	3.08±0.34
	6	3.3±0.22	3.2±0.41	3.2±0.75	2.3±0.52	2.88±0.39
Niagara F_1	0	4.0±0.10	4.2±0.41	3.5±0.55	3.0±0.10	3.54±0.20
	3	3.2±0.47	2.8±0.41	2.3±0.41	2.5±0.55	2.63±0.38
	6	3.3±0.22	2.7±0.52	2.2±0.41	2.2±0.75	2.44±0.50

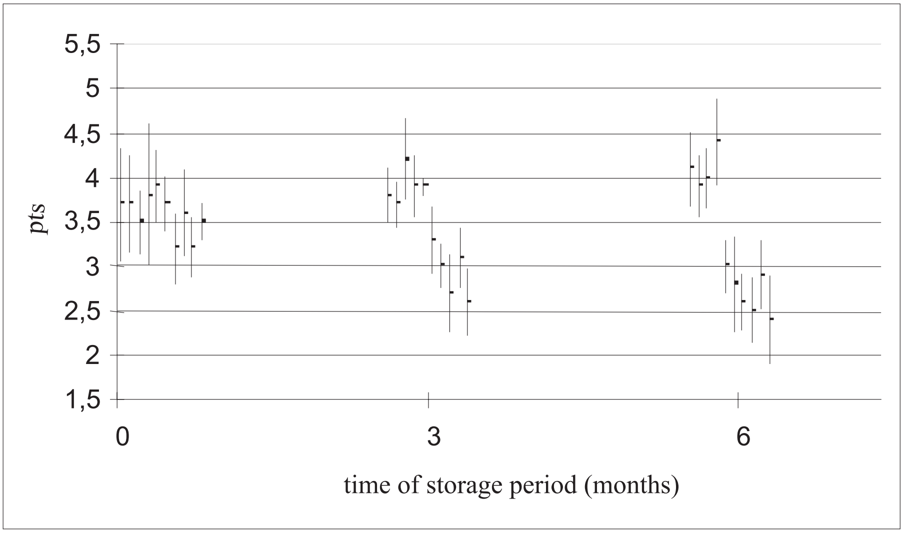

Fig. 4. The general quality evaluation of the frozen carrots cooked in the traditional way (points)

Table 5

Statistical analyses of the influence of the carrot varieties on the sensory feature of the boiled carrots

Cooking methods	Sensory assessments			
	General appearance	Smell	Consistency	Taste
in the traditional way	2.265*	1.383	4.155*	0.757
in the pressure cooker	1.451	1.884	0.817	2.646*
in the steam cooker	2.673*	1.637	3.998*	3.861*

* – statistically significant difference at α=0.05

Table 6

Statistical analyses of the influence of the carrot varieties on the sensory feature of the fresh carrots cooked in the traditional way

Time of storage period (months)	Sensory assessments			
	General appearance	Smell	Consistency	Taste
0	2.265*	1.383	4.155*	0.757
3	3.111*	4.585*	3.971*	1.686
6	5.926*	1.376	2.631*	4.336*

* – statistically significant difference at α=0.05

Table 7

Statistical analysis of the influence of the carrot species on sensory quality features of the frozen carrots cooked in the traditional way

Time of storage period (months)	Sensory assessments		
	General appearance	Smell	Consistency
0	7.519*	2.742*	0.813
3	7.547*	8.288*	10.778*
6	11.399*	14.496*	6.175*

* – statistically significant difference at $\alpha=0.05$

Conclusions

Comparing the three types of cooking methods, cooking in the traditional way is the most efficient of all the applied methods. Although the vegetables cooked in the steam cooker obtained the very high general quality, the carrots cooked in the traditional way obtained higher marks. Cooking in the pressure cooker is the least effective of all the applied methods during the experiment.

The sensory estimation of the analysed carrot samples cooked in the traditional way taking into account the storage time and the material form has shown that the raw Nigel F_1 and Nipomo F_1 carrot varieties obtained the most valuable sensory feature of all other varieties. The time of storage had the negative influence on the final quality of the raw carrots. The storage process caused that the general quality of the vegetables was lower than of the fresh carrots. During the 1st stage of the research, after harvesting the selected carrot varieties, the lowest sensory estimation had the raw Nigel F_1 carrot variety, after three and six months of the storage process the lowest sensory estimation had the raw Nerac F_1, Nigel F_1 and Napa F_1 carrot varieties. The frozen Nigel F_1 carrot variety had the lowest sensory estimation in the 1st stage of the research. The frozen Napa F_1 and Narbonne F_1 carrot varieties had the lowest sensory estimation after three and six months of the storage period.

The storage period and the freezing process caused that the general quality of the vegetables was lower than of the fresh carrots. The differences were quite significant.

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7

EVALUATION OF SOME NEW SOUS VIDE PROCESSED CHICKEN AND VEGETABLE PRODUCTS

Introduction

Consumers demand for fresh tasting, preservative free, ready-to-eat convenience meals with high dietary value require development of new minimal processing technologies such as minimally processed, ready-to-eat, extended shelf-life refrigerated foods [Rybka-Rodgers, 2001, Juneja, 2006], which include "sous vide" processed food products. "Sous vide" processing relies mainly on a mild heat-treatment and chilled storage of vacuum packed products. The "sous vide" process is a pasteurization step that reduces bacterial load but is not sufficient to make the food shelf-stable although it provides extended shelf life to the product.

Vacuum cooking is a cooking procedure developed in France in the mid-70s [Creed and Reeve, 1998]. This procedure involves heating of the raw materials vacuum-packed in a plastic film bag at a relatively low temperature and storing them until the food is reheated. Since this procedure has several advantages, it is employed by food processors in industrialized economies worldwide. When meat, poultry and seafood are processed with vacuum cooking around 60°C for a long time, the texture of the finished product is softer than that of products cooked by methods involving higher temperatures. Researchers have investigated changes of sensory and rheological properties, water holding capacity, proteins, microstructures and other properties during the vacuum cooking and/or low temperature heating of chicken [Takahashi et al., 1994, Goto et al., 1995; Murphy and Marks, 2000; Sainen et al., 2003, Nishimura et al., 2004]. The "sous vide" process of vacuum packaging food before applying low (below 100°C) temperature thermal processing and storing under chill conditions (0–3°C) is considered to offer enhanced quality and extended shelf life [Armstrong and McIlveen, 2000]. Improved flavour, due to vacuum packaging which prevents the development of oxdative off-flavours, and texture, particularly meat tenderness and juiciness has been reported [Hansen et al., 1995; Church and Parsons, 2000]. The food maintains maximum flavour because it is slow cooked for an extensive period of time at a relatively low temperature below water boiling point. From an analysis of the conventional process for cooking Korean seasoned beef, a "sous vide" processing method was developed that offers convenience and storage stability. "Sous vide" packaging resulted in better sensory quality and storage stability compared with the conventional method [Jang et al., 2005]. "Sous vide" technology has the capability to satisfy consumer demands for acceptable sensory quality beyond that of other cook-chill technology [Armstrong and McIlveen, 2000]. The aim of this study was to develop new ready-to-eat "sous vide" processed products made from poultry meat and vegetables.

Materials and methods

Broilers of the cross Ross 308 were used for the study. The average age of slaughtered parents' stock hens was 46 weeks; an average carcass weight of a bird was 2.6 kg. The slaughter and primary treatment was performed at a meat processing plant (line Stork PMT). Laying hens were stunned, then killed with a knife, bleeded for 3.37 minutes and scalded in a steam bath at 60.2±0.2 ºC for 3.47 minutes, defeathered, eviscered and chilled for 100 minutes at +1±0.5 °C. Carcasses were randomly selected for separating a fillet (*musculus pectoralis*). The obtained products were refrigerated at temperature +1±0.5 ºC. Vegetables (carrots, bell pepper, onions, garlic, and celery), fruits (sea buckthorn, orange), spices (pepper, parsley, dill, and others) and white wine were purchased from local retailers.

The study involved preparation of the raw materials, vacuum packing of the products in Polyamide /Polyethilene (PA/PE) film pouches with barrier properties (size of 200x300 mm, film thickness 60 µm), "sous vide" processing and chilling. Each sample containing chicken portion in size of approximately 130 grams and mix of various vegetables, fruits and/or spices was vacuum packed and sealed in a chamber type machine MULTIVAC A 300/16 and heat treated in a water bath "Clifton Food Range" at temperatures between 70 and 80ºC for 40 minutes. After heat treatment the samples were chilled in ice water and stored in Commercial Freezer/Cooler ELCOLD at +4.0±0.5ºC until sensory evaluation of the product. The degree of liking of new chicken products was evaluated using nine point hedonic scale [Poste et al., 1991]. In the sensory evaluation of chicken products were involved 15 panellists (2 men and 13 women, mean age 42). The tenderness of cooked chicken was determined by the Stable Miro Systems TA.XTplus Texture Analyser with load cell of 50 kg. Samples prior to test were cut in a size to fit on a platform, then positioned under the knife blade to assure cut along the meat fibres. The test speed of knife blade is set at 2 mm s^{-1}. The measurement is triggered automatically at 0.05 N. The maximum force applied to the sample was calculated as an average of 10 measurements.

Results and discussion

Sliced raw chicken was combined with fresh shredded and/or sliced vegetables (carrots, bell pepper, onions, garlic, and celery), some fruits (sea buckthorn, orange) and spices (pepper, parsley, dill, and others) in various proportions. Chicken portion in size of 130± 5 grams and mix of vegetables and spices was vacuum packed and heated in a water bath at temperature 70 or 80°C. The heating time was 40 minutes after water temperature reached the pre-set value. Chilling to meat core temperature below 10 °C in ice water occurred within 15 minutes immediately after "sous vide" treatment.

Recipe development was organized in three subsequent sets of tests. The aim of the first experimental set was the determination of the best heating time and temperature regarding the product texture, juiciness and flavour. The samples were made from cut chicken breast with carrot, garlic and/or orange additives in various proportions and salt. Poultry meat products treated at 70ºC temperature with holding time of 40 min had more tender texture and better juiciness, while products heated at 80ºC for the same time were little dry and not so tender. Texture analysis did not confirm results because significant difference among the samples was not established although there was tendency similar to the sensory evaluation giving higher maximum force required for sample cutting at 80 ºC temperature if heating time was equal (Fig. 1).

Sensory evaluation of the ingredients used in the first set of experiments excluded orange and orange garlic combination because of lower scores in liking test. Additional spices and ingredients such as dried dill, parsley, lemon pepper, white wine and some vegetables were suggested by panellists to improve the taste of new product.

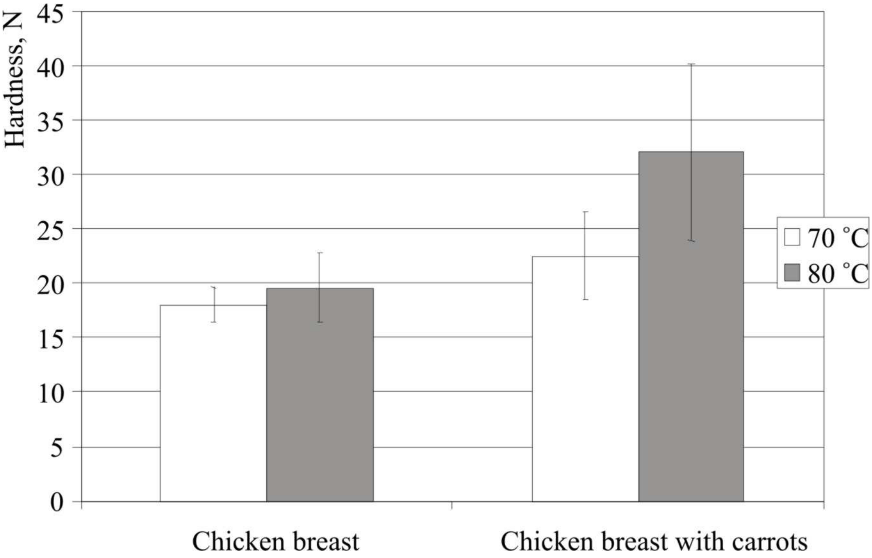

Fig. 1. Texture of "sous vide" treated chicken and chicken with carrots at 70 or 80ºC for 40 minutes

The heating temperature of 70ºC for 40 min was selected for further recipe development studies. The variations of vegetables (bell pepper, onion, celery) were added to the chicken breast prepared in white wine marinade containing wine, salt and various spices (dried dill, parsley, lemon pepper, garlic). Sensory evaluation proved favourable influence of white wine marinade on juiciness of product. The bell pepper additive was evaluated in a range from very like to very dislike, onion and celery additive was excluded from further study as these products did not get satisfactory evaluation form panellists. Based on the preliminary study the products made form combination of chicken breast with various vegetables and spices were developed for hedonic evaluation in 9 point scale.

Figure 2 shows degrees of liking of the six evaluated "sous vide" chicken breast products heated at 70ºC for 40 minutes. The obtained results show that the hedonic evaluation is from 5.0 (neither like nor dislike) to 7.13 (like).

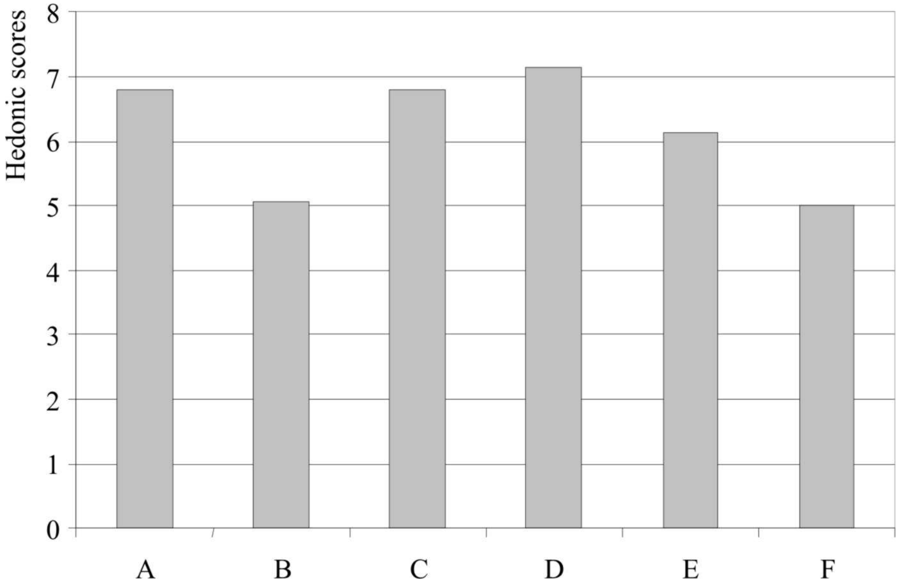

Fig. 2. Hedonic scores for six "sous vide" chicken breast products: A – chicken breast meat with spices, B – chicken breast with spices and bell pepper, C – chicken breast with spices and shredded carrots, D – chicken breast with spices, cubed carrots and parsley; E – chicken breast with spices, shredded carrots, bell pepper and parsley; F – chicken breast with spices and sea buckthorn sauce

The results of the analysis of variance show that $F_{calculated} = 6.51$ exceed $F_{critical} = 2.35$ therefore there exist significant differences in degree of liking among the studied chicken breast products ($\alpha \leq 0.05$). The highest hedonic scores received samples A (chicken breast meat with spices), C (chicken breast with spices and shredded carrots) and D (chicken breast with spices, cubed carrots and parsley). These samples had balanced aroma, good taste. Products B (chicken breast with spices and bell pepper) and E (chicken breast with spices, shredded carrots, bell pepper and parsley) got lower scores due to acceptance of the bell pepper incorporated in these samples - some panellists gave very high scores while others on contrary gave the lowest scores. The product sample F (chicken breast with spices and sea buckthorn sauce) received the lowest scores mainly due to unacceptable appearance of the product – panellists did not like the colour and texture of sea buckthorn sauce, while the taste was acceptable.

Results indicate that "sous vide" cooking resulted in acceptable sensory quality (taste, aroma, texture, and appearance) and improved storage stability of new products compared with the conventionally prepared chicken products. Sensory analysis allowed developing the suitable recipes for new products. Taste of "sous vide" processed sliced chicken with certain vegetable mix was excellent, texture and tenderness was milder, softer and more juicy therefore the ready-to-eat chicken was more acceptable than conventionally prepared product.

Conclusions

In future more extensive studies would be needed to evaluate safety issues related to use of "sous vide" technology for the preparation of specific ready-to-eat chicken and vegetable product as a function of added ingredients, storage time-temperature and heat processing conditions.

Acknowledgment

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8

THE INFLUENCE OF EMULSIFIER TYPE AND CHOSEN ANTIOXIDANTS ON STABILITY OF OIL-IN-WATER EMULSIONS

Introduction

Stability of emulsions depends on many factors, including lipid phase composition, composition and pH of water phase, emulsifier structure, presence of other molecules in the interphase, and the dispersion level of one liquid within the other [McClements and Decker 2000; Dickinson, 2001]. From a point of view of emulsion physical stability, smaller drops of dispersed phase are preferred. However, due to an increased surface area of both phases and facilitated possibility to penetrate by oxidation agents from water to lipid phase, a lower dispersion level seems to be more favorable for food emulsions to be more stable. The emulsifier type and its possible electric charge are also very important for the emulsion stability (namely at different pH values). It also refers to its oxidative stability due to a possibility of a partial penetration of pro-oxidation factors (oxygen, free radicals, metal ions) and antioxidants from water phase into the reacting substrates present in lipid phase. Furthermore, at large droplets of dispersed lipid phase, unsaturated fatty acids molecules can be easier placed within the droplet, which decreases their risk of contact with pro-oxidants present in emulsion water phase [McClements and Decker, 2000].

The aim of the study was the examination of the effects of different antioxidants addition to emulsions prepared using emulsifiers of different charge on physical and oxidative stability of 10% emulsions of sunflower oil (pH 4.0 and 5.5).

Materials and methods

Three emulsifiers were applied in the study: non-ionic Tween 40 (TW), negatively charged sodium stearyol lactate (SSL), and amphoteric soya lecithin (LEC), ethanol-soluble fraction prepared as follows: 300 g of industrial soya lecithin was extracted with 300 ml of acetone which was then evaporated with nitrogen and the preparation was extracted 3 times with ethanol (600, 400 and 200 ml). Obtained solution was vacuum-evaporated at 30°C. No. positively charged emulsifier was used in the study as none of such substances are allowed to be applied in food emulsions.

One hydrophobic antioxidant (α-tocopherol, Aldrich), one hydrophilic (L-ascorbic acid, Sigma) and one amphoteric (dephosphorylated casein, Sigma) were used to control the range of oxidation processes occurring in sunflower oil emulsions.

To determine needed emulsifier addition, low-fat oil-in-water emulsions were prepared using aforementioned emulsifiers at two pH values (4.0 and 5.5) and three different doses: 10, 12 and 15% (in relation to oil phase), in case of SSL at pH 4.0 the doses were also 17.5 and 20%. Samples were homogenized (Ultra Turrax T25) at 20500 min^{-1} during 60 s. Visual estimation of the emulsions' stability (1 and 3 days after production) was the base of the proper doses of emulsifiers choice: 12.5% and 20% in case of SSL at pH 4.0.

Final emulsions were prepared of sunflower oil containing 23.3 mg/100 g of natural tocopherols mixture (67% α-tocopherol, 33% sum of β- and γ-tocopherol) and acetate buffer (pH 4.0 or 5.5). Appropriate emulsifier and oil were weighted into beaker, then antioxidants were added (separately or in pairs) as well as 5 ml of 6.25 μM $FeCl_2$ and the total weight was made up to 100 g with appropriate acetate buffer. Samples were then stored at 37°C in the dark.

Physical stability of emulsions were determined using Turbiscan Lab Expert apparatus which also allowed to determine average particle size and through microscopic observations. Oxidative stability was measured by spectrophotometric peroxides content test (ferric thiocyanate method) and by determination of volatile secondary reaction products (HS-SPME-GC-MS).

Results and discussion

The decrease of dispersed phase particles size was observed in control emulsions made of neutral emulsifier (Tween 40) at pH 4.0 under microscope. A slight decrease of particle diameter was also apparent on a base of light back-diffusion (Table 1). The plot of back-diffusion of light illustrates the emulsion cream-formation (Figure 1). The cream layer can be observed as an increase of back-diffusion level at the upper part of the sample, which results from an increased concentration of lipid droplets. At the same time, the decrease of the back-diffusion can be seen in the middle parts of the curve, which was probably associated with a decreased concentration of dispersed phase particles. The cream-formation phenomenon is, in this case, most probably a result of flocculation that could be observed as particle aggregates under the microscope. The microscopic picture of the emulsion with Tween at pH 5.5 indicated a slight change of particle size. It was confirmed by a value of mean particle diameter. A strong particle aggregation occurred within emulsion thermostated for 14 days.

The microscopic picture of emulsion with SSL at pH 4.0 revealed that the concentration of dispersed phase particles was high directly after formation, while it decreased in the course of the experiment. The change of particle size was not apparent, although droplets of separated oil could be seen. Mean size of a particle estimated on a base of light back-diffusion increased along with the time (Table 1). Those changes allowed for supposing that coalescence occurred in that system after some time. Emulsion with SSL at pH 5.5, directly after formation, was characterized by high particle concentration and their small size. A storage had some effect on the slight particle concentration decrease, while it did not contribute to significant change in their size (Table 1).

Among emulsions produced using amphoteric emulsifier (lecithin), the most apparent changes in microscopic picture were observed for emulsion at pH 4.0. It was found that after 14 days of thermostating, larger particles appeared. They were the oil droplets separated due to a coalescence. However, mean size of particles that remained in the system in a form of dispersed phase, did not change (Table 1). The microscopic pictures of other emulsions with lecithin did not reveal apparent changes referring to both particle size, and their distribution, despite of the fact that mean particle diameter increased.

Table 1

Changes of average particle size (μm) determined on the basis of light back-diffusion

Day of experiment		1	7	14
TW 4,0	Control	4.6	4.3	4.3
	Sample with casein	5.6	5.2	5.2
TW 5,5	Control	4.1	3.9	4.1
	Sample with casein	3.8	3.7	3.7
SSL 4,0	Control	4.4	4.7	6.2
	Sample with casein	81.6	161.7	n.d.*
SSL 5,5	Control	2.3	2.3	2.3
	Sample with casein	3.1	3.1	3.1
LEC 4,0	Control	3.1	3.1	3.1
	Sample with casein	2.6	5.0	5.6
LEC 5,5	Control	6.2	10.3	10.3
	Sample with casein	9.6	16.8	16.8

* value not determined

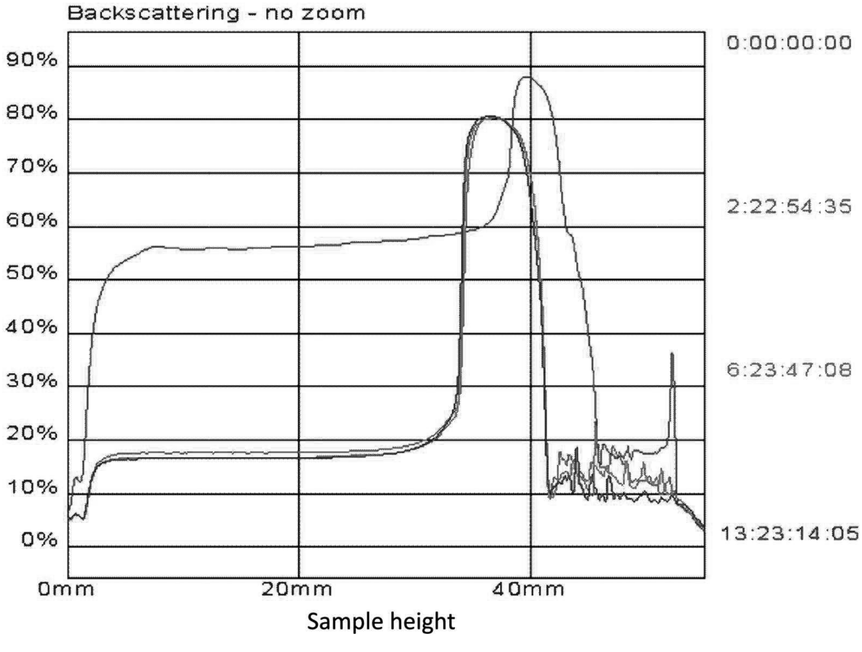

Fig. 2. Changes of light back-diffusion in relation to sample height during experiment (Tween 40 at pH 4.0, control emulsion)

As a summarizing, it can be supposed that neutral emulsifier provided with the highest stability of particle size. Amphoteric emulsifier caused the increase of particle size, while not affected the significant decrease of their concentration. Anionic emulsifier, in most cases, could not provide with emulsion physical stability, which could be seen as a coalescence (namely at the presence of casein at pH 4.0).

Subsequent stage of the experiment consisted in analyzing the oxidative stability of produced emulsions. Additions of examined antioxidants were as follows: 100 mg% casein, 50 mg% ascorbic acid, and 25 mg% α-tocopherol. Due to a fact that the sunflower oil used for studies contained a natural mixture of tocopherols, each emulsion contained additional amount of 2.3 mg% tocopherols. The autoxidation reaction was catalyzed by iron (II) ions and elevated temperature (37°C). The content of peroxides was determined by means of spectrophotometric method after 24 hrs of reaction as well as secondary oxidation products after 14 days.

Achieved results of antioxidant activity of examined antioxidants and their combinations were presented in Table 2. Casein showed the most uniform properties, and only once in a single case (lecithin as emulsifier at pH 4.0) it was a pro-oxidant. Separately applied ascorbic acid, in general, showed better activity than casein; however, in two cases in both emulsions produced using negatively-charged emulsifier, it apparently behaved as pro-oxidant. The fact in part resulted from the occurrence of the same-charged emulsifier and antioxidant, which made the latter impossible to access the lipid phase. Differences in the activities due to electrostatic effects might be even thousand-fold [Decker, 1998]. The tocopherol addition resulted in pro-oxidation effects in every system studied (the strongest in emulsions made of lecithin).

Casein showed quite good antioxidant activity (70–80%) in both systems containing Tween 40, which was only slightly worse than in the case of ascorbic acid (about 86%). The pro-oxidative effect was observed when tocopherols contents was elevated – stronger at higher pH value. Combination of tocopherol and casein apparently decreased its activity, although in the case of tocopherol plus ascorbic acid, better oxidative stability was recorded – activity of that combination reached up to 100%. Identical effect was achieved in combination of casein with ascorbic acid.

The antioxidant activity of casein is associated with the ability of free radicals quenching [Suetsuna et al., 2000]. It is a result of the presence of particular amiNo. acids (e.g. tyrosine, histidine, lysine, methionine) in particular sequence [Korhonen et al., 1998]. It was observed that in the system with neutral emulsifier at higher pH, casein showed better antioxidant capacity. It can be elucidated by the elevated antioxidant activity of some amiNo. acids, e.g. histidine and tryptophane, along with the pH increase [Chen et al., 2003]. Moreover, larger difference between system's pH from protein's pI affects the better protein solubility, hence its availability to the reaction [Szpendowski and Śmietana, 2002].

Applying the emulsifier with negative charge (SSL) considerably worsened the action of studied antioxidants. Tocopherol addition caused the strongest pro-oxidative effect (at pH 4.0) among all cases of that agent separately applied, while at 5.5 casein lost its activity and ascorbic acid showed pro-oxidative action at both pH values. Casein with tocopherol combination slightly intensified the pro-oxidative effect of the latter, whereas casein plus ascorbic acid made the moderate action of these agents when applied separately. Effects of combined tocopherol with ascorbic acid application was surprising – the activity reached up 100% at pH 4.0 and over 40% at pH 5.5, despite of the pro-oxidative action of both substances when applied separately. The antioxidant activity of the casein at pH 4.0 can be explained with the electrostatic interaction between negatively charged emulsifier and positively ionized casein [McClements and Decker 1997; Decker 1998]. A summarized positive casein charge results from the pH below its isoelectric point (pH 4.6).

Table 2

Activity of the antioxidants tested towards hydroperoxides in model systems prepared with different emulsifiers

EMULSIFIER, pH	**ANTIOXIDANTS**					
	casein (C)	α-tocopherol (T)	ascorbic acid (A)	C + T	C + A	T + A
TW, 4.0	70.1 ± 1.9^{a*}	-45.3 ± 4.6^{b}	85.9 ± 2.9^{c}	-27.5 ± 3.4^{d}	100.0 ± 0.4^{e}	100.0 ± 0.4^{e}
TW, 5.5	79.7 ± 3.1^{a}	-84.5 ± 2.7^{b}	87.0 ± 1.8^{c}	57.5 ± 2.5^{d}	95.0 ± 2.3^{e}	96.8 ± 2.4^{e}
SSL, 4.0	60.1 ± 0.7^{a}	-118.1 ± 2.8^{b}	-39.9 ± 0.9^{c}	-132.0 ± 3.6^{d}	12.0 ± 1.7^{e}	100.0 ± 1.9^{f}
SSL, 5.5	3.9 ± 1.6^{a}	-18.6 ± 3.6^{b}	-47.5 ± 3.5^{c}	-20.1 ± 3.1^{b}	-32.0 ± 2.7^{d}	41.9 ± 1.8^{e}
LEC, 4.0	-15.6 ± 3.8^{a}	-97.1 ± 1.5^{b}	30.6 ± 3.8^{c}	-104.4 ± 4.4^{d}	47.5 ± 3.6^{e}	37.1 ± 1.7^{f}
LEC, 5.5	34.5 ± 1.9^{a}	-74.2 ± 1.8^{b}	100.0 ± 1.6^{c}	-56.9 ± 2.4^{d}	99.6 ± 1.4^{c}	100.0 ± 3.0^{c}

* identical letters indicate lack of statistically significant difference in the range of a model system

Only in the case of amphoteric emulsifier (lecithin), casein showed the pro-oxidative effect at lower pH. Also ascorbic acid had three times better action at pH 5.5. With combined application of examined agents, only in the case of casein with elevated tocopherol amount, the pro-oxidative effects were observed. Addition of other antioxidants did not change the high activity of ascorbic acid at higher pH and it slightly increased it at pH 4.0. The literature data suggest that neutralizing the carboxylic groups within phospholipid molecules at lower pH and enhancing the accessibility of positively charged amine groups made impossible the antioxidative action of casein at pH 4.0, while at pH 5.5 (above pI) casein showed the antioxidative activity (35%) [Jacobsen et al., 1999].

Summing up, when measuring the emulsion oxidation level with the fatty acids peroxide contents, application of ascorbic acid at elevated (in relation to naturally occurring one in the oil) α-tocopherol content, seems to be the most favorable. Such mixture, although gave slightly worse effect than casein with ascorbic acid combination in a single case, acted very strongly in most cases and in No. combination it showed the pro-oxidative effects.

Presence of considerable amounts of hydrocarbons (with dominating 2,4-dimethylhexane), saturated aldehydes (prevailing hexanal), unsaturated aldehydes (2-heptanal, 2-octenal), as well as saturated and unsaturated alcohols (e.g. 1-octen-3-ol) was observed due to the measure of secondary products of oxidation reaction in emulsions prepared using the non-ionic emulsifier. Numerous aldehydes, hydrocarbons, alcohols, as well as saturated and unsaturated ketones along with cyclic compounds (e.g. furan derivatives) were found in emulsions containing SSL and lecithin. Hexanal, with the scent recalling grass, is the most characteristic secondary auto-oxidative product. It can be considered as an indicator of the fat oxidation [Martin et al., 2000; Stashenko et al., 2002; Sanches-Silva et al., 2004]. Also in present study, hexanal was the product formed at largest amounts. The hexanal peak area served for calculating the antioxidant activity of examined preparations at that stage of oxidation reaction. Achieved results were presented in Table 3.

Table 3

Activity of the antioxidants tested towards hexanal in model systems prepared with different emulsifiers

EMULSIFIER, pH	**ANTIOXIDANTS**					
	casein (C)	α-tocopherol (T)	ascorbic acid (A)	C + T	C + A	T + A
TW, 4.0	88.0 ± 3.4	49.3 ± 9.4	-114.4 ± 13.8	92.7 ± 0.0	-61.4 ± 4.8	75.9 ± 2.4
TW, 5.5	98.5 ± 0.4	49.3 ± 2.6	-174.8 ± 18.7	100.0 ± 1.7	-122.5 ± 1.8	55.2 ± 5.6
SSL, 4.0	20.9 ± 11.1	17.2 ± 8.2	-39.9 ± 46.5	34.1 ± 21.2	4.4 ± 4.1	-9.3 ± 17.8
SSL, 5.5	24.5 ± 6.8	7.0 ± 2.6	-9.6 ± 29.2	37.6 ± 6.3	-32.8 ± 1.8	-30.8 ± 4.6
LEC, 4.0	40.0 ± 46.7	35.9 ± 5.2	-251.2 ± 5.2	24.9 ± 5.4	-253.5 ± 15.3	-169.5 ± 10.6

Similarly as in the case of peroxides, in both model systems with Tween presence, casein showed very good antioxidant properties that got better along with the pH increase. However, action of two other substances was adverse: ascorbic acid had strong pro-oxidative features, while tocopherol addition inhibited the hexanal formation in 50%. Combination of effectively acting casein with ascorbic acid slightly decreased the pro-oxidative effect of the latter, whereas the α-tocopherol action slightly increased or did not change the high activity of the casein. Combination of tocopherol plus ascorbic acid, namely at lower pH, had quite good effect, as similar as against peroxides.

The most insignificant effects were achieved after introducing the examined antioxidants into the system emulsified using negatively ionized SSL. At both pH values of water phase, achieved values of antioxidant activity oscillated between -40 and 40%. The best effects, unlike when activity calculations were based on the peroxide contents, were achieved at combined application of casein showing the surface activity with tocopherol localized within the dispersed phase.

A moderately good effect of tocopherol effect (36% activity) and similar average casein activity was recorded in the model system emulsified with lecithin at pH 4.0 and at large result scatter, which was confirmed by calculated standard deviation value. Ascorbic acid strongly promoted the hexanal formation, both when added separately and in combination with two other agents. The system at pH 5.5 differed apparently from other ones. The control sample contained less hexanal after 14 days of thermostating than directly after formation, which may prove the antioxidant activity of lecithin fraction that was applied in that system as an emulsifier [Lee, 1997]. The fact made impossible to estimate the antioxidant activity of examined antioxidants according the formula applied for other systems. In emulsion with casein addition, No. hexanal presence was recorded, while in emulsion with casein and tocopherol combination, its level was slightly higher than in control sample. Its much higher amounts were determined in systems with ascorbic acid. At the same time, in emulsions with decreased hexanal amounts, higher 1-hexanol level was recorded. It can be supposed that hexanal present in examined emulsions was formed due to the 1-hexanol oxidation. Basing on that assumption, it could be suggested that the system with amfoteric emulsifier at pH 5.5 with casein addition was oxidized at the slowest rate. Ascorbic acid present in emulsions, both separately and in combinations, contributed to formation of many oxidation products, e.g. penthanal, 2-heptenal, 1-octen-3-one, 1-octen-3-ol, 2-penthylofuran, octanal, 3-ethyl-2-methyl-1,3-hexadiene, 2-octenal, and nonanal. Moreover, the presence of oxidized hydrocarbons and ketones, e.g. 1,1-diethoxypenthane, was characteristic for emulsions with amfoteric emulsifier.

Summarizing, for most of studied systems, the casein as well as casein with tocopherol presence the most effectively decreased the amount of hexanal produced in the medium. It was probably associated with the fact that α,β-monounsaturated aldehydes react with amine groups of proteins, e.g. ε-amine group of lysine. 2-Alkenals may react with protein's histydyl moieties. Presence of proteins containing histidine reduces the hexanal and 2-t-hexenal amounts in gaseous phase [Meynier et al., 2004], while tocopherol presence also limited the hexanal concentration in not more than 50%. The ascorbic acid addition affected the increase of the amount of formed compounds.

Conclusions

1. The neutral emulsifier (Tween 40) provided with the highest stability of the particle size at their apparent flocculation as well as the lowest amount of secondary oxidation products, while applying the anionic emulsifier (SSL) resulted in the highest worsening of the stability through a partial coalescence with the dispersed phase separation (namely at the presence of casein) and producing the largest quantities of secondary oxidation products.
2. All studied emulsions showed quick cream-formation effect due to a low viscosity of water phase.
3. Presence of ascorbic acid, separately and in combinations, efficiently inhibited the peroxide production in emulsions with Tween 40 as well as ethanol-soluble lecithin fraction, whereas it affected the increase of secondary oxidation products amounts in majority of examined systems.
4. The antioxidant activity of casein against peroxides depended on pH value and electrostatic interactions occurring in a system (in emulsion with SSL it showed antioxidant activity at pH 4.0, i.e. below its pI; in emulsion with ethanol-soluble lecithin fraction, it showed antioxidant activity at pH 5.5, i.e. above its pI).
5. The lowest number of secondary oxidation products were found in samples with casein addition.
6. Tocopherol addition caused the pro-oxidative action at the initial oxidation stage (against peroxides), whereas it considerably reduced the formation of secondary oxidation products.

7. In emulsions with Tween and SSL, at both studied pH values, casein with tocopherol combination, then separate casein, reduced the most efficiently the formation of main secondary product of oxidation reaction – hexanal, while in emulsions with ethanol-soluble lecithin fraction, casein was the most efficient. In the case of peroxides, tocopherol plus ascorbic acid mixture appeared to be the most effective and stable in action.

8. Ethanol-soluble lecithin fractions probably caused the decrease of initial amounts and inhibited further production of hexanal during autoxidation reaction (control emulsion at pH 5.5).

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9

EVALUATION OF THE QUALITY OF THE COLD STORED UHT MILK IN THE PERIOD OF CONSUMPTION

Introduction

The milk is consumed in 90.3% of Polish households, of which 62.5% drinks about 1 to 3 liters a week [Olejniczak, 2004]. Currently, most interest has a type of UHT milk, the proportion of total milk sales in Poland is 48% and growing, as mainly due to the style and pace of our lives, there is a growing interest in food for a prolonged period of suitability for consumption. UHT sterilization involves heating the fluid flow (of milk) at a temperature of 130–150° C for several seconds [Świtka et al., 1997]. UHT milk is sterilized released 99.99% of micro-organisms, including their sporulation forms [Jurczak, 1996]. The process will consist of course, on the aseptic packaging of product. On the survival of micro-organisms is also influenced by the active acidity. The greatest resistance was found when the pH between 6.0 and 7.0. At lower and higher values are dying faster [Michalski, 1992].

The durability of the product

According to the recommendations of the International Dairy Federation (FIL - IDF) established using UHT milk should be stable at room temperature for 4–8 weeks [Kruk and Czerniewicz, 1996]. In practice, sustainability is much longer - up to 6 months. In the framework of-term consumption of milk referred to as the "use by date", is listed on the packaging. This term and defines the conditions responsible for the product manufacturer. There is No. law to regulate the requirements for microbiological milk for the last day of suitability for consumption. Milk for human consumption should not contain pathogenic micro-organisms, and microbiological requirements, it is the number of micro-organisms to the product leaving the processing plant [Śmietana et al., 2004].

The sustainability of dairy products shall be determined on the basis of the organoleptic and microbiological. Evaluation of organoleptic made on the basis of taste and smell and is defined as a period shorter by one day from the time that elapses from the date of manufacture to the date on which the milk is tested disqualification based on sensory characteristics. Microbiological criterion can be defined as the time required for the development of bacteria in milk than 105–107 cfu in 1 cm^3. The content of bacteria is usually associated with the emergence of defects taste-aromatic [Pluta, 1996]. Defects of taste - odor may also occur in the case of milk with relatively high microbiological quality, but stored for a long time (2–3 days) in refrigerated conditions (at temperatures below 10 ^{o}C) as a result of the development of the psychrotrofic microflora [Kruk, 1996]. Particularly well developed in the cooled milk, bacteria of the genus *Micrococcus*, *Pseudomonas* and other Gram negative (with their characteristic lipolytic-putrid changes in the milk in the absence of an increase acidity) [Ziajka et al., 1997]. For the quality of UHT milk, it is important that there, in general, not capable of heating, psychrotrofic bacteria, there are also resistant to heat, sporulation bacteria such as *Bacillus stearothermophilus*, even surviving UHT sterilization process. In addition, a number of species of bacteria (especially of the genus *Pseudomonas*) produces significant quantities of active lipolytic and proteolytic enzymes resistant to heat, much of which can keep its activity even after heating method UHT milk. Some of them even are holding the heating at a temperature of 150°C for 27 minutes [Kruk, 1996]. These enzymes, both in raw milk and UHT milk in the later, cause the breakdown of fat, protein and milk sugar, which in turn could lead to the creation of defects and organoleptic taste and smell of an unclean, putrid, bitter, soapy, rancid or sour. Shown a negative correlation between the number of somatic cells (CC) in milk prior to sterilization and the content of casein in 120 day storage of UHT milk. CC speeds increase thus proteolysis UHT milk by the end of its validity [Fernandes et al., 2008]. Among the most active microbial species of the genus include: *Pseudomaonas*, *Micrococcus*, and aerobic and anaerobic *Clostridium* [Hattowska, 1964]. The number of heat-resistant and sporulation bacteria present in the raw material is also an important parameter in the production of UHT milk, because the effectiveness of sterilization depends inter alia on the quantities.

Effect of UHT process for milk components

Under the influence of heating milk changes in the molecular structure of its components, which lead to mutual interaction between them and the creation of atypical compounds for milk. The presence of these compounds in milk is usually reveals in his store. The main changes are of a chemical transformation of lactose: isomerisation reactions and Maillard-type reactions. The first of these transformations is the conversion of lactose (disaccharide consisting of glucose and galactose) in lactulose (another disaccharide composed of fructose and galactose), which is not absorbed or hydrolyzed in the upper gastrointestinal tract conduits [Kozikowski, 1997; Andrews, 2007]. Maillard-type reactions involve the interaction between the carbonyl groups of lactose and ε-amiNo. groups of lysine. One of the products of these developments is 5-hydroxymethylfurfural (HMF). This compound is at an early stage of transition, and its presence is easily detectable even at relatively low concentrations [Panfil and-Kuncewicz Kuncewicz, 1997, Grabowska, 2009]. The effect of Maillard-type reaction is to reduce the availability of lysine for the human body, so that nutritional value of milk proteins is reduced. Type of Maillard reaction products may also contribute to increase the acidity of milk and also change the colour, for which are responsible melanoidins compounds [Panfil-Kuncewicz, 1997; Singh, 2009]. Another indicator of Maillard reaction progress is the level of furosine in UHT milk. The content of this amiNo. acid varies with the intensity of heat treatment of milk [Świtka et al., 1997]. UHT Sterilization affects the denaturation of proteins, causing them, and thus reduces their allergic properties. Whey proteins are more sensitive to heat than casein. The whey protein is quickly denatured β-lactaglobulin, which tends to connect to the κ-casein and the result is not visible coagulation. [Kozikowski, 1997]. Heating may be expected to lower the nutritional value of milk, by the destruction of vitamins. Losses are subject to the type of applied heat treatment, the UHT milk can only be a source of thermostable vitamins [Świtka et al., 1997]. Resistant to high temperatures are vitamins A, D, E, and B2, nicotinic acid, pantothenic acid, biotin and β-carotene. Their contents during pasteurization and UHT sterilization does not change at all, or be reduced by about 10% [Kozikowski, 1997]. Losses of vitamins sensitive to light, especially such as vitamin

A and riboflavin, may occur during storage [Heeschen, 1996]. The temperature-sensitive vitamins are B1 and B6, and in the presence of oxygen in particular vitamin C, B12 and folic acid [Molska, 1994]. In anaerobic conditions, ascorbic acid, is resistant to high temperatures, is sensitive and a product of oxidation – dehydroascorbic acid. At the time of sterilization in the presence of oxygen, loss of dehydroascorbic acid may claim up to 100%. Ascorbic acid is also the largest change UHT milk during storage. During the UHT sterilization of vitamin B12 can be destroyed in 20–30%. Destruction of vitamin B12 is as a side reaction of oxidation of ascorbic acid. Overall, the UHT milk, despite some changes to the content and bioavailability of nutrients has a high nutritional value.

The objective of the study was to determine the rate of change of acidity and microbial quality of UHT milk in the period after its opening.

Material and methods

The study used 60 of packaging UHT milk from different manufacturers. The milk had the same fat content – 3.2%, and characterized by a similar date. The research was conducted in three series. Cartons of milk marked with the same letters came from the same manufacturers. Milk stored in a refrigerator at a temperature of 4°C for the entire period of testing and removing them only to the time of analysis. In each series of microbiological tests performed and measured the active acidity (pH). In a series of second and third were made in addition to the organoleptic evaluation of the practical control of microbiological changes in milk. In the first two series in the milk refrigerator closed. In the third series of the milk cartons labeled as A, B, C, D, E were kept in the refrigerator open while the F, G, H, and J closed. Research carried out after opening the package – "time 0" – in order to check the initial quality of milk, at 48 hours after opening, or after the stated period of suitability for consumption, after 96 hours (in 1 and 2 series) and after 7, 14 and 28 (in 3 series) days from the opening in order to check whether the milk is still safe.

The microbiological test performed using Petrifilm test – aerobic bacteria. He is ready to test, allowing for accurate determination of aerobic bacteria in the product. Ready nutrient gel contains soluble in cold water and tetrazole dye to colourimetric distinguish bacterial colony [Ginn et al., 1986, McAllister et al., 1987].

Results were developed using a statistical program Statistica 8.0. The analysis of variance was carried out according to the following model:

$$Y_{ij} = \mu + m_i + e_{ij}$$

where: Yij – the value of the parameter, μ – the average sample; m – fixed-effect i-th method of storing milk; eij – random error.

Results and discussion

UHT milk acidity is a measure of the freshness of the product, and to some extent a measure of the raw material used for processing and the amount of heat treatment used in the processes [Panfil-Kuncewicz and Kuncewicz, 1997]. The active acidity (pH) after opening the container of milk, was consistent with the standards and fall within the limits of 6.60–

6.80. In the first and second series of experiments the acidity immediately after opening the packaging was similar and averaged 6.61 and 6.62, respectively. In the third series (Table 1) achieved higher initial pH values. For the milk carton open 6.70, closing at 6.71. In subsequent tests of the gradual increase in acidity was observed. Changes in the acidity of milk, which occurred in the subsequent days of testing, were mainly the result of an enzymatic reaction, especially lipolysis, which is a consequence of the formation of fatty acids [Cais-Sokolowska and Pikul, 1998]. On the last day received the following pH values: the first series – 5.52, the second series – 6.50, the third series of open milk – 6.37, the third series of closed milk – 6.38. The highest acidity was obtained in a series of the third after 28 days since the opening of the milk C (open milk), up 6.24. In considering a change in pH in the study included consecutive days or a carton of milk remained open or closed in the refrigerator. Showed No. significant effect of the milk in a refrigerator at the rate of change of its acidity. However, a significant increase in the acidity of a series of third order for milk and open the closed between "0" and 48 hours of testing, respectively 0.1 and 0.12 units for milk, and open between 7 and 14 days of 0.12 units. Microbiological testing done in time "0" did not show the development of microflora in all three series. In Waliszewski et al. [1996] performed during the years 1993–1995 UHT milk with 6.5% of packages contain bacteria. Rygałło [1997] showed an increase in the total number of bacteria above 10^4/ml of milk collected from 30% of packaging UHT milk. After 48 hours from opening the package, it is stated by the manufacturers within the shelf was not observed in any sample of the growth of bacteria, indicating a well-conducted process of sterilization. In the first and second series of tests, when the milk remained closed throughout the refrigerator has not been reported in the development of bacteria in the course of the experiment. By contrast, in the third series (Table 2) the development of microflora was observed from day 7 milk in open (2 colonies in milk of C and 259 colonies in milk of E). From 14 days increased the number of samples (both in the milk as an open and closed), with a high number of micro-organisms identified as TNTC (too numerous to count), that is, as recommended by the Polish standards above 300 x 103 per ml of milk. Taking into account that in the first two series, when the milk was still closed in the refrigerator has not been to the development of micro, it can be assumed that the occurrence of bacteria in the third series was the result of secondary contamination after opening. This also applies to milk F, G, H, I, J, in the third series which was closed, although a small access of oxygen delayed development of the bacteria. You can not say with certainty whether the observed changes in microbial milk, which occurred in the third series of tests were the result of an inferior quality of raw material, whether the impact of external environment (sampling, microclimate in the refrigerator). More likely seems the second factor, due to the fact that these changes occurred only in the third series of experience and in all the examined packaging UHT milk. About qualities of a creamery products and their sustainability decide mostly microorganisms, which fall to milks after pasteurization [ZiarNo. and Czapska, 2008]. Hygienic quality of raw milk to a large extent affects the result of the pasteurization [Ravanis and Lewis 1995]. Testify to the high values of correlation coefficients between the total number of bacteria in raw and pasteurization milk shown in studies Zapletala et al. [1997]. A significant increase in the active acidity in milk is evidence of the occurrence of lactic acid bacteria (*Streptococcus*, *Lactobacilus*, *Lactococcus* and *Leuconostoc spp.*), which lost in the process of pasteurization. Together from appearing of micro-organisms in third series of investigations stepped out defects of taste of milk. These defects have also been found in other series, when there were No. development of bacteria. Flavor changes in milk occurred only after

7 days of storage in a refrigerator (Tab. 3). The taste of normal after 7 days showed 54% of milk samples from the packaging closed, and only 20% of the open. At the same time "with the bad aftertaste" were 40% of samples from the sealed packaging and 60% open. It is difficult to define the course of chemical reactions that lead to the formation bad aftertaste of the UHT milk. It is understood that the flavor of milk form a Maillard reaction product and any of the components as a result of lipid oxidation [Żbikowska and Żbikowski, 1998]. After 14 days in milk collected from the open boxes appeared the bitter taste (20% of the samples) and sour (20% of the samples), while in the milk collected from closed cartons flavors that occurred after 21 days. On the basis of existing flavors specified % of milk capable of consumption in the test days. Suitable for drinking flavored milk was considered normal and, possibly, with bad aftertaste (Fig. 1). In milk originating from open cartoons, after 7 days one did not make a note presence of bacterium, in this time milk from 80% boxes was suitable to consumptions in respect of sensory. After 14 days the number of samples too numerous to count of bacteria increased to 40%, and samples of good taste have been reduced by half compared to the state after 7 days. After 21 days of good taste was the 20% of the samples and the amount of milk samples with too numerous to count of bacteria increased to 60%. After 28 days, the milk with open cartons was not suitable for drinking in terms of sensory and 80% had too numerous to count of bacteria. The development of bacteria in milk from closed cartons walked slowly to 21 days. Better results were obtained in their research Śmietana et al. [2007]. The total number of bacteria in UHT milk after 23 days of storage in a refrigerator at home was 1.1 x10^{1} cfu / ml and it was fit for consumption.

Table 1

The changes of active acidity (pH) in the coming days of test – the third series

Milk	Hour		Day			
	0	48	7	14	21	28
A	6.77	6.63	6.61	6.53	6.47	6.46
B	6.68	6.63	6.62	6.51	6.49	6.47
C	6.68	6.58	6.58	6.44	6.26	6.24
D	6.68	6.62	6.59	6.54	6.40	6.34
E	6.68	6.57	6.58	6.35	6.34	6.32
$\overline{x}$	***6.70***	***6.61***	***6.60***	***6.47***	***6.39***	***6.37***
SD	0.04	0.03	0.02	0.08	0.09	0.10
F	6.66	6.60	6.58	6.45	6.45	6.46
G	6.73	6.59	6.55	6.50	6.37	6.29
H	6.72	6.61	6.55	6.48	6.46	6.45
I	6.73	6.58	6.55	6.42	6.36	6.30
J	6.70	6.56	6.53	6.48	6.39	6.39
$\overline{x}$	***6.71***	***6.59***	***6.55***	***6.47***	***6.41***	***6.38***
SD	0.03	0.02	0.02	0.03	0.05	0.08

Table 2

Number of colonies of aerobic bacteria in the milk – Petrifilm Test – the third series

Milk	Day			
	7	14	21	28
A	x	x	99	TNTC
B	x	x	x	32
C	2*	TNTC**	TNTC	TNTC
D	x	x	TNTC	TNTC
E	259	TNTC	TNTC	TNTC
F	x	TNTC	TNTC	TNTC
G	x	TNTC	TNTC	TNTC
H	x	x	5	TNTC
I	x	x	18	TNTC
J	x	5	x	TNTC

* – x 10^4 per ml of milk

** – too numerous to count - *> 300 x 10^3 per ml of milk*

x – lack of development of micro-organism

Table 3

The share of individual tastes in the coming days of test

Day	The percentage contribution of individual taste									
	Bitter		Acid		Slightly acidic		Bad taste		Normal	
	Z*	O**	Z	O	Z	O	Z	O	Z	O
7	0	0	0	0	7	20	40	60	53	20
14	0	20	0	20	27	20	60	40	13	0
21	6	20	7	40	53	20	27	20	7	0
28	20	20	20	60	60	20	0	0	0	0

Z – closed cartons; O – open cartons

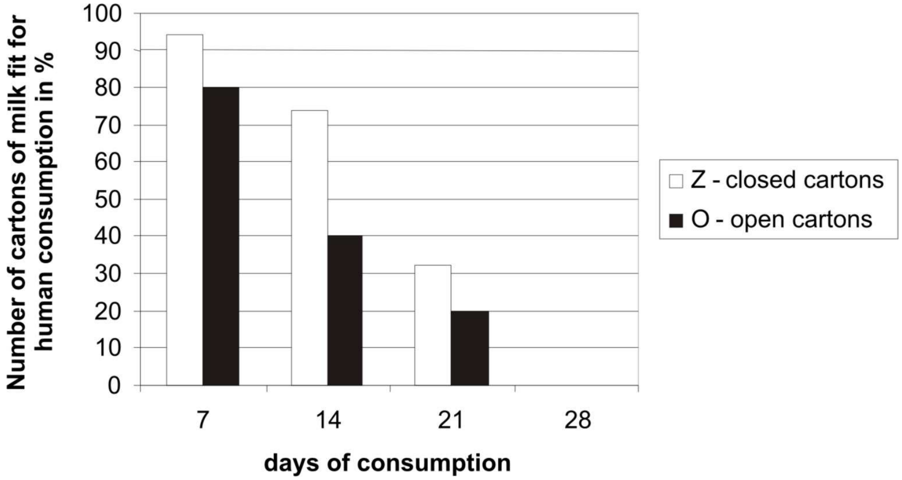

Fig. 1. Percentage of milk suitable for consumption in the coming days of test

Micro-organisms identified as *Pseudomonas spp.* is the most important group of psychrotrophic bacteria linked to the spoilage of milk and its products. It is not known exactly how the number of bacteria is responsible for the appearance of organoleptic defects. Many authors indicate that the content of 10^7/ml of psychrotrophic bacteria is most often associated with a poor rating the product [Śmietana et al., 2004]. Bacteria are unable to survive the process of pasteurization, but after processing contamination by *Pseudomonas spp.* can lead to spoilage of products such as milk, curd and butter cream. They suit enzymes (protease and lipase), many of which are characterized by a large thermal resistance, which after heat treatment may also cause taste described as bitter, rancid, unclean, etc. Most spp *Pseudomonas* does not cause fermentation of lactose and, therefore, these bacteria probably do not result in acid milk. The main types of micro-organisms causing spoilage of products are those that produce temperature-resistant spores, ie, *Bacillus* and *Clostridium spp*. *Bacillus cereus* is of utmost importance, as has the ability to very rapid growth and may have an impact even on UHT products. Other groups of organisms *Bacillus spp.* (eg *B. subtilis*, *B. megaterium*, *B. coagulans* and *B. sterothermophilis*) generally have No. impact on the products stored at low temperatures, such as UHT and canned products. Spoilage processes include production of gas, a mixture of sweet and acid coagulation, rarefaction, bitter, strange smells and sweet tastes [Jurczak, 1996; Molska, 1988]. *Clostridium spp* are bacteria forming spores that do not normally grow in the presence of air. The pace of change fat and vitamins in milk is dependent on the availability of oxygen and light. The fraction of milk fat is regarded as a source of significant changes in the organoleptic characteristics [Panfil and-Kuncewicz Kuncewicz, 1997]. As a result of oxidation and hydrolysis of fat is produced a number of substances, both the desirable and the undesirable properties. Lipolysis and the resulting overall content of fatty acids (WKT) may also affect the characteristics of taste-odor of milk.

Conclusions

Studies have shown that the development of micro-organisms in UHT milk carton closing each of which has been taken only after 14 days. Adverse changes in the milk with open cartons occurred only under the influence of bacteria from the outside. The development of micro-organisms in milk with open cartons limited its suitability for consumption of up to 7 days. A large number of bacteria in milk have contributed to the emergence of defects in the taste. When you open the packaging UHT milk should be closed each time and stored in a refrigerator.

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10

THE QUALITY OF CLARIFIED APPLE JUICE DEPENDING ON THE TYPE OF PACKAGE AND BRAND OF THE PRODUCT

Introduction

Fruit juices are products the high consumption of which is recommended on a dietary basis. They are not only a good source of vitamins and mineral elements but also many biologically active elements [Baryłko-Pikielna et al., 2002; Płocharski, 2005]. Systematic increase of their consumption in Poland results from changing habits of consumers related to the evolution of their lifestyle. Nowadays, the market offers a wide range of fruit juices – supermarket shelves offer a great variety of tastes. There is strong competition between a few main production companies, but also there is best brand visibility among consumers [Pawlak, 2004]. It has been observed that there is relatively high acceptance of juice commercials by consumers because they stress the nutritive and health values of these products. Juice consumers believe that commercials of these products communicate reliable market and dietary information [Borowska et al., 2004]. Sensory quality of juices is one of the basic factors governing the choice of a specific assortment or brand. Apart from sensory quality, other important factors are price, type of packaging and consumers' habits. Customers view the quality of juices mostly in terms of their general excellence and superiority of the brand of a purchased product. People with higher income approve of established brands; they pay attention to the image of the brand and the guaranteed high quality of the product. The price has less importance than the attachment to a certain brand [Tarant and Całujek, 2005]. Research carried on 130 respondents in 2005 showed that the choice of juices, drinks and fruit nectars mostly depends on the taste, the brand and the price of products. A factor affecting the purchase of juice proved to be the taste, which received 4.5 points on a 5-point scale, whereas the brand received 3.82 points. Over 1/3 of respondents indicated the brand to be the most important factor when purchasing juice. Among fruit juice consumers, the 1 l package proved to be the most preferred. 55% of respondents indicated this type of packaging, whereas only 20% indicated 2 l packaging. Only 10% of the respondents preferred 0.5 l packaging [Górska-Warsewicz, 2004].

The purpose of this study was to make a comparative assessment of the quality of clarified apple juice depending on the type of packaging and brand of the product available in retail sale.

Materials and methods

The research material included juice of four national producers made from apple concentrate and offered in cardboard boxes and glass bottles. In order to determine which brand of apple juice offers the highest quality to consumers as well as whether the type of packaging may influence the quality of material under examination the following analyses were carried out: 1. appraisal of packaging and labelling of tested juice by determining the tightness of hermetically closed single packages, comparing the actual capacity with that declared by the producer, and checking the labelling of unit packages. Next the sensory analysis was carried out, based on the assessment of juice by the method of sensory profiling, which took into consideration the following features: appearance (colour and clarity), fragrance (of fresh apples, acidic, sweet, bitter, sour, tart and strange), taste (of fresh apples, acidic, sweet, bitter, sour, tart and strange), and general assessment. Each feature was assessed on a 10 cm graphic scale by measuring the intensity of particular features. Ends of the scale, used for descriptive analysis, were defined in the following way: the taste and the smell from 0 – "impalpable" to 10 – "very intense", colour from 0 – "bright-straw" to 10 – "honey-yellow", clarity from 0 – "not fully transparent, opalescent" to 10 – "clear, transparent" and general grade from 0 – "bad quality" to 10 – "very good quality". The assessment of sensory features with the method of sensory moulding was carried out in a specialist sensory laboratory complying with the PN-ISO 8589:1998 normative requirements. Tests were carried out by a team of experts who deal with sensory analyses of food products on a daily basis. Eight types of apple juice, which were appropriately encrypted before the examination, were analysed. Another stage of examination consisted in the analysis of chosen physical and chemical parameters of examined juice, which included determination of the following contents: vitamin C with Tillmans' method (PN-A-04019:1998), directly reducing monosaccharides and general saccharides with the Lane-Eynon method (PN-90/A-75101/07), general essence with the refractometric method (PN-90/A-75101/02+Az1:2002) and general acidity with the measuring method (PN-90/A-75101/04 + Az1:2002), as well as calculation of the level of sucrose and sugar-free extract in the apple juice under examination. Statistical analysis of results of sensory assessment and the level of physical and chemical indicators was based on a double factor variation analysis. Conclusions were drawn on the basis of Snedecor's F-test at the level of the gravity $\alpha = 0.05$ [Greń, 1984].

Results and discussion

Table 1 presents medium assessment values of particular features of sensory value of apple juice under examination depending on the type of packaging and product brand. In the tests carried out by Baryłko-Pikielna [Baryłko-Pikielna et al., 2002] it was found that sensory quality of apple juice depends on the intensity of sweet and sour taste and their mutual relationship. The grades given to the juice of the brands under examination for particular sensory characteristics varied. Brand A juice was the best as far as the clarity and colour were concerned and had the sweetest taste. Brand B juice was characterized by the most intense smell of fresh apples, the taste of fresh apples and the least perceptible sweetness. The most intense sour smell but the least intense sour taste were found for brand C juice. Moreover, the juice of this brand also had the least acceptable colour. Brand D juice obtained the lowest result for the intensity of the smell of fresh apples and sour smell and the intensity of the taste of fresh apples. On the basis of obtained results for sharp, bitter, tart and strange taste it can be stated that the juice of brands under examination were characterised by comparable palpability of these undesirable smells. Apple juice of the brands under examination received comparable points for the taste described as sharp, somewhat bitter, tart and strange. The general result obtained for the juice under examination demonstrated that juice of brand B has the most favourable quality, whereas the least favourable were judged to be apple juices of brand C. Apple juice in glass bottles obtained higher points for clarity than that in cardboard boxes. Apple juices in glass bottles were characterized by a lighter colour in comparison to juices in cardboard boxes. Results obtained for sour and sweet smell were not much higher for juice in cardboard boxes compared to the results obtained for juice in glass bottles. The judges recognised the smell of juice described as sharp, somewhat bitter, tart and strange at a comparable level in both kinds of packaging. The judges decided that examined juices in cardboard boxes were characterized by a more intense taste of fresh apples as well as a sour and sweet taste in comparison to juices in glass bottles. The evaluation of examined juices for sharp, somewhat bitter, tart and strange taste was only slightly varied for both kinds of packaging. Based on the results of general evaluation, one can state that cardboard boxes are a slightly better type of packaging for the sensory quality of examined juices. Statistical analysis of the results of sensory evaluation demonstrated that the kind of packaging had No. statistically significant influence on examined sensory characteristics, whereas the brand of the product had a statistically significant influence on the smell of fresh apples and a strange taste of examined apple juices (Table 3). Table 2 presents the average contents of physical and chemical indicators for examined apple juices depending on the brand of product and type of packaging together with the requirements of the Polish Norm and the AIJN Code of Practice. The content of vitamin C in apple juices ranged from 2.2 to 3.1 mg/100 g. The highest content of vitamin C was found in juice of brand C, and the lowest in juice of brand D. The requirements of the AIJN Code of Practice and the Polish Norm do not define the content of vitamin C in fruit juice. The requirements concerning the content of vitamin C relate only to vitaminised juices in which the content of this ingredient should not be lower than 30 mg/100g. Juices of brands C and D had larger content of the general essence than juices of brands A and B. The requirements of the Polish Norm stipulate that the general essence should not be less than 10%, whereas the AIJN Code of Practice determines that the level of general essence should not be less than 11.2%. Each type of juice under examination fulfilled both the requirements of the PN and the AIJN Code of Practice as far as the content of general essence is concerned. Baryłko-Pikielna [Baryłko-Pikielna et al., 2002] proved in her examinations that chemical indicators of quality (general essence content, general acidity) did not demonstrate an explicit relationship with general sensory quality of juices essential for the consumer; only the level of general acidity influenced the sensory acidity of juice. General acidity was between 5.3 and 6.1 g/l in recalculation into the apple acid and between 5.1 and 5.9 g/l in recalculation into the citric acid. Brand A juices had the highest level of general acidity and brand D juices the lowest. In Table 2 general acidity is presented in recalculation into apple acid and citric acid. This is because the Polish Norm presents requirements concerning general acidity in recalculation into apple acid whereas the AIJN Code of Practice those recalculated into citric acid. According to the Polish Norm general acidity should not be less than 4.5 g/l in recalculation into the apple acid. On the other hand, the AIJN Code of Practice stipulates that apple juices the general acidity of which is between 2.2 and 7.8 g/l in recalculation into citric acid may be regarded as good quality ones. Apple juices of the brands under examination fulfil the requirements of the Polish Norm and the AIJN Code of Practice with regard to general acidity. Other tested physical and chemical indicators in apple juice included the content of directly reducing monosaccharides and total saccharides, which is not defined in the Polish Norm and the AIJN Code of Practice. The content of directly reducing monosaccharides equalled from 6.1 to 7.6 g/100 ml. Juices of brand D contained the highest amount of these ingredients, whereas the juice of brand A contained the lowest. The total content of saccharides ranged from 9.7 to 11.0 g/100 ml. The total highest level of saccharides was determined for juices of brand D, whereas the lowest one was found for brand C juice. The calculated level of sugar-free extract for juices of the brands under examination varied between 17.0 and 29.9 g/l. The lowest content of sugar-free extract was determined in brand D juices, the highest one in brand C juices. Sugar-free extract should be lower than 14.0 g/l according to the Polish Norm requirements, whereas the requirements of the AIJN Code of Practice demand that the sugar-free extract should fall within the range of 18–29 g/l. All kinds of juice of individual brands under examination meet the requirements of the Polish Norm concerning the content of sugar-free extract, but only brand A and B juice can meet the requirements of the AIJN Code of Practice regarding this parameter. The content of vitamin C in cardboard boxes oscillated around the level of 2.3 mg/100 g, and in glass bottles 3.1 mg/100 g. Juices in glass bottles contained more vitamin C than those in cardboard boxes. General acidity of apple juice in cardboard boxes was 6.4 g/l in recalculation into apple acid (6.1 g/l in recalculation into citric acid) and in the glass bottle it was 5.1 g/l in recalculation into apple acid (4.9 in recalculation into citric acid). General acidity was greater in cardboard boxes than in glass bottles. The content of directly reducing monosaccharides in cardboard boxes (7.5 g/100 ml) was similar to that of the glass bottles (7.6 g/100 ml). Determined level of total saccharides in cardboard boxes was 10.1 g/100 ml, and in glass bottles it was 10.2 g/100 ml. The calculated content of sucrose in apple juices in cardboard boxes amounted to 25 g/l, and it was minimally lower than the amount of sucrose in juices packed in glass bottles, 25.2 g/l. Sugar-free extract in examined juices oscillated around 21.9 g/l in the case of glass bottles and up to 22.9 g/l in cardboard boxes. Statistical analysis of obtained physical and chemical test results demonstrated the influence of packaging and product brand on some physical and chemical characteristics. Type of packaging had a statistically significant influence on the content of vitamin C, general essence, and general acidity. The product brand had a significant influence on the content of vitamin C, general essence, reducing monosaccharides, total saccharides, and the level of sucrose, and sugar-free extract in examined juices (Table 3).

Table1

Characteristic of sensory values of clarified apple juice depending on the type of brand of the product and packaging

Features	Brand A	Brand B	Brand C	Brand D	Card-board boxes	Glass bottles
Clarity	9,5	7,6	4,8	9,3	7,5	8,1
Colour	2,4	5,5	5,6	3,5	4,8	3,7
Fragrance of fresh apples	6,3	6,8	6,0	4,0	5,6	5,9
Fragrance acidity	3,5	3,7	4,3	3,0	3,9	3,4
Fragrance sweet	4,6	4,3	3,9	5,0	4,6	4,3
Fragrance bitter, sour and tart	0,2	0,1	0,2	0,2	0,2	0,1
Fragrance strange	0,2	0,0	0,2	0,2	0,2	0,3
Taste of fresh apples	6,2	6,3	5,0	4,9	6,1	5,1
Taste acidity	4,6	5,1	4,3	4,6	5,1	4,2
Taste sweet	5,8	4,6	4,7	5,5	6,0	4,3
Taste bitter, sour and tart	0,4	0,4	0,2	0,3	0,4	0,3
Taste strange	0,0	0,0	0,0	0,6	0,1	0,2
General grade	6,9	7,3	5,6	6,3	6,8	6,2

Table 2

Characteristic of physical and chemical parameters of clarified apple juice depending on the type of brand of the product and packaging

Features		Requirements		Brand A	Brand B	Brand C	Brand D	Cardboard boxes	Glass bottles
		Polish Norm	AIJN Code of Practic						
Vitamin C [mg/100g]		–	–	2,9	2,5	3,1	2,2	2,3	3,1
General essence [%] (m/m)		>10	>11,2	11,3	11,5	12,1	12,0	11,3	11,8
General acidity [g/l]	recalculation into the apple acid	> 4,5	6,1	5,7	5,8	5,3	5,3	6,4	5,1
	recalculation into the citricacid	–	5,9	5,5	5,6	5,1	5,2	6,1	4,9
Reducing monosaccharides [g/100ml]		–	–	7,6	7,5	7,0	6,1	7,5	7,6
General saccharides [g/100ml]		–	–	10,1	9,9	9,7	11,0	10,1	10,2
Sucrose [g/l]		–	5-30	23,8	23,3	25,2	27,6	25,0	25,2
Sugar-free extract [g/l]		>14	18-29	20,3	22,3	29,9	17	22,9	21,9

Table 3

Significance of influence type of package and brand of the product on sensory features and physical and chemical parameters clarified apple juice

Sensory features and physical and chemical parameters		Type of package	Brand
		Value of empiric F	Value of empiric F
Clarity		0,04	0,68
Colour		0,20	0,39
Fragrance of fresh apples		0,74	17,33*
Fragrance acidity		0,32	0,41
Fragrance sweet		0,49	1,10
Fragrance bitter, sour and tart		0,82	0,18
Fragrance strange		0,35	1,26
Taste of fresh apples		6,06	3,70
Taste acidity		1,06	0,12
Taste sweet		2,14	0,26
Taste bitter, sour and tart		0,82	0,74
Taste strange		1,00	121,00*
General grade		0,78	1.37
Vitamin C [mg/100g]		2506,57*	673,67*
General essence [%] (m/m)		65535,00*	67535,00*
General acidity [g/l]	recalculation into the apple acid	15,36*	0,99
	recalculation into the citricacid	14,67*	0,93
Reducing monosaccharides [g/100ml]		2,45	89,73*
General saccharides [g/100ml]		3,00	98,75*
Sucrose [g/l]		1,00	61,55*
Sugar-free extract [g/l]		3,00	89,83*
$F_{0.05}$= (1,3)		10,13	–
$F_{0.05}$= (3,3)		–	9,28

Conclusions

1. Packaging of the types of juice under examination is tight, undamaged and hermetically closed and properly marked. The real capacity of examined juices both in cardboard boxes and glass bottles is concordant with that declared by the producer.

2. The results of the sensory evaluation of apple juices in cardboard boxes and in glass bottles allows us to state that the quality of examined juices is variable. No. statistically significant influence of the kind of package was ascertained with regard to assessed sensory characteristics of apple juices. However, the brand of juice essentially influenced the assessment of the smell described as "fresh apples" and the taste described as "strange".

3. Examined apple juices differ with regard to the level of assessed physical and chemical characteristics. It was ascertained that the type of packaging and product brand had an essential influence on the vitamin C content and general extract content in the apple juices under examination. The brand of juice essentially influences the content of reducing saccharides and total saccharides as well as the level of sucrose and sugar-free extract.

4. The analysis of obtained results did not provide an explicit answer to the question which of the juices under examination, taking into regard the type of packaging and product brand, had unequivocally better quality. One can state that apple juices of the leading brands on the Polish market are characterized by good or acceptable quality.

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CHAPTER 3

SELECTED ANALYSES IN FOOD EVALUATION

1

DETECTION OF EGG YOLK IN LIQUID EGG WHITE USING MID-INFRARED SPECTROSCOPY COUPLED WITH CHEMOMETRICS

Introduction

Eggs are an excellent source of nutrients comprising proteins, lipids, vitamins and riboflavin. The annual per capita consumption of eggs was about 12.9 dozens in Canada in 2005 [Statistics Canada, 2006], and 21.6 dozens in the United States in 2006 [US Department of Agriculture, Economic Research Service, 2006]. Liquid whole eggs and liquid egg white are becoming widely available in the market; such products are growing in demand not only due to their convenience as ready-to-use ingredients for cooking or product formulation, but also due to the reduction in risk of spoilage as a result of the pasteurization process involved in their production [Froning, 2004]. However, the steps of egg breaking followed by egg yolk and white separation that are involved in the production of liquid egg white products may introduce potential carry-over of egg yolk into the egg white products.

Typical levels of yolk contamination in egg white products may range from 0.01% to 0.2% [Bergquist and Wells, 1956]. Contamination of egg yolk in egg white can negatively influence the functionalities such as foaming ability of the intended products {Froning, 2004]. Since the separation of yolk and white is often compromised by the rate of production in high-speed automated egg processing plants, rapid methodology to monitor the efficiency of separation is necessary to assure product quality.

Several approaches to monitor the level of yolk contamination in egg white products have been reported. The monomolecular surface film method proposed by Bergquist and Wells [1956] requires a high level of training to obtain reliable results. An enzymatic method based on glycerol oxidase in combination with lipoprotein lipase was proposed to determine the triglycerides in the sample, which is in turn related to the amount of egg yolk contamination [Mine, 1996]. Chemical methods based on lipid extraction followed by colourimetric determination of cholesterol have also been investigated [Nielsen, 2000]. Despite the ability of these methods to detect yolk in egg white, alternative methods that require less tedious sample preparation and analytical reagents and that can provide reduced analysis time are desired in the food processing plant as a quality assurance tool to monitor the separation performance of egg yolk and white.

Infrared spectroscopy coupled with chemometrics has gained popularity over the past decade as a rapid tool to detect adulteration as well as contamination of food products. Several studies have investigated the feasibility of using infrared technology for quality control purposes. For example, Al-Jowder et al. [1997] performed a series of studies using mid-infrared (MIR) spectroscopy coupled with chemometric tools to discriminate among chicken, turkey and pork, and to distinguish between fresh and frozen-thawed meat. The possibility of using the technique for differentiating between pure beef products and offal-incorporated beef products was investigated by Al-Jowder et al. [1999 and 2002]. Other applications of MIR spectroscopy for food analysis include the detection of adulteration of honey by D-fructose and D-glucose [Daniel Kelly et al. 2004], analysis of cider [Lobo et al., 2006], detection of phytate in raw soymilk [Ishiguro et al., 2003], classification of apple juice beverages [Gomez-Carracedo et al. 2004] as well as discrimination of white grape varieties [Roussel et al., 2003].

Although many studies have illustrated the potential of MIR spectroscopy for the analysis of various food products, to our knowledge, there has not been any published report investigating its application to eggs. The objective of this study was to examine the potential of using Fourier transform infrared (FTIR) spectroscopy in the MIR region in combination with principal component analysis (PCA) and partial least squares (PLS) for analysis of eggs. Specifically, FTIR spectroscopy was applied to detect differences among eggs from Canada (CA), eggs from the United States (US) and organic eggs from Canada (OR), and to monitor varying percentages of egg yolk contamination in egg white. In addition, spectra of samples stored for 1, 7 and 15 days at 4^{o}C were compared to determine whether MIR analysis could be used as an indicator of storage duration.

Materials and methods

Sample Preparation

12 eggs each from Canada (CA), the United States (US) and organic eggs from Canada (OR) were purchased from a local supermarket (Vancouver, BC) and stored at 4^{o}C until use. Eggs were manually cracked open, and the yolk and white were separated. Thirty samples were prepared containing known levels of egg yolk in egg white at 0, 0.5, 1, 2, 5, 10, 30, 50, 70 and 100% by weight, and were blended while held in an ice water bath for 1 minute using a Tekmar Sonic Disruptor (*Tekmar Company*, Cincinnati, OH), pulsed setting, 50% duty cycle. Prepared samples were analyzed after 1, 7 or 15 days of storage at 4^{o}C.

Spectrum Acquisition

MIR spectra were collected using a Nicolet 6700 Fourier transform infrared (FT-IR) spectrometer with attenuated total reflection (ATR) sample-handling accessories (*Thermo Electron Corporation*, Madison, WI). Samples were placed on the ATR element and the spectra were recorded from 4000 – 650 cm^{-1}. Each spectrum was acquired at a resolution of 4 cm^{-1}, corrected through the multipoint baseline correction using OMNIC professional software suite (*Thermo Electron Corporation*, Madison, WI); a total of 128 spectra were co-added to yield the spectral data for each sample. Replicate analyses of some samples to monitor precision indicate coefficient of variation values ranging from 1.4–10.3%.

Data Analysis

A total of 98 spectra (CA = 33, US = 31 and OR = 34) each comprising 1738 spectral points in the 4000 – 650 cm^{-1} region were analyzed by principal component analysis (PCA) and partial least squares (PLS) regression with full cross validation using The Unscrambler V9.1 software (*Camo Process AS,* Nedre Vollgate 8, Norway). Initial analysis showed the existence of outliers in the sample set; hence, further analysis was performed after excluding the outliers, resulting in 96 (CA = 32, US = 30 and OR = 34) and 93 (CA = 31, US = 29 and OR = 33) sample spectra for PCA and PLS analysis respectively.

Results and discussion

Spectral Characteristics

Figure 1 shows typical MIR spectra for the yolk and white of organic eggs from Canada after 7-day storage. The most notable differences between these spectra are the peaks observed in the 1700 cm^{-1} and 2800 – 3100 cm^{-1} regions of the egg yolk spectrum. These regions are typical absorption regions for fat, indicating the importance of fat being the major contributor to the spectral variation. The typical composition of hen's eggs includes 10% shell, 30% yolk and 60% white [Li-Chan et al., 1995]. Egg white contains mostly water and is the major source of riboflavin and proteins; egg yolk, on the other hand, contains water, proteins, fat, vitamins and minerals [Watkins, 1995]. This difference in chemical composition contributes to the distinct spectra of egg yolk and egg white as shown in Figure 1.

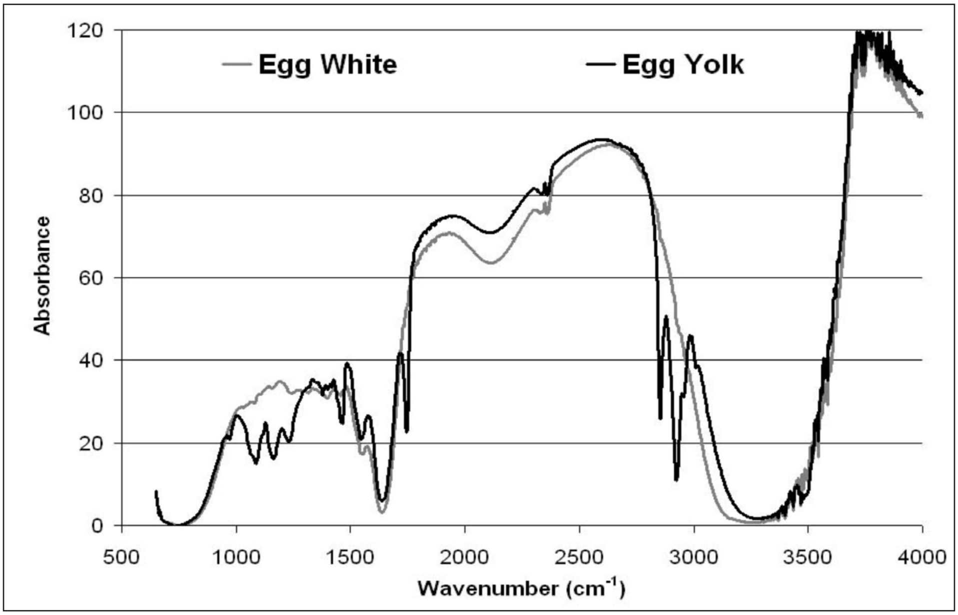
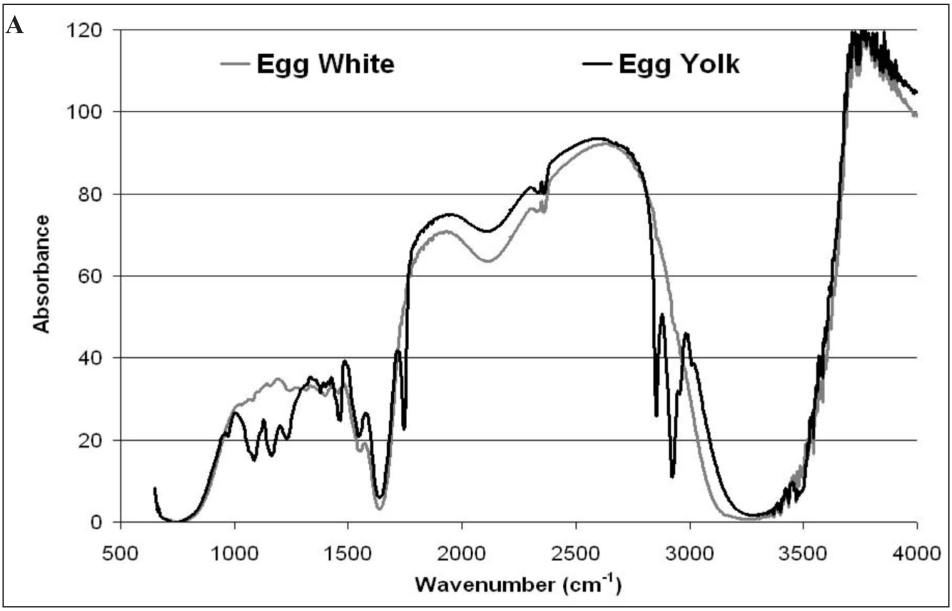


Fig. 1. Mid-infrared spectra of egg white and egg yolk of organic eggs from Canada after 7-day storage


A
Egg White
Egg Yolk
Absorbance
120
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80
60
40
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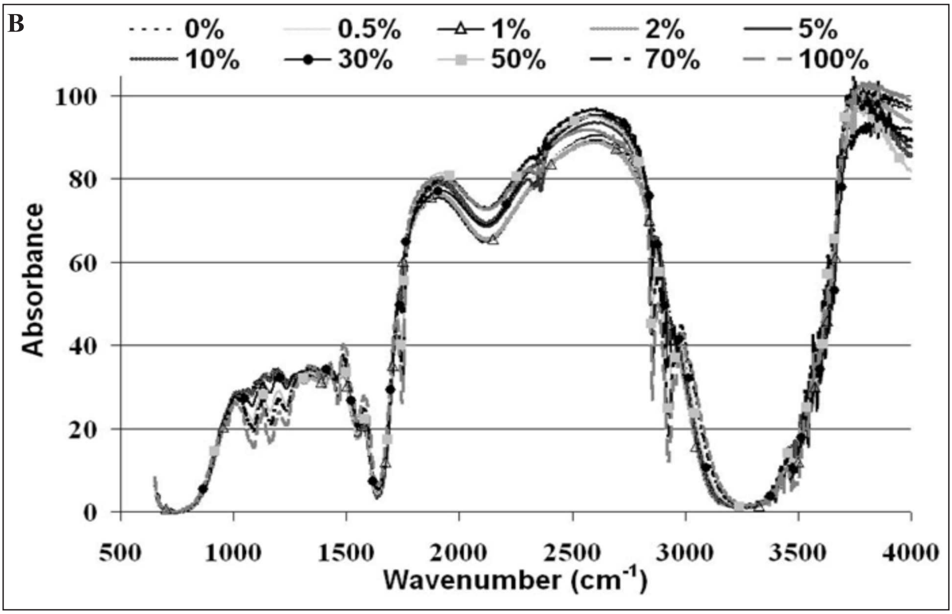
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm^{-1})


B
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4000
Wavenumber (cm^{-1})

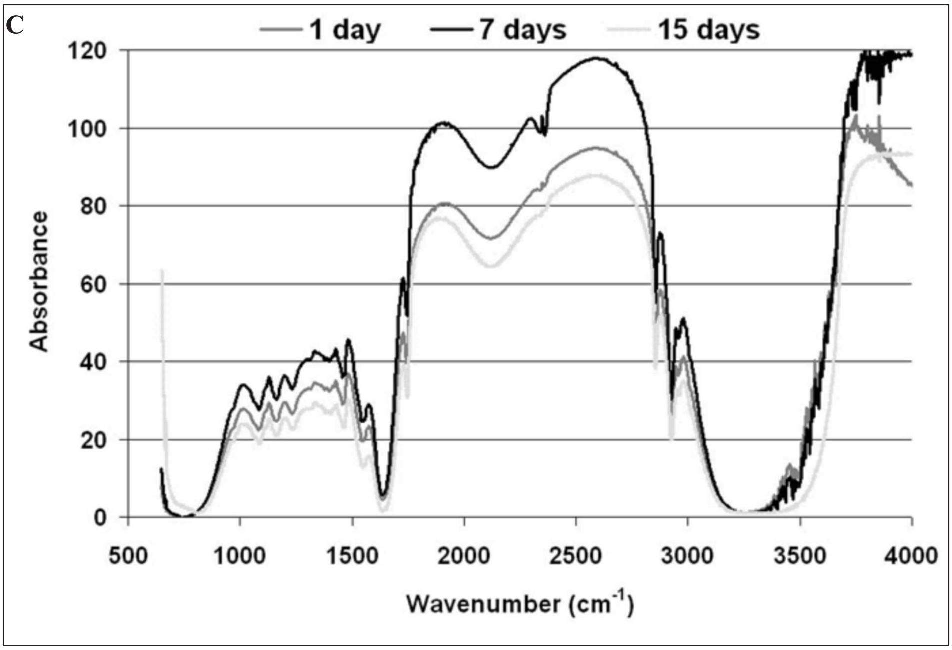

Fig. 2. Mid-infrared spectra as a function of A) eggs from Canada and the US, and organic eggs from Canada at 30% yolk by weight after 1-day storage; B) varying weight percentages of egg yolk in egg white of eggs from Canada after 1-day storage; C) different storage duration with eggs from the US at 50% yolk by weight

Figure 2A shows typical spectra of eggs from Canada (CA), eggs from the United States (US) and organic eggs from Canada (OR). In each case, several broad absorption bands were observed in the regions near 850 – 1530 cm^{-1}, 1750 – 2100 cm^{-1}, 2200 – 2850 cm^{-1} and 3500–4000 cm^{-1}. The spectra were generally similar regardless of origin of the eggs. In fact, the spectra of OR and US eggs almost coincided at every point while the spectrum of CA eggs showed slightly higher absorbance at wavenumbers below 3500 cm^{-1} and lower absorbance above 3500 cm^{-1}. Several factors, such as living conditions [Siderer et al., 2005] and diets [Nardone and Valfre, 1999] of hens, have been suggested to affect the chemical composition of hen eggs. However, in this study, based on the MIR spectral characteristics, No. obvious differences were observed, implicating either that CA, US and OR eggs were produced using similar farming practices, or that the changes were too subtle to be detected in their vibrational spectra.

On the other hand, the MIR spectra of samples with different yolk percentages (Figure 2B) demonstrated distinct variations in absorbance in the 960 – 1300 cm^{-1}, 1700 – 1740 cm^{-1}, 1770 – 2020 cm^{-1}, 2120 – 2350 cm^{-1}, 2370 – 2800 cm^{-1}, 2850 – 2900 cm^{-1}, 2950 – 3020 cm^{-1} and 3750 – 4000 cm^{-1} regions. Some of these bands represent vibrational modes related to triacylglycerol molecules. For example, bands at 930 – 1300 cm^{-1}, 1700 – 1740 cm^{-1}, 2850 – 2900 cm^{-1} and 2950 – 3020 cm^{-1} may be assigned to the C-O stretch and/or C-H bend, C=O stretch, symmetric C-H stretch, and asymmetric C-H stretch, respectively of the fatty acyl ester and hydrocarbon chains of triacylglycerol [Li-Chan et al., 2002; Yang et al., 2005; Karoui et al., 2006]. Since fat is one of the major components in egg yolks, notable differences from these regions strongly suggested that the fat content of yolk plays a major role in the spectral variation in the samples containing different levels of yolk in white.

Figure 2C displays typical spectra of samples stored for 1, 7 and 15 days at 4°C. Although the general shape of the spectrum was maintained in all three spectra, the absorbance varied drastically. Storage of eggs for 7 days increased the absorbance intensities of the entire spectrum compared to 1- day storage while 15-day storage lowered the intensities (Figure 2C). Previous study has shown that during storage of eggs, several changes, such as rise in pH, albumen thinning and increase in water content of the yolk, could occur [Karoui et al., 2006]. These changes could possibly affect the absorption in the infrared region, thus the differences observed in the spectra. Therefore, these results demonstrated the possibility of using infrared spectroscopy to monitor storage duration and product freshness.

Even though these differences could be observed from the spectra, it was very difficult to quantify or differentiate solely using human eyes. As a result, these differences were further analyzed using chemometric tools.

Principal Component Analysis

Analysis of the 96 sample spectra using PCA with full cross validation resulted in explanation of 94% of the variability in the data using three principal components, with 66, 15 and 13% attributed to PC1, PC2 and PC3, respectively. As shown in Figure 3A, the 2-dimensional scatter plot of PC1 versus PC2 showed groupings of samples based on different storage durations. High scores in both PC1 and PC2 represented samples analyzed after 15-day (long or "L") storage duration. Scores clustered around the origin in both PCs indicated samples with 1-day (short or "S") storage, while negative PC scores in both PCs or high score in PC2 and low score in PC1 corresponded to samples stored for 7 days (medium or "M") storage duration. On the other hand, PC1 and PC3 illustrated clustering of data according to low (≤10%), medium (10–50%) and high (>50%) yolk content (Figure 3B). PC3 was the best predictor of yolk content with PC3 scores greater than zero representing low yolk content, scores between zero and -100 being medium yolk content; and any scores below –100 indicating high yolk content.

Since PC1 and PC2 together reflected storage duration while PC3 was an indicator of percentage egg yolk in white, a three-dimensional scatter plot of all 3 PCs served to distinguish between samples of different storage time as well as yolk content (Figure 4). This ability to simultaneously monitor product freshness and yolk content using solely one technique could be advantageous for the egg industry.

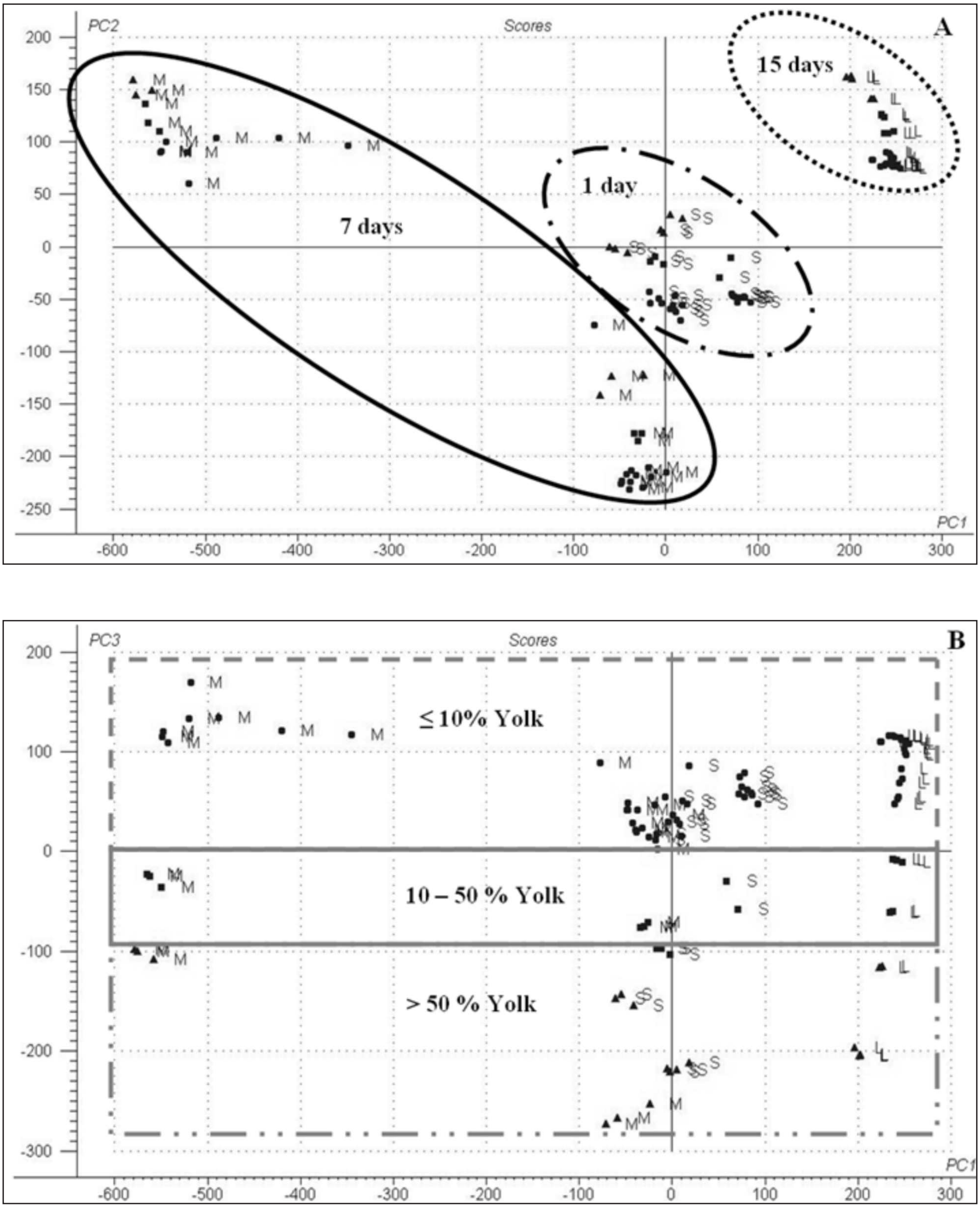

Fig. 3. Two-dimensional scatter plots of **A)** PC1 versus PC2 and **B)** PC1 versus PC3. (S, 1-day cool storage; M, 7-day cool storage; L, 15-day cool storage; ●, low yolk content, ≤10%; ■, medium yolk content, 10 – 50%; ▲, high yolk content, >50%)

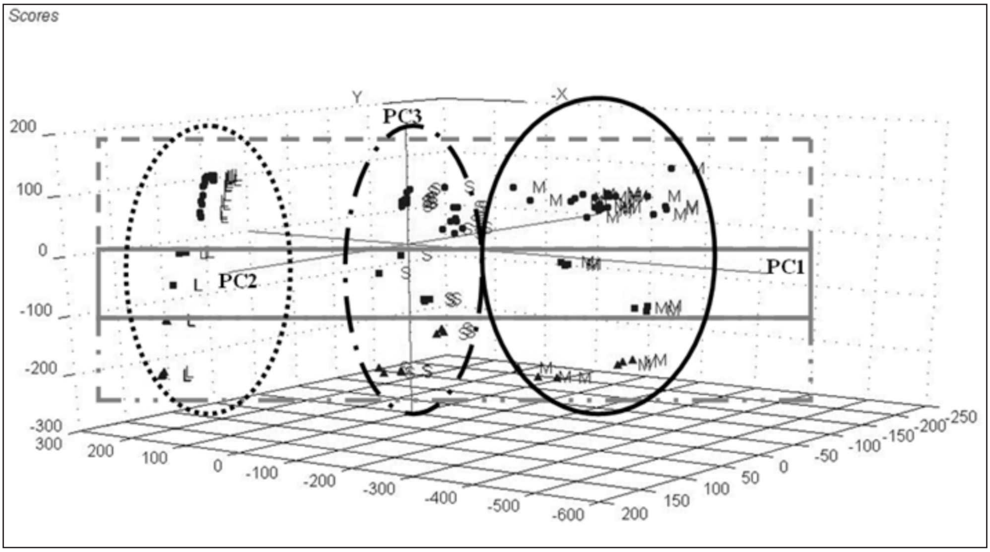

Fig. 4. Three-dimensional plot of PC1 versus PC2 versus PC3 (S, 1-day cool storage; M, 7-day cool storage; L, 15-day cool storage; ●, low yolk content, ≤10%; ■, medium yolk content, 10 – 50%; ▲, high yolk content, >50%)

Partial Least Squares Regression Analysis

Using PLS with full cross validation, the percentage of egg yolk in egg white was successfully modeled by 2 PCs. The first PC used 15% of the spectral data and was able to explain 97% of the variability in percent egg yolk content in the samples; the second PC, comparatively less powerful, required 62% of the spectral characteristics to explain only 1% of the yolk content. It was evident that PC1 was the major predictor in the model.

Several regions of the spectra showed high regression coefficients indicating their relevance to predict egg yolk content in the samples (Figure 5). These regions included 1050 – 1250 cm^{-1}, 1730 – 1760 cm^{-1}, 2830 – 2960 cm^{-1} and 3015 – 3085 cm^{-1} arising from C-O stretching and/or C-H bending, C=O stretching, symmetric and asymmetric C-H stretching, and =C-H stretching vibrations, respectively [Li-Chan et al., 2002; Flatten et al., 2005; Yang et al., 2005]. These vibrational modes could be assigned to functional groups of the triacylglycerol molecules, indicating that the fat component of yolk was an important factor in the prediction of yolk content in the model.

The PLS regression plot showed high predictive power of the model with a regression coefficient r = 0.99 and a root mean square error of prediction RMSEP = 4.5 (Figure 6). The results were promising showing the potential of using mid-infrared spectroscopy together with PLS to predict yolk content in egg samples.

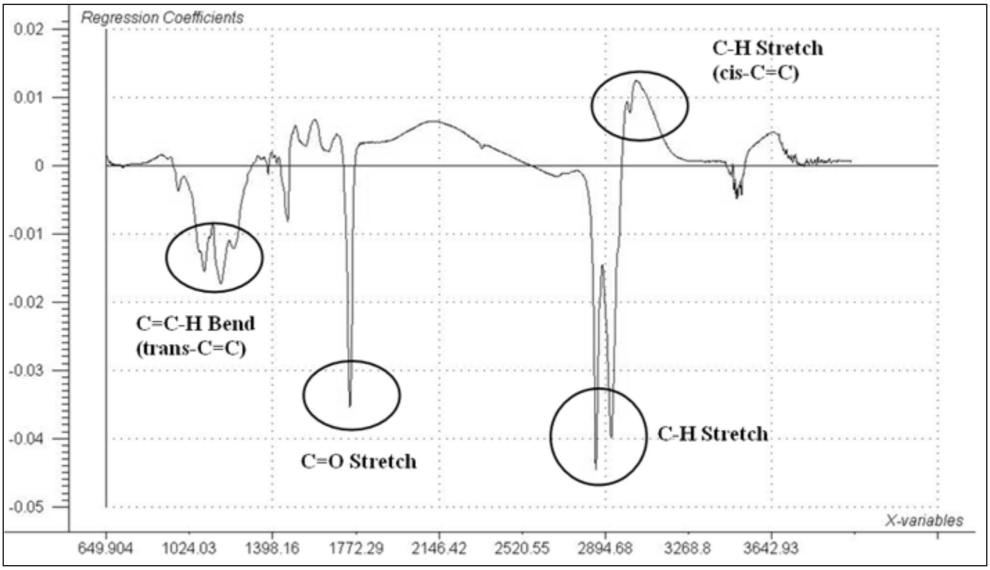

Fig. 5. Regression coefficient plot of PLS showing important spectral variables for predicting egg yolk contamination in egg white

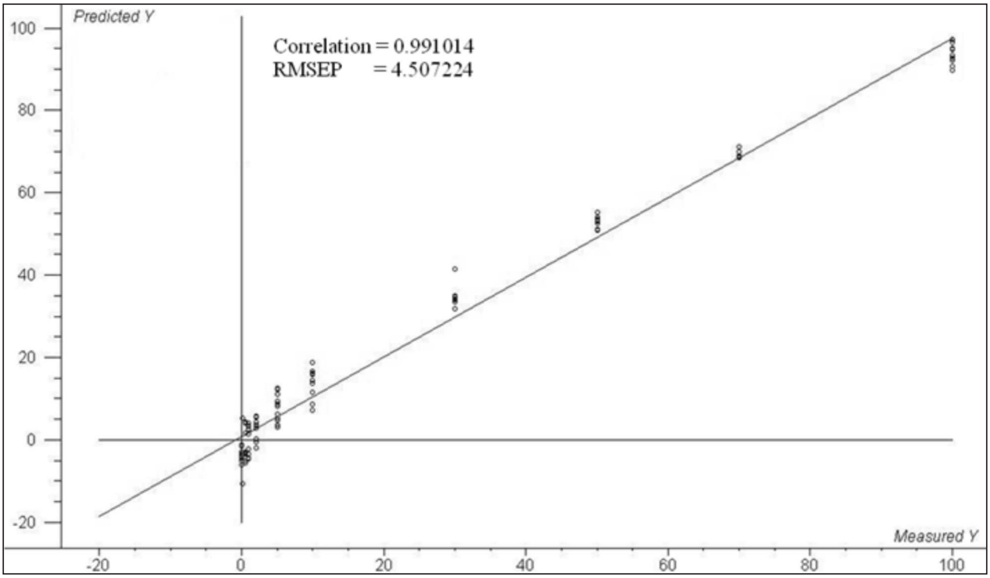

Fig. 6. Plot of predicted versus measured values for the different levels of egg yolk contamination in egg white with r = 0.99 and RMSEP = 4.5

Conclusions

This study demonstrated the potential to apply mid-infrared spectroscopy in the egg industry. Although No. spectral differences could be clearly observed to distinguish eggs from Canada and the United States, the ability of the technology in combination with chemometric tools to differentiate among egg white samples containing low, medium and high yolk contents as well as after short, medium and long storage duration may be an asset to the quality control sector of the industry. The ability of the PLS model to estimate egg yolk contamination levels in egg white products suggest potential use of mid-infrared spectroscopy as a rapid tool to monitor purity of egg white products to ensure high functionality. Further investigation should be conducted to examine a broader range of storage duration and to include more samples with yolk contamination levels below 5%, in order to better reflect the conditions likely to be encountered in the egg industry.

Acknowledgements

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2

CONTAMINATION OF RAW AND ROASTED NATURAL ARABICA COFFEES BY POLYCYCLIC AROMATIC HYDROCARBONS DETERMINED APPLYING GC-MS METHOD

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous chemical contaminants, originating from incomplete combustion of organic matter. PAHs are being found throughout the environment, in water, air, soil, and therefore also in food. Their presence in food originate mainly from thermal treatment in the preparation and manufacturing of foods [Guillen et al., 1997; SCF, 2002; Garcia-Falcón et al., 2003; CCFAC, 2005]. They have been the subject of much concern in recent years due to their toxic potential, therefore because of the genotoxic, mutagenic and carcinogenic properties (SCF, 2002).

Currently, according to the Commission Recommendation 2005/108/EC, further analyses of benzo[a]pyrene and other compounds belonging to the 15 PAHs selected by the Scientific Committee on Food are necessary in foodstuffs.

Particularly vulnerable to the contamination by PAHs are products with high fat content, which is a carrier of hydrophobic polyarenes. Taking into consideration that coffee beans contain 10 to 17% of fat, and are subjected to a roasting process, may be contaminated by PAHs [De Kruif et al., 1987; Camargo and Toledo, 2002; Houessou et al., 2008].

With the background provided, the purpose of this research was to examine contamination of selected raw and roasted natural Arabica coffees by 19 PAHs, including 4 PAHs from the group of light PAHs listed by The Environmental Protection Agency (EPA) and 15 genotoxic PAHs listed by The Scientific Committee on Food (SCF). Important issue was therefore to define influence of roasting process on PAHs level in the final product.

Materials and methods

The material investigated were raw and roasted natural Arabica coffees (Coffea arabica) imported from 5 different countries (Brazil, Peru, Ethiopia, Indonesia, Tanzania). The coffee beans were roasted at the temperature ranged from 125 to 135^{o}C for 25–26 min. using the electrical heating system. From every assortment three samples were taken to the study. All three samples of the same one product were analyzed in three repetitions.

Methodology applied for this study consisted of fat extraction, extract's clean up using silica gel column, PAHs isolation from fat matrix by gel permeation chromatography (GPC) and qualitative and quantitative PAHs determination by gas chromatography coupled with mass spectrometry (GC-MS).

The sample of coffee (7.5 g) after adding 50 ml of extraction solvent (hexane/acetone, 60:40, v/v) was placed in ultrasonic bath for 30 minutes. Subsequently solid parts were filtered off from the extract. The obtained filtrate after evaporation almost to dryness (in rotary vacuum evaporator at 35°C) was dissolved in 0.5 ml cyclohexane, subjected to clean up on silica gel column and eluted by cyclohexane. The first 10 ml of filtrate was discarded and subsequent 75 ml was collected, concentrated and dissolved in 5 ml cyclohexane. For the PAHs isolation clean-up procedure by means of gel permeation chromatography (GPC) and column TSK Gel G1000HXL (300 × 7.8 mm, 5 μm) were applied. For that purpose 1 ml of the extract obtained before was injected on the column. Chromatographic separation was performed by isocratic method with UV-VIS detector and wavelength of 254 nm applied. Mixture cyclohexane/ethyl acetate (50:50, v/v) was used as mobile phase. Samples were eluted at a flow rate of the mobile phase of 0.8 ml/min. The purified extract after solvent evaporation (almost to dryness) was dissolved in 0.1 ml toluene and analyzed by GCMS-QP 2010 of Shimadzu, consisted of a gas chromatograph GC–2010 and mass spectrometer GCMS-QP 2010. Injected sample (1 μl) was chromatographed on 30 m × 0.25 mm i.d. capillary column with film thickness of 0.25 μm (ZB-5ms Zebron, Phenomenex). The GC oven temperature was programmed from initial 92°C to 315°C. The injector temperature 265°C and splitless injection mode were applied. As a mobile phase helium with the flow of 0.74 ml/min was used. The operating conditions of the mass spectrometer were following: the ion source and interface temperature – 230 and 270°C respectively, detector voltage 1.5 kV. The quadrupole analyser measured the abundance of ions of m/z from 100 to 400 and. Electron ionization (70 eV) with selected ion monitoring mode was used, and the two most abundant ions from the molecular ion cluster were measured for each analysed compound.

PAHs identification was carried out both on the basis of GC retention time comparison with available PAHs standard solutions and using characteristic ions monitored in analyses. The standard mixture of 15 PAHs from the list of SCF (PAH-Mix 183, Dr. Ehrenstorfer): cyclopenta[c,d]pyrene (C[cd]p), benzo[a]anthracene (B[a]a), chrysene (Chr), 5-metylchrysene (5-MChr), benzo[j]fluoranthene (B[j]f), benzo[b]fluoranthene (B[b]f) and benzo[k]fluoranthene (B[k]f), benzo[a]pyrene (B[a]p), dibenzo[a,h]anthracene (D[ah]a), dibenzo[a,l]pyrene (D[al]p), benzo[g,h,i]perylene (B[ghi]p), indeno[c,d]pyrene (I[cd]p), dibenzo[a,e]pyrene (D[ae]p), dibenzo[a,i]pyrene (D[ai]p) and dibenzo[a,h]pyrene (D[ah]p) and 16 PAHs by EPA (PAH-Mix 9, Dr. Ehrenstorfer) were purchased from Witko (Łódź, Poland). The mixture of 16 PAHs by EPA served at the determination of only 4 light PAHs: phenanthrene (Phen), anthracene (Anthr), fluoranthene (Flu) and pyrene (Pyr).

In reference to the Commission Regulation (EC) No. 333/2007 performance criteria for methods of analysis for benzo[a]pyrene in foodstuffs are as follows: LOD less than 0.3 μg/kg, LOQ less than 0.9 μg/kg, precision – $HORRAT_R$ values of less than 2 and recovery from 50 to 120%. The measured validation parameters revealed that the applied method for the determination of 19 PAHs aside from fulfilling all requirements of European Union laying down the methods of benzo[a]pyrene analysis in foodstuffs, also demonstrated satisfactory validation parameters for every of the remaining PAHs.

Mean contents of the determined 19 PAHs in analysed coffees were expressed in μg/kg of the product. The obtained results were statistically analysed using Statistica 7.1 programme. To appraise the significance of the differences between the means of 19 PAHs content in the raw and roasted natural Arabica coffees, the method of multiple comparisons using Tuckey's test was applied, at significance level α = 0.05.

Results and discussion

Data on mean content of individual PAHs as well as summary content of 19 PAHs in selected raw and roasted natural Arabica coffees, analysed by the GC-MS method, are presented in Table 1.

The quality profiles of PAHs content in analysed raw and roasted Arabica coffees were characterized mainly by the occurrence of phenanthrene, anthracene, fluoranthene and pyrene (light PAHs listed by EPA). It was stated that those light PAHs constituted from 90 to 97% of all determined polyarenes in raw Arabica coffees and from 75 to 96% in their roasted equivalents. From the group of 15 PAHs, marked out for the analyses by The Scientific Committee on Food, benzo[a]anthracene and chrysene were stated in all samples. Only in case of some Arabica coffees, especially the raw ones, additionally 5-metylchrysene, benzo[b]fluoranthene and benzo[k]fluoranthene were determined. Remaining heavy PAHs: cyclopenta[c,d]pyrene, benzo[j]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, indeno[c,d]pyrene and PAHs from the group of dibenzopyrenes were not detected in any of analysed samples of both raw and roasted Arabica coffees. Moreover, it was confirmed that heavy PAHs, for which genotoxic, mutagenic and carcinogenic properties have been stated, constituted very low percentage in the total content of PAHs.

On the basis of performed statistical analysis, significant differences in the total 19 PAHs concentration were stated between individual products derived from 5 different countries both from the group of raw and roasted Arabica coffees. In the group of raw coffees the highest level of 19 PAHs contamination being equal 58.63 µg/kg was determined in coffee from Tanzania. However, the lowest sum of 19 PAHs were stated for coffees from Brazil and Peru, respectively at the level of 7.49 µg/kg and 32.71 µg/kg. Among the roasted coffees statistically the highest summary concentration of 19 PAHs being equal 14.43 µg/kg was determined in coffee from Peru. On the contrary, the lowest level was observed for coffees from Ethiopia and Indonesia, in case of which the mean content of 19 PAHs was equal to 5.35 µg/kg and 5.47 µg/kg respectively (Tab. 1).

In the case of analysed Arabica coffees derived from the four countries statistically significantly lower level of 19 PAHs contamination was confirmed for the roasted coffees in comparison with their raw equivalents. For Arabica coffee from Tanzania as a result of roasting mean content of 19 PAHs decreased from 58.63 µg/kg (characteristic of the raw coffee) to 7.83 µg/kg. The level of contamination of the raw Arabika coffee from Indonesia reached the value of 46.84 µg/kg, while after the roasting process was reduced to the level of 5.47 µg/kg. A large decrease was also observed for the coffee from Ethiopia, from the content of 46.73 to 5.35 µg/kg. Also as a result of roasting the summary content of 19 PAHs in the coffee from Peru decreased about 18 µg/kg, from the level of 32.71 µg/kg determined in the raw coffee. It was also statistically confirmed that the decrease of the total PAHs content as a consequence of roasting is even more statistically significant considering results expressed on the dry matter of the products. The roasting process of Arabica coffees was conducted at the temperature ranged from 125 to 135°C for 25–26 min. with the use of electrically heated furnace. Such a mild parameters of roasting, as well as the electrical heating system undoubtedly have substantial impact on reducing the level of PAHs contamination in roasted coffees compared with unprocessed ones.

Table 1

The content of polycyclic aromatic hydrocarbons (PAHs) in selected raw and roasted Arabica coffees [µg/kg, mean ± S.D.]

PAH	Arabica coffees									
	Brazil		Peru		Ethiopia		Indonesia		Tanzania	
	raw	roasted	raw	roasted	raw	roasted	raw	roasted	raw	roasted
Phen	4.62 ± 0.68	1.66 ± 0.37	22.98 ± 2.72	5.43 ± 0.84	33.29 ± 3.83	2.51 ± 0.51	26.85 ± 4.22	1.91 ± 0.35	31.62 ± 3.49	3.52 ± 0.77
Anthr	0.20 ± 0.05	0.45 ± 0.02	1.38 ± 0.15	0.71 ± 0.06	1.29 ± 0.29	0.15 ± 0.06	0.86 ± 0.30	0.14 ± 0.01	4.73 ± 0.63	1.25 ± 0.14
Flu	1.17 ± 0.33	2.13 ± 0.41	4.09 ± 0.68	4.29 ± 0.37	6.11 ± 0.66	1.41 ± 0.13	9.99 ± 0.71	1.08 ± 0.28	14.06 ± 1.36	1.48 ± 0.11
Pyr	1.07 ± 0.29	1.43 ± 0.29	3.20 ± 0.35	3.13 ± 0.40	4.61 ± 0.72	1.04 ± 0.08	4.69 ± 0.58	0.96 ± 0.17	6.04 ± 0.54	0.96 ± 0.16
C[cd]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B[a]a	0.16 ± 0.03	0.28 ± 0.06	0.29 ± 0.03	0.23 ± 0.08	0.32 ± 0.17	0.11 ± 0.01	0.95 ± 0.03	0.37 ± 0.16	0.62 ± 0.21	0.32 ± 0.02
Chr	0.27 ± 0.07	0.21 ± 0.13	0.48 ± 0.13	0.51 ± 0.23	0.67 ± 0.01	0.13 ± 0.08	1.67 ± 0.15	0.49 ± 0.07	1.11 ± 0.10	0.30 ± 0.01
5-MChr	n.d.	n.d.	0.08 ± 0.03	0.06 ± 0.04	0.06 ± 0.02	n.d.	0.61 ± 0.07	0.04 ± 0.02	0.23 ± 0.03	n.d.
B[j]f	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B[b]f	n.d.	n.d.	0.11 ± 0.02	n.d.	0.26 ± 0.07	n.d.	0.67 ± 0.11	0.25 ± 0.07	0.21 ± 0.11	n.d.
B[k]f	n.d.	n.d.	0.09 ± 0.02	0.08 ± 0.03	0.13 ± 0.01	n.d.	0.53 ± 0.05	0.23 ± 0.04	n.d.	n.d.
B[a]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ah]a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
I[cd]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B[ghi]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[al]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ae]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ai]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D[ah]p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ PAHs	7.49 ± 0.81^{a1}	6.15 ± 0.54^{a1}	32.71 ± 2.75^{b2}	14.43 ± 1.35^{a2}	46.73 ± 5.18^{b3}	5.35 ± 0.72^{a3}	46.84 ± 2.23^{b4}	5.47 ± 0.34^{a4}	58.63 ± 3.29^{b5}	7.83 ± 0.55^{a5}

n.d. – not detected;

The mean values of summary PAHs content marked in indices with different letters (a, b) by the same number (within one from 5 comparisons of summary PAHs content of raw and roasted coffees) indicate statistically significant difference between means at $\alpha = 0.05$ level.

In the research conducted by De Kruif et al. [1987], considering contamination of roasted coffees by PAHs, it was confirmed that the content of benzo[a]pyrene was dependent on the degree of roasting, however generally the level of this polyarene was low, as varied from 0.1 to 0.5 μg/kg of the product. According to the other studies the content of benzo[a]pyrene in ground and instant coffees ranged from less than 0.01 to 1.2 μg/kg, whereas in the heavily roasted coffees its concentration reached even 22.7 μg/kg [Lai et al., 2004; Houessou et al., 2006]. Furthermore Houessou et al. in the research dealing with contamination of roasted coffees showed that from the all determined polyarenes predominant were phenanthrene, pyrene and fluoranthene. The content of these light PAHs varied from 10.0 to 25.0 μg/kg, while the level of contamination by 11 PAHs, including 4 light PAHs and 7 consecutive PAHs from the list of The Scientific Committee on Food (from benzo[a]anthracene to benzo[g,h,i] perylene), was in the range from 21.8 to 64.7 μg/kg [Houessou et al., 2006]. The qualitative and quantitative PAHs profiles in analysed coffees, presented in this paper, therefore do not show considerable differences in comparison with the above-mentioned research.

Furthermore, the obtained results indicate a large variation in the contamination level of individual products both from the group of raw and roasted coffees. The diversity of PAHs content in natural coffees, especially the raw ones, might result from various levels of environmental pollution, as well as from the method of post-harvest drying of coffee. The above reasons for the diversification of coffees contamination by polyarenes are also mentioned by other researchers [Camargo and Toledo, 2002; Garcia-Falcón et al., 2005; Houessou et al., 2008]. Additionally, parameters of roasting, therefore the temperature and time, as well as its technology can significantly contribute to different levels of PAHs content. In the industry the coffee roasting is usually performed at the temperature of 185–190°C, however typical roasting process can be divided into three stages. The first one is the drying stage, carried out at the temperature of about 100°C, and aimed at reducing the water content of the coffee bean to a few % from the 10–12%, which are typical for raw beans. Afterwards starting from about 170°C the process of roasting begins, during which occur pyrolytic reactions. They are strongly linked with the formation of PAHs in food. Finally the process of roasting is finished off with beans chilling, when the desired roasting effect is achieved [Yeretzian et al., 2002; Rum, 2004; Franca et al., 2005; Houessou et al., 2005].

With the background provided and on the basis of the obtained results, it can be concluded that the relatively low level of PAHs contamination in analysed coffees, especially the roasted ones, arose from the applied mild conditions of roasting. However, it is generally known that the coffees which are roasted at the high temperatures and for a long time have a very dark colour, crack and in the consequence the fat is present on their surface [Matajszczyk, 2001; Yeretzian et al., 2002]. All those certainly contribute to the higher contents of lipophilic PAHs in the final product. Therefore, it could be assumed that the use of temperatures of roasting in the range of 170–240°C can result in increase in the level of PAHs contamination of roasted coffees in comparison with their raw equivalents.

Conclusions

The results showed that the level of contamination of roasted coffees was statistically significantly lower in comparison with their raw equivalents. As a result it was proved that the applied mild parameters of roasting, as well as the electrical heating system have substantial impact on reducing the level of PAHs contamination in the final product and indeed do not create conditions for PAHs formation, especially the heavy polyarenes. Moreover, the obtained low levels of PAHs content in analysed roasted Arabica coffees undoubtedly would result in low levels of contamination of their infusions.

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3

THE COMPARISON OF ANTIOXIDANT PROPERTIES OF GREEN AND BLACK LEAF TEAS

Introduction

Tea is obtained from young leaves and leaf buds of the *Camella (Thea)* plant. Dependently on the production process there are three types of tea: black, oolong, and green tea. The main production process of the black tea is an enzymatic fermentation during of which the polyphenols composed mainly of catechins, in tea lives become oxidized by the enzyme: *polyphenol oxidase*. During fermentation the amount of tannins is reduced, the caffeine is activated, also some essentials oils are activated and some new are produced. After drying the oxidized and condensed catechins make the tea leaves to be black. The green tea is not caused fermentation, the faded tea leaves are warmed up by steaming which inactivates the enzymes and makes the fermentation impossible. The oolong teas are obtained by means of a partial fermentation [Manzocco et al., 1998, Wang et. al. 2000, Dłużewska et al., 2003, Gramza et al., 2005].

In recent years tea has attracted significant attention due to reported health benefits, in particular as antioxidant, which is because of containing flavonoids. Flavonoids, i.e. catechins might make complex with metal ions, which are stimulating the reaction of oxidation, have the ability to scavenge radicals by electron transfer process, have the activity to inhibit enzymes involved in the production of reactive oxygen species [Janeiro & Brett, 2004]. The antioxidant activity of catechins is correlated with the number of OH group in a benzene ring [Heijnem et al., 2001]. The mechanisms of their action as antioxidants involve the ability of phenols to scavenge radicals by electron transfer process by which the phenol is converted into a phenoxyl radical [Janeiro & Brett, 2004, Martinez et al., 2005].

The richest in catechins are green teas (30–42% of the dry weight of leaves), black teas have much lower content of catechins (3–10%) [Benzie & Szeto, 1999]. Nevertheless black teas have also good antioxidant properties. It was found that theaflavins present in black tea possess at least the same antioxidant potency as catechins present in green teas. What is more green teas have diverse antioxidant capacity which is caused by differential composition of polyphenols, depended on the way of harvest, time of crop and the conditions of tea storage. In many papers [Yen & Chen, 1995, Lin et al., 1998, Rice-Evans et al., 2003, Fik & Zawiślak, 2004, Vertuani et al., 2004, Vittal et al., 2004] on antioxidant capacity different kinds of teas do not give the unequivocal answer which teas have better antioxidant properties. That is why the aim of this work was the comparison of antioxidant properties of the black and green teas commonly found on the polish market.

Materials and methods

The material for this work comprise of four black and four green market teas, free from artificial flavours, available on Polish market.

Quantitative descriptive analysis (QDA) was used to determine the differences in the sensory characteristics of the samples [Baryłko-Piekielna, 1975]. The sensory assessments of the samples were carried out by a panel consisting of 20 members selected and trained. Panellist rated the sensory attributes of water tea extract (aroma, taste and appearance) on continuous unstructured line scale from low intensity (0) to high intensity (10) [PN- ISO 6078–1996]. The overall sensory acceptance of water tea extract was presented on 5 points scale.

In experiment was evaluated also the content of contaminations (pieces of wood, minerals, metals, sand, vermin), share of tea dust and stalk in a single packaging, artificial colourants [Cichoń 2001], water extract [BN-78/8191-03] and caffeine by Prange-Walther's method [PN-A-94019].

The content of total polyphenols was determined in water tea extracts using Folin-Ciocalteau assay by the measurement of absorbance of colour complex arise with Folin-Ciocalteau reagent and sodium carbonate in reaction with phenolic substances [Singleton & Rossi, 1996]. The absorbance was measure on Helios β – Termo Spectronic with detection wavelength 700 nm. To determine the levels of polyphenols in the samples it was create a calibration curve with the standards (+) catechin solution. The result were formulate as a amount of total polyphenols converted into (+) catechin (mg /100 ml) of water tea extract.

The antioxidant activity of tea were estimated by Brand – Williams et al. [1995] method with DPPH (α,α – diphenyl – β – picrylhydrazyl) radical. The absorbance was measured on Helios β – Termo Spectronic, the working wavelength of maximum absorbance, used for the absorbance measurements was 517nm. The results have been obtained by using the following formula: $Q = 100*(A_0 – A_c)/A_0$, where Q is the percentage reduction of the DPPH – quenching of free radicals, A_0 and A_c are the absorbance of the control and examined samples, respectively.

Statistical analysis was performed using One Way ANOVA (Statgraphic Plus 4.1). Statistical significance was considered at $p < 0.05$.

Results and discussion

In this study, two black and two green teas derived from two producers (I and II) were analysed. The sensory analysis is one of the most important method in the estimation of the quality of tea. Taking into account the effect of tea leaves quality on their antioxidant properties the sensory analysis has been carried out. The teas were compared with respect to their flavour, taste and appearance. The flavour of all tested water tea extract was fresh (7 units for black teas, 6 units for green teas). The odd aroma was practically not detectable for all teas, except the green tea II, which differs from others by the most stale aroma (Fig. 1). Similarly the appearance of water tea extracts were high scored, except the green tea II. The water tea extracts were clear , fresh, glossy and were characterized by the colour appropriate for the tea type (Figure 2). Black teas as well as green teas had proper, fresh, mature and reach taste.

Bitterness was high scored in the green tea II (Fig. 3). In conclusion we found that the overall sensory acceptance of water tea extracts were very good or good (Tab. 1).

The good quality of analyzed teas was confirmed also by the lack of mineral contaminations, trace amount of tea dust and stalk (>10% in a single packaging). There were also No. artificial colourants in tested teas (Tab. 1). According to Polish Standards PN-ISO 1578:1996 the amount of tea extract in a dry matter should not be less than 32%. In all analysed teas the water tea extract, identified with all substances that are extracted from leaves by a hot water, was higher than in standards. The amount of water tea extract was between 35.4 and 46.9% which indicates the teas were prepared from a high quality material manufactured properly. The analysis of the caffeine content showed that green teas were richer in this substance than black teas. In black teas were more than 3 g of caffeine per 100 g dry matter while green teas contained over 4 g. The obtained results were similar to those of published by Perucka [2001]. However, in literature there are published different opinions. Taylor and Mc Dowell [1993] discovered that the content of the caffeine does not depend on technological process, that is why in green and black teas the caffeine level should be the same. From results obtained by Ostrowska et al. [2005] it can be seen that black teas have higher content of this substance than green ones.

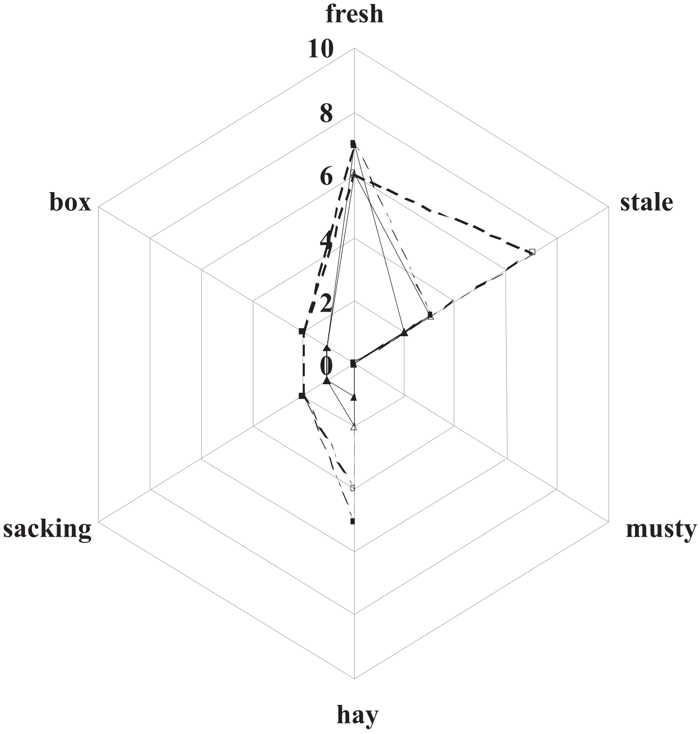

Fig. 1. Sensory analysis of odour of water tea extract

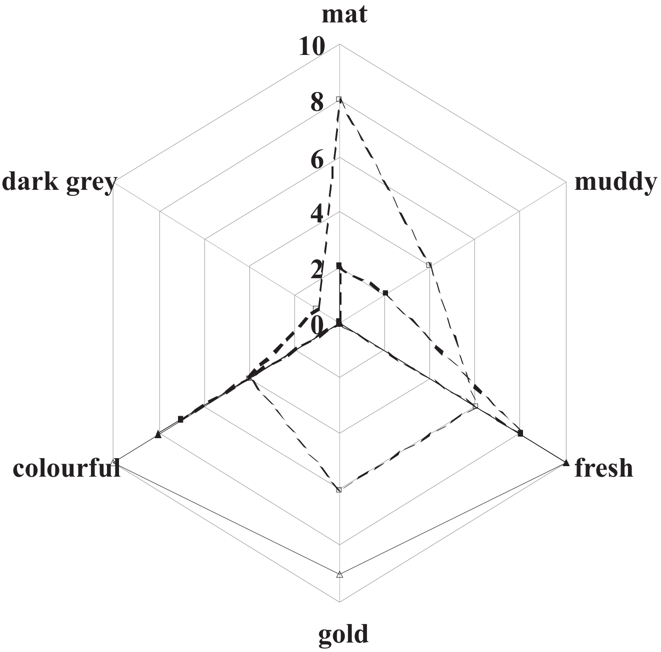

Fig. 2. Sensory analysis of apperance of water tea extract

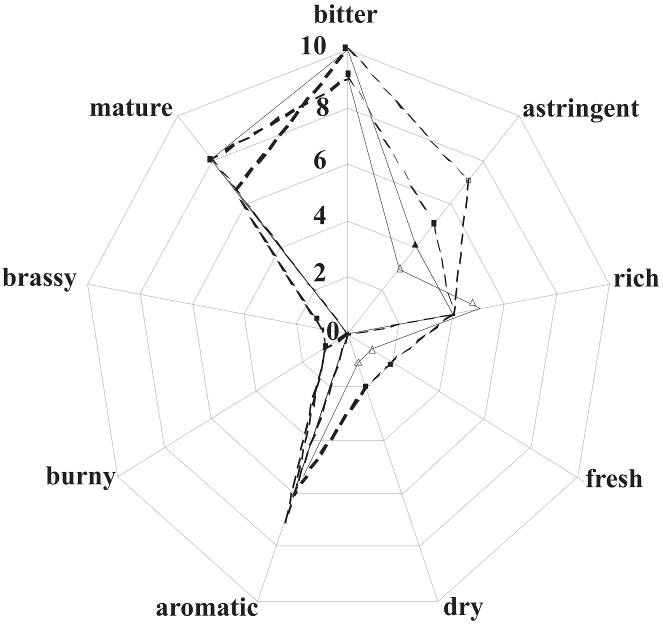

Fig. 3. Sensory analysis of taste of water tea extract

Legends to figures 1–3

Black tea I —▲—
Green tea I —△—
Black tea II – –■– –
Green tea II – –□– –

Table 1

Physicochemical properties and scores of overall sensory acceptance of teas

Type of tea	Sensory analysis of water tea extract			Artificial colourants	Water extract [%]	Caffeine [%]
	Aroma	Appearance	Taste			
Black tea I	++	++	++	none	40.40^b	3.12^a
Green tea I	+	+	0	none	35.40^a	4.52^b
Black tea II	++	++	++	none	40.70^b	3.36^a
Green tea II	0	+	+	none	46.87^c	4.89^b

++ very good, + good, 0 medium, - bad, -- very bad; mean values denoted by different superscripts in the same column differ significantly from each other ($\alpha=0.05$).

On the basis of the total amount of polyphenols in leaves and water tea extract the antioxidant activity of teas might be foreseen. The results of content of total polyphenols showed that there are significant differences between analysed teas, but it can not be concluded which type of tea have a higher content of the components (Fig. 4). It was noted significant difference between the content of polyphenols in teas made by different producers. The total polyphenols content depends on the age of tea leafs, way of harvest, climate of tea-growing, and the conditions of tea storage. In analysing teas the higher polyphenol concentration had green tea II, what might suggested that this tea will have the best antioxidant activity.

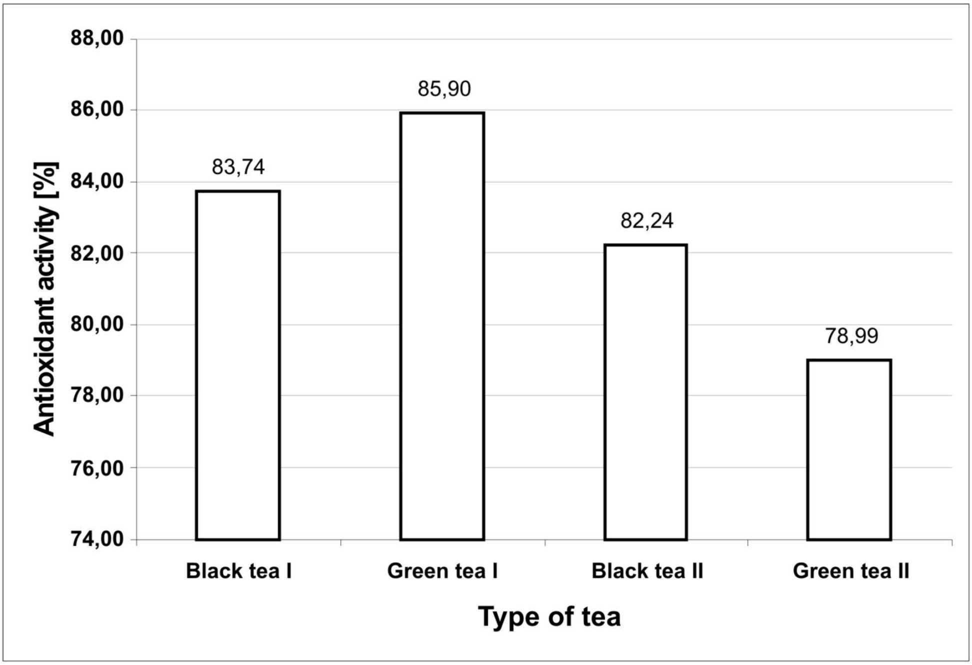

Fig. 4. The antioxidant activity of teas

The mean antioxidant activity values of analysed teas are showed in Figure 5. It was stated that a percentage reduction of DPPH was the highest in green tea I – the mean radical scavenging activity (RSC) was about 85.90%, the lower RSC had both black teas. Basic on statistic analysis it can be stated that, contrary to green teas, in the back teas No. significant differences in antioxidant activity were noted. The worse antioxidant properties of all investigated samples had green tea II. On the basis of the investigation carried out it cannot be stated definitely which type of teas has a better antioxidant properties.

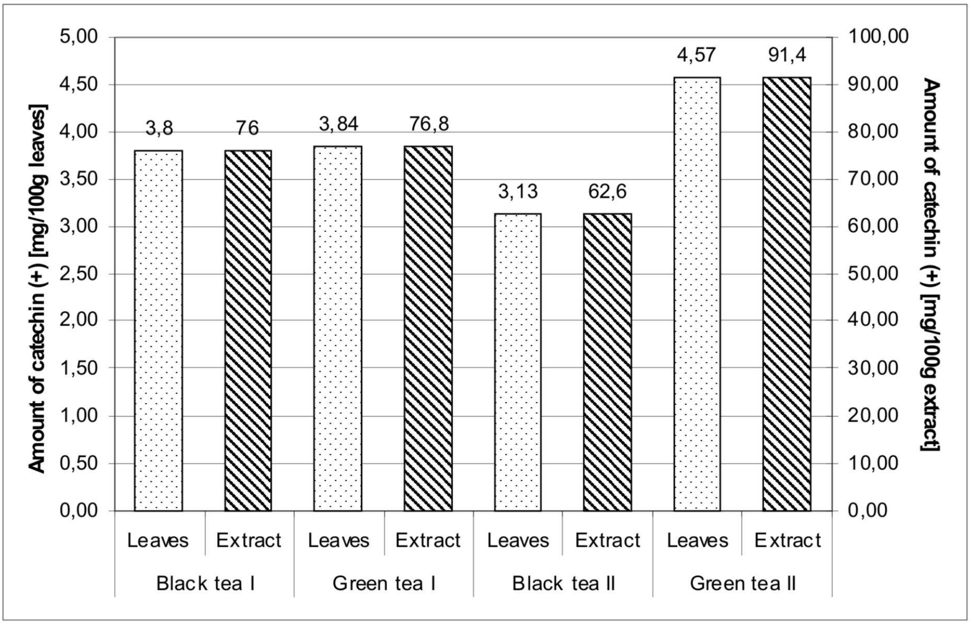

Fig. 5. The amount of total polyphenols converted into (+) catechin

In literature the opinions are on the contrary as well. Some authors prove that green teas have better antioxidant properties as compared to the black teas [Gadow et al., 1997, Lin et al., 1998, Vertuani et al., 2004]. Whereas other authors sated that fully fermented teas have higher radical scavenging activity than green teas [Yen & Chen, 1995]. The results by Fik and Zawiślak [2004] research on antioxidant properties of alcohol tea extract showed that antioxidant activity were as followed: oolong tea > green tea > black tea. The same authors confirmed that antioxidant properties can by different for teas even in the same type.

In conclusion it can be stated that antioxidant properties of tea depend more on polyphenols composition than on production conditions. The kind of catechins in tea depended on the age of tea leaves, way of harvest, climate of grove and the conditions of tea storage. Green tea II, which was characterized by the weakest antioxidant properties among analyzing teas, obtained the lowest scores in the sensory analysis. Its poor quality, that influenced the low antioxidant activity, was probably due to the reasons mentioned above. This study also confirm that theaflavins present in black tea possess at least the same antioxidant potency as catechins present in green tea.

Conclusions

1. The results of the sensory and physicochemical analysis showed that all tested teas were very good or good quality.
2. Green teas were richer in caffeine than black teas. The examined teas contained ~4g and ~3g of caffeine per 100g dry matter of tea, respectively.
3. All analyzed teas had good antioxidant properties, but the antioxidant activity of their extracts was diverse and do independent of production process runs. There were No. difference in the percentage of the reduction of the DPPH between black teas, whereas green teas varied very much.
4. In conclusion, it can be stated that antioxidant properties resulted more from the polyphenols composition in the leaves than from the fermentation process.

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4

APPLICATION OF GAS CHROMATOGRAPHY TECHNIQUE TO DETERMINE THE INFLUENCE OF CHANGE IN RAW MATERIAL CONTENT AND AGGLOMERATION ON THE CONTENT OF VOLATILE COMPOUNDS IN INSTANT COCOA

Introduction

Combining the powdered cocoa with other ingredients such as starch, sugar and powdered milk results in creation of cocoa with additions (in other words a powdered cocoa beverage). Such beverages are characterized by good solubility in milk or water. Foundations of cocoa drink content are formed by a cocoa powder mix (around 20%) and sugar (around 80%) along with such additions as lecithin, powdered milk and aromas. [Kowalska, 2003].

Mixing the cocoa powder with other ingredients in adequate proportions is the easiest way to obtain cocoa with additions. There are also more complex methods used to acquire cocoa beverage and they facilitate its further implementation and recreation. One of these methods is the instantization of powder (agglomeration, spray drying) and coating [Kowalska and Lenart, 2000].

Cocoa grain includes a broad range of flavours and aromas. Currently around 480 different aromatic compounds from 20 different chemical groups can be distinguished. This makes the coca aroma one of the most complicated aromatic compositions. The aromatic profile of cocoa powder forms a complex combination of included raw materials and processes and parameters used in its processing [Groot, 2003].

Compounds providing the characteristic chocolate smell form a valuable ingredient of cocoa grain. They form a very complex group and are composed of fractions of volatile and non-volatile compounds. They are, among others, :L – linalool (filling grains with a flowery flavour and smell), aliphatic acids (capric, capril, valeric), carbonylic derivatives and aromatic acids and lactones [Rosicka-Kaczmarek, 2006a].

The aim of this paper been to analyze the impact of the basic raw material content change, the agglomeration process and the storage time on the content of volatile compounds in powdered cocoa beverages. The extent of this paper covers the analysis of volatile compounds content in cocoa powder and mixtures and their agglomerates both directly after their production and after six months storage time.

Materials and methods

The analyzed material consisted of cocoa powder along with mixtures and agglomerates containing:

1. 10% cocoa and 90% sugar,
2. 10% cocoa, 45% sugar and 45% maltodextrin,
3. 10% cocoa, 45% maltodextrin and 45% whey,
4. 10% cocoa, 45% maltodextrin, 22.5% whey and 22.5% powdered milk,
5. 10% cocoa, 45% sugar and 45% powdered milk,
6. 10% cocoa, 45% sugar, 22.5% powdered milk and 22.5% whey.

Basic composition of the mixture included 10% cocoa and 90% sugar. The change in basic raw material content concerned the partial or full replacement of sugar with another raw material – maltodextrin, powdered milk and/or whey.

Technological methods covered the processes of mixing and agglomerating. They were conducted in the STREA 1 fluid bed agglomerator from Niro Atomizer A/S company. Mixtures were obtained by mixing of the weighed ingredients for 2 minutes after turning them into a fluidal state inside the agglomerator. All mixtures were agglomerated with water which was batched by pump to the liquid spraying nozzle – a thin layer sprayed over the agglomerated material. The content of volatile compounds was determined in obtained mixtures and agglomerates and they were subsequently placed in plastic bags with heat seals 1– the ones used by producers of powdered cocoa beverages.

The analysis of volatile compounds was conducted using gas chromatography coupled with the mass spectrometer [Frauendorfer and Schieberle, 2006]. It was preceded by incubation of analyzed material in the temperature of 50˚C for 20 minutes. The percentage share of particular volatile compounds in its whole profile was calculated on the basis of the surface area under peaks.

The analysis of volatile compounds in tested products was conducted directly after obtaining and storing them.

Results and discussion

it is difficult to evaluate which volatile compounds have significant influence upon the aroma and which do not. Not all identified single compounds exercise any significant influence upon the aroma perception because it is dependent on the concentration of scent and its intensity [Bonvehi, 2005].

Pirazines are abundant in cocoa and can be used as determinants for cocoa aroma [Bonvehi, 2005]. A compound from this group was present in analyzed products but in quantities below 0.5%.

Conducted analysis revealed 58 compounds belonging to different chemical groups, they were dominated by 14: acetic acid, 2,3-butanediol, 3-methylbutanoic acid, 3-buten-2-one, 2,2,4-trimethylpentane, thioamide derivative, benzaldehyde, tetrasiloxane, 2-furanmethanol, acetic acid amide, 1-dodecanol, dihydro-2(3H)-furanone, butyric acid, 3-hydroxy-2-butanone (Tab. 1).

Table 1

The analysis of volatile compounds in tested products was conducted directly after obtaining [**%**]

	1	7	2	8	3	9	4	10	5	11	6	12
acetic acid	87.34	64.36	90.29	74.08	87.38	83.32	85.68	86.88	90.28	89.56	89.46	82.37
2.3-butanodiol	5.87	7.56	6.98	4.01	6.28	2	6.77	3.48	7.16	4.78	6.97	3.05
izovalerian acid	0.63	1.83	0	0	0.86	0.71	1.15	0.8	0	0	0.9	0.65
3-buten-2-on	0	0	1.6	5.46	0.97	1.6	1.62	4.28	1.39	3.25	1.44	1.96
2.2.4-trimetylo-pentan	4.74	4.72	0.5	3.58	7.82	6.84	4.37	0.67	0	0	2.45	3.58

1 mixtures and 7 agglomerates – 10% cocoa and 90% sugar.
2 mixtures and 8 agglomerates – 10% cocoa, 45% sugar and 45% maltodextrin.
3 mixtures and 9 agglomerates – 10% cocoa, 45% maltodextrin and 45% whey.
4 mixtures and 10 agglomerates – 10% cocoa, 45% maltodextrin, 22.5% whey and 22.5% powdered milk.
5 mixtures and 11 agglomerates – 10% cocoa, 45% sugar and 45% powdered milk.
6 mixtures and 12 agglomerates – 10% cocoa, 45% sugar, 22.5% powdered milk and 22.5% whey.

Acetic acid and 2,3-butanediol were present in all analyzed samples both before and after the storage period. Further analysis of agglomeration. storage and content for the presence of volatile compounds was conducted on the basis of considering dominant compounds in a given raw material content. An average from two parallel repetitions was used to obtain the final result.

Such a high presence of the acetic acid could have resulted from the fact that the cocoa powder used for the purpose of testing did not undergo any alkalization process. According to Bixler and Morgan [1999] this process helps in partial reduction of the acetic acid formed mainly in the process of fermentation.

In the conducted analyses the significant volatile compounds constituted those which peak area stayed ≥5% and additionally as the result of those peaks identification the library confirmed presence of a particular compound with probability not higher than 50%.

Product containing 10% cocoa, 90% sugar was conceded as the basic mixture to which all other results obtained for different samples were compared. The highest percentage share within the volatile compounds profile was acquired for the acetic acid (around 90%) and the lowest for the 3-methylbutanoic acid (around 1%).

Conducted analyses showed the higher content of acetic acid in analyzed samples in comparison to the basic mixture. Only in the mixture no. 4 containing 10% cocoa, 45% maltodextrin, 22.5% milk, 22.5% whey the content of acetic acid was a little lower and amounted to 85.7%.

The content of 2,3-butanediol and 3-methylbutanoic acid in all mixtures increased in comparison to the standard product. In case of 3-methylbutanoic acid these differences were bigger and equalled from 36% for the product containing 10% cocoa, 45% maltodextrin, 45% whey to 82% for the product no. 4 (10% cocoa, 45% maltodextrin, 22.5% powdered milk, 22.5% whey). For 2,3-butanediol differences were set from 7% for the product no. 3 (10% cocoa, 45% maltodextrin, 45% whey) to 22% for the product no. 5 (10% cocoa, 45% sugar, 45% powdered milk).

In the mixture of 10% cocoa, 45% maltodextrin, 45% whey the content of 3-buten-2-one in relation to the mixture no. 1 was lower by around 13% while 2,2,4-trimethylpentane content increased by around 65%. For other mixtures a reversed pattern was observed. In products no. 2, 4, 5, 6 the content of 3-buten-2-one was higher than in the primary mixture and equalled on average from 24% (product no. 5) to 45% (product no. 2). In the same products the content of 2,2,4-trimethylpentane was lower in the sample no. 2 (by 89%) and equalled to 0.5% while in the product no. 4 it equalled to 4.37% and was lower by about 8%.

The statistical analysis revealed a significant influence of the raw material content change of the aforementioned mixtures upon the presence of volatile compounds.

Five dominant compounds were discovered in agglomerates: acetic acid, 2,3-butanediol, 3-methylbutanoic acid, 3-buten-2-one, 2,2,4-trimethylpentane. Acetic acid was most abundant among these compounds with its average 80% content, 3-methylbutanoic acid occurred in lowest quantities (around 1%).

In relation to the primary agglomerate which contained 10% cocoa, 90% sugar the content of acetic acid in analyzed samples was higher and equalled from 15% for the product no. 8 to 39% for the product no. 11 containing 10% cocoa, 45% sugar, 45% powdered milk up to 74% for the sample containing 10% cocoa, 45% maltodextrin, 45% whey.

Agglomerates in which the 3-methylbutanoic acid was present (9, 10, 12) contained it in lower quantities than the product of primary content. It was lower by 61%, 56%, 64% respectively. The presence of 2,2,4-trimethylpentane was detected in all agglomerates apart from sample no. 11 containing 10% cocoa, 45% sugar and 45% powdered milk. Only the agglomerate containing 10% cocoa, 45% maltodextrin, 45% whey had higher amount of 2,2,4-trimethylpentane than in the primary content sample and equalled to 6,84%. 3-buten-2-one was not detected in the primary content agglomerate but it was present in other agglomerated products in amounts from 1.6% in the sample containing 10% cocoa, 45% maltodextrin, 45% whey to 5.46% in the agglomerate no. 8 (10% cocoa, 45% sugar, 45% maltodextrin).

The statistical analysis showed the statistically significant impact of the aforementioned agglomerates' content upon the presence of volatile compounds.

There were five dominant volatile compounds found among analyzed mixtures and agglomerates: acetic acid, 2,3-butanediol, 3-methylbutanoic acid, 3-buten-2-one, 2,2,4-trimethylpentane.

Acetic acid was characterized by the highest percentage share (over 80%) among specified volatile compounds. On the other hand the lowest percentage share was demonstrated by the 3-methylbutanoic acid and its value equalled to around 1%.

In six cases the content of acetic acid was lower in the agglomerate than in mixture. The highest decrease in this acid's content (over 26%) was observed in the agglomerate with 10% cocoa, 90% sugar. In case of products containing 10% cocoa, 45% maltodextrin, 22.5% powdered milk and 22.5% whey and 10% cocoa, 45% sugar, 45% powdered milk the content of acetic acid in both mixture and agglomerate was similar and equalled to 86 and 90%.

The lower content of 2,3-butanediol (by around 29 and 18 % in comparison to corresponding agglomerates) was observed for mixtures containing 10% cocoa, 90% sugar and 20% cocoa, 80% sugar. Additionally mixtures were characterized by the lower content of 3-methylbutanoic acid in comparison to values acquired for products agglomerated with the same raw material content. In case of the first mixture this value was three times lower. The content of 2,3-butanediol in all other samples was higher in mixtures than in agglomerates. And for the product containing 10% cocoa, 45% sugar, 45% powdered milk the difference in the analyzed compound quantity between mixture and agglomerate was the lowest and equalled to 33% but for the product containing 10% cocoa, 45% maltodextrin, 45% whey the most significant difference was noted (68%). The similar tendency was observed for 3-methylbutanoic acid which was present in mixtures no. 3, 4, 6 in higher quantities than in agglomerates of the same raw material content.

Among volatile compounds present in analyzed products only two were present in all mixtures and agglomerates: acetic acid and 2,3-butanediol. A decrease in acetic acid content was observed as the result of the agglomeration process. Moreover, along with the increase of non-sugar components in mixtures – the content of acetic acid in particular agglomerates increased, however these values remained lower in relation to corresponding mixtures.

As the result of the composition change of particular mixtures it can be noted that there was one product behaving differently from all the others – mixture of 10% cocoa, 45% maltodextrin, 45% whey. The average value of 3-buten-2-one was lower in this mixture than in the remaining ones while the content of 2,2,4-trimethylpentane was higher. Moreover, the content of 2,3-butanediol and 3-methylbutanoic acid in mixtures decreased as the result of change in composition of particular mixtures. An opposite pattern took place for agglomerated samples.

Analysis concerning volatile compounds present in cocoa beverage samples of modified raw material composition was also carried out after 6 months of storage time. It was assumed that a consumer does not consume the whole quantity of instant cocoa right after opening the package but that this process usually takes a couple of months.

3 compounds which were detected in primary analyses were also present among dominant compounds in mixtures and agglomerates after 6 months of storage time. They were: acetic acid, 2,3-butanediol and 3-methylbutanoic. They reacted in a similar way as before storage but the differences between mixtures and agglomerates were slightly smaller. Moreover, along with the increase in raw material content used to replace sugar in agglomerates also a decrease in benzaldehyde content could be noted.

The statistically significant influence of the raw content change, agglomeration and storage was noted on the basis of acquired results.

There were 12 dominant compounds found in analyzed samples stored for 6 months. They included: acetic acid, 2,3-butanediol, thioamide derivative, benzaldehyde, tetrasiloxane, 2-furanmethanol, 3-methylbutanoic, acetic acid amide, 1-dodecanol, dihydro-2(3H)-furanone, butyric acid, 3-hydroxy-2-butanone (Tab. 2).

Acetic acid was dominant among the aforementioned compounds. It was present in the amount of around 65% in the agglomerate no. 7 to around 90% in mixtures no. 2 and 5. In most of the analyzed products agglomeration caused a decrease in the acetic acid content in comparison to mixtures of the same raw material content. Only samples no. 4 and 6 (with respectively 10% cocoa, 45% maltodextrin, 22.5% whey and 22.5% powdered milk and 10% cocoa, 45% sugar, 22.5% powdered milk and 22.5% whey) did not demonstrate any significant difference in the content of acetic acid between mixtures and agglomerates.

Analysis of the 2,3-butanediol and 2-furanmethanol content in most samples showed a lower amount of analyzed compounds in agglomerates in comparison to mixtures of the same raw material composition. In case of 2,3-butanediol on average by around 12% and it was situated within range from 6.41% for the agglomerate no. 15 to 14.82% for the product number 11.

Table 2

The analysis of volatile compounds in tested products was conducted after storing them [**%**]

	1	7	2	8	3	9	4	10	5	11	6	12
A	61.55	60.39	58.08	58.37	75.72	68.23	68.26	64.57	74.43	71.41	74.7	77.23
B	14.82	14.82	15.42	12.83	6.53	6.41	8.2	7.53	9.02	8.54	9.3	7.25
C	1.2	2	0	0	0.64	2.11	1.97	2.87	0.87	1.74	1.36	1.16
D	1.64	2.14	1.88	1.74	0.64	0.58	0	0	0	0	0	0
E	1.98	1.82	2.08	3.62	0.92	1.55	1.44	1.86	0.68	0.97	0.76	0.6
F	1.51	1.05	2.72	1.88	0.78	0.77	0.86	0.66	0.7	0.7	0.96	0.75
G	3.25	4.72	4.48	3.52	3.02	2.37	3.98	2.37	0	0	3.51	2.95
H	1.43	1.44	0	0	0	0	0	0	0	0	0	0
I	8.28	5.88	9.08	12.2	2.37	4.64	2.95	3.49	1.72	2.5	2.34	1.79
J	3.61	4.25	5.46	4.27	1.92	1.8	1.78	1.47	1.47	1.6	2.05	1.72
K	0	0	0	0	4.98	4.93	6.17	6.38	5.48	5.05	2.84	4.34
L	0	0	0	0	1.2	1.33	1.62	1.56	1.42	1.28	0	0

A) acetic acid
B) 2,3-butanediol
C) thioamide derivative
D) benzaldehyde
E) tetrasiloxane
F) 2-furanmethanol
G) 3-methylbutanoic
H) acetic acid amide
I) 1-dodecanol
J) dihydro-2(3H)-furanone
K) butyric acid
L) 3-hydroxy-2-butanone

1 mixtures and 7 agglomerates – 10% cocoa and 90% sugar.
2 mixtures and 8 agglomerates – 10% cocoa, 45% sugar and 45% maltodextrin.
3 mixtures and 9 agglomerates – 10% cocoa, 45% maltodextrin and 45% whey.
4 mixtures and 10 agglomerates – 10% cocoa, 45% maltodextrin, 22.5% whey and 22.5% powdered milk.
5 mixtures and 11 agglomerates – 10% cocoa, 45% sugar and 45% powdered milk.
6 mixtures and 12 agglomerates – 10% cocoa, 45% sugar, 22.5% powdered milk and 22.5% whey.

In case of 2-furanmethanol, however, products marked with numbers: 9 and 11 contained on average around 0.7% of this compound both in mixture and agglomerate. In the remaining products marked 7, 8, 10, 12 a decrease in 2-furanmethanol content was noted by 23% on average in relation to mixtures.

The thioamide derivative was present in nearly all analyzed products and its average content in mixture was lower than in an agglomerate. The greatest differences were achieved for the sample with 10% cocoa, 45% maltodextrin, 45% whey where the content of this compound increased 3 times. Sample marked with no. 8 (10% cocoa, 45% sugar, 22.5% milk, 22.5% whey) was an exception along with its corresponding agglomerate in which the percentage share of thioamide derivative decreased by 15% in relation to the mixture.

Benzaldehyde was present in 5 products. A decrease in its content due to agglomeration process was noted in 3 mixtures combining cocoa and sugar. This difference was the highest (nearly 3 times) in a product containing 20% cocoa, 80% sugar. In case of mixtures with 10% cocoa, 45% sugar, 45% maltodextrin and 10% cocoa, 45% maltodextrin, 45% whey its content was higher in a mixture than in an agglomerate by 7 and 9% respectively.

A similar situation was observed for tetrasiloxane and 1-dodecanol in analyzed products. Mixtures marked with no. 1 and 6 contained more of these compounds than the corresponding agglomerates. For tetrasiloxane these differences were 8% and 21% respectively but for 1-dodecanol – 29% and 24%. For the remaining samples the situation was reversed.

Products containing 10% cocoa, 90% sugar and 20% cocoa, 80% sugar were noted for significantly higher content of 3-methylbutanoic and dihydro-2(3H)-furanone in an agglomerate than in mixture. In case of this last compound a similar situation could be observed in relation to the product with 10% cocoa, 45% sugar, 45% powdered milk. For the remaining samples a reversed pattern was observed.

Butyric acid and 3-hydroxy-2-butanone were present in samples 3, 4, 5, 6 and corresponding agglomerates. In case of the butyric acid in products containing 10% cocoa, 45% sugar, 45% powdered milk (number 5) its quantity in the mixture was higher than in the agglomerate by around 8%. The most significant difference between a mixture and an agglomerate was obtained for a sample containing 10% cocoa, 45% sugar, 22.5% powdered milk and 22.5% whey and it amounted to 53%.

3-hydroxy-2-butanone in product containing 10% cocoa, 45% maltodextrin, 45% whey had 11% lower value than in an agglomerate. The quantity of this ketone as a result of agglomeration decreased by 4 and 10% in products containing 10% cocoa, 45% maltodextrin, 22.5% powdered milk, 22.5% whey and 10% cocoa, 45% sugar, 45% powdered milk.

The statistical analysis demonstrated statistically significant influence of agglomeration upon volatile compounds content after storage period in each type of analyzed mixture.

For stored products it was observed that the agglomeration allowed to keep volatile compounds in analyzed products to a greater extent. It was best visible on example of acetic acid. Mixtures which did not undergo this process contained on average 25% less acetic acid. As the result of agglomeration, however, its average loss amounted to around 20%.

Conducted analyses demonstrated a dominant share of four volatile compounds: acetic acid, 2,3-butanediol, dihydro-2(3H)-furanone and butyric acid. The value of acetic acid decreased as in case of mixtures as a result of storage while the quantity of 2,3-butanediol increased. The content of acetic acid in all agglomerates remained on the average level of 70%. Dihydro-2(3H)-furanone was present only in an agglomerate containing 10% cocoa, 90% sugar and its value increase over 4 times as the result of storage. Butyric acid however, was present in samples 9, 10, 11, 12 and its value also increased due to storage – 8 times on average.

Statistical analysis demonstrated a significant influence of storage time on the content of volatile compounds in all analyzed agglomerates.

Conclusions

1. There were 14 volatile compounds dominant in conducted analyses belonging to different groups. They included: aldehydes, ketones, alcohols and acids. The two compounds detected in each of the analyzed products were: 2,3-butanediol and acetic acid (which had the highest percentage share in the volatile compounds' profile).
2. The partial or full replacement of sugar with other ingredients influenced the profile change of dominant compounds. In most of the analyzed products introduction of a new raw material resulted in the increase of acetic acid and 2,3-butanediol content.
3. Agglomeration process caused a decrease in dominant volatile compounds content in most analyzed products in comparison to mixtures of the same composition. Only two products (no. 4 and 5) which contained powdered milk demonstrated an opposite pattern.

4. A decrease in loss of volatile compounds as a result of agglomeration process was observed in stored products in relation to non-agglomerated mixtures which was most visible on example of acetic acid.

5. A statistically significant influence of raw material content change, agglomeration and storage upon the presence of volatile compounds in analyzed products was observed.

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5

PHYSICOCHEMICAL PROPERTIES OF HONEYDEW HONEYS

Introduction

The honey is a natural sweet substance produced by honey bees *Apis mellifera* from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature [Directive, 2001].

The honey-bee honey, consumed mainly in unprocessed form, is a pure natural product, the quality of which depends on a forage it was collected from – nectar or honeydew. The geographical and botanical differentiation of Poland may affect the forage characteristics and thus, the honey quality. Depending on a raw material it was produced from, three honey types are distinguished: nectar, honeydew, and mixed (honeydew-nectar or nectar-honeydew).

Honeydew is a plant juice processed by insects, mainly aphids. Depending on the tree type, honey-bees collect honeydew from, the leafy-tree or coniferous-tree types are recognized. The honeydew honeys present in Poland are mainly produced from the fir and spruce honeydew [Bornus, 1989]. Its dark colour, both before (from green-tea to almost black) and after crystallization (brighter, dark-brown, grey-green) is characteristic. In general, honeydew honeys are smooth in flavor and less sweet than nectar ones. Honey made of coniferous-tree honeydew are nice and spicy scent, and smooth and slightly sweet flavor with resinous aftertaste. Honeys achieved from leafy-tree honeydew are characterized by less apparent scent and sharper aftertaste [Popek, 2002; Rybak-Chmielewska, 1986].

The aim of present study was to determine the quality of honeydew honeys, their evaluation referring to obligatory legal acts, as well as to verify their authenticity.

Material and Methods

The study material consisted of 6 honeydew and nectar-honeydew honeys. Three samples were made of leafy-tree honeydew, while two were nectar-honeydew, including one with declaration of leafy-tree honeydew. Five of six examined samples originated from Poland, whereas one was declared as "made in EU".

The physicochemical tests made on the study material included determinations of: water content by means of refractometry (PN-88/A-77626), sucrose, glucose, and fructose contents by means of enzymatic method [Boehringer Mannheim, 2008], calculating the glucose to fructose ratio, specific electrical conductivity measure (PN-88/A-77626), total ash content

[Codex Stan, 1981], polyphenols content by means of Folin–Ciocolteu's method [Kumazawa et al., 2002], antioxidant capacity against $DPPH^{\cdot}$ radicals [Yen, Chen, 1995], and analysis of volatile substances profiles.

The ethanol extracts of honeys were prepared to determine the polyphenols contents and antioxidant capacity: 3 g of honey was added into 30 ml of ethanol and shaken for 1 hour in automatic shaker, then the solution was remained for 24 hrs at room temperature. Next, extracts were filtered. The total polyphenols contents were determined on a base of colour reaction invoked due to Folin-Ciocolteu's reagent and sodium carbonate addition. The absorbance of produced colour complex was measured at λ=700 nm wavelength. Results were expressed as recalculated onto gallic acid. The antioxidant capacity of analyzed extracts was determined by measuring their ability to neutralize the stable synthetic $DPPH^{\cdot}$ radicals. The method consists in adding the anti-radical substances into the methanol $DPPH^{\cdot}$ solution that shows the absorbance at λ=517 nm at its radical form. The antioxidant activity against $DPPH^{\cdot}$ was calculated on a base of the formula:

$$A = [(A_K - A_{spec})/A_K] \times 100\%,$$

where: A – anti-radical activity [%], A_K – absorbance of the control, A_{spec} – absorbance of the sample.

The technique of micro-extraction into SPME solid phase through adsorption the substances on a Divinylobenzene/Cerboxen/Polidimetylosiloxan fiber was applied to extract volatile compounds from honey over-surface phase. The analysis of volatile compounds was carried out in gas chromatograph coupled with mass spectrometer (SHIMADZU GCMS-QP2010S). The gas chromatograph was equipped in capillary column of DB–5 type 30 m × 0.25 mm × 0.5 μm (non-polar phase with 5% share of phenyl groups).

Gas chromatograph (GC): carrier gas – helium, carrier gas flow rate – 1.0 cm^3/min, sample injected in *splitless* system. The column temperature program: initial temp. 40°C, isotherm 3 minutes, increase by 5°C/min till temp. 180°C, and final isotherm for 10 min, temperature of injection chamber 230°C.

Mass spectrometer (MS): temperature of ion source 175°C, interface temperature 175°C, detector voltage 0.9 kV, working in *Scan* system, molecular ions of determined substances were monitored within quadrupole filter sweeping range 35–600 m/z.

Aliquots of 5 g honey, 3 g sodium chloride (after de-aromatization), and 20 cm^3 previously boiled and cooled water, were added into the glass de-aromatized bottle. The bottle was hermetically closed and components were stirred on an electric stirrer. The system was conditioned for 20 min at 30°C. Volatile compounds were adsorbed from the system by exposing the SPME fiber over the sample for 20 minutes at 30°C. Then, the fiber was thoroughly placed at the same position as before extraction to achieve maximum result reproducibility. After that, the fiber was placed within the injector chamber, where it was remained for 1 minute to desorb volatile substances into the capillary. The method was optimized experimentally.

Identification of compounds was made on a base of comparisons of achieved mass spectra with those collected in a library Wiley 7N2 and NIST 147. The GC-MS Lab Solutions software was used. Comparison of the studied compounds contents can be possible on a base of their separated peaks areas.

Results and discussion

Water is an important component of a honey and it is carried from the forage. Nectar may contain even up to 70%, while honeydew 50% of water. It is evaporated during the honey production. Water contents in examined honeys (Table 1) ranged from 17.5 to 22.1%. Level of water content in honeys permitted by Polish Norm [1988], EU Directive [2001], and Nutritional Codex [Codex Stan, 1981] amounts to 20%. That value was exceeded in 2 samples: No. 1 and No. 5; in the first case, the result was slightly different from the norm (20.1%), while in the second, the exceeding was 2.1%. Higher water content may indicate that the honey was centrifuged from the honeycombs at improper time, when the water had not been evaporated yet and had been immature [Szczęsna, 2003]. Studies performed by Popek [1997] revealed water contents for the honeydew honey at the level of 16.1%, i.e. the same value as that achieved by PersaNo. Oddo et al. [2004]. Čelechowská [2001] reported slightly lower water content (15.6%). Values recorded in present study and by particular researchers are more or less distinct to one another, which may result from different sample origins and different climatic conditions in a year the honey was collected.

Table 1

Physicochemical characteristics of honeydew honey

Lp.	Sample honey	Moisture [%]	Sucrose [%]	Glucose [%]	Fructose [%]	G+F [%]	F/G	Conductivity [mS/cm]	Ash [%]
1	Honeydew coniferous	20,1	6,6	21,9	26,0	47,9	1,19	1,06	0,56
2	Nectar-honeydew	17,5	4,6	32,5	33,1	65,5	1,02	0,56	0,24
3	Honeydew coniferous	18,9	4,4	32,1	31,1	63,2	0,98	0,83	0,54
4	Nectar-honeydew	19,7	3,0	30,1	35,9	66,0	1,19	0,56	0,27
5	Honeydew deciduous	22,1	2,6	27,9	38,4	66,3	1,38	0,65	0,27
6	Honeydew coniferous	18,9	4,3	25,8	34,4	60,2	1,34	0,74	0,39

Sucrose is present abundantly in forage, but it is decomposed onto simple sugars (glucose and fructose) due to enzymes contained in honey-bee saliva. Therefore, the sucrose content in a honey decreases in relation to that in forage, whereas the quantities of simple sugars increases [Wojtacki, 1988]. Results on sucrose content achieved for all studied samples (Table 1) were similar. However, a single of six samples exceeded permissible level, that according to legal acts on honey quality, should not be higher than 5%. Polish Norm [1988] permits slightly higher sucrose contents, but when considered as sucrose plus melecitose combined content. Honey No. 1 containing 6.6% of sucrose was inconsistent with norms. PersaNo. Oddo et al. [2004], in their study upon sucrose content in honeydew honeys, recorded 0.8%. The sucrose amount in samples examined by Čelechowská [2001] oscillated around 2.5%. Analysis carried out by Sorkun et al. [2002] revealed the sucrose quantity (5.9%) that exceeded obligatory limits. All above researchers applied HPLC technique to determine the sucrose content in honeydew honey samples, and despite of this, their results differ to one another, which may be a result of different maturity of examined honeys. However, data achieved in present study – with a help of enzymatic method – are comparable to those recorded by majority of authors.

The level of **glucose plus fructose sum** in analyzed honeydew honeys (Table 1) amounted to over 60%. Only in sample No. 1, it was lower (47.9%). According to standardization documents for honeydew honeys, determined value should not be lower than 45%. Polish Norm is more restrictive, because permissible levels for nectar-honeydew honeys are set for No. less than 65%, while for honeydew honeys – not less than 60%. Here analyzed samples of nectar0honeydew honeys (No. 2 and No. 4) met requirements of Polish Norm, because glucose and fructose sum amounted to 65.5 and 66.0%, respectively. Among other samples, No. 1 did not reach the level permissible by Polish Norm [1988]. It is probable that the honey was falsified with sucrose (exceeded value), e.g. by feeding the honey-bees or it was collected from the beehive too soon, and hence complete sucrose decomposition into simple sugars could not occur [Bąk and Wilde, 2004].

When analyzing the reducing sugars, the **fructose to glucose ratio (F/G)** should be also considered. These values are not included in legal acts; however, they are characteristic for a given honey type. In the case of examined honeys, the F/G coefficient values ranged from 0.98 to 1.38. A proper range for that parameter described by Curyło et al. [1978] should be from 1.03 to 1.3. Among recorded data, only two samples got into the recommended range: No. 1 and No. 4, while other values slightly differed from the limits. Results on reducing sugars for honeydew honeys recorded by Yanniotis et al. [2005] indicated that fructose to glucose ratio was 1.19, whereas PersaNo. Oddo et al. [2004] reported that parameter as amounting to 1.25.

The **electrical conductivity** is a parameter that makes possible to estimate the honey's botanical origin, i.e. indicates the forage type it was produced from. Its value depends on the amount of minerals in honey. The higher minerals quantity, the higher electrical conductivity, as well. Measurements of the electrical conductivity made in present study revealed its variations for particular honey samples (Table 1). Samples No. 1 and No. 3 were characterized by electrical conductivity higher than 0.8 mS/cm, hence they met requirements for honeydew honey, for which legal norms set the level as not lower than 0.8 mS/cm. Other honey samples revealed electrical conductivity below 0.8 mS/cm; however, in the case of No. 2 and No. 4 (nectar-honeydew honeys), they are good results at the level of (both samples) 0.56 mS/cm. Thrasyvoulou and Maniks [1995] examined honey samples made of pine-tree and fir-tree honeydew and recorded following values of electrical conductivity: 1.26 mS/cm and 1.40 mS/cm, respectively. Tsigouri et al. [2004] achieved similar results: 1.24 mS/cm and 1.44 mS/cm for the same types of honey samples. When comparing here achieved values with literature data, it becomes apparent that here analyzed honeys were characterized by much lower electrical conductivities.

The **ash** content in examined honeys ranged within 0.24–0.56% (Table 1). The parameter is not included in legal documents, but is closely associated with the electrical conductivity. Values recorded by other researchers were similar to those here achieved. Popek [2000] reported the ash content in honeydew honey as 0.56%. Similar level – 0.53% – was achieved by Čelechovská [2001]. Nevertheless, there was a difference of ash contents in pine-tree and fir-tree honeydew honey samples in studies made by Thrasyvoulou and Maniks [1995]: 0.6% and 0.9%. The percentage of ash content is closely associated with specific electrical conductivity. It was confirmed by high correlation coefficient (0.942) for studied honeys (Figure 1). Furthermore, the ash content was also lower in honey samples No. 4 and No. 5 that did not reach the minimum values of electrical conductivity. Majewska [1999] also recorded a significant correlation between above parameters – the correlation coefficient for dark honeys was calculated as 0.982.

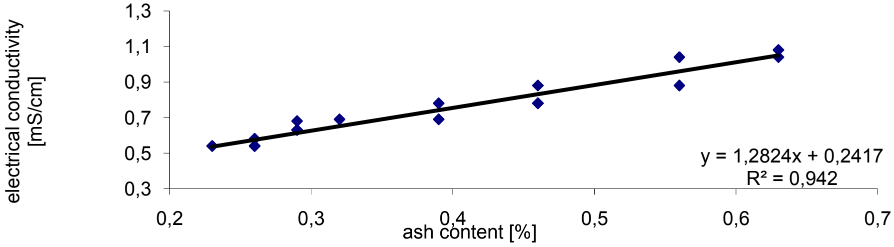

Fig. 1. Relationship between ash content and electric conductivity in honeydew honey.

Antioxidant capacity is determined by honey's composition, among which polyphenols, peptides, organic acids, enzymes, Maillard's reaction products, and other compounds are important [Al-Mamary, 2002].

The polyphenols contents (Figure 2) in examined honeydew honeys oscillated within the range from 16.85 to 72.39 recalculating onto mg of gallic acid /100g honey. The antioxidant activity (Figure 2), against DPPH˙ radicals amounted to about 9.88–20.32%. Comparison of both parameters reveals a great differentiation of achieved results. In samples No. 1 and No. 4, polyphenols content was slightly higher than antioxidant capacity. Higher differences could be observed in other samples. Probably, it resulted from determination also other compounds than polyphenols only. High level of polyphenols in honey No. 2 may resulted from high proline content, while much lower antioxidant activity against DPPH˙ radicals was perhaps associated with the honey type (nectar-honeydew). A similar situation occurred in the case of sample No. 4, that showed similar antioxidant capacity against DPPH˙ radicals as No. 2. The nectar honeys are usually characterized by lower antioxidant capacity as compared to the honeydew ones.

Contents of polyphenols recorded in studies performed by Med et al. [2005] was at the level of 113.05 mg gallic acid /100 g honey. Moreover, the honeydew honey was characterized by the highest polyphenols content among all analyzed samples. Analyses made by Ouchemoukh et al. [2007] also confirmed the highest polyphenols quantities in honeydew and nectar-honeydew honeys, amounting to 1304 and 714 mg gallic acid/100 g honey. Beretta et al. [2005] found much lower levels of polyphenols (25.56 mg gallic acid/100g honey). The antioxidant activity recorded by Beretta et al. [2005] was slightly lower than here achieved results and amounted to 8.48%. Baltrušaitytė et al. [2007] reported the antioxidant activity against DPPH˙ radicals for coniferous-tree and leafy-tree honeydew as 93 and 90.2%, respectively. Studies made by Bertoncelj et al. [2007] revealed the antioxidant capacity against DPPH˙ radicals for 8.2%. A significant divergence between above results may resulted from different origins of the samples, referring to forage collection time and place.

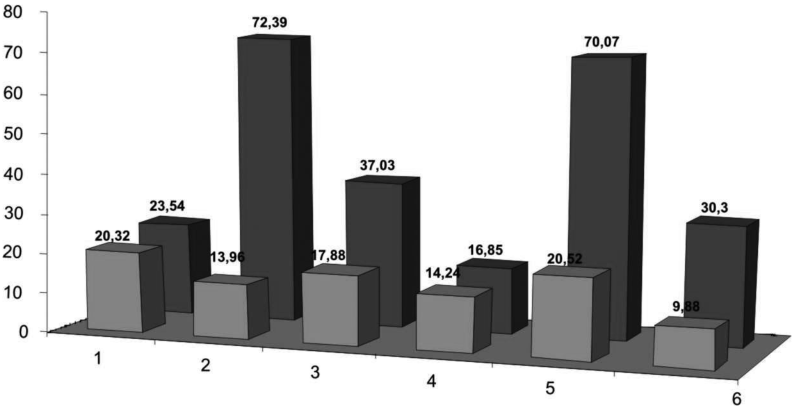

Fig. 2. Comparison antioxidant capacity (blue) and polyphenols contents (brown)

The qualitative analysis of **volatile substances profile** of examined honeydew honeys applying SPME GC-MS technique made possible to identify 104 compounds (Figure 3), including: aldehydes, alcohols, esters, furanes, ketones, acids, benzene derivatives, and carbonates. Research performed by Plutowska and Wardencki [2007] upon honeys confirmed the occurrence of above compounds.

Recorded results indicated that analyzed honeydew honeys had slightly different compositions of volatile fraction. It should be on mind that studied samples contained coniferous-tree or leafy-tree honeydew, and moreover, honeys No. 2 and No. 4 were nectar-honeydew type, although the nectar type was unknown. Therefore, slightly different nectar flow type, the honey was produced, was the reason of different volatile substances presence. However, these differences allowed for characterizing and evaluating the authenticity of studied samples, despite of quite great variations in identified compounds. It can be done on a base of the presence (or absence) of only one or several compounds occurring in only one type of analyzed honey. Part of volatile substances could not be reliable markers, because they were found also in other samples. Therefore, they were not taken into considerations during chromatograms analysis.

Achieved results allow for accepting the following compounds as characteristic for leafy-tree honeydew honeys: phenyl-tert-buthyl-isopropoxyborate and oxido-cis-limonene, because their shares were quite high. Also 3,7-dimethyl-1,5,7-octatriene-3-ol, occurring also in honey No. 2 containing nectar and leafy-tree honeydew, can be considered as characteristic. Moreover, No. nonenal and 2-methylofurane were not identified in sample No. 5, although it was detected in honey No. 2, thus there was a suspicion that the honey contained the coniferous-tree honeydew instead the leafy-tree one. 1-iodomethoxyprop-2-yn was identified in samples No. 2 and No. 4 being the nectar-honeydew honeys, which may indicate the presence of the same nectar forage. According to obtained data, the coniferous honeydew may be characterized by 2-methylbutanal. The qualitative analysis allowed for botanical distinguishing of determined samples and confirmed that it is useful to verify the botanical authenticity of honeys. Therefore, the detailed qualitative analysis of chromatograms for particular honey types, on the base of which the authentication of other samples could be possible, would be useful, as well. In addition, enhancing of these studies by quantitative analysis would make possible to differentiate samples referring to their geographic origins.

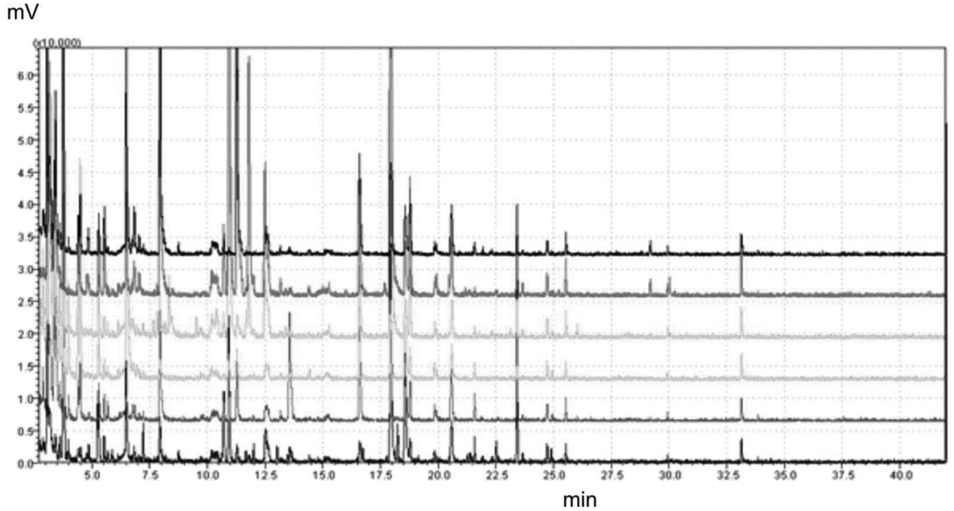

Fig. 3. Chromatogram profiles obtained by GCMS analysis of honeydew honey (in turn from above honey No: 4, 2, 3, 1, 6, 5).

Conclusions

1. A set of physicochemical determinations make possible to evaluate the quality of tested honeydew honeys at the high level and allows for confirming the authenticity or indicates some falsifications.
2. Honey No. 1 did not reach its limits referring to several parameters, which suggests that it was collected from a beehive too soon, thus a proper water amount was not evaporated and enzymatic sucrose decomposition process was not completed.
3. Not meeting the limits of electrical conductivity by honey samples No. 5 and No. 6 suggests that the nectar honey characterized by much lower content of minerals, was added into them.
4. A strict dependence between specific electrical conductivity and crude ash content suggests the equivalence of both methods for mineral sum determination, due to which analysis time can be much shortened, when conductometry is applied to determine the ash content.
5. Applied qualitative analysis of volatile substances profile made possible to botanical differentiate of examined products and confirmed that method of micro-extraction to solid phase (SPME) applying gas chromatography coupled with mass spectrometry (GC-MS) appeared to be useful in estimating the botanical authenticity of honeydew honeys.

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6

DETERMINATION OF BIOTIN CONTENT IN MOLASSES AND YEAST SAMPLES

Introduction

Biotin (vitamin H – for German word "Haut" – skin) is one of the most important cofactors involved in central metabolism pathways of eukaryotic and prokaryotic cells. The organic substances that are necessary for proper growth of yeast have been subject of investigations since the time of Liebig and Pasteur in XIX century. At the turn of twentieth century, biotin was first discovered as a growth factor that was required by some yeast strains. The unknown growth factor was initially termed "bios" [Wilidiers, 1901]. 30 years later, the vitamer was purified from egg yolk by Kogl and Tonnis. Biotin was also discovered in liver in 1931 and isolated by Gyorgy and his colegues in 1941. The structure of biotin ($C_{10}H_{16}N_2O_3S_1$) was established in 1942 by the group of de Vigneaud and Melville [Vigneaud et al, 1941]. It's molecular mass is 244.31 g/mol and consists two fused rings: an imidiazol (ureido) and sulfur-containing (tetrahydrothiophene), and the latter is extended via a valeric acid side- chain attached in a cis-configuration with respect to the ureido ring. Biotin is reported to melt with decomposition at 233°C. It is soluble in water to the extent about 20 mg/100mL at 25°C, more soluble in hot water or dilute alkali and ethanol. Biotin can be regarded as a thermostable compound, even in moderately acid solutions – this feature may be used in biotin extraction from biological samples by means of acid hydrolysis (2–3M of H_2SO_4) at high temperature (120°C) [Oura and Suomalainen, 1978, 1982].

In living cells biotin acts in the prosthetic group of biotin dependent enzymes catalysing carboxylation, transcarboxylation and decarboxylation reactions. Those enzymes are: EC 6.4.1.1 Pyryvate carboxylase (gluconeogenesis, lipogenesis, anaplerotic reactions); EC 6.4.1.2 Acetyl-CoA carboxylase (fatty acid synthesis); EC 6.4.1.3 Propionyl-CoA carboxylase (propionate metabolism); EC 6.4.1.4 Methylcrotonyl-CoA carboxylase (catabolism of leucine); EC 6.4.1.5 Geranolyl-CoA carboxylase (degradation of isoprenoid compounds); EC 2.1.3.1 Methylmalonyl- CoA carboxyltransferase (propionate metabolism); EC 4.1.1.3 Oxaloacetate decarboxylase (oxyloacetate decarboxylation); EC 4.1.1.41 methylmalonyl-CoA decarboxylase (succinate decarboxylation); EC 6.3.4.6 Urea carboxylase (hydrolysing) (urea catabolism). Three of them function in baker's yeast, namely pyruvate carboxylase, acetyl-CoA carboxylase and urea carboxylase [Oura and Suomalainen, 1978, 1982].

Although the *S.cerevisiae* strains used as baker's and distiller's yeast vary somewhat in their requirements for vitamins, biotin is an essential growth factor for all industrially employed *Saccharomyces cerevisiae* strains. At about 1950 the producers of baker's yeast began to pay attention to the nutrient content of their raw materials and added pure biotin to their media. It's is generally known that to obtain maximal cultivation yield, biotin must be supplied at about 29 µg of D(+) biotin per 100g of cultivation medium made from sugar beet molasses [Burrows, 1970].

Analytical techniques reported so far in the literature for determining biotin can be divided into four main categories: microbiological methods, biological methods (bioassays), physicochemical methods and binding assays. Microbiological methods are highly sensitive (10^{-9} g/L) methods based on the principle that growth of many microorganisms depends upon presence of biotin in the culture media. Many microorganisms have been applied to microbiological assays for biotin e.g. *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Neurospora crassa* and *Esherichia coli*. Bioassays are usually less sensitive than microbiological ones. Determination is for example based on animal (rat or chicken) growth curve or by assaying of biotin dependent enzyme activity. Physicochemical methods include: spectrophotometric, colourimetric, polarographic, LC, TLC, HPLC and GC. During last 40 years many attempts have been made to apply binding assays to the determination of biotin. Biotin binding assays are based on the same principle as immunoassays, which are widely spread in clinical laboratory practice, differing from them only in the fact that they utilize a specific binding protein instead of an antibody. Binding assays have many advantages such as high sensitivity, rapid and convenient performance. In all binding assays known so far, the specific binding protein used is a glycoprotein avidin or the streptavidin. Both of them exhibit an extremely high K_{aff} and high specificity in their binding to biotin molecule [Genghof et al., 1948; Suomalainen et al., 1965; Livaniou et al., 2000].

Aim of this was to determinate biotin content in various samples of baker's yeast and molasses used for yeast production.

Materials and methods

8 Molasses samples (M1-M8) were obtained from 3 beet-sugar factories located in Poland. 8 various samples of baker's compressed yeast (D1-D8) manufactured in 2 yeast factories (located in Poland) were purchased in local groceries. For biological determination of biotin *Saccharomyces cerevisiae 225* strain from Centre of Industrial Microorganisms Collection, Institute of Fermentation Technology and Microbiology, Lodz was used.

Apparent sucrose content in molasses samples were measured with use of saccharimeter. Apparent extract of molasses was assayed by means of Balling aerometer [Polish Standards, 1978]. Dry matter in yeast biomass was determined gravimetrically in laboratory weighting dryer [Polish Standards, 1997].

All chemicals used were analytical grade.

All assays were done in triplicate

Biotin extraction

Biotin was extracted from yeast biomass and molasses by means of autoclaving samples with 2N H_2SO_4 (10 mL/1g of sample) in 120°C for 1 hour. After cooling down and pH regulation (to pH = 6) hydrolyzates were mixed with 1g of powdered active carbon and mixed by means of magnetic stirrer for 1hour at room temperature. Mixture was then filtered on paper filters and biotin was eluted from charcoal with use of mixture of ethanol (96%):water: ammonia (25% NH_4OH) in volumetric proportions 10:10:1. Eluates were then concentrated to

10 mL by means of rotary vacuum drier at 55°C. Such prepared samples were stored at -18°C for further biotin determinations [Genghof et al., 1948; Suomalainen et al., 1965, Scheiner and Ritter, 1975; Szopa, 1976, 1980].

Microbiological assay

Biotin content was determined by microbiological assay with *Saccharomyces cerevisiae 225* strain, using mineral solidified growth medium [Suomalainen et al., 1965; Szopa, 1976] and auxonographic method [Genghof et al., 1948] using paper filter discs [Schleicher and Schuell 2668].

ELISA – systems biotin assay for biotin determination

During this experiments "ELISA – systems biotin "Microtitre plate test for the determination of Biotin" manufactured Riedel -deHaen was used.

The biotin enzyme immunoassay test is based on high affinity of avidin to biotin. The walls of the microtitre plate were coated with avidin. Biotin standard or sample solutions and an alkaline phosphatase – biotin conjugate (AP) were added. Free and enzyme-labeled biotin competed for the binding sites of the avidin (competitive enzyme immunoassay). After washing out the unbounded substances detection of biotin was carried out by adding substrate solution (p-Nitrophenyl phosphate – PNPP), the bound enzyme conjugate converts the colourless solution into yellow end product. After stopping this reaction the measurment was carried out photometrically at a wavelenght 405 nm. The solution extinction was inversely proportional to the biotin concentration in sample or standard.

Results and discussion

Main aim of this study was to assay biotin content in compressed baker's yeast and molasses samples. Some additional assays (apparent extract, sucrose content and dry matter) for better sample's description were also made. All results are presented in Tables 1 and 2.

Biotin content in molasses varies from 0.9 to 2.51 µg/100 g (Table 1). There are small differences between results obtained by means of microbiological and ELISA method. Results obtained with use of biotin-avidin binding method are slightly lower (up to 9.71% in case of M8 sample) than obtained during traditional microbiological estimations. Results obtained with use *S.cerevisiae 225* strain are similar to those obtained by Szopa [1980, 1982] and Raczyńska-Cabaj [Raczyńska-Cabaj et al., 2000]. There is also close correlation to results presented by Burrows [Burrows, 1970]. Biotin content in molasses depends on many factors from beet cultivation and their storage, technology of sugar extraction to fine molasses' storage conditions. It can be clearly stated that biotin levels in beet molasses' samples should be at least 10-times higher to fulfill demands of yeast producers. This fact confirms necessity of biotin supplementation for efficient baker's yeast obtaining.

Highest biotin concentration in yeast samples (Table 2) was noticed for D7 sample (43 µg/100 g of d.m. Lowest value (25.1 µg/100 g of d.m.) was observed for D1 yeast. Results obtained by means of ELISA were, as previously observed for molasses, lower (up to 6.7% for D7 yeast) than obtained with use of *S. cerevisiae 225* strain. Range 25.1–43 µg of biotin/100 g of yeast d.m. is typical for compressed baker's yeast biomass and can be seen in many publications [Ali, 1976; Burrows, 1970; Scheiner & Ritter, 1975; Szopa, 1976, 1980]. As for molasses there are many factors affecting biotin content in the final product. Among them: biochemical features of specific yeast strain, composition and supplementation of cultivation media with biotin can be enumerated.

Table 1

Apparent extract, sucrose content and biotin concentration in various molasses' samples

Molasses sample	**Apparent extract [°Blg]**	**Sucrose content (polarimeter) [%]**	**Biotin [µg/100g d.m.] Assayed by means of method:**		**Difference between microbiological and ELISA result [%]**
			microbiological	**ELISA**	
M1	72.3	48.3	2.3	2.2	-4.35
M2	75.1	49.8	2.22	2.21	-0.45
M3	80.2	52.3	1.86	1.76	-5.38
M4	70.8	47.6	2.51	2.37	-5.58
M5	77.9	50.9	2.17	2.15	-0.92
M6	72.3	47.7	0.9	0.85	-5.56
M7	74.7	48.2	2.16	2.05	-5.09
M8	73.2	48.6	2.47	2.23	-9.71

Table 2

Dry matter and biotin concentration in various compressed yeast samples

Yeast sample	**Dry matter content [%]**	**Biotin [µg/100g of d.m.] Assayed by means of method:**		**Difference between microbiological and ELISA results [%]**
		microbiological	**ELISA**	
D1	28.7	25.1	24.45	-2.59
D2	29.8	34.18	32.18	-5.85
D3	33.2	40.84	40.1	-1.81
D4	30.3	35.69	33.91	-4.98
D5	29.3	38.23	36.7	-4
D6	32.2	32.57	31.45	-3.44
D7	32.1	43.0	40.25	-6.39
D8	29.6	29.18	28.43	-2.57

Comparing two methods of biotin assay it's clearly seen that results obtained with use of microbial auxonographic method are generally several percents higher than those obtained with novel ELISA method based on avidin-biotin affinity. To elucidate this fact focus on a principles of two methods must be done. ELISA method is based on high specific affinity of biotin to avidin and only biotin and some it's structural analogs like selenobiotin or dethiobiotin are able to make irreversible complexes with avidin located on the microtitre plate's walls. Microbiological method is based on use an auxotrophic to biotin *Saccharomyces cerevisiae 225* strain. Assumption of this method is that biotin promotes growth of microbial strain, but in fact not only biotin promotes *Saccharomyces sp.* growth instead of biotin. There are some other compounds like: 7-keto-8-aminopelargonic acid; 7,8-diaminopelargonic acid;

oleic acid; palmitoleic acid and aspartic acid which can completely replace biotin in biotin free-medium [Suomalainen and Keranen, 1963]. As a result it's obvious that microbiological method is less sensible to distinguish pure biotin from its biological analogs than ELISA method. If comparing those two methods for samples analysis containing only pure biotin the same results for both methods may be obtained but when analyzing hydrolyzates containing mixture of various compounds featuring biotin-like activity it's possible to obtain slightly different results with both methods for the same sample. It can be assumed that with more specific method i.e. ELISA the readings for the same sample are lower than those obtained with less specific microbiological method since in the second one method more compounds can represent biotin-like activity.

Microbiological assay takes 6–12times more time (inoculum and media preparation, sterilization and at last seeding and waiting for culture growth) than avidin-biotin affinity based method but there is one big advantage of microbiological method supporting it's still actual existence for over 50years – costs. Coarsely calculated: evaluation of biotin content by means of ELISA method for 20 samples costs about 400PLN. In case of microbiological method costs are much lower – approx. 50PLN per 20 samples.

Conclusions

Comparing two methods of biotin assay it's clearly seen that results obtained with use of microbial auxonographic method are generally several percents higher than those obtained with novel ELISA method based on avidin-biotin affinity. Microbiological method is less sensible to distinguish pure biotin from its biological analogs than ELISA method. There is one big advantage of microbiological method supporting it's still actual existence for over 50years – costs. Elisa method assays were about 8-times more expensive than microbiological assays.

Thanks to the results obtained during this researches it can be concluded that both methods have some advantages and disadvantages and both can be used for biotin assay in the molasses and yeast biomass.

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7

UTILIZATION OF ANTI-GLIADIN ANTIBODIES FOR HORDEIN DETECTION IN BEER

Introduction

Celiac disease is a common autimmune disorder caused by genetic, environmental and immunological components. The proline- and glutamine-rich alcohol soluble proteins (prolamins) from wheat rye, barley and oats produce harmful effect of celiac disease or gluten sensitive enteropathy in humans by causing characteristic changes in intestinal mucosa [Kagnoff, 2007]. Patients so affected have to avoid eating these grains and replace them with rice, corn, soy, potatoes they needs completely gluten-free foods. Due common contamination of food by gluten it is not easy to maintain a diet with zero gluten content. Therefore characterization and quantification of gluten proteins in foodstuff is crucial to avoid celiac damage [Ciclitira et al., 2005].

Gluten is unique protein because it contains approximately 15% proline and 35% glutamine residues [Stern et al., 2001]. This high proline and glutamine content prevents complete proteolysis by gastric and pancreatic enzymes. The toxic for celiac patients oligopeptides build up in a small intestine. Particularly one peptide, 33-mer is highly proteolytically resistant, and is a principal contributor to gluten immunotoxicity [Shan et al., 2005]. However because the gluten is a complex mixture of proteins its toxicity is not fully understood.

For several years foodstuff based on enzymatically modified cereals, such as beers, were considered safe for celiac disease patients, on the basis that No. native hordein proteins can generally be found in beer. However, this opinion was formed without any evidence that this was the case. It is true that barley native hordeins decrease in amount during brewing [Sheehan & Skerrit, 1997] but it is also well know that during the malting and fermentation processes, the barley hordeins are broken down into peptides [Chandra et al., 1999]. Peptides resulting from enzymatic digestion are often quite water soluble so that they remain in the beer throughout the complete processing to the final product. This degraded peptides have been mostly studied because of their contribution to beer quality such as foam formation and haze [Evans et al., 2003].

A barley hordein might have a polypeptide chain including 300 amiNo. acids in its sequence, yet it is reasonably well established by experiments that polypeptides with as few as 13 amiNo. acids residues in the chain can still retain toxicity for celiac patients. These small pieces of the original proteins can have very different properties from the original larger proteins but can still be dangerous for celiac patients because of presence toxic gliadin-like epitopes [Jin et al., 2009; Ciclitira et al., 2005].

Codex Alimentarius Commission clearly defined that the quantitative determination of gluten in food and ingredients shall be based on an immunologic method or other method providing at least equal sensitivity and specificity. The antibody used should react with the cereal protein fractions that are toxic for persons intolerant to gluten and should not cross-react with other cereal proteins or other constituents of the food or ingredients. The detection limit should be 10 mg gluten/ kg or below. Recommended method is the sandwich R5 ELISA developed by Menez [Codex Alimentarius Commission, 2008]. However even this recommended method has some limitation. It is able to quantify native and heated gluten, but overestimate barley hordein. It also is unable to accurately quantify hydrolyzed gluten which may contain peptides toxic for celiac patients [Moren et al., 2008; Thompson & Menez, 2008, Janssen, 2006].

This immunochemical methods are sensitive, simple but efficiency is influenced by several factors: methods of antibody production and selection, the test format, and the choice of the gluten extraction method [Denery-Papini et al., 1999]. Also it should be noted the ability of assays to measure gluten's toxicity towards celiac disease patients [Lester, 2008].

There are some evidences from analytical methods involving antibodies prepared to gliadins that there are peptides in beer that react with these antibodies [Kanerva et al., 2005, Ellis et al., 1994]. It is not proved beyond any doubt that the peptides in beer are actually toxic to celiac patients. The amount of harmful peptides is likely to be small, but there is No. satisfactory analytical data that defines the amount exactly. Two different methods of gluten determination in food are endorsed by Codex Alimentarius Comission and Association of Analitical Communities but none of them is able to accurately detect and quantify barley prolamins as well as peptides obtained after processing [Thompson & Menez, 2008].

The purpose of the present study was to evaluation the practical efficiency of SIGMA polyclonal anti-gliadin antibodies for the detection of hordein proteins in beer by ELISA.

Materials and methods

Materials.

Anti-gliadin antibodies

Anti-gliadin polyclonal antibodies from rabbit (Sigma G 9144) and anti-gliadin-peroxidase conjugate antibodies from rabbit (Sigma A 1052) were used for enzyme-linked immunosorbent assay (ELISA). Although both antibodies were developed in rabbits using purified gliadin as immunogen they also reacts with prolamins fractions of rye, barley, soy and oats.

Beer samples

24 beer samples were analysed by direct and indirect ELISA method for checking present of gluten protein. Beer samples were purchased in local supermarkets. On the basis of alcohol content beers were divided on three groups: light, lager and strong.

Hordein from Malt

Barley malt sample pilsen type was used for hordein preparation. Extraction was performed according method described by Kanerva et al. [2005]. A 5 g of ground barley malt was extracted with 25 mL of 1M NaCl by shaking for 30 minutes followed by centrifugation 9000×g at 10°C for 20 minutes. In this step the water-soluble albumins as well as saline-soluble globulins were extracted. The remaining pellet was washed with 20 ml of distilled water and centrifuged 10 minutes 9000×g at 10°C. Than the precipitate containing the hordein fraction, was extracted three times with 25 mL of a 55% v/v aqueous solution of izopropanol at 60°C by shaking manually for 30 minutes. The supernatant containing hordein fraction was evaporate to dryness during 3 days at room temperature. The dried hordein fraction was frozen. Before using was dissolved in a 70% v/v aqueous solution of ethanol.

Analysis.

ELISA

Hordein contents in beers were analyzed by direct and indirect enzyme-linked immunosorbent assay (ELISA) with commercially available polyclonal anti-gliadin antibodies.

Microtitrate plates were coated by 100 µl of antigen: hordein, gliadin, or beer sample for 2 hours at room temperature. Plates were washed and free binding sites were blocked by overnight incubation of the plates with 1% bovine serum albumin (BSA) in phosphate buffer (PBS) pH=7,4 at 4°C. The plates were washed again and 100µl of antibodies were added: in direct detection 1000-fold diluted solution of anti-gliadin antibody conjugated with peroxidase (Sigma A 1052) in indirect detection 3000-fold diluted solution of anti-gliadin antibodies (Sigma G 9144). Dilutions of antibody were prepared with PBS, with the addition of Tween-20 and 1% BSA. After incubation of the plates for 2h at room temperature and rinsing with PBS containing Tween-20 in indirect technique second antibody Anti-Rabbit IgG peroxidase conjugate developed in goat (Sigma A 8275) diluted 4000-fold were used. After that in both techniques the bound peroxidase activity was determined by reaction with ortophenylenodiamine and absorbance was measured at $\lambda = 490$ nm using plate reader (Asys-HiTech UVM 340).

Electrophoresis

The protein content of samples analyzed by the electrophoresis was determined by Bradford [1976] method. BSA was used as a standard.

The electrophoretic separations were achieved in OWL the Emperor Penguin System P8DS-3 vertical electrophoresis unit. Gels after staining were scanned and analyzed with GelScan 1.45 computer program.

The electrophoretic analyses were carried out according to the Laemmli [1970] discontinuous buffer system. Separation gels (0,8 mm thick, 10×10 cm) consisted of a 4% polyacrylamide stacking gel and a 12% polyacrylamide resolving gel. Samples were mixed 1:1 (v/v) with the sample buffer, in order to achieve a final protein quantity of 5 µg. The molecular weight marker SIGMA Dalton Mark VII-L Standard Mixture was used as a standard. Separations were carried out at 70 V in the stacking gel, and at 170 V in the resolving gel. During separation, the electrophoresis system was cooling by tap water. Gels were stained using CBB-R250.

Imunoblotting

After electrophoresis separated proteins were transferred onto PVDF membrane (Immobilon P transfer membranes, Millipore) using the SCIE-PLAS semi-dry electroblotting units V10-SDB. Transfer was running at 64 mA during 2,5 hours at 4^{o}C. Next, the unoccupied sides on the membrane were blocked by overnight incubation with 1% bovine serum albumin (BSA) in phosphate buffer (PBS) pH = 7,4 at 4^{o}C. Than the membrane was incubated with solutions of antibody in direct detection with 1000-fold diluted solution of anti-gliadin antibody conjugated with peroxidase (Sigma A 1052) in indirect detection with 3000-fold diluted solution of anti-gliadin antibodies (Sigma G 9144). Dilutions of antibody were prepared with PBS, with the addition of Tween-20 and 1% BSA. After 1,5 h incubation at room temperature and rinsing with PBS containing Tween-20 in indirect technique second antibody Anti-Rabbit IgG peroxidase conjugate developed in goat (Sigma A 8275) diluted 4000-fold were used. The last step was detection of peroxidase activity by reaction with 3,3$^{'}$–5,5$^{'}$-tetramethylobenzidine (Sigma T 0565) in both techniques.

Statistical analysis.

All statistical analysis were performed using software Statistica v.8.0. All ELISA measurement were made in duplicate by measuring the same sample on 2 separate ELISA plates on the same day in parallel experiments. One-Way analysis of Variance at the 5% significance level was used for confirmation of the repeatability of the results.

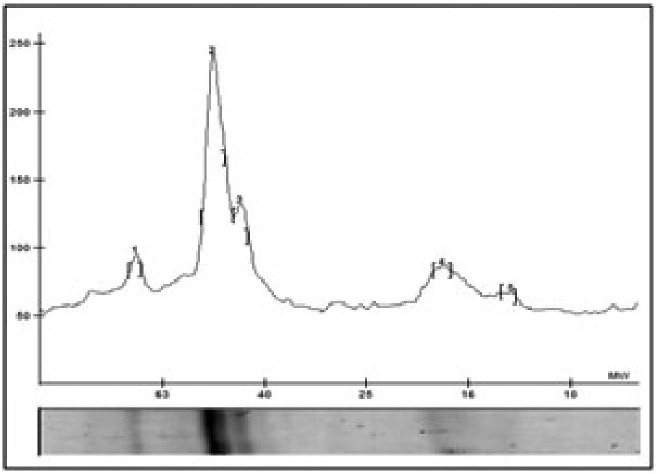
Results and discussion

Barley hordein is the main protein fraction in barley seeds, accounts up to half of the total protein in the mature grains and may be classified into four groups named B, C, D and γ hordeins based on their electrophoretic mobility and amiNo. acid composition. The B (30–45 kDa) and C (45–75 kDa) fractions account respectively for 70–80% and 10–12% of the total hordein, while the D (>100 kDa) and γ (<20 kDa) fractions are minor components respectively 2–4% and 1–2% total hordein [Qi et al., 2006, Shewry & Tatham, 1990]. During malting the most high molecular weigh proteins are broken [Chandra et al., 1999]. In barley malt hordein fractions form 10 to 70 kDa were identified [Figueredo et al., 1999].

Gliadin, the wheat prolamin, is commercially available, while hordein is not. To investigate composition of obtained from malts sample hordein preparation SDS-PAGE analyze was carried out. Electrophoretic patterns are presented on Figure 1.

In hordein preparations the five protein bands were identified after electrophoretic separations with molecular weigh from 13 to 71 kDa, the most intensive was protein band 50,5 kDa. Separated proteins belongs to B, C and γ hordein zones. No. bands were observed in the D hordein zone. Howard et al [1996] referred that high quantities of B and/ or C hordeins can be correlated to poor malt extract values.

Immunoreactivity of rabbit polyclonal anti-gliadin antibodies labeled with horseradish peroxidase (Sigma A1052) and unlabeled (Sigma G9144) were tested with electrophoretically separated hordeins. Figure 2 illustrates immunoblots obtained in direct and indirect technique. The results indicated that both used antibodies present cross-reactivity against hordeins from barley malt. The several hordein protein bands were react with antigliadin antibodies in direct and indirect technique. The membranes were strongly coloured, and No. distinct protein bands could be discerned. The sample would have to require further dilution to separate the protein. This was beyond immediate interest, however, the detection of proteins in hordein preparation obtained from malt samples being sufficient for detecting hordeins.



Band number	1	2	3	4	5
Molecular weigh [kDa]	71,0	50,5	44,5	17,9	13,2

Fig. 1. Electrophoretic pattern of hordein preparation

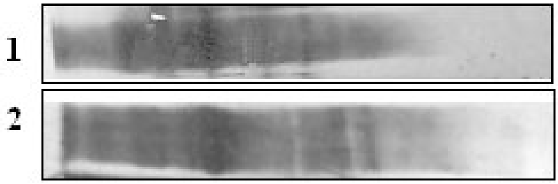


Fig. 2. Immunoblots of hordeins preparation obtained with
1. Direct detection (Ab 1 - SIGMA A1052)
2. Indirect detection (Ab 1 - SIGMA G9144; Ab2 - SIGMA A8275)

To obtain quantitative data about possibility of anti-gliadin antibodies capacity to detect hordeins direct and indirect ELISA methods were utilized. Producer of antibodies declared that anti-gliadin antibodies cross react with prolamins from different cereals, such as rye, barley, soy and oats. For quantitative detection of hordeins by ELISA with anti-gliadin antibodies it is preferable to use as a control standard proteins purified from barley. Contrary prolamin polymorphism may be responsible for differences in protein composition between samples and standard [Denery-Papini et al., 1999]. To compare affinity of polyclonal anibodies for wheat gliadin and hordein the different amount of studied proteins were immobilized and detected with SIGMA A1042 as well as SIGMA G9144 antibodies in direct and indirect

ELISA. The standard curve for detection of gliadin as well as hordein in direct and indirect ELISA with polyclonal anti-gliadin antibodies are shown on Figure 3.

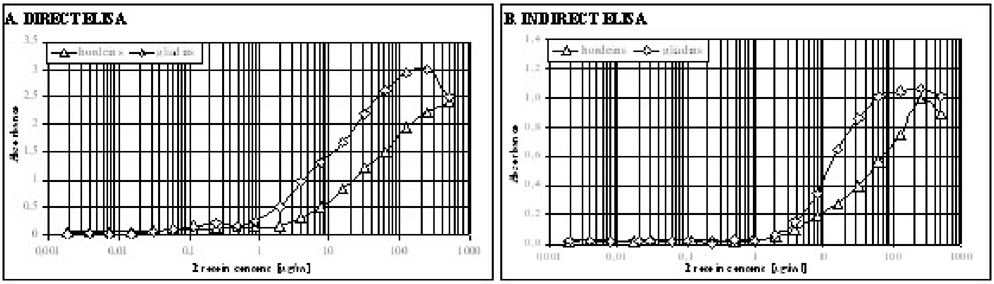


Fig. 3. Standard curves of the detection of gliadin and hordein by:
A) direct ELISA with use of rabbit polyclonal anti-gliadin antibody labeled by horse radish peroxidase Sigma A 1042,
B) indirect ELISA with use of rabbit polyclonal anti-gliadin antibody Sigma G 9144

In both methods measured absorbance was higher for gliadins compare to hordeins preparation. It was because utilized polyclonal antibodies were raising against wheat gliadins, and they only cross react with prolamins from different sources. Range of calibration curve was 1,9 to 125 x 10^{-6} g/mL for gliadin and 1,9 to 500 x 10^{-6} g/mL for hordein whereas for indirect respectively 1,9 to 125 x 10^{-6} g/mL and 1,9 to 250 x 10^{-6} g/mL. Also measured absorbance in direct technique was higher than in indirect technique. The obtained results shown that it is possible to detect hordeins from barley malt by direct and indirect ELISA with anti-gliadin antibodies.

During brewing hordeins from malt are broken by malting enzymes. Nevertheless gliadin-like epitopes have been detected in both malt and beer. All beers are banished from diet of celiac patients by the standard that they are No. made from gluten-free raw material [Ellis et al., 1994].

Twenty four beers were analyzed by direct and indirect ELISA with anti-gliadin polyclonal antibodies. On the basis of alcohol content beers were divided into three groups: light, lager and strong. The absorbance of hordein detected in direct and indirect technique are presented in Table 1. All obtained results in both techniques are different but very low, near the limit of detection. The antibodies utilized in ELISA methods are designed and intended to measure the gliadin content of wheat-based food samples. They cross react with hordeins isolate from barley malt, but during brewing process hordeins go through extensive hydrolysis which can effect to their ability to react with antibody of the assay. Cross-reaction of anti-gliadin antibody with peptides from other prolamin families is due to binding to peptide having significant sequence homology [Skerritt et al., 2000]. After enzymatic hydrolysis hordeins are degraded and gliadin-like epitope might change or even "disappear".

Table 1

Absorbance of hordeins in the beer samples detected by direct and indirect ELISA

Beer sample		Hordein absorbance	
		Direct detection	Indirect detection
Light	Carter	0,054 ± 0,006	0,053 ± 0,005
	Karmi Black	0,081 ± 0,001	0,055 ± 0,001
	Karpackie Pils	0,060 ± 0,001	0,052 ± 0,001
	Zdrojowe	0,112 ± 0,007	0,090 ± 0,001
	Redd’s	0,085 ± 0,007	0,068 ± 0,006
	Łaskie	0,078 ± 0,011	0,049 ± 0,001
Lager	Noteckie	0,056 ± 0,001	0,050 ± 0,001
	Noteckie Eire	0,070 ± 0,004	0,067 ± 0,005
	Fasberg	0,078 ± 0,001	0,043 ± 0,002
	Czerwony Smok	0,078 ± 0,013	0,073 ± 0,001
	Lech Mocny	0,070 ± 0,013	0,058 ± 0,002
	Lubuskie	0,053 ± 0,008	0,051 ± 0,001
	Junak	0,070 ± 0,005	0,050 ± 0,001
	Ciechan Mocne	0,075 ± 0,002	0,051 ± 0,005
	Goolman Strong	0,054 ± 0,001	0,062 ± 0,005
	Warka Strong	0,080 ± 0,003	0,064 ± 0,002
	Złoty Strong	0,072 ± 0,011	0,060 ± 0,001
	Okocim Mocne	0,077 ± 0,003	0,054 ± 0,002
	Argus Strong Premium	0,066 ±0,003	0,053 ± 0,003
	Mocne Eksportowe	0,068 ± 0,001	0,088 ± 0,003
	Perła Mocna	0,078 ± 0,002	0,060 ± 0,002
	Black Boss Porter	0,058 ± 0,003	0,053 ± 0,005
	Kaper	0,071± 0,004	0,050 ± 0,003
	Żywiec Porter	0,068 ± 0,002	0,068 ± 0,004

Conclusions

The direct and indirect ELISA with studied anti-gliadin polyclonal antibodies can be utilized in detection of native barley malt hordeins but poorly suited for determination of hordein in beers. During brewing the hordeins from malt are hydrolyzed to peptides which are not deteced by studied anti-gliadin antibodies.

For analysis of gluten protein in beer antibody specific for a malt barley prolamin peptides are necessary, but unfortunately such antibody isn’t commercially available, yet.

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8

DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY IN CHEESE BY FLUORIMETRIC METHOD

Introduction

The quality of cheese depends on milk, which the cheese is made on. Except of the sensory properties, relate with fat containing, whey protein, activity of enzymes is very important for health safety. Contamination or addition of raw milk to pasteurized milk can cause hazard of pathogens contamination of the product. Wrong process of pasteurization can influence on final product by denaturate of whey proteins, inactivate enzymes, destroy beneficial bacteria or chemical changes which can have an effect on increase or decrease of substrate availability for bacteria and enzymes. Because of that the heat treatment of milk for cheese production should be limited. The lowest limit of proper process of heat treatment is defined as pasteurization, and the rating of this is activity of alkaline phosphatase (ALP), which is inactivated during the process, and its presence indicates that the process was wrong [Ardo, 1999; Yoshitomi, 2004]. Approximately 30–40% of ALP in milk is associated with the fat globule membranes, the rest of the enzyme is bound to the lipoproteins in skimmed milk fraction [Raynal-Ljutovac, 2007]. Legislation of the European Union adopts the level of 350 $mU \cdot L^{-1}$ of ALP activity as safe for consumption of milk and milk-based drinks (Commission Regulation (EC) No. 1664/2006). For cheese this limit has not been established yet. To avoid a danger of cheese poisoning it is required that the cheese should be made only from pasteurized milk [Rosenthal, 1996]. However, it is necessary to establish the limit to check product and process of manufacture. According to French Food Safety Agency (AFFSA) tentative limits for cheese made from pasteurized milk ranged from 2 to 11 $mU \cdot g^{-1}$ [Desbourdes et al., 2008]. There is an ability of making an error in analyzing the positive result of determination of ALP in cheese, caused by cheese microflora. It is possible to discriminate native ALP, which is less heatstable from ALP produced by microorganisms by testing after repasteurization at 62.8°C by 30 min. The positive result of ALP activity is caused by microflora [Rosenthal, 1996].

The methods of determination of ALP in dairy products are based on colourimetric methods: by reaction which uses as a substrate phenyl phosphate (Kay and Graham Method, Scharrer Method), p-nitrophenyl phosphate (Aschaffenburg and Mullen Method), phenolphthalein monophosphate (Kleyn Method). The intensity of colour developed is proportional to the activity of ALP. There are also instrumental methods, like chemiluminescence, amperometric, or fluorimetric [Marshall, 1993; Yoshitomi, 2004, Salter and Fitchen, 2006; Fox and Kelly, 2006]. Official reference method for the measurement of ALP activity is the fluorimetric method (ISO 11816-1 and ISO 11816-2). It is applicable for measurement of ALP in cow, ovine and goat milk, whole, semi-skimmed and skimmed milk, milk-based drinks, and cheeses. It is suitable for the determination of ALP activity in raw milk and milk after heat treatment with ALP activity over 2,000 $mU \cdot L^{-1}$ after dilution of the sample. The Advanced Fluorophos assay is based on a monophosphoric ester substrate, 2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole phosphate called Fluorophos, which, when acted upon ALP, is converted to a highly fluorescent product. Fluorimetric measurement of alkaline phosphatase activity is measured at 38°C over a 3-min period when using the Fluorophos. This includes pre-incubation of substrate and sample to ensure proper temperature, followed by multiple kinetic readings of the reaction rate. The enzyme activity is calculated automatically in $mU \cdot L^{-1}$ (for milk and milk-based drinks) or $mU \cdot kg^{-1}$ (for cheese). Unit of alkaline phosphatase activity is amount of alkaline phosphatase enzyme that catalyses the transformation of 1 µmol of substrate per minute per litre or gram of sample (Rocco, 1990, ISO 11816-1).

The aim of this study was revalidation of the assay used for determination of alkaline phosphatase activity in cheese. Moreover, the ALP activity in different types of cheeses from retail outlets of various producers was monitored.

Materials and methods

Materials

In the validation method Gouda cheese was used. In addition, 15 samples of cheese were examined. Eight of them were samples of hard cheese, 4 samples of soft cheese (Brie and Camembert), 2 samples of cottage cheese and one sample of goat milk cheese. Pasteurized milk containing 3.2% of fat was used for dilution of the cheese samples. The cheeses and pasteurized milk were from retail outlets. Raw milk for fortification of the samples was from a rural farm.

Reagents

- *Fluorophos substrate* - non-fluorescent aromatic monophosphoric ester substrate, 2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole phosphate (Fluorophos),
- *Substrate buffer solution* to dissolve Fluorophos substrate - diethanolamine (DEA) buffer solution, c(DEA) = 2.4 $mol \cdot l^{-1}$, with pH 10.0,
- *Working calibrator solutions,* Fluoroyellow - (2'-[2-benzothiazolyl]-6'-hydroxy-benzothiazole) in DEA buffer,
    - *Calibrator solution 'A',* containing 0 $\mu mol \cdot l^{-1}$ of Fluoroyellow,
    - *Calibrator solution 'B',* containing $17.24 \cdot 10^{-3}$ $\mu mol \cdot l^{-1}$ of Fluoroyellow,
    - *Calibrator solution 'C',* containing $34.48 \cdot 10^{-3}$ $\mu mol \cdot l^{-1}$ of Fluoroyellow,
- ALP-Daily Instrument Control
- Positive, Negative and Phosphacheck-N control samples

Equipment

- *Fluorophos filter fluorimeter,* with thermostatically controlled cuvette holder, capable of operating at 38°C±1°C.
- *Analytical balance*

- Homogeniser Ultraturrax
- *Cuvettes,* disposable, non-fluorescent glass, of diameter 12 mm and of length 75 mm.
- *Pipettes* of capacity 2 ml and 0.075 ml
- *Vortex mixer*
- *Water bath,* capable of maintaining a temperature of 63°C ± 1°C and 95°C ± 1°C.

Preparation of samples

Cheese sample was prepared as directed in ISO 11816–2. Cheese portion was mixed by blender. Sample of cheese weighed 0.5 g was taken into the beaker. The 10 ml of ALP free milk was added to the sample and homogenized with Ultraturrax. ALP free milk was obtained by heating at 95°C for 5 min of whole pasteurized milk. The samples were fortified with raw cow milk to establish 4 levels of ALP activity: 2, 270, 900, 2,500 mU·g^{-1}.

Verification of instrument performance

Quality control tests:

- The daily A/D (analog/digital) test used to check the proper functioning of the equipment by measuring the accuracy of the A/D conversion channel and monitoring the A/D channel for drift over time or temperature;
- The daily instrument control test using ALP-Daily Instrument Control used to monitor any electronic or optical drift in the fluorimeter.
- Control tests: Positive, Negative and Phosphacheck-N controls
    - *Negative control test* include a negative control test with each batch of samples. The instrument reading shall be less than 10 mU·L^{-1} to indicate No. fluorescence activity detected.
    - *Positive control test* include a positive control test with a ALP activity level of 500 ± 75 mU·L^{-1}
    - *PhosphaCheck-N-test* the reading should be < 40 mU·L^{-1}.

Testing the samples for ALP activity

The cheese samples were examined on channel which was calibrated before for cheese. The test portion of 0.075 ml was added to glass cuvetes containing 2 ml Fluorophos substrate which was incubated before at 38°C for 10 min. The contents of the cuvette were immediately mixed on the vortex. Samples were tested on Fluorophos FLM 200 (Advanced Instruments Inc.). Results were calculated automatically. ALP activity in samples of each level of ALP were measured by 3 analysts, in 6 repetitions.

Moreover, 15 samples of cheese derived from retail outlets were examined in 2 repetitions.

Scaling of results

The values indicated in apparatus were in mU·kg^{-1}. To get the final result in mU·g^{-1}, the result in mU·kg^{-1} is recalculated allowing of dilution of sample and divided by 1,000.

Interpretation of results

The precision of the method was determined by assignation of repeatability and reproducibility. Moreover, the values of standard deviation and coefficient of variation of repeatability and reproducibility were assigned and compared for each level of ALP activity. Values of repeatability and reproducibility obtained in the study were compared with criteria established for interlaboratory comparisons which are included in standard ISO 11816-2.

The results of ALP activity of cheeses samples from retail outlets was compared to the tentative limits in cheese proposed by AFFSA.

Results and discussion

The values of the precision of the method, like repeatability and reproducibility in each level of ALP activity, fitted the criteria established for interlaboratory comparisons which are included in standard ISO 11816-2. The values obtained in the study were presented with reference to the values from standard ISO 11816-2 in the Table 1. The values of the repeatability depended on ALP activity level and were equal adequately to 0.37; 26.20; 80.82 and 213.19 mU·g^{-1} and the reproducibility reached the levels of 0.48; 32.78; 108.60 and 342.28 mU·g^{-1}.

The coefficient of variation of the repeatability ranged from 3.24 to 5.95%, and the coefficient of variation of the reproducibility ranged from 4.26% to 7.74%. The values of standard deviation and coefficient of variation of repeatability and reproducibility at each level of ALP activity were presented in the Table 2.

The values of measurement at each level of ALP activity for individual analysts presented on the diagrams (Fig. 1–4). The diagrams display divergences between the values according to analysts.

The most important level of ALP activity is the lowest one, because the products made on milk after complete pasteurization, or without any contamination and addition of raw milk, should range from 2 to 11 mU·g^{-1} of ALP activity according to type of cheese. The tentative limits for soft cheese range from 2 to 6 mU·g^{-1}, for hard cheeses - from 2 to 11 mU·g^{-1}. Soft cheeses made from thermised milk had higher tentative limits of ALP activity, range from 83 mU·g^{-1} (for Brie cheeses), to 490–1,317 mU·g^{-1} (for Camembert). The ALP activity in soft cheeses made from microfiltratet milk should be on the range 555–1,542 mU·g^{-1} [Desbourdes, 2008].

Moreover, 15 samples of cheese from retail outlets were examined. Eight of them were samples of hard cheese, 4 samples of soft cheese (Brie and Camembert), 2 samples of cottage cheese and one sample of goat milk cheese. All cheeses were made from pasteurized milk. ALP activity of 7 hard cheeses fited to the tentative limits and ranged from <0.5 to 3.52 mU·g^{-1}. Only one sample had higher ALP activity on the level of 19.9 mU·g^{-1}. Soft Brie cheese (3 samples) had ALP activity between <0.5 and 4.66 mU·g^{-1}, however, Camembert (1 sample) had much higher ALP activity (88 – 90 mU·g^{-1}). Tentative limits for cottage cheese haven't been established, but the ALP activity in examined samples ranged from 1.41 to 2.18 mU·g^{-1}. One sample of goat milk cheese had the ALP activity ranged 6.8–6.9 mU·g^{-1}. The values of ALP activity obtained in this study are presented on the graph (Fig. 5).

Table 1

The values of repeatability and reproducibility obtained in the study, with reference to the values from standard ISO 11816-2

	LEVEL OF ALP ACTIVITY [mU/g]							
	2		270		900		2,500	
	Obtained value	PN-EN ISO 11816-2	Obtained value	PN-EN ISO 11816-2	Obtained value	PN-EN ISO 11816-2	Obtained value	PN-EN ISO 11816-2
REPEATABILITY	0.37	2	26.20	60	80.82	155	213.19	520
REPRODUCIBILITY	0.48	2	32.78	75	108.60	500	342.28	980

Table 2

The values of standard deviation and coefficient of variation of repeatability and reproducibility at each level of ALP activity

	LEVEL OF ALP ACTIVITY [mU/g]			
	2	270	900	2,500
REPEATABILITY				
Standard deviation (S_r)	0.13	9.36	28.,86	76.14
Coefficient of variation CV_r [%]	5.95	3.40	3.24	3.30
REPRODUCIBILITY				
Standard deviation (S_R)	0.17	11.71	38.79	122.24
Coefficient of variation CV_R [%]	7.74	4.26	4.35	5.29

The fluorimetric method of determination of ALP activity in cheese according to ISO 11816-2 is still on the stage of verification and validation in laboratories, so in the literature there are No. examples of results of this method. The methods which are used for monitoring of ALP activity in cheese are colourimetric, for example Scharer's method, where ALP activity is shown as μg of phenol equivalent/0.25 g of cheese. A value below 1 μg of phenol/0.25 g of cheese indicates that the pasteurized process was proper [Rosenthal, et al., 1996]. Other method is fluorimetric method, based on substrate 4-methylumbelliferyl phosphate, which release a highly fluorescent product, similarly as in Fluorophos method, but with results expressed as units of fluorescence which corresponds to ng of fluorescent product and ALP activity in examined sample [Yoshitomi, 2004].

The results of ALP activity in various type of cheese has been presented during the 11th Workshop of the EU CRL for Milk and Milk Products (CRL MMP). The Italian researches illustrated that extra hard cheeses had the ALP activity on the level of 4 $mU \cdot g^{-1}$, semi-hard cheeses 0.8 mU·g^{-1} pressed cheeses 0.6–0.9 mU·g^{-1} [Pellegrino, Rosi, 2008]. In French research of 39 samples of soft cheese – Camembert from pasteurized milk mean results of assay was 2.83 mU·g^{-1}, the single results ranged from 1 to 5 mU·g^{-1}. The Camembert from thermised milk had mean value of ALP activity on the level of 729 mU·g^{-1}, and the Camembert from micro-filtrated milk had a higher mean activity than thermised, equaled 930 mU·g^{-1}. In 22 samples of cheese Brie from pasteurized milk ALP activity ranged from <2 to 6, and the mean value was 2.93. Brie from thermised milk obtain value of ALP activity on the level of 83 mU·g^{-1}. The tentative limits of ALP activity in cheese are based on this study [Desbourdes, et al., 2008].

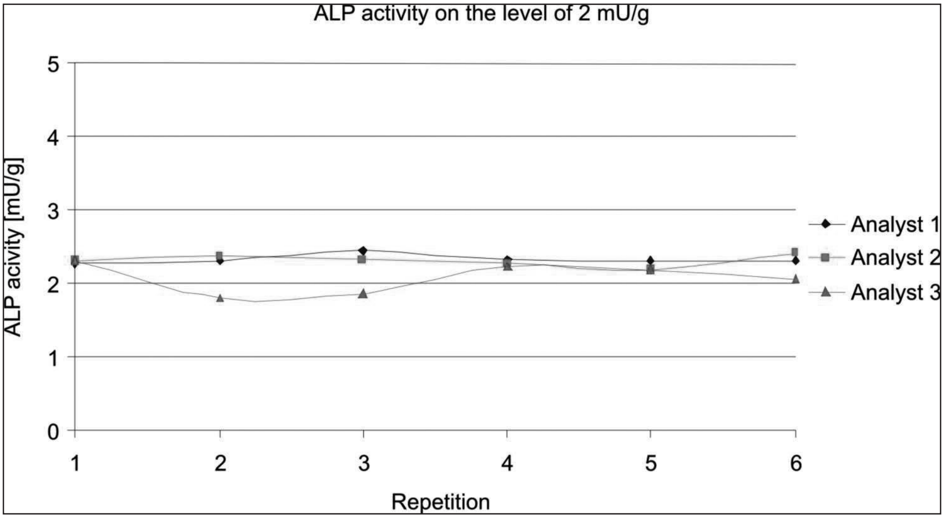

Fig. 1. The values of measurement on the level of 2 mU·g-1ALP activity for individual analysts

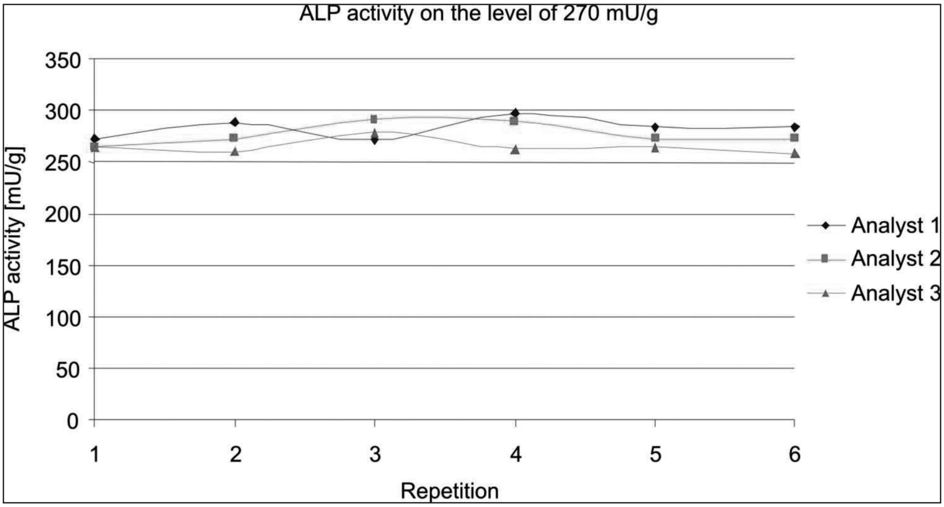

Fig. 2. The values of measurement on the level of 270 mU·g-1ALP activity for individual analysts

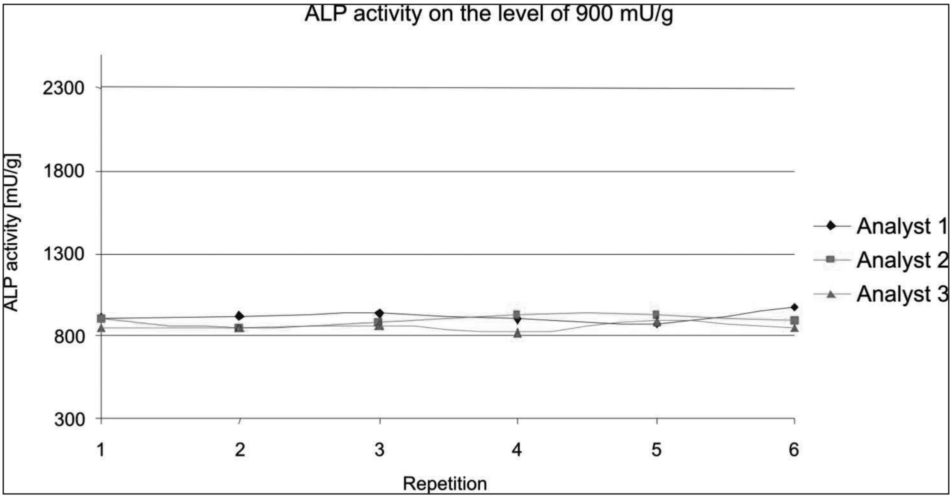

Fig. 3. The values of measurement on the level of 900 mU·g-1ALP activity for individual analysts

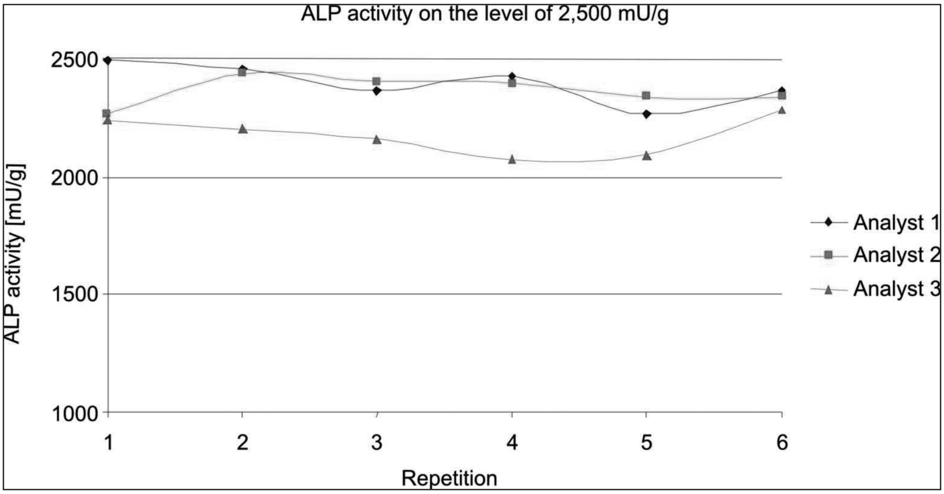

Fig. 4. The values of measurement on the level of 2,500 mU·g-1ALP activity for individual analysts

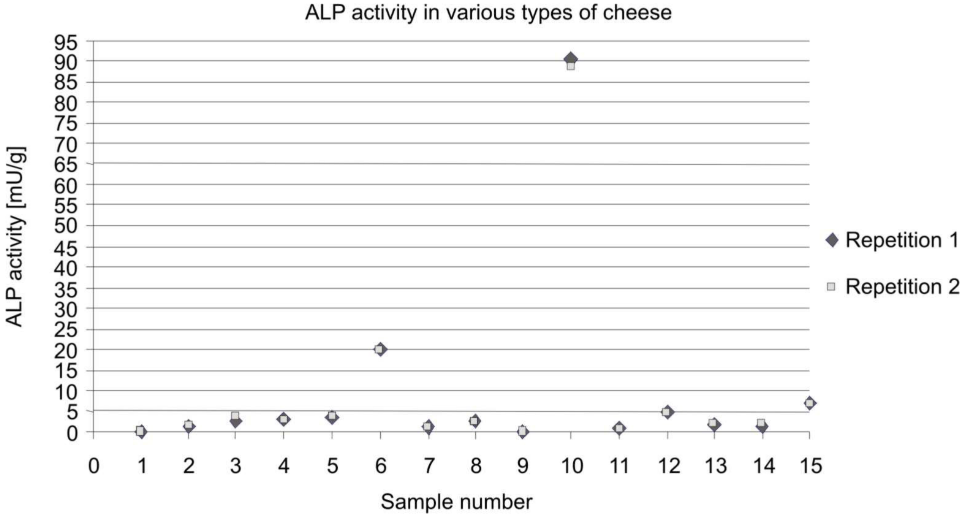

Fig. 5. ALP activity in cheese: sample numbers 1–8: hard cheeses; 9–12: soft cheeses; 13–14: cottage cheeses; 15: cheese of goat milk.

Conclusion

In spite of initial stage of determination of ALP activity in cheese by reference fluorimetric method, this method was validated and verified in laboratory and requirements of precision of the method included in ISO 11816:2 were fitted. Monitoring of ALP activity in cheeses from retail outlets is carried on.

The comparison of results of ALP activity in our study with the results presented in 11th Workshop of the EU CRL MMP, the most of examined cheeses fitted to the tentative limits. In spite of all producers declaration that they use for cheeses production only pasteurized milk, ALP activity of two samples were significantly higher from the others. The reason can be related to ALP of microbiological origin, or on the ground of type of cheese – Camembert (the cheese which had 90 mU·g^{-1}), of mold origin.

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9

PREDICTION OF THE BITTER TASTE STUDIED BY COMPUTER MODELING

Introduction

Humans may sense taste when the specific taste substance is directly contacted with the receptors located in taste buds. Receptors cannot be studied empirically by means of a microscope in respect of shape and functioning since they are biodynamic forms, changing their shapes depending on their environment and losing their properties when prepared *in vitro*. Not until recently has it been possible to stimulate the shape of receptors by means of special kinds of software. [Gawęcki and Baryłko-Pikielna, 2007].

Owing to the achievements of bioorganic chemistry in the recent years, has it been possible in the recent years to explore the biochemistry of the formation and transmission to the brain of signals sent by the sweet and the bitter taste receptors [Wong et al., 1996]. Recent literature reports indicate that we are approaching the moment when cloning the receptors of the two tastes at the molecular level becomes possible [Lindemann, 1999].

After a series of approximations and forecasts, has it been possible in the recent years to find certain relationships between the spatial structure of a taste compound and the impact of the taste compound upon a hypothetic receptor (that is, hypothetic in terms of shape). Those relationships are part of the notion of chemoreception, i.e. a group of physical and chemical phenomena that occur as the taste compound interacts with an appropriate receptor, producing a specific taste sensation in the human brain [Tinti and Nofre, 1991].

To fully explain the phenomenon of chemoreception of selected bitter compounds an effective, complete identification of the surface of the agonist (a bitter compound) [Temussi et al., 1984] , and the receptor is required. Those surfaces must complement one another in terms of size, shape, and function. It is not possible at the moment to comprehensively analyze and give details of every component of such identifying analysis at a time.

Therefore, this paper proposes a more narrow description of effective identification at the molecular level, e.g. in terms of size and shape in the complementary arrangement: agonist – receptor, and glucophore-receptor [Jasiczak et al., 2000].

Many attempts have been made in the past decade to give an interpretation of the sweet and bitter taste on the basis of the molecular structures of sweet (glucophore) and bitter compounds (agonists) [Jasiczak et al., 2005]. The first correlation among sucrose, saccharine, chloroform etc. was successfully found by Shallenberger and Acree who proposed that the perception of sweetness was due to intermolecular hydrogen bonding with the receptor site [Shallenberger and Acree, 1967, Shallenberger, 1998]. Temussi and co-warkers described activity entities of bitter compounds by analogy to the Schallenberger theory of sweet taste.

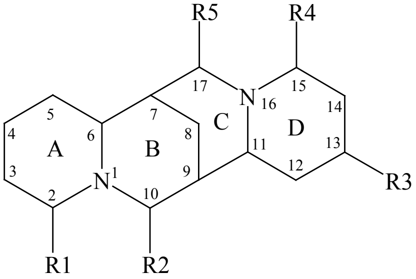
Temussi constructed the three-dimensional receptor model for the bitter taste and defined the active entities of agonists [Temussi et al., 1978, 1984, 1991].

The topological model of the bitter taste receptor was built by analogy to the shape of Temussi's taste receptor of sweet taste.

Experimental

Materials

The selected sparteine derivatives [Borowiak and Wolska, 1996] were used in the study of the prediction of the taste. Special attention has been put to sparteine monolactams in witch some hydrogen atoms in defininte position were selectively oxygen (Figure 1). These alkaloids may occur in two conformations in the form of ring C. They are the "boat" and "chair" conformations (Figure 2) [Wysocka and Brukwicki, 1996].



R_1=H, R_2=H, R_3=H, R_4=H, R_5=H Sparteine (1)*
R_1=O, R_2=H, R_3=H, R_4=H, R_5=H 2-oxosparteine (2)*
R_1=H, R_2=O, R_3=H, R_4=H, R_5=H 10-oxosparteine (3)
R_1=H, R_2=H, R_3=O, R_4=H, R_5=H 13-oxosparteine (4)
R_1=H, R_2=H, R_3=H, R_4=O, R_5=H 15-oxosparteine (5)*
R_1=H, R_2=H, R_3=H, R_4=H, R_5=O 17-oxosparteine (6)

Fig. 1. Structure of sparteine monolactames

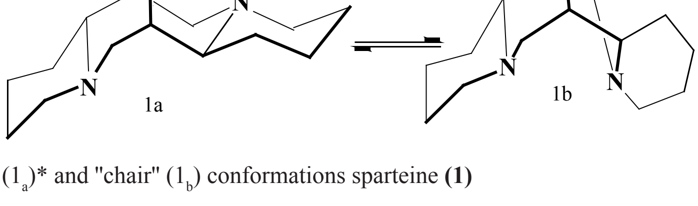


Fig. 2. "boat" (1_a)* and "chair" (1_b) conformations sparteine **(1)**

Methods

The study on sparteine monolactams proceeded in two independent directions: sensory analysis and computer projections.

In sensory analysis, bitterness indices were found for the alkaloids. Owing to a team of professionals, graphical scales depicting the intensity of bitter taste of the test compounds relative to the bitter taste standard were prepared, using organoleptic scaling method. (Polish standards based on the ISO standards)

Computer projections of the structure of the test sparteine derivatives into a molecular matrix of the bitter taste receptor were made using Chem-X software.

Conformation dynamism calculations, molecule geometry and molecular mechanics methods were used for molecule construction. On the basis of computer programme, the minimum energy conformers of active compounds were created. Two options: the energetic (MM and VdW) and quantum mechanics (MOPAC) were used for the optimization of the structures of the fitted molecules.

On the basis of model bitter compounds, a molecular matrix of the bitter taste receptor was prepared and was divided into test sectors [Jasiczak et al., 1999, Skolik, 2003]. Hydrophilic active sites, having a nucleophilic nature, of the test compounds were identified. Maps of the molecule protection potential were prepared in order to find the most convenient structure positioning in the bitter taste receptor model. The resulting structures of the test compounds were fitted in the molecular matrix of the bitter taste receptor. The effect of interactions of these compounds with the walls of the molecular matrix of the bitter taste receptor was evaluated.

In the final step of the study, the two independent directions of the study, i.e. sensory analysis and computer projections, were compared in order to define the chemoreception of the taste of those compounds and define a new systematic of sparteine monolactams with respect to their taste.

Results and discussion

Sensory analysis

The degree of bitterness for each of the alkaloids was determined by sensory analysis and results were compared with the prediction of taste based on computer analysis.

The sensory analysis has been made according to the Polish Standards: taste daltonism, sensibility treshold and difference threshold. The scaling method (Skolik, 2006] was used to express the variation of bitter taste in terms of quantity. The compounds were tested by 10 persons using the rank order test. Results were grouped, averaged and arranged to obtain a graphical scale describing the effect of bitter taste (Figure 3) for the respective sparteine derivatives and show the percentage index of the bitter taste of the compounds (Table 1). Bitter indexes of individual sparteine monolactams were found against a standard. Compound with extremely high bitter taste was quinine **(7).**

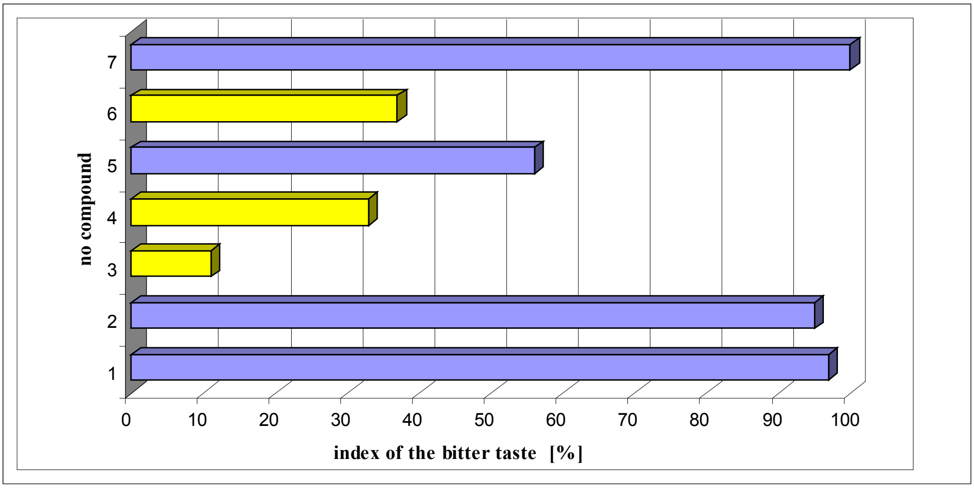


Fig. 3. Graphical scale sparteine derivatives **(1–6)**, standard **(7)**

Table 1

Percentage index of the bitter taste of alkaloids **(1–7)**

COMPOUND	INDEX OF THE BITTER TASTE [%]
Quinine **(7)** – standard	100
Sparteine **(1)**	97
Lupanine **(2)**	95
15-oxosparteine **(5)**	56
17-oxosparteine **(6)**	37
13-oxosparteine **(4)**	33
10-oxosparteine **(3)**	11
Water **(0)** – solvent	1

Computer analyses

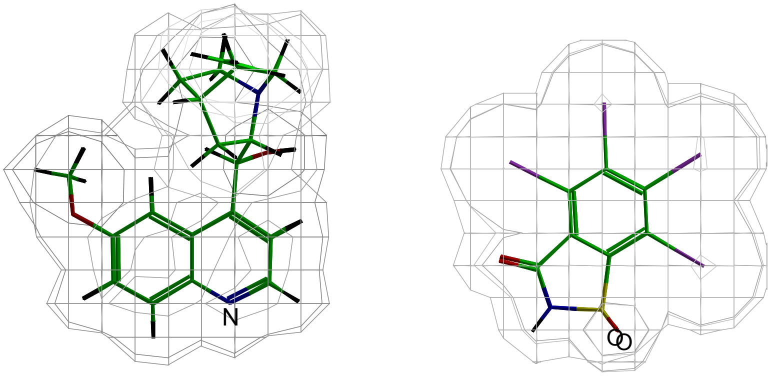
For computer analyses of sparteine monolactams investigated, a computer program Chem X was used. Computer projections of the alkaloid structures in the molecular bitter taste matrix in the procedure that included the following steps:

- design of a 3D structure of the molecule,
- energy optimisation in the MM,
- construction of VdW (van der Waals) net,
- quantum mechanical optimisation with the use of the MOPAC software,
- calculation of molecule charges and electrostatic potentials EP in the compound with the use of INDO/S software,

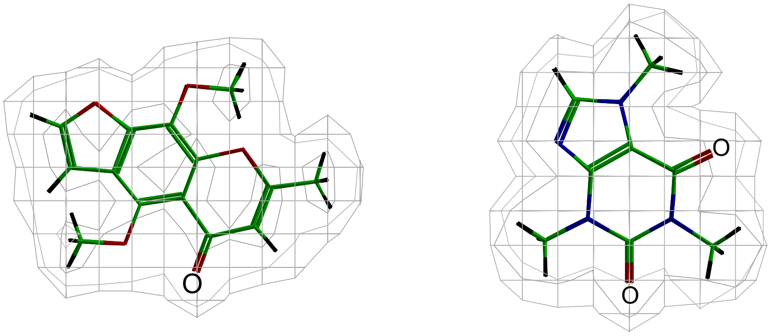
- construction of a map of the molecule's protective potential to identify the strongest electrophilic or nucleophilic centre, recognised as the active centre,
- superposition of the resulting structure of the compound in question on the VdW (van der Waals) net of the model molecules of quinine **(7)**, tetraiodosaccharine **(8)**, kheline **(9)**, and coffeine **(10)** (Figure 3).

Modeling of the molecular matrix (mould) of bitter taste receptors

The topological model of the bitter taste receptor was built by analogy to the shape of Temussi's taste receptor of sweet taste. Molecular mould was obtained by superposition of the agonists with the largest possible rigid area in the plane of AH-B (centers): quinine **(7)**, tetraiodosaccharine **(8)**, kheline **(9)**, and coffeine **(10)** (Figure 4) [Skolik et al., 2002].



Quinine **(7)** Tetraiodosaccharine **(8)**



Kheline **(9)** Coffeine **(10)**

Fig. 4. Potential map with active centers agonists **(7–10)**.

The calculated electrostatic potentials of those compounds enabled them to be fitted one in the other (Figure 5). Spatial orientation of model compounds was obtained by positioning them so that their appropriate electrophylic-nucleophilic sites were facing their complementary sites that are found in the hydrophilic part of the receptor. For the resulting supermolecule, van der Waals' total area was found which may be considered to be the critical area of a receptor unit. A solid line marks the critical area, the range of interaction of model antagonists at the distance of van der Waals radiuses with the walls of the hypothetic receptor. The resulting topological form was referred to as the **molecular matrix of bitter taste receptor** (Figure 6). The obtained map of the molecular mould was divided into sectors A-D.

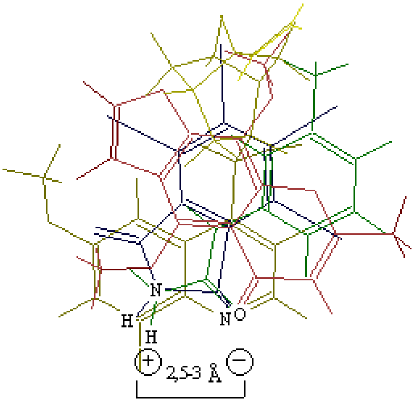


Fig. 5. Superposition agonists: quinine **(7)** in yellow
tetraiodosaccharine **(8)** in blue
kheline **(9)** in red
coffeine **(10)** in green.

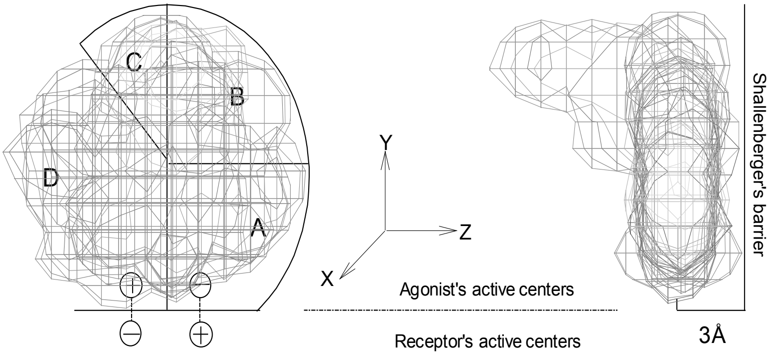


Fig. 6. Matrix-model of the molecular mould of the bitter taste compounds and its division into four sectors A-D in y-z plane and x-z plane according to Schallenberger spatial barrier

The following regions were isolated within the molecular matrix:

- *region of strong nucleo-electrophylic sites of the matrix* (in the plane of the Y-axis), in which interactions between the polar groups of the hydrophilic portion of the antagonist and the hydrophilic portion of the matrix are observed, which result in the antagonist being anchored in the receptor and sterical positioning of the remaining portion of the compound,
- *A, B, C, sectors* in which there occur interactions between the antagonists, brought about by hydrophobic forces, and complementary interactions of the n/e and e/n type with the walls of the molecular matrix describing the bitter taste receptor,
- *walls of the matrix in Sector A* which, when stimulated by interactions with the antagonists, have a significant effect on the structure of intensity of bitter taste (so-called "profile" of deep bitter taste),
- *open space in Sector D of the molecular matrix* which, having No. critical dimension, frequently enables very large molecules to penetrate into the bitter taste receptor.

Computer fitting sparteine monolactams in to the molecular mould

The main important factor in the chemoreception of taste is the conformation of the sparteine derivatives (Figure 1,2). The sparteine monolactams **(1,2,5)** with ring C in boat form fit well into the molecular matrix of the bitter taste receptor (sector A and partially B) which results in the sensation of bitterness of very high intensity (Figure 7). The full-chair sparteine monolactams **(3,4,6)** does not fit into the moleculae mould of tne bitter taste and does not stimulate the molecular matrix of the bitter taste receptor (Figure 8).

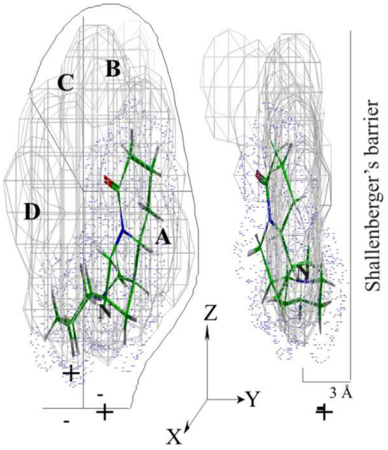


Fig. 7. Projections of lupanine **(2)** molecule with dot surface map (blue contours) were fit to the molecular matrix of bitter taste receptor

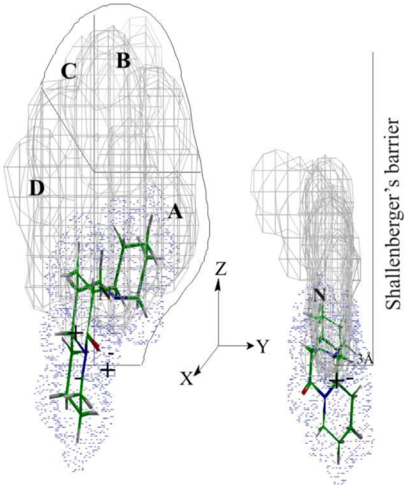


Fig. 8. Projections of aphylline **(3)** molecule with dot surface map (blue contours) were fit to the molecular matrix of bitter taste receptor

Conclusions

Studies on the relationship between bitter taste and some stereochemical aspects of sparteine derivatives confirmed our latest assumption concerning the model of the bitter taste receptor. The results of the sensory tests support these conclusions.

To better understand the interactions that may occur between the structure of compounds and their tastes, the chemoreception of the sparteine monolactams by receptor sites in the human body was examined.

To explain the chemoreception phenomenon of sparteine derivatives in a comprehensive manner, the surfaces of both the compounds with bitter taste and the receptor were investigated efficiently and thoroughly. Those surfaces were observed to complement each other in terms of size, shape, and functions.

Apart from *chair* conformers sparteine monolactams, all of the alkaloids demonstrated high bitterness indexe of over 56%. In future, those alkaloids may be accepted as the bitter taste standards, specifically sparteine **(1)**, which is a structure flat enough not to reach the bottom of the molecular mould of the bitter taste receptor, and it has the same effect on the receptor as quinine (in sector A).

The findings for a series of sparteine monolactams were categorized, averaged, and arranged to provide scales describing the effect of bitter taste and were used to provide a new systematics of those compounds based on their taste.

This paper has contributed to improving the cognitive value of computer modeling and indicated sparteine monolactams which are responsible for its bitter taste.

Our investigation showed that the obtained semi model of the bitter taste receptor (e.g. molecular mould) can help chemists to predict taste for synthesised or isolated compounds (not only alkaloids). The correlation between the predicted taste and the structure of some compounds can also help in stereochemical discussion on respective compounds.

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10

DETERMINATION OF ACRYLAMIDE CONTENT IN FRIED POTATO PRODUCTS

Introduction

Acrylamide is present in fried, baked and dried starch-containing products which have been cooked at temperatures > 120^{0}C, e.g. chips, French fries, bread, biscuits, etc. [Rosén & Hellenäs, 2002; Tareke et al., 2002; Grob et al., 2003; Jung et at., 2003; Granda et al., 2004]. Acrylamide content of chips, reported by researchers, may range from 100 to 6500 μg·kg^{-1} [Becalski et al., 2003; Svensson et al., 2003, Taeymans et al., 2004; Kita et al., 2008] from 50 to 1900 μg·kg^{-1} in French fries [Becalski et al., 2003; Svensson et al., 2003; Fiselier & Grob, 2005; Palazoğlu & Gőkmen, 2008; Tajner-Czopek et al., 2008].

Due to the toxic effects of acrylamide, extensive studies have been carried out in order to get to know the structure of this compound and the mechanism of its formation [Taeymans et al., 2004; Claeys et al., 2005]. At the same time, researchers and food manufacturers conduct studies aimed at finding the ways to reduce acrylamide content in food products. Besides, research is also focused on improvement and modifications of the methods used for acrylamide content determinations for more precise and accurate measurements. The first methods used for these purposes were: high-performance liquid chromatography (HPLC) with ultraviolet detector [EPA, 1994] and gas chromatography electron-capture detector (GC-ECD) with derivatization [EPA, 1996]. However, these methods proved to be useful for simple aqueous solutions, but not suitable for measuring trace amounts of acrylamide in a complex matrix, such as processed food. The majority of methods reported for the analysis of acrylamide are based on derivatization of bromination by GC-ECD [Takatsuki et al., 2003]. During the derivatization procedure, the ethylenic double bond in acrylamide is substituted with potassium bromine or potassium bromated to produce 2,3-dibromopropionamide (2,3-DBPA) [Nemoto et al., 2002]. Precise analytical methods are required for determinations of acrylamide content in various processed food products and foodstuffs [Kim et al., 2007). Most recently, gas chromatography mass spectrometry (GC-MS) [Takere et al., 2000] and liquid chromatography-tandem mass spectrometry (LC-MS-MS) [Rosen & Hellenäs, 2002; Kim et al., 2007], have been used for acrylamide determinations. The main differences between the methods used for this purpose can be seen in a variety of preparatory procedures for acrylamide extraction, aqueous extract purification, derivatization, and the use of various internal standards. Researchers have also studied the use of HPLC-MS-MS and GC-chemical ionization mass spectrometry (GC-CI-MS) to remove problems in both the derivative reaction and dehydrobromation [Andrzejewski et al., 2004]. 2-methylacrylamide and $^{13}C_3$-acrylamide are used as internal standards in GC-MS analysis [Becalski et al., 2004] and $^{13}C_3$- acrylamide and d_3-acrylamide are available for LC-MS-MS [Rosén & Hellenäs, 2002]. The LC-MS-MS method employing $^{13}C_3$-acrylamide is easier than GC-ECD and GC-MS, but the problems of poor recovery efficiency of acrylamide-d_3 and $^{13}C_3$-acrylamide during the extraction and purification of a sample matrix have not been solved [Nemoto et al., 2002]. The HPLC method has the advantage that the transfer of acrylamide into an organic solvent or the removal of water after aqueous extraction is not necessary [Hoenicke et al., 2004].

Selection of the method for acrylamide content determination depends on the type of potato product under investigation. When chips and French fries or any other potato products containing fat are studied, it is important to that fat extraction is followed by acrylamide extraction, with respect to the weight of the sample and all the requirements and of LC-MS-MS parameters, taking into account some modifications at certain stages, especially elimination of fat extraction with the use of methanol at 65^{0}C [Pedersen & Olsson, 2003] or hexane [Kita et al., 2004] thereby reducing the measurements of acrylamide content in defatted chips and French fries.

The purpose of the present study was to determine acrylamide content of potato chips and French fries by adapting the procedures reported in literature, using liquid chromatography tandem mass spectrometry LC-MS-MS.

Materials and methods

Materials. The material selected for the study consisted of French fries and chips made from potatoes containing 21.80 % of dry matter and 0.17% of reducing sugar content.

Preparation of French fries and potato chips.

French fries. The potatoes were washed and peeled using a carboround peeler and next cut into strips sized 1x1 cm, 6–7 cm long. Next, they were blanched in water at 80°C for 5 minutes. The French fries were fried in rapeseed oil (3.5 l per 200 g of potato strips). One-cycle frying was repeated 10 times (at 140°C) and acrylamide content was determined in each of them. Four samples of French fries were selected for the determinations of the "recovery rate"of acrylamide. Frying temperature was different for each sample: 130°C, 150°C, 175°C and 190°C. The experiment was carried out in 9 replications. In this way, French fries of varied acrylamide content were obtained. Acrylamide content was determined with and without internal standard.

Potato chips. The potatoes were washed and peeled using a carboround peeler. Next, they were sliced into 1.2 mm thick slices and fried in rapeseed oil (3.5 l per 100 g of potato chips) to the moisture content < 2%. Frying of chips was performed in 10 replications (at 180°C) and acrylamide content was determined in each sample. Two samples of chips were selected for the determinations of the acrylamide "recovery rate". For this purpose, the chips were fried at 175°C and 185°C, in 9 replications. In this way, chips of varied acrylamide content were obtained. Acrylamide content was determined with and without internal standard. Acrylamide content of the French fries and chips was determined using an LC-MS-MS equipment.

Analysis.

Reagents. All reagents were of analytical grade unless otherwise stated. Acrylamide (2-propene amide) and d_3-acrylamide (standard solution) were purchased from Fluka, Switzerland. Methanol HPLC-grade (methanol) was purchased from Merck (Darmstadt, Germany), water Milli-Q (deionized water) from a Millipore purification system (Millipore, Bedford, MA, USA) and HPLC-grade acetonitrile from Lab-Scan (Ireland). Two Supelco SPE columns (Bellfonte PA, USA) were used for purification of the samples. The upper one (MCAX) of 300 mg and 3 ml in volume was placed directly on the lower one (DSC C-18 of 1g and 6 ml in volume). The two columns were connected with each other by an adapter.

Standard solutions and calibration standard solutions. Preparation and calibration of a standard solution were performed by modifying the method described by [Rośen & Hellenäs, 2002; Takatsuki, Nemoto et al., 2003; Aurand & Trinh, 2005; Brunton et al., 2007]. The stock solutions of acrylamide and d_3-acrylamide-standard solution as well as calibration standard were prepared in acetonitrile concentration of 500 $mg \cdot L^{-1}$ (500 ppm) (Fluka Co.). The standard solution was protected from light and stored in a refrigerator. The stock solutions of standards 500 $mg \cdot L^{-1}$ (500 ppm), were prepared in acetonitrile and refrigerated. A working standard solution, for spiking samples as well as for the standard curve, was obtained by dilution in acetonitrile. Concentrations for the standard curve were 0, 1, 5, 10, 30, 50, 100, 500, 1000, 1500 and 2000 $ng \cdot ml^{-1}$, all with acrylamide-d_3 at 50 $ng \cdot ml^{-1}$ for French fries and potato chips.

Sample preparation for the LC-MS-MS analysis. The samples were prepared by modifying the methods described by [Rośen & Hellenäs, 2002; Aurand & Trinh, 2005; Brunton et al., 2007; Pedreschi et al., 2007; Zhang et al., 2007]. The samples of ground, non-defatted French fries, each weighing 4g (0.0001 g) and chips, each weighing 2 g (0.0001 g) were prepared following the procedure described in *2.2.* The French fries and the chips were ground using a GM 200 cutting mill (Retsch, GmbH, Germany) at a speed of 2000 $rev \cdot min^{-1}$ for 16 seconds (2 x 16 seconds). The samples were next placed in 50 ml Falcon centrifuge tubes and added 40 ml of deionised water. Parallely, the samples containing 200 µg/L of internal standard (akrylamid-d_3), at a concentration of 10 $\mu g \cdot ml^{-1}$ were prepared. The samples were shaken using a Multi Reax shaker (Heidolph Instruments GmbH & Co.KG. Germany) for 10 minutes. Next, the samples were centrifuged in 3 KI 5 centrifuge (Sigma) at a speed of 9500 $rev \cdot min^{-1}$ and 4°C for 15 minutes.

Solid phase extraction (SPE). Solid phase extraction (SPE) was performed by modifying the method described by Aurand & Trinh, [2005], Eerola et al., [2007] and Kim et. al. [2007]. 1 ml of transparent (clarified) solution (IA) was placed in SPA columns, which had been previously conditioned with 1ml of methanol and 1ml of deionized water, and vacuum-dried (Vac Elut 20 Manifold, Varian, Walut Creek, CA., USA). The samples in the columns were washed with 1 ml of deionized water. The upper SPE columns (MCAX) and the filtrate were removed. Acrylamide from the lower SPE columns (C-18) were eluted with 2 ml of methanol and the filtrate obtained was nitrogen-dried (at 30°C). The dry residue was dissolved in 500 µL (0.5 ml) of deionized water and placed in an autosampler using liquid chromatography-tandem mass spectrometry (LC-MS-MS). The final acrylamide content of the finished product was expressed in [$\mu g \cdot kg^{1}$] = [ppb]. Figure 1 shows a scheme of acrylamide extraction from potato fried products.

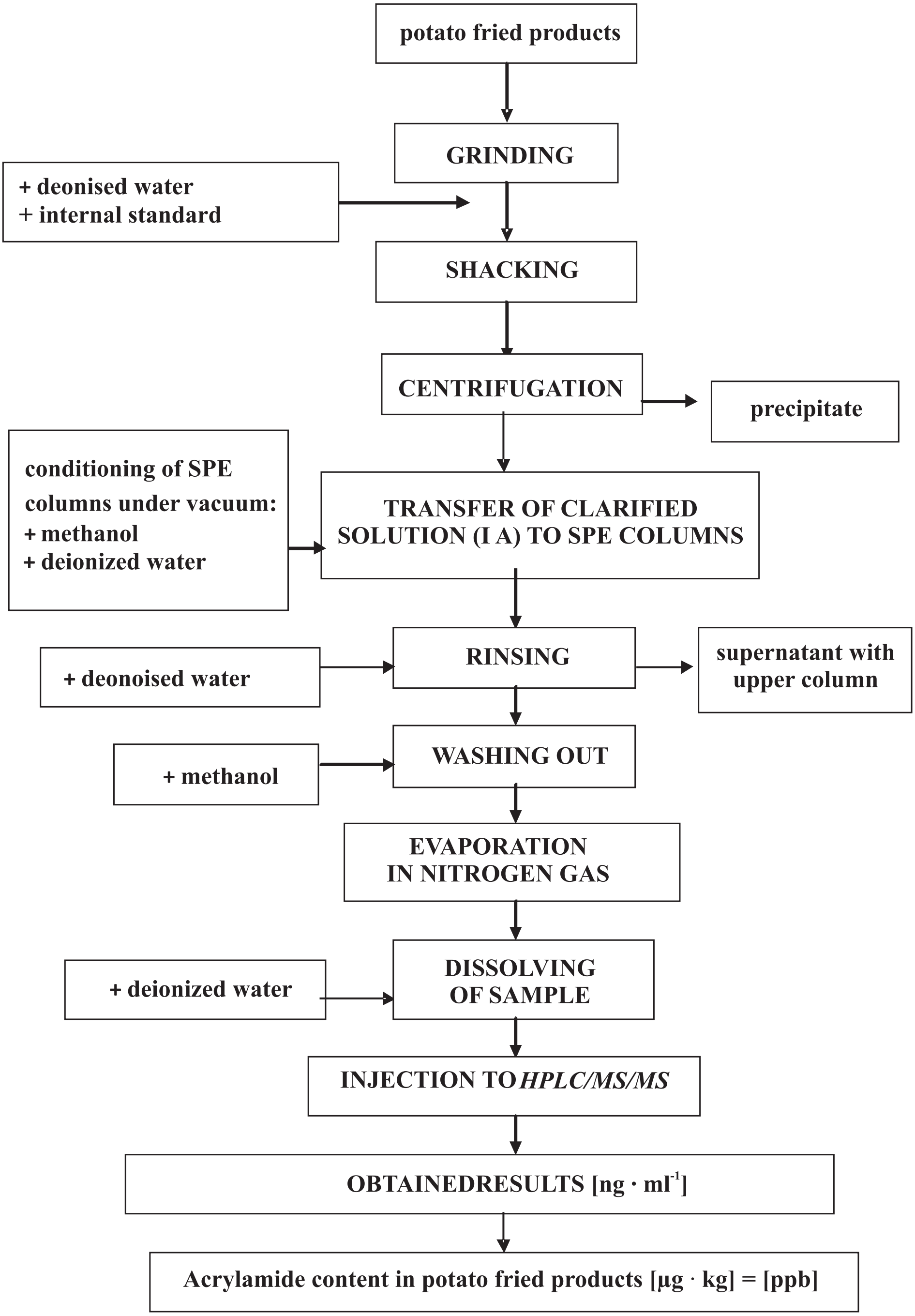

Fig. 1. The scheme of acrylamide extraction from potato fried products

Equipment and LC-MS-MS analysis. An improved liquid chromatography-tandem mass spectrometry (LC-MS-MS) method was developed with modification of methods published by other authors [Takatsuki et al., 2003; Aurand & Trinh, 2005; Gőkmen et al., 2005; Eerola et al., 2007] for the determination of acrylamide in potato food products. The acrylamide content of French fries and potato chips was obtained using LC-MS-MS 1200 L (Varian, Walnut Creek, CA., USA) performed using a triple quadruple with interfacial electrospray (ESI) a HPLC system Model a ProStar of a Varian, Walnut Creek, USA. HPLC-MS-MS 1200L system was equipped with one ProStar 210 Pump and ProStar 430 Autosampler. (Nitrogen generator, domnick hunter, model G 4510 E, UK). The samples were separated by the Pursuit XRs 3 u C-18 column (150 x 2.0 mm) – with a Metaguard Pursuit XRs 3 u C18 column (2.0mm, Varian, Walnut Creek, CA.,USA), using the mobile phase of 0.5% methanol in aqueous 0.1% acetic acid, at a flow rate of 0.2 $mL \cdot min^{-1}$. The volume of each injected sample was 20 μl.

MS-MS condition for the determination of acrylamide. The LC-MS-MS was operated in the positive electrospray mode, needle voltage 5000V, nebulizing gas (compressed air or N_2) 54 psi, capillary voltage 25V, drying gas (N_2, 99.5%, 400^{o}C, 22 psi). The collision cell gas (Ar 99.999%) pressure was 1.86 mTorr) and the detector voltage was set to 1300V. Acrylamide was determined by multiple reaction monitoring (MRM). The MRM mode was performed by monitoring the $72 \rightarrow 54.9$ *m/z* transition for acrylamide (collision energy-10V) and $75 \rightarrow 58$ *m/z* transition for acrylamide-d_3 (collision energy -10V). In all the MRM transitions, the dwell and inter scan delay times were 1 sec, respectively, SIM width 0.7 atm. The methods detection of limit values were calculated for S/N = 3 for the summed signals of both fragment ions.

Statistical analysis. The data of acrylamide content determination were analyzed statistically using a Statistica v. 8.0. programme. Standard methods for determining the acrylamide content of French fries and chips were used in 10 replications in order to compare the results obtained with the use of LC-MS-MS. The analysis of variance ($p<0.05$) was used for confirmation of the repeatability of the results. The determinations of the recovery rate [%] of acrylamide in the samples of French fries and chips were performed in 9 replications. All mean results are shown in tables.

Results and discussion

Fat extraction is not only time consuming, but also adversely affects acrylamide determinations of fried potato products [Pedersen & Olsson, 2005]. However Grob et al. [2004] and Tanaka et al. [2004] reported that using Soxhlet extraction with methanol at 65^{0}C for several days, acrylamide was formed from coextracted precursors and thus Soxhlet extraction suffered from de novo formation of acrylamide. Elimination of fat extraction using the LC-MS-MS method for acrylamide content determinations, replaced by the analysis of non-defatted samples could markedly reduce the length of acrylamide content determinations.

In recent years, the procedures used for acrylamide content determinations in fried potato products have been modified by analysing defatted samples and the use of aqueous acrylamide extraction from the samples of French fries [Pedreschi et al., 2008] and chips [Kim et al., 2007]. The results obtained in the present study show that the use of non-defatted samples, aqueous acrylamide extraction, and purification with the use of two SPE columns:

DSC-MCAX and DSC-18 [Aurand & Trinh, 2005], markedly reduced the time needed for the determinations. Besides, the results obtained using the LC-MS-MS method were repeatable.

Two groups of potato fried samples were selected (French fries – temperature of frying 140°C and chips – 180°C) to compare various analysis errors based on variable sample matrixes and acrylamide concentration. Table 1 shows that repeatability for French fries with the use of LC-MS-MS method modified by the authors of the present study accounted for 96% at standard deviation of 5.53, while with chips it reached 95% at standard deviation of 25.67. As has been found, this method is suitable for determining acrylamide content of fried potato products. The analysis of variance at $p<0.05$ showed that the results were repeatable, since the accuracy reached 95% [Stanisz, 1998]. Kim et al. [2007] obtained reapeatability of potato chips at 0.4%, of bread at 9.0% and coffee at 3.1%. Author [Kim et al., 2007] report that repeatability was clearly dependent on residual concentrations and the characteristics of samples.

Table 1

Repeated analysis of acrylamide content in fried potato products, using LC-MS-MS

Assay No. (replications)	French fries 140°C	Potato chips 180°C
1	324	1396
2	322	1441
3	311	1391
4	320	1394
5	325	1435
6	318	1448
7	311	1433
8	325	1372
9	313	1398
10	321	1409
Mean±SD	319±5.53	1412±25.67
Statistical analysis of replications [%]	96	95

Table 2 shows the results of acrylamide content of French fries and chips fried at different temperatures. The acrylamide content and the recovery rate [%] were determined by comparison of the concentration of this compound in the samples containing standard d_3-acrylamide and d_3-acrylamide-free samples. The recovery rates determined for French fries were found within the ranges from 94 to 104% and for potato chips within the ranges from 97 to 105%. All the results are within acceptable limits. Matissek & Raters [2005] report that the recovery rate in potato chips was 94%, while Park et al. [2005] obtained 108% with potato chips and 105% with French fries. Eerola et al. [2007] reported the ranges from 98 to 108%. Kim et al. [2007] obtained 102.2% recovery rate in potato chips, but authors used $^{13}C_3$-acrylamide – international standard and d_5-3-chloropropanediol – as a recovery standard prior to purification after extraction of acrylamide in sample. Authors [Kim et al., 2007] explain that using of suitable solution as a recovery standard was important to determine the quantitation of acrylamide by LC-MS-MS method. Table 2 shows that acrylamide content of French fries and chips increased with the increasing temperatures of frying, while the recovery rates were decreasing.

Table 2

Acrylamide content determined in fried potato products[1)]

Sample	Frying temperature	Acrylamide content [μg·kg^{-1}]			Recovery rate [%][2)]
	[^{0}C]	Before spiking (without standard)	Spiked amount (standard addition)	After spiking (after standard addition)	
French fries	130	80	77	160	104.0
	150	493	500	973	96.0
	175	925	875	1759	95.0
	190	1153	1000	2062	94.0
Average					97.0
Potato chips	175	896	500	1420	105.0
Average	185	1642	1000	2612	97.0
					101.0

[1)] Values are given as mean, n = 9

[2)] Recovery rate [%] = $\dfrac{\text{(After spiking – Before spiking) x 100}}{\text{Spiked amount}}$ [Kim et al., 2007]

Conclusions

The use of non-defatted French fries and chips (No. fat extraction) and aquaeous acrylamide extraction from the samples (the acrylamide content in potato chips was between 896–1642 μg·kg^{-1} and in French fries 80–1642 μg·kg^{-1}), especially purification with the use of two SPE columns (DSC-MCAX and DSC-18) markedly reduced the length of time necessary to determine acrylamide content of the finished product and did not affect repeatability of the results when the LC-MS-MS was used. The results of acrylamide content of French Fries were repeated in 96% and those of chips in 95%, therefore, the LC-MS-MS method was considered suitable for these determinations. The changes in the recovery rate (%) of both French fries and chips were found within almost the same ranges and the recovery rate was decreasing with the increasing temperatures of frying.

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11

STUDIES ON APPLICABILITY OF DIFFERENTIAL SCANNING CALORIMETRY (DSC) TO DETECT BUTTER ADULTERATION WITH PALM OIL

Introduction

Butter is a product, which name is legally protected, i.e. in accordance with the appendix to the Ordinance of the EEC Council no. 1898/87 it is a product produced solely from milk. A similar definition is contained in Polish Standard PN-A-86155:1995, where in the production of butter it is admissible to use only pasteurized cream, starters and other as well as natural pigments. All other products containing an admixture of foreign fats of animal or plant origin may not be called butter. Butter fat is relatively expensive (approx. 10–12 zlotys/kg) if we compare it to the price of e.g. palm oil, amounting to approx. 3 złotys/l. An addition of cheaper fats in order to reduce the price of butter and at the same time neglecting the need to inform the consumer on that fact on the label is adulteration of food. According to the definition which was given in the Act on the commercial quality of agri-food products (the official Gazette Dziennik Ustaw of 2008 no. 214, item 1346) an adulterated agri-food product is "a product, which composition does not comply with the regulations concerning commercial quality of agri-food products or a product, in which changes were introduced concerning labeling, aiming at concealment of its actual composition...". The problem of adulteration in the dairy sector is a matter of giving honest information on the label and applying adequate nomenclature.

In recent years intensive works have been conducted aiming at the development of effective methods to detect butter adulterations. Stołyhwo and Rutkowska [2007] in their study presented a review of methods facilitating the detection of the presence of foreign fats in butter. One of the described methods is the analysis of fatty acid composition using gas chromatography. It is a relatively simple method, although burdened with certain limitations connected with changes in acid composition, depending on the season of the year. Gas chromatography is also used to detect adulterations of butter based on the composition of triacylglycerols. In some laboratories methods based on the determination of tocochromanols or phytosterols by HPLC are also used to detect the presence of foreign vegetable fats in butter.

A relatively simple technique which is not very expensive or labour-intensive is differential scanning calorimetry (DSC), facilitating analyses of various food components, including proteins, carbohydrates and fats. Studies on processes of fat melting and crystallization using this technique consist in the measurement of the amount of released or absorbed heat. In world literature on the subject we may find examples of studies on the application of DSC to detect adulterations in different fats. The method to analyze fats using DSC is based on the fact that every fat has its characteristic composition of fatty acids and triacylglycerols, which are unique for a given type of fat, being manifested in the characteristic melting or crystallization curve, referred to as a thermogram.

Marikkar et al. [2003] investigated palm oil and its mixture with lard, poultry fat and suet. They ran the crystallization process for 100% palm oil and its mixture with suet, lard or poultry fat. When comparing individual thermograms significant differences were observed in the process of crystallization (changes in peak temperatures and peak areas) with an increasing percentage of a given animal fat. In studies on chocolate adulteration different types of bitter chocolate were compared, depending on the proportion of cocoa butter [Fesser et al., 2005]. Depending on the amount of cocoa butter in chocolate and the amount of used cacao butter substitutes (replacers and equivalents) different thermograms were obtained for melting and crystallization. Coni et al. [1994] investigated adulteration of butter with animal fats, particularly poultry fat, using DSC. Based on obtained thermograms of melting and crystallization it was shown that there are considerable differences for butter, poultry fat and mixtures of butter with fat in terms of values of peak temperatures, peak areas and start transition temperatures. Aktas and Kaya [2001] used DSC to detect adulterations of butter with animal fats and margarine. Based on thermal curves of melting and crystallization they showed differences in the course of these processes depending on the content of added fats in butter. They stated that adulteration of butter with suet was easier to detect than that with vegetable fat.

The aim of the presented study was to investigate applicability of DSC to detect adulterations of butter resulting from an addition of palm oil.

Methods

Thermal analysis was conducted using a DSC 7 differential scanning calorimeter by Perkin-Elmer. The "Gostyńskie" butter, earlier analyzed by HPLC to detect adulterations with vegetable oils, was used as pure, unadulterated butter. A sample of dehydrated butter was used in DSC measurements. A sample of dehydrated butter, in the amount of approx. 12 mg weighed to an aluminum dish of 20 µl was placed in the calorimeter capsule. In order to melt all polymorphic forms the sample was first heated at 50^{o}C for 2 min. and next the analysis of crystallization was run at a temperature range from 50^{o}C to -40^{0}C at a cooling rate of 5^{o}C/min. The successive analysis was conducted for the melting process at a temperature range from -40^{o}C to 50^{o}C, at a heating rate of 5^{o}C/min. The reference sample was an empty capsule. Analyses were conducted on samples of butter, palm oil, and mixtures of butter with an addition of palm oil at 5, 10, 20, 40, 60 and 80%.

Based on obtained thermograms the following parameters were analyzed:

- for crystallization : maximum transition temperature T_{K1} T_{K2},[^{0}C], onset transition temperature -T_{onset} [^{0}C], the difference in temperatures between two peaks $\Delta T = T_{K1} - T_{K2}$, enthalpy of crystallization ΔH [J/g]
- for melting: maximum transition temperature T_{T1} , T_{T2} [^{0}C], clearing temperature T_{sk}, the difference in temperature between two peaks $\Delta T = T_{K2} - T_{SK}$, enthalpy of melting ΔH [J/g].

Results and discussion

The effect of an addition of palm oil on temperature and enthalpy of butter melting

As a result of conducted analyses of the melting process by calorimetry using DSC7 thermograms were obtained for dehydrated 100% butter, dehydrated butter with an addition of 5, 10, 20, 40, 60 and 80% palm oil and 100% palm oil (Fig. 1). When analyzing the thermogram presenting a sample of 100% butter (Fig. 1) two peaks may be observed: T_{T1} and T_{T2}, which is consistent with previously presented results by Aktas and Kaya [2001]. Peaks T_{T1} and T_{T2} were identified in a sample of pure butter and in samples containing a 5, 10, 20, 40 and 60% addition of palm oil, while above this amount peak T_{T2} disappears and only peak T_{T1} remains (Fig. 1). It was observed that values of temperatures T_{T1} and T_{T2} and proportions of areas and heights of peaks T_{T1} and T_{T2} change with an increasing amount of palm oil added to butter.

Temperatures of peaks T_{T1} and T_{T2} decrease with an increasing addition of palm oil, for pure butter T_{T1} and $T_{T2,}$ amounting to 2.82°C and 11.24°C, respectively, while for a sample with a 60% addition of oil it was 1.04°C and 8.66°C. The ratio of the area (ΔH) and height of the first to the second peak decreased with an increasing amount of palm oil in butter. Moreover, an increase was found for end temperature of the melting process, referred to as clearing temperature (T_{SK}), from 32.31°C for pure butter to 38.57°C for pure palm oil. In relation with the observed phenomenon of an increase in temperature (T_{SK}) and a reduction of temperature $T_{T2\,,}$ another parameter, being a difference in temperatures $\Delta T_T = T_{SK} - T_{T2}$, was also analyzed.

Moreover, the study analyzed a dependence between the amount of palm oil added to butter and a change in values of such parameters as temperatures (T_{T1}, T_{T2}, T_{SK}), the difference in temperatures - ΔT_T (Fig. 2) and enthalpy: ΔH_{T1} and ΔH_{T2} (Fig. 3). The investigated dependence was described using a correlation coefficient r as well as a regression equation and the coefficient of determination – R^2 (at a significance level $p = 0.05$), results of correlation dependences are presented in the form of graphs (Figs. 2, 3).

For all analyzed parameters of temperature (Fig. 2) dependences were linear in character. For parameters T_{T2}, T_{SK} , ΔT_T coefficients of correlation exceeded 0.95, only for parameter T_{T1} it was below that value, amounting to $r = -0.8384$. For T_{T1} and T_{T2} correlations were negative, i.e. with an increase in the amount of palm oil these temperatures decreased, while for T_{SK} ΔT_T showed a positive correlation.

Figure 3 presents a dependence between the amount of palm oil in butter and changes in value of enthalpy ΔH_{T1} and $\Delta\ H_{T2}$, corresponding to two identified peaks of the melting process. The correlation coefficient for enthalpy of the first peak was $r = -0.94$, which indicates a strong negative dependence, the higher the addition of palm oil, the higher the value of enthalpy for the first peak. Correlation for enthalpy of the second peak was weaker ($r = -0.65$), but it was calculated only for the samples of 100% butter, butter with an addition of 5, 10, 20 and 40% palm oil, since only for these samples it was possible to calculate peak areas.

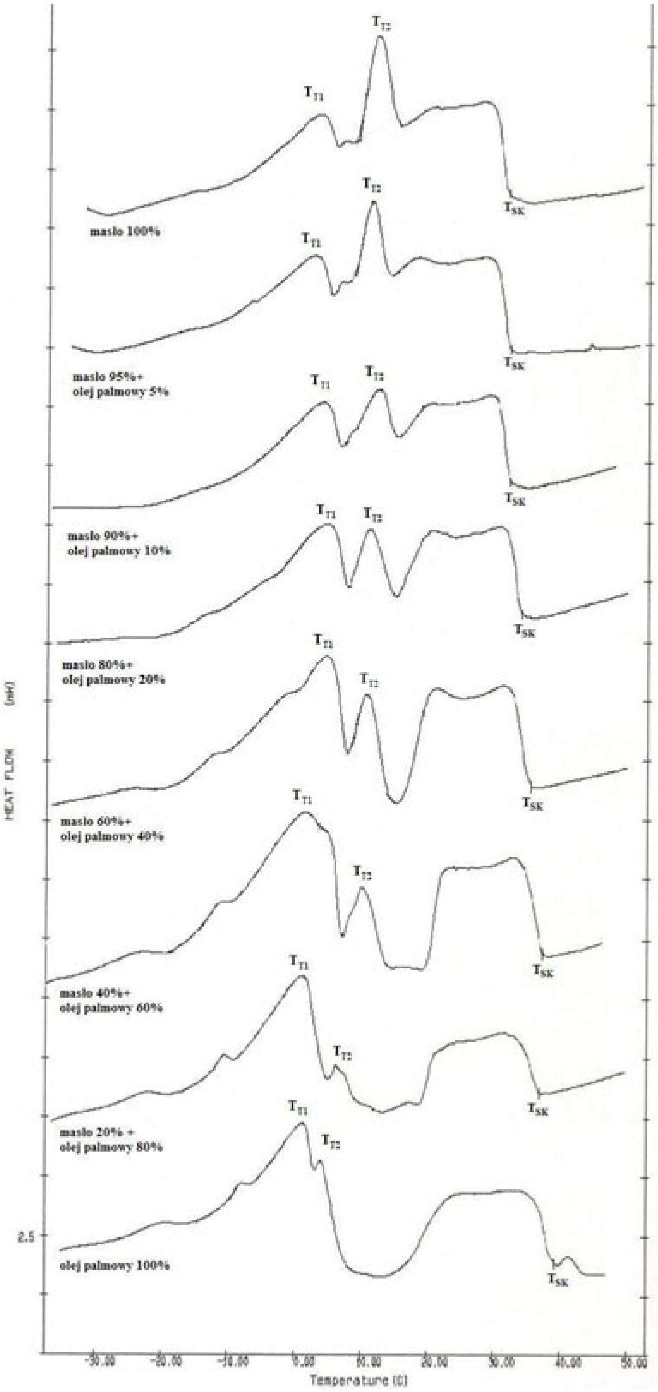

Fig. 1. A comparison of thermograms for melting of dehydrated butter modified with palm oil (heating rate: 5^0 C/min). B – butter, PO – palm oil

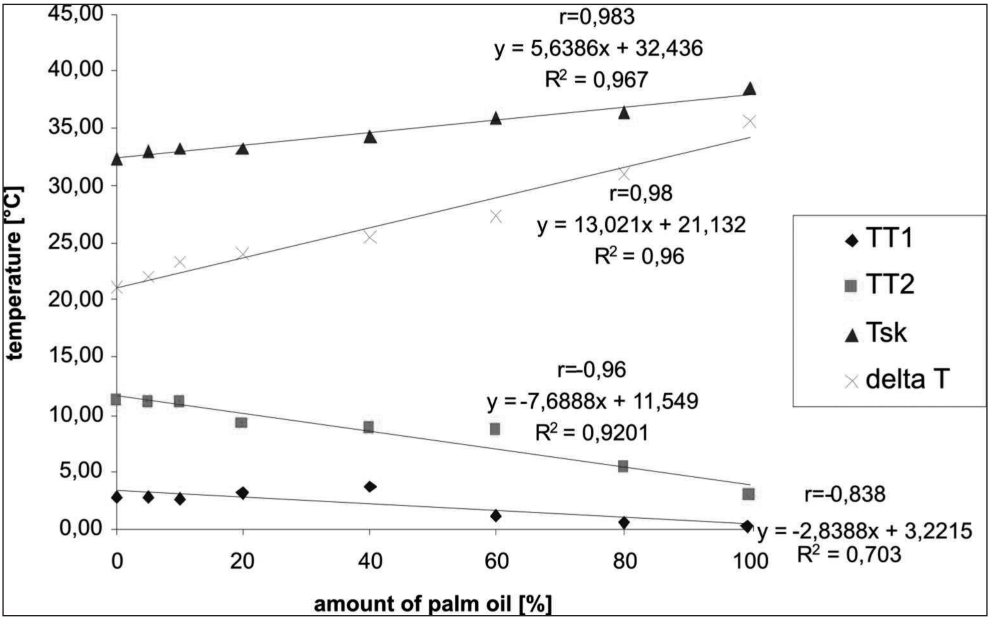

Fig. 2. The effect of an addition of palm oil on changes in temperatures of the melting process – T_{T1}, T_{T2}, T_{SK} and ΔT_T

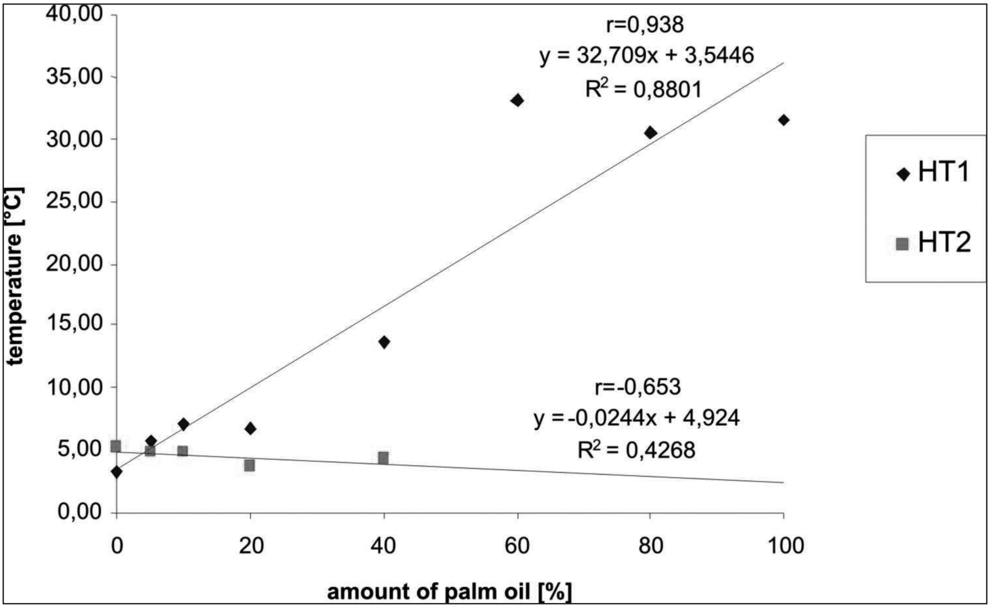

Fig. 3. The effect of an addition of palm oil on changes in enthalpy of melting – ΔH_{T1} and ΔH_{T2}

The effect of an addition of palm oil on temperature and enthalpy of butter crystallization

Similarly as for the melting process, analyses of the crystallization process were conducted on samples of 100% dehydrated butter, dehydrated butter with a 5, 10, 20, 40, 60 and 80% addition of palm oil as well as 100% palm oil (Fig. 4). On all obtained thermograms two peaks were observed, which shape and height changed markedly with an increase in the amount of added palm oil. Moreover, the distance between peaks also changed, i.e. the two peaks T_{K1}, T_{K2}, became more distant from each other, while for pure butter they are very close. In the sample containing a 40% addition of palm oil these peaks became distinctly separated. Based on the thermogram of pure butter values of individual parameters were determined, i.e. onset temperature of the crystallization process T_{onset}, of 9.81°C, temperature of peaks T_{K1}, T_{K2} of 8.61°C and 4.67°C, ΔT_T of 3.95°C. It was found that with an increasing amount of palm oil the following parameters increased: T_{onset}, which for pure palm oil was 17.8°C, parameters of temperatures of the first peak T_{K1} (15.6°C for pure palm oil) and ΔT_T of 18.15°C. Temperature of the second peak T_{K2} decreased with an increase in the percentage content of palm oil in butter up to a temperature of -2.55°C for palm oil.

Similarly as for the melting process, a dependence was determined between the addition of palm oil to butter and changes in temperatures and values of enthalpy of crystallization (T_{K1}, T_{K2}, T_{onset}, ΔT_K, ΔH_{K1} and ΔH_{K2}). Results are presented in the form of graphs (5 and 6).

Based on correlation coefficients ranging from 0.98 to 0.99 strong linear dependences were determined for parameters of temperature T_{K1}, T_{K2}, T_{onset}, and ΔT_K. For the temperature of the second peak T_{K2} a negative correlation was found ($r=-0.9851$), i.e. the higher the addition of palm oil, the lower the temperature of the second peak. In the other cases correlations were positive.

In case of the dependence between the amount of added palm oil and values of enthalpy (ΔH_{K1} and ΔH_{K2}) much lower correlation coefficients were recorded ($r=0.80$). In case of enthalpy of the first peak ΔH_{K1} it was possible to determine the values only for the samples of butter with a 60 and 80% addition of palm oil and 100% palm oil (in these samples a marked separation of these two peaks was observed).

Summing up the obtained results it may be stated that both for the process of melting and crystallization thermograms differ considerably in the shape of peaks and their parameters (temperature and enthalpy). Based on many replications it may be stated that each type of fat has a thermogram of melting and crystallization processes, characteristic of its composition. Using the analysis of correlations it was attempted to determine quantitative changes in values of temperature and enthalpy, depending on the amount of palm oil added to butter. It was established that values of all analyzed temperatures exhibit high correlation coefficients. Parameter ΔTT, which takes into consideration changes in values of both temperatures, is particularly suitable for the determination of an addition of palm oil. Correlation coefficients for this parameter were lowest, at 0.96 for the process of melting and 0.98 for crystallization. The parameter of enthalpy proved to be a definitely inferior parameter in the assessment of the amount of palm oil added to butter. Results were characterized by high scatter and lower correlation coefficients, which may result from problems with the integration of areas of individual peaks, which were not always distinctly separated. Further studies with a bigger number of replications are required to develop the detection threshold for this analytical technique.

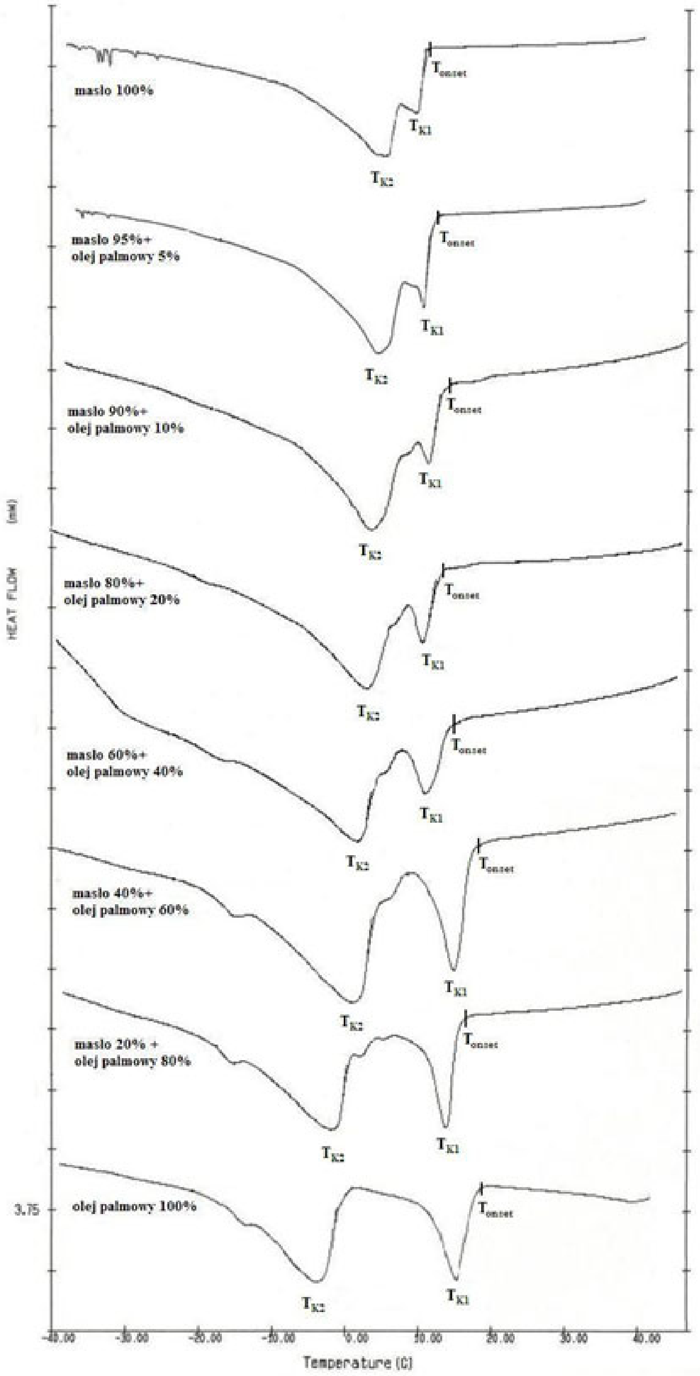

Fig. 4. A comparison of thermograms for the crystallization process of dehydrated butter modified with palm oil (heating rate: 5^0 C/min). B – butter, PO – palm oil

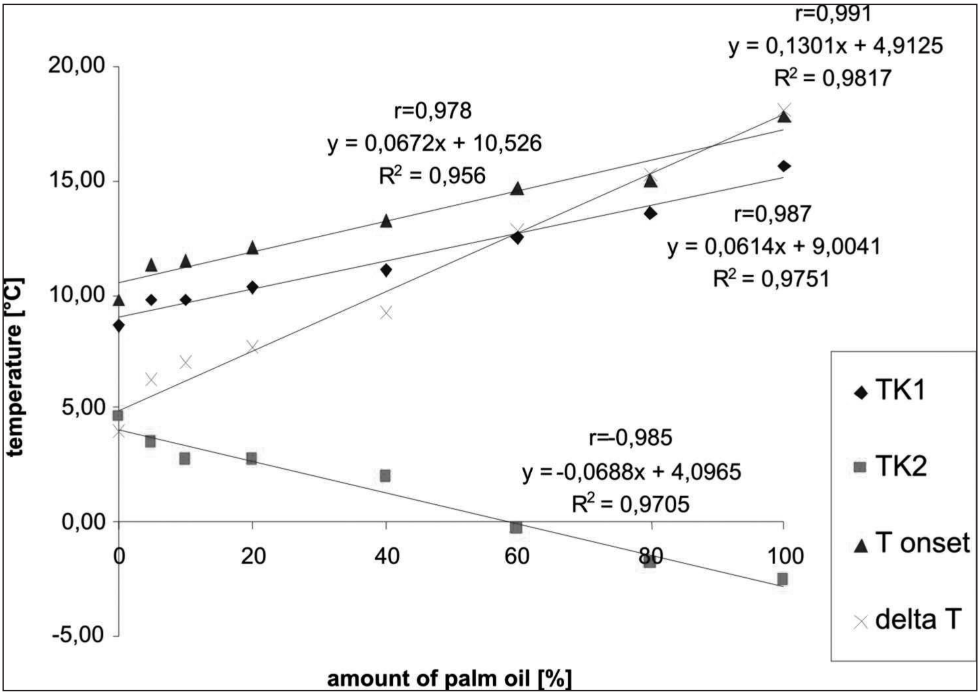

Fig. 5. The effect of an addition of palm oil on changes in temperature of crystallization – T_{K1}, T_{K2}, T_{onset} and ΔT_K

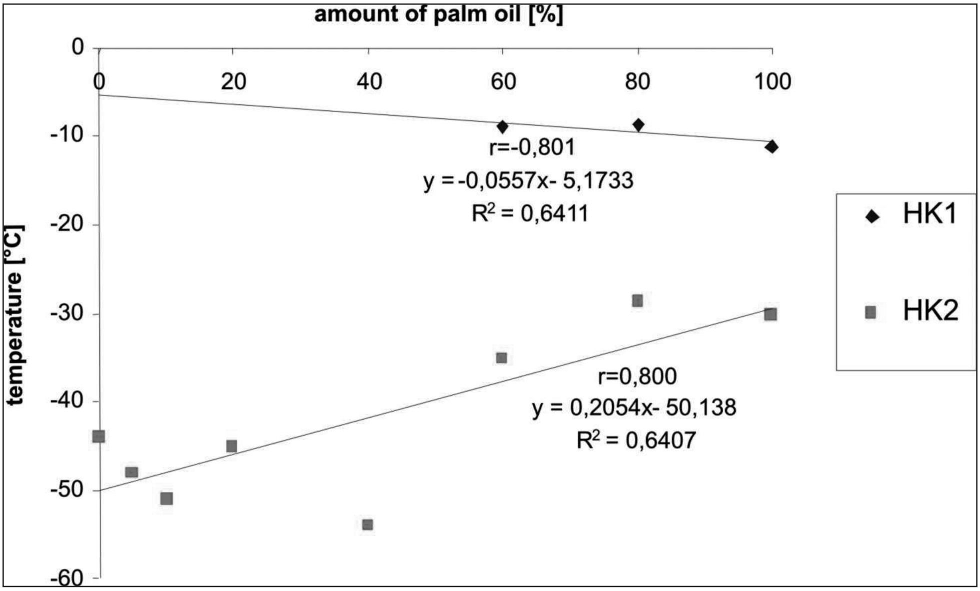

Fig. 6. The effect of an addition of palm oil on changes in enthalpy of crystallization – ΔH_{K1} and ΔH_{K2}

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12

AN ATTEMPT TO APPLY MEDIUM PRESSURE LIQUID CHROMATOGRAPHY TO SEPARATE LYSOZYME OLIGOMERS IN MODIFIED ENZYME PREPARATIONS

Introduction

Lysozyme is an enzymatic protein, commonly found in nature, characterized by highly useful properties, which are likely to find a much more extensive range of practical applications. A special role should be attributed to modified hen egg lysozyme, since the newly documented, extended antibacterial properties of lysozyme pertain first of all to its dimer form [Ibrahim et al., 1994; Kiczka, 2004; Leśnierowski and Kijowski, 2007; Leśnierowski, 2007]. Modified enzyme may be used not only in food industry to preserve foodstuffs, but also in medicine, veterinary medicine and pharmacology, e.g. as an agent against different infections, as a natural antibiotic or as an agent stimulating the immune system.

It results from conducted research that the presently used methods of enzyme oligomerization yield preparations, in which the amount of lysozyme dimer ranges from 30 to 40% [Ibrahim, 2003, Vogt and Winter, 2006, Leśnierowski, 2007, Cegielska-Radziejewska et al., 2009, Leśnierowski et al., 2009]. Although such an amount in samples definitely increases the range of the enzyme antibacterial action [Kijowski et al., 2000, Leśnierowski et al., 2001, Cegielska-Radziejewska et al., 2003, Leśnierowski et al., 2004, Cegielska-Radziejewska et al., 2008], it does not guarantee complete utilization of its potential. It seems necessary to develop methods increasing the proportion of dimer in produced preparations. It was attempted in this study to increase the proportion of dimer in modified preparations, purifying them with the use of medium pressure liquid chromatography.

Material and methods

Analyses were conduced on preparations of lysozyme modified by the thermo-chemical method [Leśnierowski, 2007].

Separation of samples of modified lysozyme into oligomeric fractions was run according to the methodology given by Leśnierowski [2007], applying medium-pressure liquid chromatography in a column packed with an acid ion filler and gel exchanger characterized by good affinity to proteins. In the analyses a module set for preparation liquid chromatography (B684-B688) by Büchi (Switzerland) was used. Columns with a capacity of 100 and 1800 ml were used, the stationary phase was Fractogel® EMD SO_3- by Merck and Sephadex™ G-75 by GE Healthcare Bio-Sciences AB.

Hydrolytic activity of lysozyme and its antibacterial activity against Gram-negative bacteria were analyzed by spectrophotometry [Leśnierowski, 2007].

In order to verify the objectivity of results they were analyzed statistically using the *STATISTICA PL v.7.0* software.

Results

In the first stage the analyses of fractions of modified lysozyme were run using a chromatographic column with a length of 230 mm and diameter of 15 mm packed with a sorbent Fractogel EMD by Merck with grain diameter of 20–40 µm. The concentration of the enzyme introduced to the column was 2.5%. Analyses were conducted using thermo-chemically modified lysozyme, which contained over 35% dimer and slight amounts of trimer (below 10%). As a result of the chromatographic process it was separated into two fractions, corresponding to successive peaks in the diagram (Fig. 1a). Thus produced preparations F1 and F2 (corresponding to the first and second fractions) considerably varied in terms of proportions of monomer and dimer, as well as differed from the oligomeric compositions of modified lysozyme preparation (sample 1) (Figs. 1b-d). The chromatographic process caused an enrichment of the first fraction (F1) in dimer, while in the second fraction (F2) the main component was monomer. As a result two preparations were obtained, which contained approx. 72% dimer and 28% monomer, and 77% monomer and 23% dimer, respectively (Tab. 1). Thus chromatography facilitated an over two-fold increase in the proportion of dimer in the sample. Such a considerable increase in its proportion in the preparation should enhance antibacterial properties of the enzyme, since this oligomeric form is responsible for the occurrence of the so-called specific activity extending the spectrum of lysozyme action [Ibrahim, 2003, Leśnierowski and Kijowski, 2007]. Conducted analyses showed that hydrolytic activity is attributed to the monomer form of lysozyme. It turned out that after chromatographic separation in the monomer-enriched fraction its value increased in comparison to the modified sample, while in the dimer-enriched fraction this value decreased (Tab. 1).

Presented results indicate that medium-pressure liquid chromatography may successfully be used to produce modified lysozyme considerably enriched in the dimer fraction. However, further studies are required, first of all aiming at the improvement of the rate of preparation purification and the production of a preparation containing an even bigger amount of dimer, the selection of more effective and cheaper sorbents and an improved efficiency of the chromatographic process. The problem of trimer, which appeared in many preparations of modified lysozyme, still remains open. So far little is known about trimer, particularly its antibacterial activity. Although scarce studies indicate that trimer may also exhibit such activity, it needs to be further documented [Leśnierowski, 2007]. It is required to isolate a pure trimeric form and subject it to microbiological analyses. Chromatographic methods may play a decisive role in the production of such a preparation; however, the efficiency of separations first needs to be improved. The present low recovery rate of separated fractions results in the situation when from a sample with a slight trimer content, following the chromatographic process, it is not recovered at all or it appears only in trace amounts. Slight amounts of trimer were obtained in the second stage of presented studies, in which the chromatographic column packed with a gel carrier Sephadex™ G-75 was used to isolate fractions from modified lysozyme. As a result of chromatographic separation three lysozyme fractions were produced (Fig. 2, Tab. 2). Separation of dimer and trimer was dependent on the conditions of the chromatographic process, and first of all on the amount of sample introduced onto the column. At the preparation concentration of 5 mg/ml, separation into fractions was poor, whereas a reduction of concentration to 1 mg/ml markedly improved this effect (Fig. 2). However, at such a reduced concentration the efficiency of the process was very low and further analyses of preparations containing microgram amounts of individual fractions were seriously hindered and reduced the accuracy of measurements.

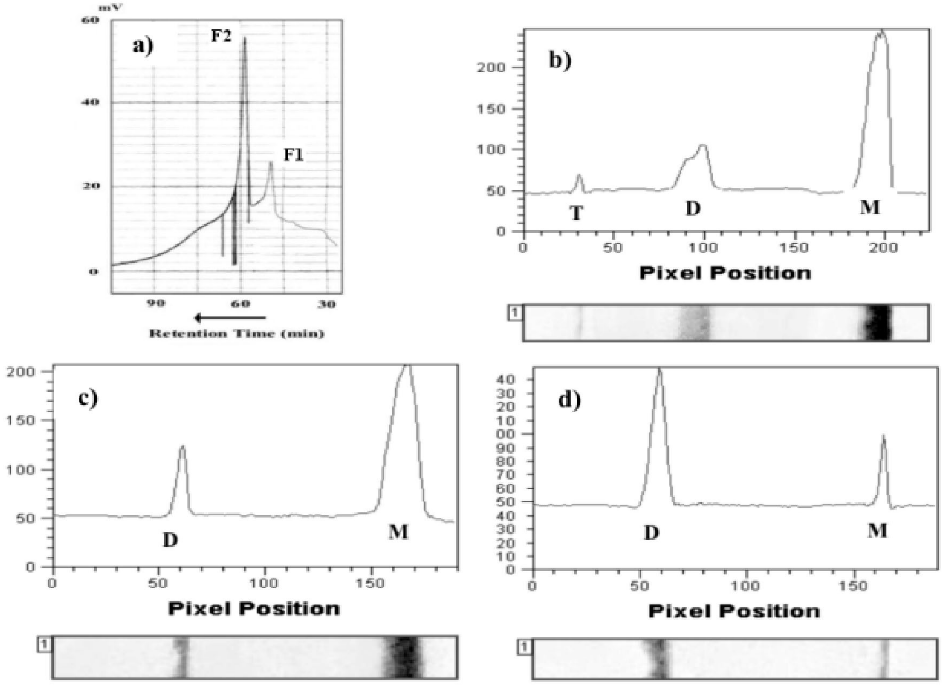


Fig. 1. Chromatogram (diagram a) and electrophoretic separations and densitograms obtained through purification of thermo-chemically modified lysozyme (diagram b) on a column filled with weak-acid cation exchanger Fractogel by Merck: diagram c – fraction 2 (F2), diagram d – fraction 1 (F1)

Table 1

Percentages of oligomeric forms and hydrolytic activity of lysozyme modified thermo-chemically (sample 1) and obtained as a result of the chromatographic process pf the first (F1) and second (F2) fractions

Sample	Monomer [%]	Dimer [%]	Trimer [%]	Hydrolytic activity [U/mg]
1	52.6^{b}	38.1^{b}	9.3	7920^{b}
F1	27.6^{c}	72.4^{a}	–	6150^{c}
F2	76.7^{a}	23.3^{c}	–	8460^{a}

a-c – different letters in columns denote a significant difference for means at p≤0.05

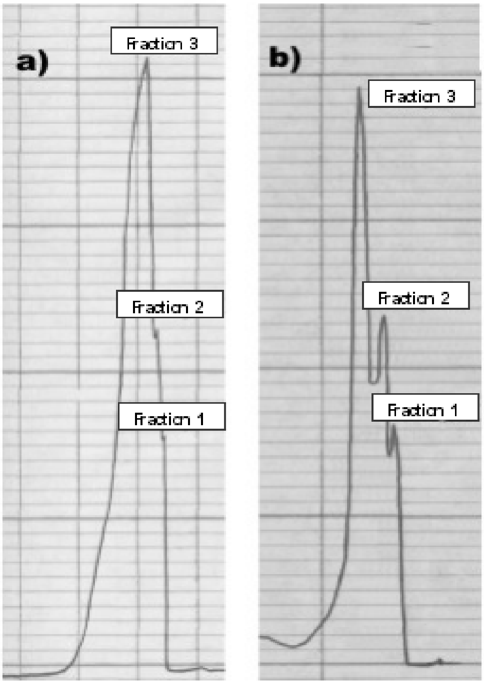


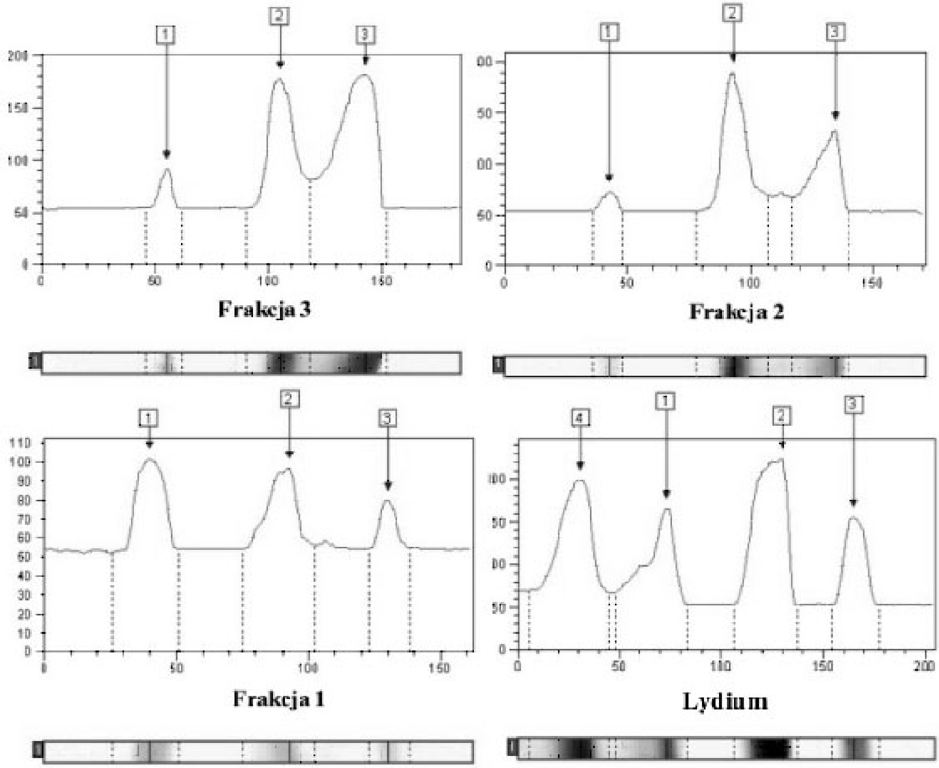
Fig. 2. Chromatograms obtained as a result of attempted separation of modified lysozyme using gel filtration on a Sephadex G-75 carrier by GE Healthcare AB, lysozyme concentration in solution: a) 5 mg/ml, b) 1 mg/ml

Table 2

Percentages of oligomeric forms, hydrolytic activity and activity against Gram-negative bacteria of modified lysozyme and obtained as a result of the chromatographic process of fractions

Sample	Percentage of oligomers			Hydrolitic activity [U/mg]	ΔA
	Monomer	Dimer	Trimer		
Modified lysozeme	31.4	35.3	32.9	5060	0.014
Fraction 3	16.3	35.3	48.5	450	0.009
Fraction 2	28.3	60.8	10.9	2030	0.018
Fraction 1	60.7	31.7	7.6	6310	0.006

Results collected in Table 2 confirmed the attribution of hydrolytic activity to the monomeric form of lysozyme, since its volume decreased with an increase in the amounts of oligomers in the sample. Conducted tests of the antibacterial action (Δ*A*) obtained after chromatographic separation of fractions showed that the highest activity against Gram-negative bacteria *Pseudomonas fluorescens* was exhibited by the preparation containing the biggest amount of dimer (Fraction 2) and it was so even despite the considerably reduced hydrolytic activity (Tab. 2). Good effectiveness against these bacteria was also found for the first fraction (Fraction 1), where trimer was the primary component, which may indicate its antibacterial properties. In order to finally confirm this fact it is necessary to test preparations containing pure oligomers, and such have not been produced to date. Studies concerning the application of chromatographic methods to produce preparations of pure lysozyme forms need to be further intensively developed, which create a prospect for the production of pure oligomers and as a consequence – the possibility to obtain new information on the enzyme itself.


Frakcja 3

Frakcja 2

Frakcja 1

Lydium

Fig. 3. Electrophoretic separations and densitograms of Lidium preparation (lysozyme dimer) and lysozyme fractions obtained as a result of gel filtration: 1 – trimer; 2 – dimer; 3 – monomer; 4 – tetramer

Conclusions

1. It was shown that the application of medium pressure liquid chromatography facilitates the separation of obtained fractions in modified lysozyme. Under optimal separation conditions produced preparations contained over 70% dimer.
2. The application of a gel carrier made it possible to isolate a trimeric fraction from modified lysozyme.
3. It was shown that hydrolytic activity of lysozyme is attributed to the monomer form of the enzyme, since its volume decreased with an increase in oligomer contents in the sample.

4. A higher effectiveness was shown for the action of the dimer-enriched preparation against Gram-negative bacteria as a result of the appearance of the so-called specific antibacterial activity.

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CHAPTER 4

QUALITY MANAGEMENT, MARKETING AND CONSUMER PREFERENCES

1

THE COMPARISON OF FATTY ACIDS AND STEROLS CONTENT IN *ARABICA* COFFEE BEANS DERIVED FROM FIVE COUNTRIES

Introduction

World's coffee production is mainly located in South America and Brazil is the leader of coffee production. Regardless of health hazard coffee consumption remains very high especially in Europe. Only two from 70 species of coffee are cultivated and are economically significant, these are *Coffea arabica* and *Cofea canephora,* known as *arabica* and *robusta*, respectively. Coffee is consumed in a form of beverages made from roasted beans [Belitz and Grosh, 1999; Martin et al., 2001]. Coffee production process is very complex and one of the very important steps is roasting to extract the delightful aroma of coffee. Roasting is conducted at 200–250ºC. There are four phases of roasting: drying, development, decomposition and full roasting. First composition changes of coffee beans are observed at 50ºC, proteins denatures and water evaporates. Due to pyrolysis of organic compounds browning occurs above 100ºC and volatile compounds are released at 150ºC. The decomposition phase leads to formation of coffee aroma at 180–200ºC [Belitz and Grosh, 1999]. As a result of these processes the chemical composition of coffee beans becomes significantly altered. The alteration is due to decomposition of hydrocarbons, reactions of hydrocarbons with proteins, partial breakdown of fats, proteins, tannins and formation of hundreds of aromatic compounds, that constitute coffee oil [Świderski, 1999].

During the roasting fat fraction of coffee changes less significantly in comparison to proteins and hydrocarbons. Therefore, an attempt was made to classify species of coffee by analysing the fat fraction. The average fat content of roasted coffee ranges from 11 to 17% [Vila et al., 2005]. Coffee fat contains mainly triacylglycerols (75%), free and esterified sterols (2%), free fatty acids (1%) and other components like terpene esters and acylglycerols [Nikolova-Damayanova et al., 1998; Vila et al., 2005]. The main fatty acids present in coffee oil are: myristic (C14:0), palmitic (C16:0), palmitoleic ($16:1\Delta^9$), stearic (18:0), oleic ($18:1\Delta^9$), linoleic ($C\ 18:2\Delta^{9,12}$), linolenic ($18:3\Delta^{9,12,15}$), arachidic (C 20:0), eicosenoic ($C\ 20:1\Delta^{11}$), and behenic acids (C 22:0) [Khan and Brown, 1953; Nikolova-Damayanova et al., 1998; Martin et al., 2001; Dussert et al., 2008]. Sterolic fraction contains mostly sitosterol, campesterol and stigmasterol [Carrera et al., 1998; Valdenebro et al., 1999; Speer and Kölling-Speer 2006].

The purpose of the study was to find the differences between fatty acid and sterol content in *arabica* coffee beans derived from five different countries before and after roasting.

Materials and methods

The study was carried out on five *arabica* coffee beans that were grown in five different countries: Brazil (B), Costa Rica (CS), Columbia (C), Guatemala (G), Ethiopia (E). Green and roasted beans were analyzed. Thermal procedure was performed using roasting equipment (PROBAT). In the beginning of roasting process coffee beans were kept in temperature of 190^{o}C for 20 minutes and then in 210^{o}C for 3 minutes. During the process coffee beans were constantly mixed. After the second cracking the temperature was increased about 20^{o}C and heating has finished within 3 minutes.

Analysis of fatty acids. Coffee samples were ground and powered. 1 g of coffee was dissolved in 6 mL of acetone. Then 1 mL of acetone extract was taken and 100 µL of internal standard heneicosanoic acid (60 ppm) were added, afterwards extract was evaporated to dryness under nitrogen and the residue was dissolved in 1,5 mL of hexane. The mixture was saponificatedby adding 0,5 mL of sodium hydroxide solution in methanol (2 N) in room temperature, 1–2 hours. 200 µL of hexane layer was transferred into 1,5 mL vial and 1 mL of hexane added, 1µL of mixture was collected for GC-MS analysis. A BPX90 capillary column was used to separate fatty acids with helium as a carrier gas, a flow rate of 0,9 mL/min. The injector temperature was 230°C, and the column temperature was programmed as follows: 50ºC in the beginning for 2 min, subsequent increase to 230ºC at the rate of 2.5ºC/min maintained for 5 min. The interface temperature for GC-MS was 220°C. Temperature of ion source was 200°C ionization energy was 70V. The total ion monitoring (TIC) was used to detect fatty acids (m/z ranged 50–500). The internal standard heneicosanoic acid was used to quantify fatty acids. Tree replicates per each sample were analysed.

Analysis of sterols. Coffee samples were ground and powered. 1 g of coffee was dissolved in 6 mL of acetone. Then 4 mL of acetone extract was taken and 100 µL of internal standard 5α-cholestane (4 ppm) was added, afterwards the extract was evaporated to dryness under nitrogen and the residue was dissolved in 1,5 mL of hexane. The mixture was saponificated by adding 0,5 mL of sodium hydroxide solution in methanol (2 N) in room temperature, 1–2 hours. 200 µL of hexane layer was transferred into 1,5 mL vial and after evaporation to dryness under nitrogen, the residue was dissolved in 100 µL of pyridine and 100µL BSTFA with 1% TCMS and left to remain in the dark for 24 hours to complete derivatization. Then, 1 mL of hexane was added and 1µL of mixture was collected for GC-MS analysis. A DB5ms capillary column was used to separate sterols with helium as a carrier gas, a flow rate of 0,9 mL/min. The injector temperature was 230°C, and the column temperature was programmed as follows: 50ºC in the beginning for 2 min, subsequent increase to 230ºC at the rate of 15ºC/min, to 310ºC at the rate of 3ºC/min maintained for 10 min. The interface temperature for GC-MS was 240°C. Temperature of ion source was 220°C, ionization energy was 70V. The total ion monitoring (TIC) was used to detect sterols (m/z ranged 100–600). The internal standard 5α-cholestane was used to quantify sterols. Tree replicates per each sample were analysed.

Data analysis. The obtained results were statistically worked out using STATISTICA 8.0 programme. To appraise the significance of the differences between the means sterol and fatty acids content in particular lard samples, Tuckey's test was used, at significance level $\alpha = 0.05$. Principal Component Analysis (PCA) were carried out using STATISTICA 8.0 programme.

Results and discussion

Obtained results of fatty acids and sterol content in coffee beans before and after roasting are presented in Table 1 and Table 2.

Fatty acids were analyzed by gas chromatography in *arabica* coffee beans originating from Brazil, Ethiopia, Guatemala, Costa Rica and Columbia. Seven peaks were identified in chromatograms of coffee beans before and after roasting. Palmitic (16:0), stearic (18:0), oleic ($18{:}1\Delta^{9}$) and oleic ($18{:}2\Delta^{9,12}$) acids constituted higher amounts of fatty acids in all coffee. Coffee oil extracted from green and roasted coffee oil contained palmitic (34.1–37.6%), stearic (6.2–7.9%), oleic (6.8–11.6%), linoleic (42.6–47.6%), arachidic (1.9–3.1%), linolenic (1.2–1.5%), and behenic acids (0.3–0.6%). Composition of fatty acids in coffee beans obtained in the present study was compliant with previous reports concerning *Coffea arabica* composition [Khan and Brown, 1953; Vila et al., 2005; Dussert et al., 2008]. For example Khan and Brown [1953] investigated fatty acids content in blends of Brazilian, Colombian and Venezuelan coffee. They stated that coffee oil contained the highest amount of linoleic (46.3%), palmitic (32.0%) and oleic (8.2%) acids. Fatty acids profile of Brazilian *arabica* coffee determined by Oliviera et al. [2006] was very similar to linoleic acid and represented 44% of all fatty acids, palmitic 34% and oleic only 9%. The Abyssinian green coffees were also investigated, it contained higher amount of palmitic (39–40%) and oleic acid (9–10%), but lower amount of linoleic acid (38–39%) [Tawfik and Bader, 2005].

Fatty acids content [mg/g of fat] presented in this study provides more information about differences between coffee originating from five countries. Summary of the calculated amount of the fatty acids of coffee oil is given in Table 1. Those results show that country of origin of a particular coffee type affects the fatty acid content as tested by ANOVA. Also there are significant differences in the fatty acids composition between green and roasted beans.

PCA was applied to the data matrix of fatty acids content in green and roasted coffee. It appears that PC1 and PC2 explain up to 98.0% of the total variance of fatty acids content in green coffees, being 61.3% explained by PC1 and 26.7% by PC2. PC1 had a strong negative correlation coefficients of fatty acids content in analyzed coffees. PC1 distinguished Columbian, Costa Rica coffee samples from other, they have lower content of stearic, oleic and linolenic acid. PCA was also applied to fatty acid content in roasted coffee. PC1 and PC2 explained 91.0% of the total variance. To visualize the trends of the data the scores for samples were represented in the space of the two principal components (PCs) obtained from PCA [Figure 1]. As can be seen, all *arabica* samples derived from Costa Rica and Guatemala are at the positive side of PC1 while *arabica* from Colombia and Brazil appear at the negative side. Also it can be observed, that all *arabica* samples derived from Ethiopia and Guatemala are at the positive side of PC2 while Brazilian and Colombian samples of roasted coffee appear at the negative side. Therefore, a complete separation of varieties was achieved from PCA. The most differentiating compounds is oleic and linoleic fatty acids.

Table 1

Fatty acids content[mg/g of fat] in coffee beans derived from five different countries before and after roasting

Country	Fatty acids content [mg/g of fat]							
	C 16:0	C 18:0	C 18:1Δ^{9}	C 18:2$\Delta^{9,12}$	C20:0	C 18:3$\Delta^{9,12,15}$	C 22:0	Σ
	Green coffee beans							
B	306.6 ± 1.1^{a}	68.0 ± 2.3^{a}	74.4 ± 0.3^{a}	381.2 ± 0.1^{a}	24.1 ± 1.1^{a}	10.7 ± 0.9^{a}	4.6 ± 0.3^{a}	869.8 ± 3.9^{a}
E	350.9 ± 1.5^{b}	62.0 ± 0.8^{b}	70.4 ± 1.1^{ab}	436.0 ± 1.3^{b}	18.4 ± 0.2^{b}	$11.2 \pm 0.1a^{b}$	4.1 ± 0.04^{a}	952.9 ± 4.7^{b}
G	312.4 ± 4.3^{c}	66.7 ± 0.4^{a}	73.5 ± 8.1^{ab}	380.1 ± 6.1^{a}	27.0 ± 0.6^{c}	12.7 ± 1.3^{b}	5.2 ± 0.5^{b}	877.7 ± 0.3^{a}
CS	269.8 ± 2.1^{d}	47.3 ± 3.8^{c}	52.2 ± 5.0^{c}	364.7 ± 10.7^{c}	19.1 ± 1.4^{bd}	9.7 ± 1.7^{a}	3.4 ± 0.2^{c}	766.2 ± 0.7^{c}
C	309.0 ± 3.7^{ac}	57.7 ± 0.5^{d}	65.9 ± 2.0^{b}	354.5 ± 7.7^{c}	20.4 ± 0.01^{d}	10.7 ± 0.2^{a}	3.5 ± 0.03^{c}	821.6 ± 13.1^{d}
	Roasted coffee beans							
B	275.4 ± 0.1^{a}	64.1 ± 4.6^{a}	94.1 ± 2.9^{a}	343.8 ± 6.0^{a}	17.7 ± 0.01^{a}	9.6 ± 0.6^{a}	3.1 ± 0.1^{a}	807.9 ± 14.3^{a}
E	282.8 ± 0.7^{b}	54.0 ± 0.7^{b}	66.1 ± 0.8^{b}	363.5 ± 2.3^{b}	15.3 ± 1.2^{b}	11.4 ± 1.0^{a}	2.3 ± 0.4^{a}	795.5 ± 7.2^{b}
G	268.9 ± 1.8^{c}	50.4 ± 0.02^{b}	54.6 ± 1.0^{c}	343.3 ± 2.8^{a}	15.6 ± 0.5^{b}	8.5 ± 0.1^{b}	2.7 ± 0.1^{b}	744.0 ± 6.3^{c}
CS	205.6 ± 1.5^{d}	38.9 ± 0.6^{c}	46.8 ± 1.0^{d}	265.5 ± 1.1^{c}	13.5 ± 0.2^{c}	8.3 ± 0.02^{b}	2.1 ± 0.1^{c}	580.9 ± 4.4^{d}
C	293.1 ± 1.3^{e}	59.7 ± 1.7^{d}	77.3 ± 4.3^{s}	365.5 ± 2.2^{d}	18.0 ± 0.2^{d}	12.5 ± 0.1^{c}	2.9 ± 0.03^{d}	829.1 ± 9.8^{e}

[a,b,c] letters in columns represents statistical differences between groups in relation to fatty acids content in coffee beans that derived from five different countries, $P \leq 0,05$

Table 2

Sterol content[mg/g of fat] in coffee beans derived from five different countries before and after roasting

Country	Campesterol	Stigmasterol	Sitosterol	Σ
	Green coffee beans			
B	2.0 ± 0.1^{a}	5.0 ± 0.5^{ab}	6.9 ± 0.9^{a}	13.8 ± 0.7^{a}
E	4.0 ± 0.4^{b}	7.6 ± 0.1^{c}	12.0 ± 0.8^{b}	23.7 ± 1.0^{b}
C	2.8 ± 0.1^{c}	5.0 ± 0.1^{a}	8.9 ± 0.2^{c}	16.8 ± 0.1^{c}
CR	2.0 ± 0.1^{d}	4.4 ± 0.2^{b}	7.5 ± 0.2^{a}	13.9 ± 0.3^{a}
G	2.7 ± 0.3^{c}	5.1 ± 0.5^{a}	9.6 ± 0.3^{c}	17.4 ± 1.1^{c}
	Roasted coffee beans			
B	1.7 ± 0.1^{s}	3.9 ± 0.4^{a}	6.3 ± 0.5^{a}	11.8 ± 1.0^{a}
E	3.3 ± 0.2^{b}	6.3 ± 0.4^{b}	8.3 ± 0.5^{b}	17.8 ± 0.5^{b}
C	2.5 ± 0.1^{c}	4.0 ± 0.1^{a}	7.6 ± 0.3^{c}	14.0 ± 0.4^{c}
CR	2.3 ± 0.1^{c}	4.7 ± 0.1^{c}	7.6 ± 0.1^{c}	14.6 ± 0.2^{c}
G	2.6 ± 0.1^{d}	5.3 ± 0.3^{d}	8.9 ± 0.9^{d}	16.8 ± 0.4^{d}

^{a,b,c} letters in columns represents statistical differences between groups in relation to sterol content in coffee beans that derived from five different countries, P≤0,05.

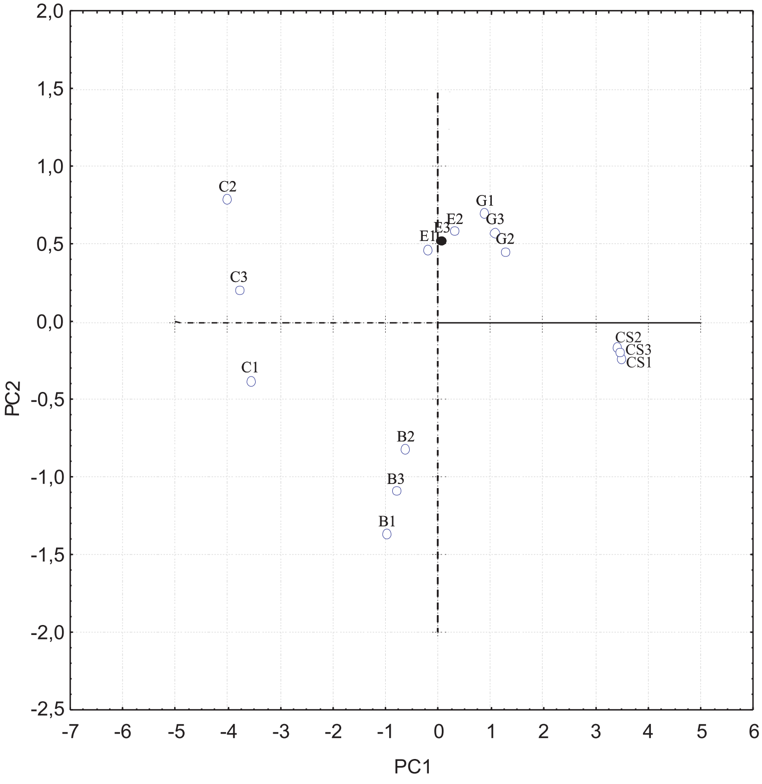

Fig. 1. Scores plot for the first PCs

Sterol content was analyzed in all coffee samples. Coffee fat contained sitosterol, stigmasterol and campesterol (Table 2). Green coffee contained on average from 13.8 to 23.7 mg of sterols in 1 g fat. The highest content of sterols was found in samples of green *arabica* coffee from Ethiopia (23.7 mg/g of fat). Lower content of sterols were characterized by coffee from Guatemala, Colombia (16.8–17.4 mg/g of fat) and Brazil and Costa Rica (13.8–13.9 mg/g of fat). Roasted coffee samples contain similar content of sterols in comparison with green coffee (11.8–17.8 mg/g of fat). The total sterol content decreased after the roasting process (3.5–24.9%). Only coffee beans from Costa Rica contained higher amount of sterols after roasting. The sterol fraction extracted from green and roasted coffee contained mainly sitosterol (50%), stigmasterol (30%) and campesterol (20%). Collected results confirmed other studies carried out on sterol content in coffee beans [Carrera et al., 1998; Kamm et al., 2002; Speer and Kölling-Speer, 2006]. Dussert et. al. investigated sterol content in mixture of *arabica* and *robusta* coffees. Composition of sterolic fraction of roasted coffee blends was as follows: sitosterol (48–52%), stigmasterol (18–19%), campesterol (15–17%), Δ^5avenasterol (2–8%), sitostanol (1.5–2%), Δ^7stigmasterol (1–2%), Δ^7avenasterol (1–2%) [Dussert et al., 2008). Carrera et al. [1998] also determined sterols in *arabica* and *robusta* and they obtained very similar result, because sitosterol represented 44–55%, stigmasterol 20–23% campesterol 14–18% of sterolic fraction. Sterolic fraction of *arabica* green coffee originating from Brazil contained 45% of sitosterol, 37% of stigmasterol and 14% of campesterol [Nikolova-Damayanova et al., 1998].

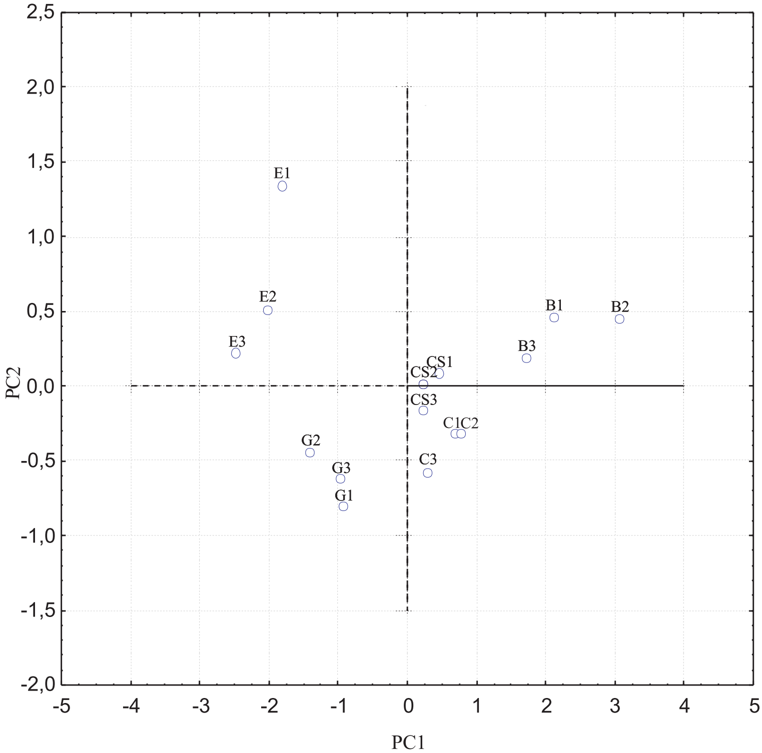

Fig. 2. Scores plot for the first PCs

PCA was applied to the data matrix of sterol content in green and roasted coffee. In the case of green coffee it appears that PC1 and PC2 explain up to 98.9% of the total variance, being 92.7% explained by PC1 and 6.1% by PC2. PC1 had a strong negative correlation coefficients of sterol content in analyzed coffee. PC1 distinguished Ethiopian coffee samples from other, it has higher total sterol content. PCA was also applied to sterol content in roasted coffee. PC1 and PC2 explained 96% of the total variance. To visualize the trends of the data the scores for samples were represented in the space of the two principal components (PCs) obtained from PCA (Figure 2). As can be seen, all *arabica* samples derived from Costa Rica, Columbia and Brazil are at the positive side of PC1 while *arabica* from Ethiopia and Guatemala appear at the negative side. Also it can be observed, that all *arabica* samples derived from Ethiopia and Brazil are at the positive side of PC2 while Guatemala and Colombia samples appear at the negative side. Therefore, a complete separation of varieties was achieved from PCA. The most differentiating compounds are sitosterol and stigmasterol.

Conclusions

The content of seven fatty acids and three sterols in *arabica* coffee samples derived from five countries, both green and roasted, has been determined. Analysis of fatty acids and sterols composition can be used as tool to distinguish the country of origin of a particular coffee type. PCA allows for a complete separation of origin of *arabica* coffee with the most differentiating factors being steric, oleic, linoleic acids. Sterol content especially sitosterol and stigmasterol were found to be the most differentiating variables.

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2

FOOD QUALITY MANAGEMENT SYSTEMS

Introduction

The concepts of the quality management are of a utilitarian character, i.e. they carry practical benefits. This means that these concepts must be adjusted to practical needs, which leads to the formation of practical guidelines (rules) of the quality management. To address this issue we present certain aspects of the quality management, which are important for the food production. The quality management in the food production is of high importance because of the commonly accepted need for consumers' safety (health protection) – often regulated by law.

Nowadays among consumers the choice of quality and safety criteria becomes more important. The lack of a clearly defined quality criterion leads to the quality definition as a product characteristics and the degree of consumer's needs fulfilment. The goal of modern quality management systems is to fulfil the consumers' requirements concerning quality. It is the food consumers' (who express their higher quality demands) opinion that the existing law regulations are not sufficient. That is why the food producers, in order to be more competitive, implement new quality management systems, quality certificates and programmes of quality culture. All actions increasing the food quality should be treated as profitable for food consumers [Jeznach, 2008].

Narrowing the aspect of food quality to its safety is No. longer sufficient. All characteristics of the food product important for the consumer (buyer and user) should be taken into account. In accordance with this conception, the consumers make a decisive verification of quality and their opinions must be taken into account and applied to the management system.

According to Kołożyn-Krajewska and Sikora [1999] if we understand quality in this narrow meaning (q), then the quality control equals the technical control of the manufacturing process or the work-stand. The product, being a result of the manufacturing process, is assessed only in its technological aspect. Still many producers in Poland apply only this kind of product control. When the production rate is low and the products sell, these producers are able to function. But the food products supply being high and supplementary products becoming available, the mechanism of competitiveness makes the quality management compulsory. The quality control (Q) compared to the technical control is much more progressive. The application of the prophylactic actions, self-control and statistical methods leads to higher quality standards. This concept of product and trade is based on the assumption that customers prefer high quality and innovative products with best characteristics. The production policy is not based on customers' preferences and choice criterion. It is the sales policy which is constantly perfected and developed. Products are created by designers (engineers) and their idea is of importance not the actions of the competitive companies. These actions are not focused on customers and their requirements – this characterizes the marketing orientation.

In answer to this situation changes in the quality management took place. These changes were visible in works on the ISO 9000 series of standards. At first these standards were the directives of requirements for various kinds of business activity. Now the progressive management based on the process approach to quality is proposed.

Total Quality Management – the concept and its use in the food production

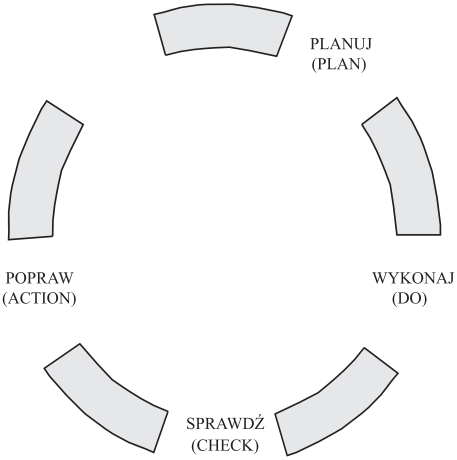
Karaszewski [2005] and Kłos [1998, 1999] reviewed different more or less detailed definitions of the Total Quality Management (TQM). In some definitions only the main elements were included and in other the techniques and tools were presented. In this paper we will use the TQM definition, which combines the management and marketing elements [Altkorn, 2001]. These two elements make it utilitarian.

The quality management (management trough quality, total quality management, TQM) is a total set of management functions covering all substantial and non-substantial elements of the manufacturing industry and inter- and intra-processes influencing the product quality. All members of the staff are involved through the team work, self-control and qualification improvement. The goal is to achieve a sustainable success – a source of customers' (and also the company's and its workers') satisfaction and the society welfare. To achieve this goal a kind of marketing based on long-term strategies is needed – the strategies considering market changes on a different scale.

A vast part of the quality management development must be granted to the researchers (working both on the theory and practical approaches) from the United States, Japan and Western Europe. The dynamic development of these countries and regions economies led to the practical and modelling progress in the quality management [Skrzypek 1996, 1997, 2000; Steinbeck, 1998; Szczepańska, 1998, Chabiera et al., 2000; Zalewski, 2002; Hamrol and Mantura, 2006].

William Edwards Deming is regarded the father of TQM concept. He was a well known American statistician, although he is perhaps best known for his work in Japan. He addressed the issue of the importance of the statistical methods application in the production quality control systems. He was interested in the elimination of non-controllable processes changes and in the creation of dynamic approach to the quality management. His concept of the product and process quality control is illustrated by the so-called Deming cycle (Figure 1). This cycle is a chronologically organized scheme for actions concerning business processes (a continuous feedback loop). In this model the quality is understood as a degree of homogeneity and infallibility at the possibly low costs and the fulfilment of the market requirements. For the marketing purposes the adjustment of the quality to the market requirements is beneficial and the guidelines are: "do not choose your product, choose your customer" or "do not be bound with your product, be bound to your customer". The product and its quality are not valuable by themselves, it is the customers' appreciation that makes them valuable.

The Deming cycle presents the manufacturing processes and products according to the following scheme (known also as PDCA cycle):



Source: K. Szczepańska, 1998: Kompleksowe Zarządzanie Jakością TQM [Total quality managment TQM]. Wydawnictwo Normalizacyjne Alfa-Wero, Warszawa, s. 26

Fig. 1. Deming cycle(PDCA)

To implement the PDCA cycle one needs a new organization of work, clear and understandable standards and expectations, and means for all these goals realization. The engagement of the managing crew and the rest of the staff are needed to achieve the goal of new quality for the first time. The fourteen rules formulated by Deming are good guidelines to achieving this goal.

Joseph M. Juran understands quality as the utility usefulness. This means that the customers are decisive for the quality formulating processes as they decide about the product utility. Juran's idea of quality is very important for this paper purposes to formulate the guidelines for dealing with the customer. Juran states it is very important to be constantly aware of the customer's needs. In terms of company marketing this means a marketing orientation towards recognizing and fulfilling the customers' needs. The knowledge of customers' needs is used in the projecting stage, the production preparation, purchase of stock, distribution, trading, installing, using and conservation, and even assessment of users' satisfaction. All this is very important on the modern market (called the customers' market) for an effective producers' and distributors' performance.

Juran points to the necessity of identifying the cause of the problems encountered as a mathod for avoiding faults, as well as the necessity of taking into account the quality costs (with their full classification) as a factor motivating the managing crew and drawing their attention to the organisation's quality problems. This model and total way of quality management were considered when the ISO 9000 series of standards was created.

Juran's conception and the achievements of Armanda V. Feigenbaumaand and Philip B. Crossy enable us to formulate the most important elements for the food producing companies wishing to achieve and sustain success on the market:

- Quality is a natural expectation of the consumer.
- The goals and environment for a constant quality improvement for all staff members must be created. This means building the quality culture for all workers and customers.
- The attitude of the management crew and other staff members towards quality must change. This means: the implementation the quality policy, creating of the quality management systems and self-control.

As the presented ideas are of universal character they may be applied to all possible stages of product (including food) quality creation.

Although there are specifications, certain conditions and controls as to food quality for the consumer safety means it is not enough in the light of the quality management idea. According to the quality management concepts the customers' needs and requirements (including individual ones) are the main rule. Only when the company applies the quality management, it may prevail against competitive companies on the market in the short and longer terms.

Genesis and development of the ISO series 9000 of standards

The ideas and concepts of the specialists in the quality management presented in the previous part of the paper led to the formation of the quality philosophy TQM. This philosophy influenced the ISO series 9000 of standards.

The ISO series 9000 of standards was formulated by ISO/TC (Technical Committee) 176. The oldest standard (ISO 8402) was published in 1987 (*Zarządzanie jakością i zapewnienie jakości. Terminologia [Quality management and quality ensuring. Terminology]*). This standard contained a definition of the quality as all object characteristics bound with the fulfilment of given and expected needs. The definition combined different ways of the quality understanding. It mentioned the product characteristics but in the context of consumers'/buyers' requirements. As this definition was of marketing character it was popular in different circles. It had an aspect of modern thinking about quality, but created a possibility for the existence of various market subjects.

The amendment of the ISO series 9000 of standards by CEN on 15 December 2000 took into account the idea of quality development. To improve the organization functioning eight rules (of the best managing practice) of the quality management were used:

- Focus on customers (organizations are dependent on their customers, so they need to understand the present and future customers' needs, fulfil their requirements and even exceed their expectations),
- Leadership,
- Involvement of people,
- Process approach,
- System approach to management,
- Continuous improvement,
- Factual approach to decision making,
- Mutually beneficial supplier relationships.

The presented changes in the ISO series 9000 of standards show the system approach to organization and administrative matters, but this is not enough for the optimal improvement of the product quality. This optimal improvement requires the quality policy approach – the TQM.

It is common now that various management systems of the company are integrated. The new ISO standards are formulated in the way to enable their use with another management system, e.g. environmental management system.

Family of the ISO series 9000 of standards

The family of the ISO series 9000 of standards contains:

- ISO 9000:2006 – Quality management systems – Fundamentals and vocabulary; Polish equivalent PN-EN ISO 9000:2006, is an introduction to the new standards of the quality management and defines the basic terms used in the standards. It plays an important role in the understanding and application of other ISO series 9000 standards.
- ISO 9001:2000 – Quality management systems – Requirements; Polish equivalent PN-EN ISO 9001:2001, includes the decisions and requirements for the quality management systems.
- ISO 9004:2000 – Quality management systems – Guidelines for the performance improvements; Polish equivalent PN-EN ISO 9004:2001, contains guidelines for the improvement of the quality management systems and by this of the whole organization. The improvement of all processes in the organization concerns the process efficiency and effectiveness. The satisfaction of the customer and other parties: the working staff, owners, distributors, and society are taken into consideration.

In the standards the requirements for the product and for the quality management system are separated. In comparison to previous standards some changes in the terminology were made. Now the word "management" is used instead of the word "fulfilment". This change is meant to highlight the new approach based on the TQM philosophy. The quality and customers' satisfaction is not only to be fulfilled but continuously improved.

Role of the ISO series 9000 of standards in creating food quality

The ISO series 9000 of standards performs a very important role in the international trade. The ISO standards help in the barriers elimination and the implication of management systems throughout the world. They are commonly accepted by all organizations as the basis of the management systems creation. This means they may be used in various companies producing and distributing food products.

Implementing of the management system based on the ISO series 9000 of standards is not obligatory but it gives the organization an advantage over competitive companies. This advantage comes from better fulfilment of customers' expectations and the guarantee of the product high quality. This is an effect of the cooperative functioning of all the elements. It is not the quality increase itself that gives the advantage. Without the quality increase the customers' expectations cannot be met. The focus on the customers and their needs being the main rule of the quality management system seems to be a good solution. On the modern marketing-oriented market the customer plays the key role. Meeting customers' expectations is a way to customers' satisfaction and in the same time the marketing success. This success is not understood as a solitary act of sale, but it includes making bonds with the customer (loyal customer) who makes conscious choices. This also helps the organizations to gain knowledge about the present and future customers' expectations, which is necessary for the quality management.

In this sense the ISO series 9000 of standards directing and systemizing the food production company actions is the key element of creating the food product quality in accordance with the producer's abilities and consumer's requirements.

Customers requirements fulfilment in the quality management system

In the presented standards the elements concerning customers' needs in the quality management systems are emphasized. As these needs are emphasized in the standards and also in the marketing policy and quality management, the actions should be focused on the customers' needs. The goal of establishing the family ISO 9000 series of standards was to help the organizations of various types and sizes in implementing and successful use of the quality management systems.

However, the requirements included in the family ISO 9000 series leave some freedom of interpretation [Berdowski and Berdowski, 2004]. This is a result of focusing on the customers' role and their individual requirements as to the food quality management.

The element of customers' role, if important and put forward in the standards, is not so well implemented in practice. It seems very important to highlight this element for food producers and distributors.

The help in increasing of the customers' satisfaction justifies the reason for using the quality management systems. The customers want products to meet their expectations and fulfil their needs. These needs and expectations are reflected in the product' specification and are called the customers' requirements. They can be specified by the customer in the trade agreement or defined by the organization itself. In both cases it is the customer who decides whether to accept the product or not. That is why the food producing companies are continuously focused on their processes and products improvement as the customers' requirements change. In the quality management systems the food producing organizations are encouraged to perform analyses of the customers' requirements, and to establish processes leading to products accepted by the customers, and to control these processes. The quality management system creates a framework for increasing the probability of customers' satisfaction and satisfaction of the other interested parties. This enables the organization and its clients to trust in the ability of making the regiment fulfilling product.

To create and implement the quality management system one must at first establish what are the needs and expectations of the customer. It is in accordance with a total management system in which the customers' requirements are established at entering the system and their satisfaction is checked at the system exit.

The quality concept is defined as the degree to which the inherent set of characteristics fulfils the requirements. According to the PN EN ISO 9001:2001 standards a characteristic is a distinctive feature, which may be inherent or assigned, and also qualitative or quantitative. In the standard there is a following classification of characteristics:

- physical (e. g. mechanical properties, electric properties, chemical and biological properties),
- sensual (e. g concerning scent, flavour, sense of touch, eyesight and hearing),
- behavioural (e. g. politeness, honesty, truthfulness),
- time related (e. g. punctuality, reliability, availableness),
- ergonomic (e. g. psychological features or these concerning people safety),
- functional (e. g. simplicity of preparation).

The qualitative characteristic is the inherent (constant) distinctive feature of the product, production process or system concerning the need or expectation being agreed, customary or obligatory.

Requirements and profits of the ISO series 9001 of standards implementation

The ISO 9001:2000 standards can be used to evaluate – by internal and external parties including the certification bodies – the ability of the organization to fulfill the customers requirements (those regulated by law and organizations' own policy). The quality management system in the ISO 9000 standards is based on the process approach. Using this approach means to identify the relation between the resources use and management and to implement such processes in the organization to gain a total (optimal) fulfilment of the customers' requirements.

The resources management requires the human resources (well trained, motivated, and aware of tasks and goals), infrastructure (buildings, installations, equipment, software, additional services, etc.), and work environment (climate).

The customer's requirements are formulated at the system entrance to be reflected in the final product. In the quality system management it is continuously improved to gain the customers' and other parties' maximal satisfaction. To reach this goal in the production processes the measurement and the analysis of the customer's requirements and the product improvement is carried out with the companies' resources use and managers' responsibility. This is only possible when there is an information flow between the customer and the responsible managers. The responsibility of the managers' crew consists of: proving engagement in the development and improvement of the quality system management, which leads to the quality policy focused on the customers and their needs, the goal planning, appointment of the person from the leading management responsible for these actions and periodical controls of the management system.

The certification process of the quality management systems in food production companies (PKD DA), based on the data of the biggest certification firms, was not a mass process in Poland and is illustrated above.

Only 88 food production companies had the up-to-date certificates for the quality management system implementation with the ISO series 9001 of standards and these were big companies from meat production, dairy industry, fruit and vegetables and confectionery branches. The management system for the food safety by PN-EN ISO 22000:2006 standards combines both the requirements of the HACCP and Good Practice policy in each organization from the food industry. This standard, adjusted to the ISO series 9000 of standards, is quite a novelty so only 28 certificates have been given so far.

Table 1

The number of the food production companies which received certificates from certification firms (PCBC, ZSJiZ i TUV) for implemented and used non-obligatory quality management systems

Branch	ISO 9001	ISO 22000
Meat production	15	2
Fish production	2	1
Dairy industry	6	1
Fruit and vegetables	15	3
Cereal and mill industries	6	3
Bakery	5	0
Confectionery	4	1
Pasta producers	4	2
Tee and coffee processing	4	2
Alcohol, wine and mead making	8	0
Mineral water and soft drinks	2	1
Delicacies	3	3
Deep-frozen foods	4	2
Concentrates	4	5
Spices	6	2
TOTAL	**88**	**28**

Source: calculation based on data from PCBC, TUV, ZSJZ (WWW.rwtuv.pl, WWW.zsjz.pl, WWW.pcbc.gov.pl

Table 2

Evaluation of profits coming from ISO 9001 standards implementation in the examined food production companies

Profits	Number of scores
Improvement of organization functioning and management	4.60
Starting the process of the continuous improvement of actions	4.00
Increase of customers trust	3.80
Systemizing the action procedures with a precise designation of inter-relations	4.60
Increase of competitiveness and prestige on domestic and foreign market	4.00
The costs decrease (better organization, costs reduction, resource management system, effectiveness increase)	3.80
Better inter and out communication (good information flow about the tasks and the tasks realization)	4.40
Organizations development, change of the attitude towards the quality in the company, awareness of the staff concerning the company goals	4.20
Decrease of the number and cost of the complaints	4.00
Customer’s (consumer’s) satisfaction	3.60

Source: research mine

The Polish companies offering their products and services on the domestic market and the European and other markets must be competitive for other foreign companies. They need to gain knowledge about the changing requirements and needs of the customers. This includes a need for the safety, high quality and reasonable price. To fulfill such requirements a continuous improvement of quality is needed, which demands self-aware and competent managing crew and other staff members. The modern management methods and application of the TQM philosophy are also needed.

The improvement of food production company organization functioning and management, and systemizing the action procedures with a precise designation of inter-relations were the most important profits coming form the ISO 9001 standards implementation — the score was 4.60 (a scale from 1 to 5).

Still the consumers' satisfaction and trust increase have been evaluated as the least of profits. The decrease of costs was not noted, either. This is a result of not carrying out the evaluation and the analysis of the quality costs.

Conclusions

To ensure the food products quality and safety is much more difficult and complicated than in the case of other products. This is mainly caused by the increase of the customer awareness leading to much higher expectations concerning the quality of purchased an consumed food. The goal of the non-obligatory quality management systems is to obtain the optimal quality expected by the consumer.

The quality is formed by all the parties engaged in the production cycle, so all the actions leading to the food quality improvement should be considered profitable from the consumers' point of view. The consumers establish the expected degree of the quality and they verify the quality at the final stage. This forces the food production companies to implement the quality management systems, gain certificates, and implement the quality policy (quality culture). All these actions increase the product quality and safety and increase the company's competitiveness on both the domestic and international market.

The food production companies' interest in implementation of the non-obligatory quality management systems with accordance to the ISO 9001 standards shows that actions for the systemizing of the procedures and the functioning improvement to gain proof of safe food production are undertaken. These actions open the way for the companies to the international trade and the cooperation with trade networks. Still the low evaluation of the profits coming from customers' satisfaction is somewhat worrying. This is the result of insufficient concern about this very important element in the process of quality improvement. Further research is needed in this area.

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3

MARKET BEHAVIOUR OF THE WROCLAW CONSUMERS OF ENRICHED EGGS

Introduction

Consumer preferences related not only to the life-style, but also to eating habits has been dynamically developing in recent years. The trend is conditioned by numerous economic, social and cultural factors. The change of the model of organisation of life, resulting from professional mobility, leads to an increased susceptibility to civilisation metabolic diseases related to improper eating habits. This drives scientists to look for new methods of food improvement which may fully satisfy the demand for the indispensable food components [Balas et al., 2004; Daniewski et al., 1997]. Eating habits and diet preferred by Polish consumers do not always follow the guidelines recommended by experts [Szostak, 2003]. Insufficient amounts of food components (especially of marine origin) may result in the deficiency of substances characterised by high chemical and biological activity, esp. of polyenic fatty acids [Ziemlański and Budzyńska-Topolowska, 1991; Simopoulos, 2000]. As observed by Dybkowska et al. [2004], saturated fatty acids dominate in a typical Polish diet. High amounts of linolic acid were also observed. The supply of unsaturated polyenic acids is 13 times lower than recommended (0.1 g/day for adults) [Kolanowski et al., 2004].

A conscious customer who looks for innovative and pro-health products increases the demand for new generation products, i.e. food products which are characterised by a channelled and desired influence on human body. The offer of food industry should thus be directed towards consumers whose awareness of the effect of eating habits on human health is rising and whose requirements concerning new generation products are growing.

Intensive development of new type of food market was also observed in the context of egg products. New terms, such as "functional food" or "designed food" have been appearing in world literature since the beginning of the 90’s of the 20th century [Van Elswyk et al., 2000; Anton et al., 2006; Froning, 2006; Sparks, 2006]. Such products are produced using common methods and the material is often obtained from individually organised animal farms, plantations in special conditions or selected varieties and breeds [Świderski and Kolanowski, 2003].

Hen eggs containing an increased amount to biologically active compounds are considered new generation food products. Research results proved that layer hens are biotechnological objects which, through proper feeding system, can change the composition and participation of components in the modified product [Horrocks and Yeo, 2000]. The process of enriching consumer eggs with vitamins, mineral compounds and indispensable polyenic fatty acids, esp. from the n-3 family, is highly beneficial. Food products containing higher than normal amounts of biologically and chemically active substances are becoming a group of products which are sought for and popular among customers.

From the biological and medical point of view, n-3 fatty acids (esp. EPA and DHA) are of special importance [Connor, 2000; Kidd, 2007]. Polyunsaturated fatty acids (DHA, EPA) play an important role in human nutrition, especially in countries where animal fats and hydrogenated vegetable oils are dominant in the diet and fish consumption decreased. Omega – 3 fatty acids help to reduce the incidence of such life-style diseases as coronary artery diseases, hypertension and diabetes, dermatitis [McNamara, 2000; Kris-Etherton et al., 2002; Calder, 2004]. Experimental studies have provided evidence that incorporation of n-3 fatty acids modify inflammatory and immune reactions, and are potential therapeutic agents for inflammatory and autoimmune diseases [Simopoulos, 2006]. Omega-3 fatty acids lower plasma triglyceride levels, particularly in people with hypertriglyceridemia. This action apparently results from suppression of the secretion of very-low-density lipoprotein (VLDL) and inhibiting the synthesis of cholesterol and triglycerides in liver [Demonty et al., 2006]. DHA fatty acid is an important structural component of the brain (60% of the brain is made up of DHA) and is beneficial for mood management (nervous system growth and development) [Horrocks and Yeo, 2000; Linko and Hayakawa, 1996]. Diet with deficiencies of n-3 fatty acids may be linked to attention dyslexia, schizophrenia, anorexia nervosa, Alzheimer's disease and dementia. DHA acid is also essential for maintenance of learning and memory. Consuming foods rich in omega-3 fatty acids appears to reduce the risk of macular degeneration [Anderson and Connor, 1994; Suh and Clandinin, 2006]. A study showed (Bagga et al, 1997] that polyunsaturated fatty acids have been associated with lower cancer risks and slow tumour growth (colourectal, breast and prostate cancer).

The possibility of multiple enrichment of eggs with these acids and other bioactive substances opens a new marketing strategy leading to production of a "new generation" of eggs with nutraceutical and biomedical features [De Mester 2006, Juneja 2006].

Innovation activities in this area are related to the design of new technologies for both obtaining egg material and its processing as well. The consumer must be made aware about the importance of research leading to the modification of the composition of food products. At the lack of sufficient information, the contemporary customer may not be eager to purchase an innovative product. Thus, in order to create trust and new eating habits of consumers, the materials which can be easily and naturally modified should be improved. Such economic activity should be accompanied by nutritional education which encourages the customers to change their eating routine and style of life which is necessary in pro-health prophylactics.

The aim of the study was to determine the level of consumers' knowledge about the quality of enriched eggs, their nutritional value as well as to determine the frequency of consumption of eggs enriched with polyenic fatty acids.

Material and methods

The study using a questionnaire was conducted in the city of Wrocław, in the period from November 2008 to January 2009. 215 persons, selected on purpose, were interviewed.

The first part of the questionnaire contained questions helpful in determining the consumers' preferences concerning eggs enriched with polyenic fatty acids and the factors deciding about their purchase. Moreover, the questions concerned the level of respondents' knowledge about enriched eggs, they were open-type questions and gave the respondents possibility to freely express their opinions. The second part contained general questions which helped define the demographic and social profile of the respondents. It was possible to give several answers to some questions, thus the total number of answers is higher than the number of respondents. The sample of respondents was selected on purpose.

Results

Table 1

Social structure of respondents

Sex:	**Number of replies**	**[%]**
Female	127	59
Male	88	41
Total	215	100
Age:		
19–29	69	32
30–39	46	21
40–49	41	19
50–59	35	16
>59	24	12
Total	215	100
Education:		
Higher	95	44
Secondary	82	38
Vocational	33	16
Primary	5	2
Total	215	100
Social status:		
Employee (private sector)	80	37
Employee (public sector)	54	25
Student	43	20
Entrepreneur	8	4
Unemployed	16	7
Retired	14	7
Total	215	100

Table 2

Respondents' opinions about enriched eggs

Question:	Number of replies		**[%]**	
Do you shop for all your family?	Yes	154	Yes	72
	No.	24	No.	11
	Occasionally	37	Occasionally	17
Total	215		100	
Do you buy eggs enriched with omega-3 acids?	Yes	42	Yes	20
	No.	84	No.	39
	Occasionally	34	Occasionally	16
	Seldom	55	Seldom	25
Total	215		100	
Why do you buy eggs enriched with omega-3 acids?	Health	71	Health	54
	Price	16	Price	12
	Fashion	6	Fashion	5
	No. reason	38	No. reason	29
Total	131		100	
Do you know how omega-3 acids influence human body?	Yes	92	Yes	43
	No.	123	No.	57
Total	215		100	
From what source do you learn about eggs enriched with omega-3 acids?	Television	6	Television	7
	Press	7	Press	8
	Internet	36	Internet	39
	Acquaintances	14	Acquaintances	15
	Other	29	Other	31
Total	92		100	
Do you think that information about eggs enriched with omega-3 acids is sufficiently available?	Yes	16	Yes	7
	No.	114	No.	53
	Don't know	85	Don't know	40
Total	215		100	

Discussion

The changing trends which create the style of life are reflected in the level and structure of the consumption of food products. The Polish food market is well stocked and access to various types of products is easy. This gives the consumers a possibility to enrich their diet by adding to it products with pro-health qualities, which are beneficial for health and proper functioning of human body [Grzybowska-Brzezińska and Pilarski, 2005]. When a customer makes a decision to buy a specific product, he pays attention not only to its price but, more and more frequently, to the quality, nutritional value or simplicity of its use.

At the beginning of the 21st century, the stereotypical vision of women as the only shoppers started to disappear. This fact was confirmed by own study, in which 59% of participants were females. Young people, aged 19–29 constituted majority of the respondents (32%). The group of persons at the age of over 59 was the least numerous (12%). Of the 215 persons who were interviewed, 89% shop for their families and 17% do it occasionally. Only a small number of persons declared that they had primary education (2%). Persons with higher and secondary education constituted 82% of those interviewed. 62% declared their status as that of employed, including 37% working in the private sector. The unemployed and retired persons constituted very small groups (7% each).

The appearance of pro-health, "bio" food products enriched with biologically active components on the market should lead to the increase of consumer awareness so that decisions about buying a specific product are made consciously. The study conducted in the years 2005/2006 [Trziszka et al., 2006] showed that the expectations of an average consumer of eggs were low and limited mainly to the external features of eggs. The consumers preferred L-size eggs with hard, clean and brown shells. Price was the main feature taken into consideration by many customers before buying eggs. It was shown in own study that 39% of the interviewed persons do not pay much attention to what eggs they buy, do it routinely and do not buy eggs enriched with omega-3 acids at all. 61% of the respondents declared that they had some knowledge on that matter. 25% of them bought enriched eggs seldom and 16% did it occasionally. Over half of the group (54%) who bought enriched eggs did it because of their composition and pro-health features, 43% gave a positive answer to the question about their knowledge about the influence of n-3 fatty acids on human health. Own research showed that the Internet was the most frequently indicated source of information (39%). Verbal recommendations from friends were another channel of information (15%). Television (7%) and press (8%) did not widely promote the knowledge about the possibility to design food products, esp. to enrich consumption eggs with long-chain fatty acids.

Studies conducted in the year 2000 in Poland and Great Britain showed that, when buying eggs, the consumers who do not have enough information about their quality pay attention to such factors as price, fashion, look, position on a shelf and packaging. Lack of knowledge and groundless stereotypes were reflected in the preferences of customers buying eggs [Dybowski, 2002]. The analysis of consumers' behaviour conducted in Denmark and the Netherlands confirmed that price is the main factor taken into consideration when selecting egg products. However, own study showed that only 12% of the respondents paid attention to the price of enriched eggs. Nutritional value was the most important factor for 54% of the persons who were interviewed. The fashion for healthy style of life and healthy food had an influence on the decision to buy enriched eggs in case of 5% of the respondent. The remaining 29% of the persons who were interviewed were unable to answer why they selected eggs enriched with omega-3 acids.

The problems related to supply, demand as well as prices and relations between these factors are taken into account in the analysis of the market of consumption eggs [Szybiga, 2000]. All links of the food chain, from the producer to purchase centre, distribution, processing and catering as well as the final consumer are analysed. Specialist knowledge and reliable information provided to the final consumers is vital for the development of the egg sector. Unfortunately, information about enriched eggs reaches mainly the persons who are interested in rational nutrition, who are interested to know what is happening in this field of industry. There are No. effective methods to promote the products among large groups of consumers although enriched eggs are relatively less expensive than other products of animal origin [Trziszka et al., 2006].

There are numerous campaigns conducted in EU countries to educate the consumer about the quality of eggs and to promote trust in this material. Large amounts of money are spent on campaigns aimed at an increase of consumption of eggs and egg products and they result in an increased demand for egg material. Such actions are rarely taken in Poland. The lack of organisations associating all the links in the poultry production chain, which could promote Polish products, increase their sale and consumption, is also visible [Trziszka et al., 2006].

Conclusions

1. A growing group of consumers makes conscious choices when buying food products. Their composition and nutritional value of is less important than price.

2. The study showed that, when buying enriched eggs, over half of the respondents from the selected group were concerned about their nutritional value and pro-health qualities.

3. Based on the study, it may be concluded that although enriched eggs are gaining popularity, the consumers' knowledge about enriching eggs with omega-3 acids is still not sufficient and requires further study.

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4

QUALITY TRAITS OF TABLE EGGS IN TERMS OF CONSUMER REQUIREMENTS

Introduction

Hen eggs demonstrate an excellent nutritional value and are used in numerous, traditional food products, since they contain many valuable components such as highly – digestible proteins, lipids, vitamins, minerals and other substances of nutritional and biotechnological properties which have been studied for a long time [Ball, 2004; Froning, 2004; Juneja, 2004; Trziszka et al., 2005; Anton et al., 2006]. Dynamic changes have been observed in the eating habits, quality requirements and food products wholesomeness and for those reasons the co-operation among consumers, food producers and researchers aimed at linking the qualitative and quantitative food attributes with the current needs of the human populations becomes inevitable [Pingel and Jeroch, 1997; Trziszka, 2000]. The main efforts have now been directed to the quality improvement being the main goal to be reached. The consumers expect to be offered poultry products wholesome and palatable [Stadelman, 1999; Sparks, 2006].

The purpose of this study was the comparison of egg quality traits in selected two-strain hybrids developed on the basis of local strains of laying hens.

Material and methods

Local breeding strains of laying birds: paternal K-44 and K-66 (Rhode Island Red) and maternal A-22 and A-88 (Rhode Island White) maintained in the Duszniki Laying Hen Breeding Farm Ltd. Co., within the Poultry Experimental centre of the National Institute of Poultry Production were used in this study.

The experimental material comprised eggs from the following two-strain hybrid layers: KA-62, KA-42, KA-68 and KA-48 which were kept under the same environmental and feeding conditions. The eggs for experiments were laid by birds being at 36 weeks of age. One hundred eggs were taken from each studied group of birds. The electronic device Egg Quality Micro-Technical (EQM) Services and Supplies Limited (England) was used in the determination of the following egg traits: weight of whole egg (g); albumen (g), height of the outer thick albumen (mm); Haugh units (H.U.); yolk weight (g), yolk colour (points acc. to La Roche colour scale); shell weight (g) and shell colour (% of reflected light). Shell thickness without outer membrane was measured with Mitutoyo (Japan) micrometer with the accuracy to 1 µm. The air space was measured with manually operated ovolux lamp and using the mm scale. The egg shape was expressed by % index, i.e. the with width to length ratio. The egg shape measurements were carried out with the accuracy to 0.5% using Shape-Meter N.V. Van Doorn-De Bilt (The Netherlands) having measurement scale from 65 to 85%. The pH value of albumen and yolk was determined with Metler Toledo pH-meter. The experimental results were used for the calculation of percentage content of albumen, yolk and shell in the total egg weight. Chemical analyses were used to determine the percentage content of crude protein, water, ash in the albumen and yolk, as well as lipids in the yolk. Crude protein content was determined by Kjeldahl method (acc. to PN-75/A-04018 Standard), water content acc. to. PN-A-86509:1994 Standard, whereas of ash by the method of Krełowska-Kułas [1993]. Lipid content in the egg yolk was determined by Soxhlet method acc. to PN-A-86509:1994 Standard. The experimental results were subjected to statistical analysis of variance.

Results and discussion

The analysis of egg physical traits (Table 1) revealed that eggs from KA-62 birds had the largest weight (62.97 g) and the highest index parameter (78.78%) as well as the highest percentage albumen content (59.33%)and the lowest yolk content (27.53%). The percentage egg shell content ranged from 8.72% (KA-48) to 9.33% (KA-68) and the noted differences were statistically significant. The eggs from two-strain hybrid laying hens exhibited similar air space of 3.74mm on average and No. statistically significant were found. The variation coefficient of air space was relatively high and amounted to 12.8% on average, but in the case of the other traits did not exceed 8.1%.

The analysis of egg albumen traits (Table 2) revealed that the most required quality parameters were noted in KA-62 eggs which demonstrated the largest 7.63mm and Haugh units (86.14). Attention has to be paid to the differentiated albumen height in eggs from all groups of birds (variation coeff. from 13.3% to 16.8%). The pH values ranged from 8.77 (KA-68) to 8.99 (KA-42). In the case of Haugh units only No. statistically significant differences were noted. The same situation was observed in the content of crude protein and water content in egg albumen. It was found that egg albumen of the KA-48 hens demonstrated the highest crude protein content (9.88%) followed by significantly ($p \leq 0.05$) lowest ash content (0.84%). Water content ranged in the albumen from 88.69% (KA-62) to 88.92% (KA-68).

The analysis of yolk physical traits (Table 3) revealed the highest and statistically significant ($p \leq 0.05$) weight of yolk (17.72g) in the KA-68 birds and was followed by the darkest colour (8.52). On the other hand, the yolk of KA-42 eggs showed the lowest weight (17.04g) and the lightest colour (7.95). The pH value was on a similar level of 6.54 on average, and the observed difference amounted to 0.03 only. No. statistically significant differences ($p \leq 0.05$) were noted.

Significantly differentiates traits ($p \leq 0.05$) in the basic chemical composition of egg yolk were in the two-strain hybrids observed. The noted highest difference in the percentage content of crude protein was found in the egg yolks of hybrids and reached 0.85%, in the eggs laid by KA-42 birds was the lowest and statistically significant ($p \leq 0.05$). It was observed that hybrid layers developed with the use of A-22 strain produced eggs having yolks containing significantly higher ($p \leq 0.05$) lipid contents. The noted difference ranged from 0.78% to 1.01% towards hybrids originating from A-88 hens. In the case of percentage ash and water content significant ($p \leq 0.05$) differences also occurred and reached 0.19% and 1.04%, respectively.

Table 1

Physical traits of eggs from Polish two-strain hybrids of laying hens

Trait		KA-68	KA-62	KA-48	KA-42
Egg weight [g]	$\bar{x}$	61.98^{a}	62.97^{a}	62.79^{a}	58.96^{b}
	s	3.83	3.14	4.59	3.04
	v	6.2	5.0	7.3	5.2
Egg index [%]	$\bar{x}$	77.39^{b}	78.78^{a}	78.33^{a}	78.43^{a}
	s	2.92	2.55	3.66	3.20
	v	3.8	3.2	4.7	4.1
% content of albumen	$\bar{x}$	57.89^{b}	59.33^{a}	58.81^{a}	58.00^{b}
	s	2.27	2.47	2.45	2.30
	v	3.9	4.2	4.2	4.0
% content of yolk	$\bar{x}$	28.56^{ab}	27.53^{c}	28.12^{b}	28.92^{a}
	s	2.07	1.61	1.50	1.90
	v	7.3	5.8	5.3	6.6
% content of shell	$\bar{x}$	9.33^{a}	9.05^{b}	8.72^{c}	8.94^{b}
	s	0.53	0.73	0.83	0.78
	v	5.7	8.0	9.5	8.7
Egg chamber height, mm	$\bar{x}$	3.77	3.72	3.80	3.68
	s	0.45	0.45	0.40	0.59
	v	12.0	12.2	10.6	16.1

Explanations:
$\bar{x}$ – mean value for the experimental group
s – standard deviation
v – variation coefficient (%)
^{a,b} – means statistically significant difference at $p \leq 0.05$

Differences were observed in the examined traits of shell quality (Table 4). The shells of the highest weight (5.78 g) and thickness (368.71 μm)were noted in the eggs from KA-68 birds. Layers from all experimental groups delivered brown shell eggs. Significantly darker shells exhibited KA-62 eggs and their colour can be described as brown (34.06). On the other hand the eggs from the other bird groups were of light brown colour (from 36.22 to 36.81). The variation coefficient of that trait was high and ranged from 14.1 to 18.8%.

The presented results of this study confirm those reported by other authors. Faria et al. [2007] examined two bird lines of white and brown feathering and found that eggs from white feathered hens demonstrated by 3.87% lower egg albumen content and by 2.38% higher yolk content. Studies by Dottavio et al. [2005] on egg quality from Fayoumi, White Leghorn and Rhode Island Red strains demonstrated a higher content of egg shell by 4.03% and of yolk by 3.59% whereas a lower egg albumen content by 3.28% on average. Silversides et al. [2006] reported that differences in the quality traits of eggs were dependent on hen origin and, among others, the statistically significant difference ($p \leq 0.05$) in the egg weight varied from 52.45 g (Brown Leghorn) to 66.86 g (ISA-Brown). In this study definitely lower differences were observed in the egg physical traits in comparison with those reported by cited authors. It can result from the fact that the genetic material used in the creation of hybrids was less differentiated.

Table 2

Physical and chemical traits of egg albumen from Polish two-strain hybrids of

Trait		KA-68	KA-62	KA-48	KA-42
Physical traits					
Weight [g]	$\bar{x}$	35.87^{b}	37.38^{a}	36.99^{a}	34.21^{c}
	s	2.37	2.74	3.92	2.43
	v	6.6	7.3	10.6	7.1
pH	$\bar{x}$	8.77^{c}	8.84^{bc}	8.91^{ab}	8.99^{a}
	s	0.40	0.46	0.47	0.49
	v	4.6	5.2	5.3	5.5
Height [mm]	$\bar{x}$	7.57^{ab}	7.63^{a}	7.43^{ab}	7.26^{b}
	s	1.27	1.18	0.99	1.11
	v	16.8	15.5	13.3	15.3
Haugh units	$\bar{x}$	85.93	86.14	85.16	85.08
	s	7.68	6.93	6.24	6.94
	v	8.9	8.0	7.3	8.2
Chemical traits					
% of protein	$\bar{x}$	9.73	9.87	9.88	9.74
	s	0.68	0.56	0.87	0.52
	v	7.0	5.6	8.8	5.3
% of ash	$\bar{x}$	0.94^{a}	0.95^{a}	0.84^{b}	0.96^{a}
	s	0.13	0.09	0.16	0.09
	v	13.6	9.4	18.7	9.2
% of water	$\bar{x}$	88.92	88.69	88.73	88.89
	s	0.62	0.67	0.75	0.60
	v	0.7	0.8	0.8	0.7

laying hens

Explanations:

$\bar{x}$ – mean value for the experimental group

s – standard deviation

v – variation coefficient (%)

^{a,b} – means statistically significant difference at $p \leq 0.05$

Strahle et al. [2005] demonstrated that the height of air space in the egg is affected not only by age of laying hen but first and foremost by bird origin. In the examined two periods of study the height of air space in four bird strains (LT-5, L-2, LT-5, LSL-1) varied from 2.22 mm to 2.72 mm (14 days old eggs) while from 3.28 mm to 4.06 mm (21 days old eggs). In this study the noted difference reached only 0.12mm in the one-day-old eggs. It has to be mentioned, however, that in the case of fresh eggs the examined trait was rather high (3.68–3.80). Silversides and Budgell [2004] demonstrated high variation of the analysed quality traits of eggs within the studied hen strains (Brown Leghorn, ISA-Brown and Babcock) and the noted differences attained: 10.88 g in albumen weight; 1.7 g in yolk weight; nearly 2 mm in albumen height and nearly 2.0 in pH value of albumen. Monira et al. [2003] demonstrated in Haugh units a difference which reached 11.01. In the case of White Rock strain the Haugh unit was very great and amounted to 99.21. In this study considerably lower values were noted and the differences in quality traits of albumen were: 3.17 (weight); 0.22 (pH); 0.37 mm (albumen height) and 1.06 (Haugh units). The observed differences indicated in the hybrids a genetic potential which can be adequately utilized in breeding work on laying hens. Ahn et al. [1999] analysed egg yolk in White Leghorn hens and found that the concentration of hydrogen ions was 6.27 and yolk colour reached 9.53 in the La Roche scale. In this study the concentration of hydrogen ions was by 0.27 lower and of yolk colour by 1.52 point lower, on average. Anderson et al. [2004] reported in CS5, CS7, CS10 and CCS hen strains a difference of 0.56g among weight of the analysed egg shells. In this study that difference was similar and attained 0.51g. On the other hand, Silversides and Budgell [2004] and Silversides et al. [2006] noted in shell weight a difference by nearly 2g which indicated an appreciable variation of that egg trait.

Table 3

Physical and chemical traits of egg yolk from Polish two-strain hybrids of laying hens

Trait		**KA-68**	**KA-62**	**KA-48**	**KA-42**
Physical traits					
Weight [g]	$\bar{x}$	17.72^{a}	17.33^{bc}	17.63^{ab}	17.04^{c}
	s	1.93	1.27	1.17	1.23
	v	10.9	7.3	6.6	7.2
pH	$\bar{x}$	6.54	6.53	6.53	6.56
	s	0.27	0.28	0.29	0.46
	v	4.1	4.2	4.4	7.0
Colour, La Roche scale	$\bar{x}$	8.52^{a}	8.42^{ab}	8.26^{b}	7.95^{c}
	s	0.91	1.02	0.86	0.91
	v	10.7	12.1	10.4	11.4
Chemical traits					
% of protein	$\bar{x}$	17.04^{a}	17.09^{a}	17.34^{a}	16.49^{b}
	s	0.81	0.81	1.01	0.75
	v	4.7	4.7	5.8	4.5
% of lipids	$\bar{x}$	31.83^{b}	32.71^{a}	31.70^{b}	32.58^{a}
	s	0.70	0.43	0.61	0.54
	v	2.2	1.3	1.9	1.6
% of ash	$\bar{x}$	3.29^{b}	3.32^{b}	3.48^{a}	3.32^{b}
	s	0.29	0.24	0.31	0.21
	v	8.9	7.1	9.0	6.4
% of water	$\bar{x}$	47.44^{a}	46.40^{b}	46.91^{ab}	47.02^{ab}
	s	0.82	0.71	0.84	0.77
	v	1.7	1.5	1.8	1.6

Explanations:

$\bar{x}$ – mean value for the experimental group

s – standard deviation

v – variation coefficient [%]

^{a,b} – means statistically significant difference at $p \leq 0.05$

Table 4

Physical traits of egg shell from Polish two-strain hybrids of laying hens

Trait		KA-68	KA-62	KA-48	KA-42
Weight [g]	$\overline{x}$	5.78^{a}	5.69^{a}	5.46^{b}	5.27^{c}
	s	0.43	0.47	0.52	0.50
	v	7.4	8.3	9.5	9.5
Thickness [µm]	$\overline{x}$	368.71^{a}	357.57^{b}	340.67^{c}	342.42^{c}
	s	23.58	28.25	32.11	28.36
	v	6.4	7.9	9.4	8.3
Colour [pkt]	$\overline{x}$	36.22^{a}	34.06^{b}	36.81^{a}	36.35^{a}
	s	6.82	4.081	5.90	5.50
	v	18.8	14.1	16.0	15.1

Explanations:
$\overline{x}$ – mean value for the experimental group
s – standard deviation
v – variation coefficient (%)
^{a,b} – means statistically significant difference at $p \leq 0.05$

In 1999 Ahn et al. determined the chemical traits of eggs from Single Comb White Leghorn – Delta, H&H, W-36 and W-37 laying hen strains. They found that eggs from W-37 line demonstrated not only the highest percentage content of albumen (12.03%) and yolk (51.41%), but also the highest crude protein content in the yolk (17.00%) followed by the lowest level of lipids (30.44%). H&H eggs exhibited the lowest albumen content (11.51%) while eggs of Delta layers the lowest content of egg yolk (50.45%). In the yolk of W-36 eggs the highest level of lipids (31.05%) was noted. The experimental results reported by Ahn et al. [1999] confirmed the results of this study that bird genotype affects univocally the chemical composition of the egg contents. Tharrington et al. [1999] also thoroughly analysed the contents of eggs in bird of various genotype and noted significant differences among the examined egg traits in four laying hen lines: CS5, CS7, CS10 and CCS. They found that egg of CS5 birds showed not only the highest crude protein content in the whole egg (11.90%) but also of crude protein in the yolk (51.53%) and of lipids in the yolk (33.08%). The eggs laid by CS10 birds demonstrated the highest crude protein content in the albumen (10.60%) whereas the lowest one the eggs of CCS hens which were also found to have egg yolk of the lowest lipid content (32.40%) and the lowest crude protein in the albumen (10.29%). Also their studies confirmed the significant effect of bird genotype on the chemical traits if the egg contents.

The results of this study demonstrated that eggs of KA-62 birds due to high egg weight and the greatest percentage content of albumen having most required quality traits can be useful in the processing industry for the manufacture of egg products. On the other hand the eggs laid by KA-68 hybrid birds exhibit certain traits required by the egg consumers, e.g. thick, brown coloured shell and lower lipid content in the yolk. For those reasons those layers are most suitable for a back-yard flocks producing eggs for individual consumers. The research papers reviewed here indicate that both chemical and physical traits of consumption eggs are affected by bird origin. In the light of presented scientific literature the results of our study indicate that the local strains of laying hens can be used in the development of hybrid birds producing table eggs of highly required quality traits.

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5

SOCIAL MEDIA TOOLS ENHANCING COMPETENCES IN FOOD SCIENCE EDUCATION

Introduction

Throughout the history, different educational tools have been used. Some of the old inventions like blackboards have proved their usefulness, and still remain in use. Today the variety of learning technology available is overwhelming. The lifespan of a single educational tool might be short – new, improved tools displace it before it has gained ground in schools and universities. This fast cycle might be frustrating if too much time and effort is felt to be wasted for learning new tools. On the other hand, this fast pace of change is also emphasising the importance of competences in information and communication technologies. Getting used to new technologies during the studies also enhances the general learning competence which is perhaps the most important competence in working life. It is still at the responsibility of faculties and teachers to select and use tools which are safe, convenient to use and which improve learning process and learning outcomes.

Many believe that web has advanced to a second phase characterized by social media. The predominantly "read only" web has changed to web 2.0 in which anyone can publish and share content and collaborate with others. Networking and collaborative working are taking an important role in work, leisure and education.

Food sector is experiencing the same change which is also changing the qualification requirements in the working life. Industrial manufacturing of food has become more automated. Labour is No. longer needed for routine work but for more complex and variable tasks. Work is often done in multidisciplinary and international teams and even the smallest enterprises need to be prepared for the international competition. Communication with the customers and the media has changed as well as the tools for handling the customer feedback.

The aim of this article is to encourage educators in food science to become familiar with the possibilities of social media tools to enhance the key competences of the students.

Process of defining generic and subject-specific competences in food science

Competences are to be understood as dynamic combinations of know-how – composites of knowledge (attributes), skills and attitudes [European Commission, 2004]. They present what students are expected to have gained by graduation. Competences can be subject specific or generic. The key competences can be defined on several levels. During the Bologna process, European institutions for higher education have been carrying out core curriculum analyses and described competences at European level, national level and degree programme level. Learning outcomes have been described at study year level and for each course unit. The objective has been to create such a collection of competences that is easy to comprehend, can be learned by heart and which actually steers and directs teaching and learning processes in practice [Anon., 2007].

Key competences for lifelong learning presented by the European Commission [2005] are 1) communication in the mother tongue, 2) communication in the foreign languages, 3) mathematical competence and basic competences in science and technology, 4) digital competence, 5) learning to learn, 6) interpersonal, intercultural and social competences and civic competence; 7) entrepreneurship and 8) cultural expression. Key competences are those which all individuals need for personal fulfilment and development, active citizenship, social inclusion and employment [European Commission, 2005]. Science universities have done core-skill analyses based on knowledge. Universities of applied sciences have built their own generic and subject-specific learning outcomes. For example, as a collaborative effort of a nationwide expert group in Finland, six generic competences were defined: learning competence, ethical competence, communicative and social competence, development competence, organisational and societal competence, internationalisation competence [Anon., 2007].

The subject-specific competences have been defined in European or national programme-specific groups. These competence lists have been published for example by Delft University of Technology, Eindhoven University of Technology and University of Twente [Meijers et al., 2005] and ISEKI Food Network [Anon., n.d.]. Finnish universities of applied sciences defined six subject specific competences for food science graduate at bachelor's degree: competence in living and other biological materials, competence in bioprocesses, safety and quality competence, technological and economical competence, mathematical and scientific competence and managerial competence.

Although the competence lists produced by different work groups might have slight differences, in principle there seems to be a common understanding on the goals which food science education is having. The more laborious task is to carry out a change from faculty-centered and lecture-based teaching to learner-centered model which aims to acquiring the generic and subject-specific competences and helps the learners to achieve the competence-based qualifications needed in working life.

Possibilities of Blended Learning

Traditionally eLearning (online learning, web based learning) has been used especially in distance education, enabling education and training that would not otherwise have been accessible for the target group. Distance learning has reported to be comparable to face-to-face learning, at least in terms of student engagement in effective educational practices [Chen et al., 2008]. eLearning can also be effectively used during the face-to-ace education.

Blended learning is a design approach that assesses and integrates the strengths of face-to-face and eLearning [Garrison & Vaughan, 2008]. It can be used to redesign the learning experience in ways that can enhance the traditional values of higher education. A study of Rovai and Jordan [2004] provided evidence to suggest that blended courses produce a stronger sense of community among students than either traditional or fully online courses. We have the same experience with undergraduate food technology students who have participated blended learning courses during the past four years. These students have participated regularly face-to-face learning sessions but communication technologies have been utilised before, during and after face-to-face sessions. Digital learning objects, web lectures, self-assessment tests and discussion forums have saved valuable contact time for discussions and individual tutoring. These technologies have given the students an opportunity to practice, independently on the time and place, the core skills, also with the aid of interactive animations and videos showing the most common laboratory practises.

Social Media Tools

Most educational institutions took their first steps in their eLearning practices at the turn of the century, when eLearning platforms (virtual learning environments) like WebCT, Optima, Blackboard and Moodle became available. These platforms helped to organise courses and learning. In the beginning, the platforms were often mainly used as storages for course materials, but gradually teachers learned to utilise also other options like news and discussion forums, wikis, tests and assignment and evaluation tools. The use of social media tools in education, including food science education is today about at the same position than the use of eLearning platforms in their infancy.

Social Media, also called Web 2.0, has been claimed to revolutionise the thinking and ways of action in the society. Wikipedia, one of the plain representatives of social media, is describing social media as follows: "Social media is content created by people using highly accessible and scalable publishing technologies. At its most basic sense, social media is a shift in how people discover, read and share news, information and content. It’s a fusion of sociology and technology, transforming monologues (one to many) into dialogues (many to many) and is the democratization of information, transforming people from content readers into publishers".

The change from Web 1.0 to Web 2.0 can be seen for instance in the following examples, as formulated by O’Reilly [2005]: typical content for Web 1.0 was Britannica Online, personal websites and content management systems whereas typical for Web 2.0 is Wikipedia, blogging and wikis. Publishing has changed to participation and stickiness to syndication.

For universities and other educational institutions social media is a chance, but it has also caused suspicion. Subsequent to social media, the amount of collaboratively produced information has increased enormously. Social web enables publication lacking the checking of the correctness of the information. Access to information is not any more a limiting factor. The critical evaluation of the reliability of the information becomes more and more important. Information retrieval skills are getting highlighted in today’s information society.

Today, there is already a wide variety of different social media tools available [Anderson, 2007; Vaughan, 2009; Kalliala & Toikkanen, 2009] and new tools are created frequently. Some of the social media applications have already gained a huge success and they have millions of users daily. These tools are often used for commercial and political purposes but many social media tools have also been tested and adopted to educational use. The use of social media tools can support especially the development of generic competences like interpersonal, intercultural and social competences, digital competence, learning competence and communication competence. All of these contain several sub-competences. Examples of sub-competences which in particular can be enhanced using social media tools are presented in Table 1.

Table 1

Social media tools used in education and examples of sub-competences that can be enhanced using these tools (modified from Vaughan [2009], Guitert and Romeu [2009] and Anderson [2007])

Social Media Tool	**Description**	Examples	Competences
Social bookmarking	– Sharing personal collections of URLs on a web-based server – Ability to re-use and re-purpose existing collections of links – Tagging of resources helps to develop relationships between concepts and people	Delicious, Furl	Planning and managing the process of finding information
Blogs	– A Web-based public diary with dated entries, usually by a single author, often accompanied by links to other blogs – Reflective writing and reading activity – Opportunity for students to receive external feedback and to make contributions to the dialogue in their field of study – RSS subscription to other blogs to receive automated content updates	Blogger, Edublogs	Presentation and dissemination of digital information Working in social and professional networks
Wikis	– A collection of Web pages that can be edited by anyone, at any time, from anywhere	Wikiversity, Wikipedia, Wikispaces, Pbwiki	Collaborative teamwork and project-based work Planning and management of network Presentation and dissemination of digital information
Social networking & Learning Management Systems	– Focuses on building and verifying of online social networks for communities of people who share interests and activities – Additional "communication channel" to reach students (i.e. RSS feeds from institutional learning management systems)	Ning, Elgg, LinkedIn, Facebook, MySpace	Collaborative teamwork and project-based work
Social media sharing	– Simplify the process of posting and sharing content on the Web (i.e. text, audio, images and video) –Provide a wealth of re-usable media resources for learners and educators	Flickr, YouTube, Slideshare	Presentation and dissemination of different types of digital information
Synchronous communication and conferencing	– Synchronous communication opportunities (i.e. text messaging, audio, video)	WebEx, Skype, Adobe Connect Pro	Interpersonal communication Net-based teamwork and project-based work
Virtual worlds	– Synchronous interaction in 3D immersive worlds	Second Life	Net-based teamwork and project-based work

Enhancing competences by social media tools

Planning and managing the process of finding information

The need for updating one's knowledge constantly is considered self-evident. Researchers and teachers are accustomed to use printed and electronic library databases for searching scientific information from refereed journals. Information retrieval portals available in many libraries provide good opportunities for comprehensive literature search and it is important that students learn to use those effectively already in the beginning of their studies.

In addition to traditional publication channels, many forms of social media are producing information in a way that sometimes seems uncontrollable. On the other hand, social media is also providing tools for searching information from the expanding information "flood". The challenge today is to learn to filter the useful and correct information and news from those not so necessary, and perhaps incorrect.

News readers (RSS, acronym for Really Simple Syndication, Rich Site Syndication, Rich Site Summary and RDF Site Summary) can be useful both to teachers and students. For example, in the beginning of a food technology course students can be asked to look for newsfeeds about food industry. This helps to immediately link the new knowledge to the current events, news and discussion in the food sector. Many food industry enterprises are following both "official" news concerning their own and competitors' company and products as well as the criticism and comments presented in different forums. News readers may help in this task.

The basic way of organising navigation in internet has been the use of bookmarks (favourites). A teacher may share useful links via sending the students a link list or via an eLearning platform. The weakness of both methods is that updating the links lists might be laborious. By using social bookmarking (applications like Delicious and Furl), favourites can be shared between people with common interests. Bookmarks can be saved to a public Web site and they can be tagged with keywords. Tagging and the fact that a bookmark can belong in more than one category are adding value to social bookmarking compared to traditional bookmarking systems. Individual bookmarks can be designated as public or private. Visitors to social bookmarking sites can search for resources by keyword or by person [Anon., 2005].

Collaborative Teamwork and Project-Based Work

Social media tools are created especially for collaborative working, therefore also in education they can best be utilised in teamwork. Typically students have been reports and seminar works together so that one produces the first draft with a word processor, sends it by e-mail to the next one who improves the text and sends it to the third one, and so on. A wiki is a collection of web pages that can be edited by anyone, at anytime from anywhere. By using wikis different versions are handled automatically. The free encyclopedia Wikipedia is the most famous wiki to date. Although anyone can edit Wikipedia and the correctness of its content is not critically reviewed (the facts that teachers often point out), students seem to have learned to use it as a first information source in quick searches. Although many doubts have been presented against the idea of public wikis, they seem to be surprisingly useful. One reason for this is the possibility to view the history of the modifications and to restore earlier versions of the text. The undesired modifications can therefore be easily removed. Many organisations are using private wikis.

Wiki is a suitable tool when the aim is to reach a common understanding between different people. Wiki tools at the eLearning platforms are still too cumbersome for publishing, but they are handy for example when students need to agree on the laboratory work teams, seminar topics or when they need to collect the results that different work teams have got for their laboratory experiments to one place.

The student teams in HAMK University of Applied Sciences have processed articles to Wikipedia and other public wikis and students at the next course have continued their work. Motivation of the students has increased when they have received comments also from people outside the course. Web publications have been written in a wiki collaboratively, especially in projects.

Many students use social networks like Facebook and some of them have spontaneously started to collaborate there with a study team. Educational institutes have usually preferred communities like Elgg which is open-source software and which can be administrated by the organisation.

Presentation and Dissemination of Different Types of Digital Information

Web contains many different forms of multimedia. During the past few years, the use of videos in internet has become extremely popular. Many teachers have found surprisingly many useful video clips for educational purposes from YouTube. Thanks to tagging, the possibilities to find also multimedia content (for example videos from YouTube, photos from Flickr and podcasts from Odeo) that interests the user have greatly improved.

Educational videos made by teachers themselves are usually shared via own institution web pages or eLearning platforms. Recording a lecture of a visiting expert on video makes the content available for several student groups. Production of that kind of video is simple and fast. Useful videos are also those that show action that would otherwise be difficult to explain. If a lot of time and effort is put to record, edit and narrate education videos, it is recommended to choose topics that will remain relevant in the near future. The authors have produced videos showing basic laboratory practices and food technology students have utilised these videos for several years.

Podcasting is often an alternative for a video. Podcasting has been utilised for example during language courses integrated to other subjects. Students have interviewed experts from industry about selected topics using a foreign language (usually the native language of the interviewee). Podcasts have been analysed both during the language course and during the subject course.

During the past few years, blogs have become enormously popular. They are Web-based public diaries with dated entries, usually by a single author, often accompanied by links to other blogs. Blogs are basically tools for individual publishing and some students have decided to use those for documenting their own learning, opinions and how they are building knowledge. If a teacher encourages students to write blogs during the course, it is important first to give pre-reading materials which students can use while forming their own opinions. Blogs work best as tools for reflection [Kalliala and Toikkanen, 2009]. At the authors' course students have prepared a portfolio by reflecting their studies by blogging. Different blogs were linked together, the students followed the projects of others and commented their work.

Various software [Camtasia, WebEx and Adobe Connect Pro) can be used for preparation of web lectures. Subjects that are often needed to be repeated to students are practical topics for short web lectures. If the students follow these lectures before face-to-face sessions, valuable contact time can be saved for questions and discussions. Quizzes available at most eLearning platforms are useful tools for self-evaluation and they help teachers to ensure that each participant of the course has studied the most important contents. The advantage is that they are always available for repeating.

Interpersonal Communication and Net-Based Teamwork

Communicative and social competence of the students is built in the multiple face-to-face learning occasions, especially when teamwork is included. Later in working life graduates are likely to operate also in virtual teams and they will need to communicate using different synchronous and asynchronous technologies. This will be easier if communication in virtual environment is a common practice already during the studies.

Discussion forums are more practical tools for simple discussion about a certain topic than blogs. eLearning platforms include their own discussion forums. The first year biotechnology and food engineering students have used these forums for example for discussion about GMO food. They found useful that the forum was available during the whole half-year course and it was possible to add opinions and links as soon as they learned something new about the topic. If participation to the discussion forums has an effect on grading, it is important to inform students of the evaluation criteria.

As most young students regularly use instant messengers like Skype, they are also good tools for tutoring the students. When students and teacher should share documents, online conference system like WebEx and Adobe Connect Pro is more practical. In addition to managing courses and keeping remote lectures, at the authors departments WebEx has been used tutoring the food technology students during their work placement and thesis work preparation, especially when the students are working in industry far from the institution. Although in general many fully online conferences have been organised, in food technology field these are but a few. Online conference systems are also very practical tools for net-based teamwork when all the participants can share the same document, discuss and process the work together. They have also been used in international projects between food education institutes.

Online conferences have many advantages: When No. conference venue is needed and participants do not need to travel, time and money is saved. Environmental aspects are also supporting the use of virtual conferences and meetings. However, the unquestionable disadvantage is that these synchronous communication and conferencing sessions are lacking the sense of togetherness. Meetings might be effective but usually everyone has an urge to quit the session as soon as possible. Thus the networking aspect of conferences remains unreachable.

A huge step forward has been the possibility for synchronous interaction in three dimensional immersive worlds. At the moment, higher education institutions are mainly using Second Life (Linden Lab). Universities and schools have set up virtual campuses and are getting familiarised with the possibilities of the 3D-virtual learning environment. Anyone (having access to a computer that fulfils the system requirements) can create an avatar and explore the world. The most noticeable experience from those who have participated educational sessions in virtual worlds has often been the much stronger feeling of presence compared to any other virtual learning environment. So far this is actually the only major advantage of using virtual worlds in education that has been proposed. However, there is potential for more and it is interesting to see the future progressin this sector.

Some people are using Second Life as a game, for some it is a social life and increasing number of people are working there. Advertisers, politicians and different kind of service providers are present. Organisations have run inworld seminars and symposia that have contained also informal programme and served as a place for networking.

Subject-Specific Competences

The power of information technology in biosciences is best seen in applications in bioinformatics. eLearning tools have potential to enhance the subject-specific competences of the food technology students also. Some examples were discussed in our earlier article [Pirttijärvi and Kullaslahti, 2007].Videos showing manufacturing processes of food products and simulations enabling the user to practise the use of equipment and control the processes have been produced in educational institutes, sometimes in cooperation with food industry. They are useful in building the competence in bioprocesses but their production is extremely laborious. The increased interest in social media might encourage industry to produce more digital content to the web.

The fast expanding of virtual worlds seems inevitable and more and more people will find their way there. The more potential clients spend their time in virtual worlds and in other social media environments, the more attractive it is for companies to be visible there as well. Virtual worlds are natural environments for example for videos and different kind of simulations that might interest the clients and reference groups. This raises also opportunities for educational institutions: digital content introducing the principles of the food production chain, raw materials, manufacturing processes, quality management etc. that might become available can also be useful at the basic food technology courses. Some of these materials could be developed further to provide more detailed information that can be used for training the staff of the company and for example for students intending to do their work placement in the company. Multinational companies like Coca-Cola and Unilever are already present in Second Life.

Learning entrepreneurship is extremely well applicable to virtual worlds. Students have set up and run enterprises in Second Life, got real clients and gained economical and managerial competence.

From a teacher's point of view, to invest a lot of time and effort for preparation high level, pedagogically meaningful and user-friendly web-based learning object is motivating if the number of users is relatively high. The authors have tutored a virtual course in food hygiene. Hundreds of students from different Finnish universities of applied science and from different fields have taken part to the course annually in order to gain the basic hygiene proficiency required from everyone handling food in their work. Preparing and updating web lectures and sharing those through eLearning platform has made the training available at any time. If students become interested in virtual worlds, this concept could be built in Second Life.

The specialisation courses in food technology have often only few students. Social networking tools could join students majoring in the same topic from different countries. In addition to subject-specific competences, this type of cooperation could greatly enhance the internationalisation competence, necessary for the productive career. Strategic decisions made in European higher education aim to promote student and faculty mobility. The recent strategies also challenge higher education institutions to add international courses utilising e-learning to education. For example, strategy for the internationalisation of higher education institutions in Finland [Ministry of Education, 2009] states that Finnish higher education institutions will by the year 2015 incorporate a module supporting internationalisation in all their degrees. This module can be completed with a mobility period or high-quality international courses.

Conclusions

Collaborative working has been an issue in web-based learning for a long time, but the communication has been mainly between the student and the computer. The true formation of networks and social media sharing has a change to real collaboration between people. The challenge of the teachers is to successfully integrate social media to the curriculum. It is important to define, what needs to be improved in the learning process and only after that start thinking what kind of tools might be useful to reach the aims. The changes we make in our educational practices should all improve learning.

A prerequisite for learning is that students are engaged to their work. Social media tools might provide environments where students like to stay. Some teachers have found that the use of the same virtual environments in which their students are already spending their free time had improved engagement of students to the aims of the course. However, this is not necessarily always a good decision and needs to be carefully considered in each case.

If several social media tools are utilised in a single course, it is useful to link those to one site, for example to a eLearning platform. This creates a safe and logical environment for the student to work.

What makes a tool as a social media tool is the way it is used. eLearning platform may be used as a simple material bank or it can be used as a versatile forum for student networking and collaboration. The same is true with online conferencing systems and even virtual worlds: the way of use is setting the value of the applications.

Conceptualisation of social media might be difficult by only reading articles. The best way is to explore the web services and test the tools. Teachers that are interested in the possibilities of social media tools in education have formed social communities like Sometu (sometu.ning.com) in Finland. Finnish educational institutes have an archipelago, EduFinland (edufinland.fi), in Second Life. This joint concept fosters cooperation and coordination of use and learning and development of Second Life as a tool and environment for education.

To evaluate the usefulness of social media tools teachers should proceed with small steps. It is useful to bring into play only a one or two new tools at a time, collect feedback, evaluate the course format and next time transform the course accordingly. Today we have enough possibilities, technologies and tools to relatively easily build joint courses where students from different countries can participate. This does not require heavily organized projects or funding. Motivated teachers from the same field and some technical assistance from the ITC department are enough. Joining forces and resources brings added value to all of those participating.

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6

EFFECTIVE RISK COMMUNICATION AT THE FOOD MARKET

Introduction

Risk communication has always been a difficult task, but for a long time food consumption was not its target. The need for well designed strategies of food risk communication (henceforth ‘FRC’) has emerged in the last three decades, following a wave of food scares including BSE, dioxin residues, salmonella in eggs or melamine in baby formula milk. Before the mid 1970s, food risk was neither political, scientific nor societal concern [Knowles et al., 2007]. Nowadays, people are more worried about food hazards than ever before, despite the industry’s and regulators’ efforts to persuade the public that the global food supply chain has never been better controlled. This failure has become a challenge for academics and practitioners to identify the origins of the problem and set the correct communication strategies.

According to the European Food Information Council (EUFIC), although the current food safety system is highly sophisticated, consumers’ perceptions of food has changed as a result of some poorly communicated food scandals, so that they are increasingly concerned by even the smallest hazards. Second, many consumers believe that the food industry and regulators cannot control the food supply effectively, questioning their capacity to fully protect their interests. This perception is reflected in public rejection of some modern technologies of food production, such as genetic engineering [Frewer, 2000]. On top of that, the role of the media has changed significantly from passive communicator to opinion-forming system. The contemporary consumer is bombarded by large amounts of information concerning health, environmental and ethical food issues from a variety of sources [Smith, 1999]. The above processes affect consumption and buying behavior and have negative impact upon the producer or retailer [Knowles et al, 2007].

The aim of the present paper is to bring together the existing knowledge on FRC, starting from the ‘risk analysis’ framework, recommendations concerning effective communication of risks and review factors influencing this process, notably public trust, public perceptions of food risks and the role of media. We assume that FRC is successful if followed by desired changes in opinions, awareness and/or behavior of recipients of risk messages. The contemporary FRC strategies and factors influencing their effectiveness are described in a variety of sources. They include organizations responsible for dealing with food safety internationally, such as FAO (www.fao.org), EFSA (www.efsa.europa.eu), EUFIC (www.eufic.org) as well as numerous scientific studies published in peer-reviewed journals. We do not attempt to provide a comprehensive review in this area, but rather indicate that this problem has been meticulously researched by various sources, and our aim is to refer to the most recent and interesting findings.

Risk analysis framework and food risk communication (FRC)

The term "risk analysis" was first defined during the 1986–1994 Uruguay Round of GATT in attempt to systematize efforts in dealing with different food safety hazards[1]. It was established that all countries trading food internationally should conduct risk analysis, as stated subsequently in Multilateral Trade Agreements, which entered into force on January 1, 1995. From then on, all members of the WTO are obliged to implement effective and transparent system of risk analysis, and the guidelines of how to build such a system have been provided by the Codex Alimentarius Commission (CAC) [Kołożyn-Krajewska and Sikora, 2001]. According to the Codex, "risk analysis" comprises three major activities (elements): risk assessment (determining the degree of risk involved), risk management (defining measures required to alleviate the risk), and risk communication (ensuring that all stakeholders are involved in the process). This framework has become a basis for formulating and harmonizing food safety regulations in Europe [Tyszkiewicz, 2000].

Risk assessment is based on scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards. It involves four steps: (1) hazard identification, (2) hazard characterisation, (3) exposure assessment, and (4) risk characterisation. *Risk management* is the process of weighting policy alternatives to accept, minimize or reduce risks estimated in the process of risk assessment, and finally to select, implement and monitor the preferred management decisions. It consists of four consecutive steps: (1) risk evaluation (i.e. identification of safety problem, establishing risk profile, ranking hazards), (2) risk management option assessment (identification of available options, selection of preferred management option – also based on risk-benefit analysis, making final management decision), (3) implementation of management decision, and (4) monitoring and review.

Risk communication has been originally defined as an interactive process of exchange of information and opinions on risk among risk assessors, risk managers, and other interested parties [FAO/WHO, 1995]. The initial concepts of risk communication assumed that this process is shaped by the conclusions of risk management, which in turn result from the outcome of risk assessment (Figure 1). More recent models assume integration between all three elements of risk analysis (Figure 2), adding that public risk perceptions should be treated equally important to technical estimates. Such development reflects recognition of the importance of a dialogue on food safety between key stakeholders in the food chain [Houghton et al., 2008].

Fig. 1. Previous model of food risk analysis

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[1] Initially, the CAC addressed two types of foodborne hazards: biological and chemical. In 2000, a supplement report to CAC has been produced, providing guidelines for assessment and management of GM foods specifically.

Source: www.safefoods.nl

Fig. 2. Contemporary model of food risk analysis

Current recommendations for risk communication strategies

In the 1970s, risk communication efforts focused on aligning public views with those held by experts regarding the acceptability or rejection of a particular hazard [Frewer, 2004]. Such models of risk communication were based on the assumption that scientific and technical estimates are the only possible sources of 'correct' risk information, while the public is a passive receiver of risk information [Scherer, 1991]. This attitude was described as "expert-lay discrepancy" or "knowledge-deficit" model, indicating that the "lay-public" concerns are irrational because they result from an incomplete knowledge. If only the public would be willing to learn about risk issues, they would perceive a hazard differently. Accordingly, providing the consumer with technical estimates was the most obvious reaction to the problem. However, as the proponents of such model have not been successful, they started to acknowledge the fact that lay perceptions of risks are rooted in more than scientific criteria [Hansen et al., 2003]. In response to this, academics and practitioners have worked to develop a new formula for risk communication, taking into account public views of risks and trust in risk communication sources (www.eufic.org).

According to Fessenden-Raden et al. [1987] reactions to risk information are determined by the characteristics of 'audience', 'message' and 'messenger'. Audience characteristics refer to both 'community' (i.e. attitudes towards institutions responsible for handling the risk) and 'individual' (i.e. past experiences with subject and sources of information, prior knowledge about the risk, family health). Factors to consider with regard to messenger include, *inter alia*, the quantity of sources providing risk information (involving too many messengers may result in conflicting messages) and experience of the messenger to communicate effectively. Pertaining to messages, aspects such as simplicity of information and conflicting risk messages should be taken into considerations. The subsequent studies in this area have in fact evolved around these three aspects. Numerous sources refer to food safety issues to be communicated [Frewer, 2004; Frewer, 2000], focusing specifically on communicating risks and benefits of gene technology [i.e. Frewer et al., 2003; Siegrist, 2000; Frewer et al., 1996], while some other provide recommendations of risk communication in general.

Bier [2001] in his publications concerning state-of-the-art of the effective methods of communicating risk analysis results addressed two types of audiences: lay people [2001a] and regulatory decision makers [2001b]. With regard to the lay audience, Bier [2001a] suggested some strategies to consider for enhancing the effectiveness of risk communication in three phases: (1) planning risk communication efforts, (2) designing risk communication messages, and (3) stakeholder participation[2]. Regarding the risk communication to decision makers, Bier [2001b] made a remark that a dialogue between risk assessors and risk managers are equally important to that with the general public, yet little research has been done on this subject. In addition to the typically considered key issues in risk decisions, that is legal requirements, possible adverse effects of a hazard, available variants for reducing risk, extent of concern about the issue expressed by various groups and reliability of information on which the decision will be based, he referred to methods of effective presentation of technical data on uncertainty, variability/randomness and dependence, as well as provided recommendations concerning format of risk messages.

Other sources of knowledge and recommendations on the FRC for practitioners can be found on-line as issued by various international organizations dealing with food safety, i.e. EFSA, FAO, EUFIC. According to EFSA's document on Risk Communication Strategy and Plans [2006), "effective risk communication based on the highest level of scientific expertise" is required to restore and maintain consumer confidence in food. Accordingly, the EFSA's objectives in risk communication is to act "as and expert and trusted source" of information on food and feed safety issues, ensuring that messages are relevant, understandable and address food safety concerns. Therefore, scientific excellence should be a prerequisite for the development of effective communications. The EFSA strategy of risk communication begins from understanding consumer and public perception of food and risks, followed by "bridging the gap" between science and the public, providing support for key actors in their communication efforts and finally promoting coherent risk communication within the risk assessment and management interface. EFSA itself has been described as risk communicator that "influence the risk communicators" [EFSA Risk Communication Strategy and Plans, 2006].

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[2] In the phase of planning of risk communication, the following activities should be undertaken:
(i) determining legal / organizational requirements constraining the risk message;
(ii) defining the purpose of risk communication, i.e. educating, motivating, building trust, reaching agreement;
(iii) matching risk communication strategy to the purpose at hand, i.e. simple messages to raise awareness of a hazard, tools such as diagrams to educate people, persuasion techniques to motivate;
(iv) characterizing the audience, i.e. education, attitudes, concerns, openness;
(v) determining sources of audience information, by doing own research such as focus groups, or by reviewing the existing knowledge.

The underlying recommendations for designing risk communication messages include:
(i) perceiving risk communication as an opportunity to demonstrate trustworthiness;
(ii) listening to the audience concerns before conveying the new information;
(iii) using proper comparisons with other risks in the messages,
(iv) making risk information comprehensible to the audience, by i.e. using graphical representations of probabilities, providing clear explanations of potentially unfamiliar terms (with examples of both what a term means and what it does not mean), and addressing and explain the audience misconceptions.

In order to enhance the stakeholder participation, the following strategies should be considered:
(i) making sure there is a stakeholder commitment to the problem;
(ii) clarifying the scope and purposes of any stakeholder involvement in the process;
(iii) deciding whether participants will have a voice in final decisions.

FAO outlines procedure of effective food risk and crisis communication strategies, according to a sequence of steps, beginning from gathering background and information needed, followed by the preparation and assembly of risk messages, their dissemination and distribution, and finally a follow-up review and evaluation of their impact. It is also recommended to consider public concerns regarding various food risks (i.e. familiarity). The FAO also produced specific guidelines for the FRC within the risk analysis framework. They refer to various levels of setting up the communication strategies, namely 'international', 'national', 'industry' and 'local' levels. 'International level' involves international organizations such as FAO/WHO, intergovernmental organizations such as Codex, and national governments on bi- and multi-lateral bases. At this level, the importance of the effective FRC is emphasized by its critical role in determining the equivalence of food control measures in different countries, alongside the improvement of organizational infrastructure for internal and external communication. At the 'national level', national Codex Committees should be responsible for communicating issues involving risk to consumers, local food industry and the national authorities involved in risk analysis. 'Industry' should be more proactive in supporting or initiating non-profit information centers to provide science-based information on food safety and nutrition to the public, educators, health professionals, government and the media. Finally, 'local' authorities should take advantage of being closer to the local population and therefore being regarded as more trustworthy and credible source of risk information [FAO Food and Nutrition Paper, 1998].

EUFIC (www.eufic.org) provides general eleven on-line recommendations[3] on how to communicate risks effectively. These recommendations focus on aspects such as identification of the audience of risk messages, formulating appropriate content, building public trust in information source and being "proactive" in cooperation with media. Perhaps the most controversial suggestion states that communicators should not always reveal all technical details, especially if they are highly uncertain, because it may cause public confusion and consequently, reduce credibility.

Key factors influencing effectiveness of the FRM

It can be observed that almost all discussions concerning effective risk communication strategies are conducted with reference to three aspects: (1) role of media in risk communication, (2) consumer perceptions of risks, and (3) public trust.

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[3] (1) Identify target audience of risk messages (which of the stakeholder groups should be informed); (2) Craft an appropriate message dependent upon the nature of risks to be communicated (i.e. technical or naturally occurring, voluntary or involuntary, familiar or unfamiliar), taking into account costs and benefits, and pick the most appropriate tool for disseminating the message (i.e. press conference, internet release); (3) Do not amplify risks, especially those naturally occurring and perceived by the public as attenuated, in order to avoid public distrust in the source of information; (4) Do not involve too many scientific bodies to avoid conflicting messages; (5) Be proactive in risk communication to earn public trust; (6) Do not always reveal all technical details, especially in case of scientific uncertainty, because it may lead to public confusion and consequently, to distrust; (7) Avoid mentioning brands, except for the situation when a potentially hazardous specific product must be recalled; (8) Proactively seek media contact with specific editors and inform them about the ongoing work; (9) Communicate message to the media via a trusted source (i.e. government body); (10) Communicate message to the media by a competent and experienced person; (11) Avoid creating a "communication vacuum" which may lead to unnecessary rumors and speculations. To prevent this, hold press conferences as short as possible (i.e. 2–3 hours instead of 18).

The mass media has become particularly active in its reporting and their role has changed considerably throughout the years. A couple of decades ago, the media was just an instrument used by policy makers or other stakeholders to transfer the information to the masses, while today journalists shape the public viewpoints. Food related risks are considered a socially sensitive issue, for everyone has to eat and all foods carry a degree of inherent risk. Thus, any information about food safety combined with intensive media reporting contribute to a widespread public anxiety [Fitzgerald & Campbell, 2001].

Risk perception can be defined as a way in which consumers perceive risk and interprets risk information, and depends upon a variety of factors. There are numerous publications concerning this issue. Perhaps the most popular way of analyzing risk perceptions is psychometric approach [i.e. Fife-Shaw & Rowe, 1996; Sparks & Shepherd, 1994]. The core assumption of this method is that the lay people perceive risks multi-dimensionally, addressing a number of factors, namely: uncertainty, familiarity, dread, catastrophic potential, controllability, natural or manmade, risk to future generations, potential benefits, inter alia [Slovic, 1999; Hansen et al., 2003]. The research using the psychometric approach has led to the development of the perceived food risk index PFRI [Fife-Shaw & Rowe, 1996]. As it was pointed out by Sjoberg [2000], perceived risk has become a focus of interest of policymakers and researchers. A number of large-scale international research projects have been realized in which consumers' views on food risk and its management was one of the central issues to investigate (i.e. Safefoods – Food-CT-2004-506446, 2004-2007) or the only focus (i.e. Eurobarometer research on 25 member states commissioned by EFSA & DG SANCO, 2005).

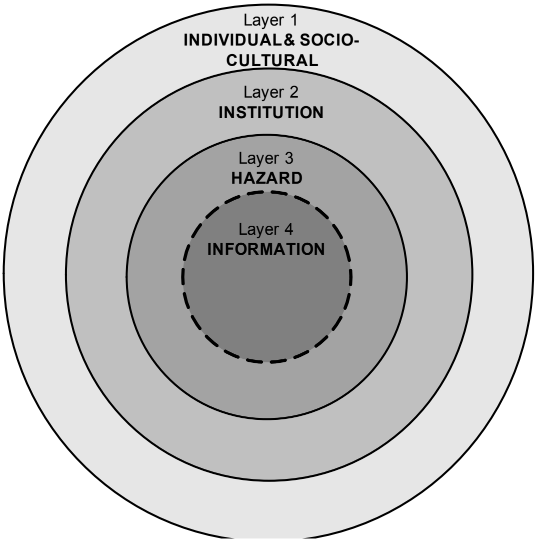
Public trust is a prerequisite for effective communication, therefore it has also been the subject of substantial research and main focus of various research projects (i.e. Consumer Trust in Food – QLK1-CT-2001-00291, 2001–2004). A comprehensive and thorough review of mechanisms and factors underlying public trust can be found in the study by Chryssochoidis et al. [2008]. Based on the extensive review of literature, they posited four groups of trust-related dimensions: information-related, hazard-related, institutional-related and socio-cultural and personality ones, to come up with a concept of "trust-layers" (Figure 3). Understanding these dimensions is key to earn and maintain public trust, and consequently, improve food risk communication.

Layer 1, socio-cultural and personality, represents the most generic context in which the attribution of trust is made, dependent on different conditions, such as family, economic and political situation, shaping one's confidence. *Layer 2* reflects "institutional trust" in: (a) actors responsible for risk analysis; (b) sources of information about risk. Two key dimensions have determine institutional trust, namely 'ability' and 'willingness' to protect the public from risk and provide correct information. 'Ability' comprises competence-related qualities, such as "knowledge", "competence", "expertise", as well as "dependence" – related attributes, such as e.g. "freedom" to take actions. 'Willingness' includes features related to "goodwill" or "motives" of an institution, such as "concern", "care", "openness", "honesty", "fairness" and "vested interest". *Layer 3* assumes that the same source can be trusted in dealing with some risks (i.e. microbial) while distrusted in case of some other (i.e. GMOs). However, across the research studies, perception of hazards has been conceptualized differently with relation to trust, either being influenced by the level of pubic trust (i.e. Siegrist, 2000), or as a factor influencing public trust itself [i.e. White et al., 2003], suggesting that the direction of the trust-hazard relationship works possibly two-way. Finally, *Layer 4* reflects the role of information in building public trust. Three information-related factors have been described: (i) content-related: trust in the source of information or the subject reported; (ii) source-related: trust in a message; (iii) amount-related: large amount of messages can be perceived as trust-building factor ("openness") or right the opposite ("sensitization").

Conclusions

The present paper overview the existing knowledge on risk communication at the food market, starting from its role in the ‘risk analysis’ framework (risk assessment, management and communication). The paper overviews state-of-the-art knowledge on effective communication of risks found in the literature, with related recommendations for practitioners by various food safety organizations (FAO, EFSA, EUFIC).

Over years, the idea of risk communication developed from simply passing an information from knowledgeable institutions to "ignorant" consumers, to complex interactive exchange of information and opinions between all stakeholders throughout the whole risk analysis process. These stakeholders include scientists, industry, consumer organizations, activist groups and the consumer, *inter alia*. Consequently, risk communication plays a special role in risk analysis, therefore it is important to follow all principles that contribute to its success.



Source: Chryssochoidis et al [2008]

Fig. 3. Layers of trust-related factors

First, the exchange of information should involve all stakeholders at each stage of risk communication. Second, this process should not account solely on technical estimates, but must reflect upon the consumer concerns regarding various foods. Third, building public trust should be the prerequisite of establishing communication channels. Inviting "lay" consumers to take part in the "real" debates concerning food safety issues and listening to their concerns can be an example of positive fulfillment of these three assumptions. Fourth, it is necessary to acknowledge the media impact on the consumer opinions and subsequent behavior. Although the media are blamed for risk amplification and raising public anxiety, they are in fact a very powerful tool that can be used to achieve positive goals, such as increased education on food risk among the lay public. Despite a tendency of food risk researchers to separate and contrast "risk knowledge" and "risk perceptions", we postulate that well informed consumers are more likely to get involved in risk communication and make more aware choices. For example, there should be a TV national educational program about food safety and production issues, covering a variety of aspects, from those associated with hygiene and production practices to modern production technologies (i.e. GMO). This issue requires further attention, especially from practitioners.

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7

FACTORS DETERMINING CONSUMER PREFERENCES IN RESPECT OF YOGHURTS AVAILABLE ON THE LOCAL MARKET

Introduction

Dairy products constitute a wide array of products differing in terms of assortment, nutritive value, shelf life and price. Yoghurts, belonging to a group of fermented dairy drinks, constitute one of the most diversified groups. A consumer may chose between plain and flavored yoghurts, with various consistency – from solid, through jelly-like to liquid, with a different fat content, with pieces of fruits, with the addition of cereal grains, probiotics, coenzyme Q10, ginseng and many other substances, in cardboard packages, bottles and cups of different volume. The competition between producers of yoghurts is severe, hence an increasing number of dairy plants tend to recognize expectations of consumers and their market behaviors in order to extend their offer and to satisfy expectations of the customers. When designing new products or when modifying the already existing ones, a target client should be determined taking into account such aspects as: psychical, social and economic diversity of consumers, as well as their genetic and cultural determinants, educational status, life experience and social affiliation. All these factors make companies pay an ever increasing attention to consumer surveys. Consumers determine the type and direction of an economic activity of the suppliers, thus affecting the condition and development of the entire economy [Smyczek and Sowa, 2005]. In companies managed to promote quality, client's satisfaction constitutes a measure of success. A measurement of client satisfaction consists in the determination of how a client perceives the activity of product supplier [Hill and Alexander J, 2003]. Such a measurement is a multi-stage process of differentiated activities aimed at enabling objective, accurate and comprehensive recognition of a selected fragment of reality [Churchill, 2002]. Analyses of consumer satisfaction provide extensive knowledge in the following areas: how do the customers evaluate the product, how do the customers perceive the company, what factors of cooperation with the company are important to the customers, and how do the customers evaluate products of company competitors. Such an analysis enables determining strong and weak points of products and the company, indicates areas that should be improved in the company in terms of functioning, points to factors that affect loyalty of the customers as well as determines means of establishing strong relations with a customer [Churchill, 2002].

Attention should also be paid to the fact the ISO standard 9001:2008 *Systems of Quality Management. Requirements*, – highly popular in the dairy industry, – puts emphasis on explicit recognition of needs and expectations of a customers, and then on verifying whether they have been fulfilled. The standard obliges to the constant monitoring of information on customer satisfaction, which should be confirmed in the conducted quantitative and qualitative assays. One of the forms of analyzing customers satisfaction is a survey study in the form of a questionnaire, which provides data on customers requirements in respect of products as well as enables later translation of those requirements into particular specifications of products, instructions and technical parameters of processes.

The objective of the undertaken study was to collect data on preferences and expectations of consumers in respect of yoghurts. Data collected from the respondents enabled determining selection criteria of yoghurts, i.e. the meaning of consistency, flavor, unit package, additives or price of yoghurts to a consumer, as well as brands preferred, frequency of purchase, choice factors and preferred flavors of yoghurts.

Material and methods

Analyses were conducted with the method of survey using a self-completed questionnaire. The questionnaire consisted of 28 questions, including 5 referring to the social status of respondents. The surveyed population covered 500 respondents differing in terms of: gender, age, education, social status and size of the place of living. The population was selected with the method of non-randomized sample selection (the so-called "convenient sample").

The exact survey was preceded by a pilot study conducted on a sample of 100 persons from the target group, in order to identify drawbacks and inaccuracies that might have occurred while elaborating the contents of questions and forms of replies.

Results and discussion

The structure of the surveyed population was presented in Table 1 in respect of gender, age groups (5) and education (2). The results obtained were subjected to a quantitative analysis.

All respondents appeared to buy dairy products, including 55% of women who declared to buy dairy products 3–4 times a week on average, and 21.4% of men who purchased those products 1–2 times a week on average. The most frequently purchased dairy products included: sour cream, drinking milk, hard cheese and yoghurt. The frequency of yoghurts purchase was higher in women than in men (Fig. 1), which is likely to result from the fact that women pay greater attention to health-promoting values of food products.

Both to women and men, the key factors at making a decision on the purchase of yoghurt included: additives (e.g. pieces of fruits), flavor and price. The semi-liquid consistency of yoghurt was indicated as the preferred one by 52% of the surveyed women and men.

Analyses of the factors determining a decision on the choice of yoghurt demonstrated that both women and men were driven most of all by the flavor values of yoghurts (29%), followed by their health-promoting values (26%), and common availability at a reasonable price (Fig. 2). Respondents at the age of 25–34 years pointed additionally to the convenience of consumption. This factor was of great significance owing to the fact that most of the respondents from that age group were either employees (45.5%) or students (46.4%), and yoghurts constituted to them a convenient and fast form of satisfying hunger.

Flavors of yoghurt most frequently chosen by the women were: strawberry (14%) as well as cherry, vanilla and blueberry (9% of answers for each). In turn, the men most often reached for yoghurts with strawberry (19%) and peach flavor (12%) or for plain yoghurts (12%). These are traditional flavors, i.e. first that had appeared on the market of yoghurts and ever since have been accepted by most of the society. It may be observed, in turn, that consumers were somehow reluctant in reaching for such flavors as: tiramisu, cappuccino, stracciatella or rafaello, which results from the fact that they are still uncommon and that consumers remain loyal to already proven flavors.

The appearance of a unit package was of little significance to the respondents, which has been indicated in answers of 47.2% of the women and 25.6% of the men. It turned out that the package did not fulfill its promoting function in any special way. It is also worth adding that out of the characteristics of a unit package, the least important to the respondents was its detrimental effect on the environment, which indicates indifference of the respondents in terms of the care over management of the spent packages. In contrast, the most significant appeared to be data contained in the label, which – according to the respondents – should be comprehensive and clear. The most useful unitary volume of yoghurt was 330 g and 500 g in the opinion of men and 125 g in the opinion of women. This may easily be explained since women may satisfy their nutritional needs with a lesser volume of yoghurt, whereas in the case of men such a volume is insufficient.

Table 1

Characteristics of the surveyed population

Group of consumers			**Number [%]**	
GENDER	women		**63.6**	
	men		**36.4**	
AGE	15–24 years	women	14.6	**22.8**
		men	8.2	
	25–34 years	women	13.8	**22.0**
		men	8.2	
	35–44 years	women	10.0	**17.2**
		men	7.2	
	45–54 years	women	15.2	**23.2**
		men	8.0	
	55 years and more	women	10.0	**14.8**
		men	4.8	
EDUCATION	15–24 years	occupational/ secondary	12.8	**22.8**
		higher	10.0	
	25–34 years	occupational/ secondary	5.4	**22.0**
		higher	16.6	
	35–44 years	occupational/ secondary	8.8	**17.2**
		higher	8.4	
	45–54 years	occupational/ secondary	17.2	**23.2**
		higher	6.0	
	55 years and more	occupational/ secondary	12.8	**14.8**
		higher	2.0	

Source: Own study

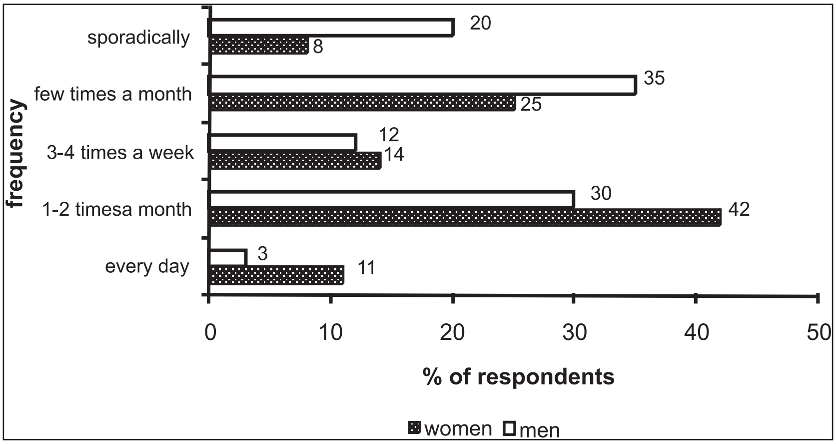

Fig. 1. Frequency of yoghurts purchase

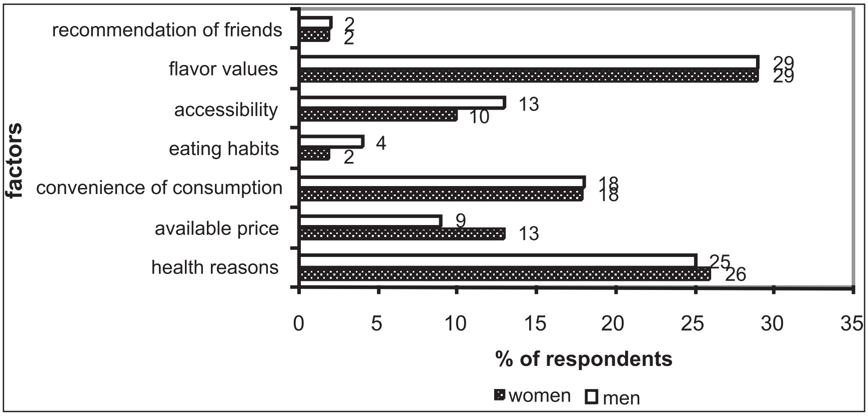

Fig. 2. Factors determining the selection of a purchased yoghurt

When asked whether they happen to meet promotion and advertise of yoghurts, in most of the cases the respondents replies positively, i.e. 40% of women and 28.8% of men. Based on the replies provided, it may be observed that the most effective form of advertisement is a TV commercial, which has the greatest range of impact, and advertisement in the form of tasting experienced by the respondents while doing shopping (Fig. 3). The respondents declared that those forms of advertisement convinced them to the purchase of a promoted yoghurt only to a little extent (19.8% of women and 11% of men). It may be explained by the fact that the consumers are distrustful of what is declared by the producers of yoghurts using various marketing tools.

In analyzing the age structure of the surveyed population, the highest frequency of yoghurts purchase was observed in the age groups of 15–24 years and 25–34 years, whereas the lowest one – amongst respondents at the age of 35–44 years – who were buying more often hard cheese than yoghurts. The frequency of yoghurts purchase amongst women over 55 years of age was considerably higher than amongst the men (Fig. 4). Yet, it should be emphasized that both women and men over 55 years of age declared to buy yoghurts not for themselves but for their children and grandchildren. The results obtained are consistent with those achieved in a study by ZiarNo. and Mauzer on a group of 150 citizens of Warsaw as well as with those reported by Nowak et al. who surveyed a group of 310 respondents [Ziar-No. and Mauzer, 2009].

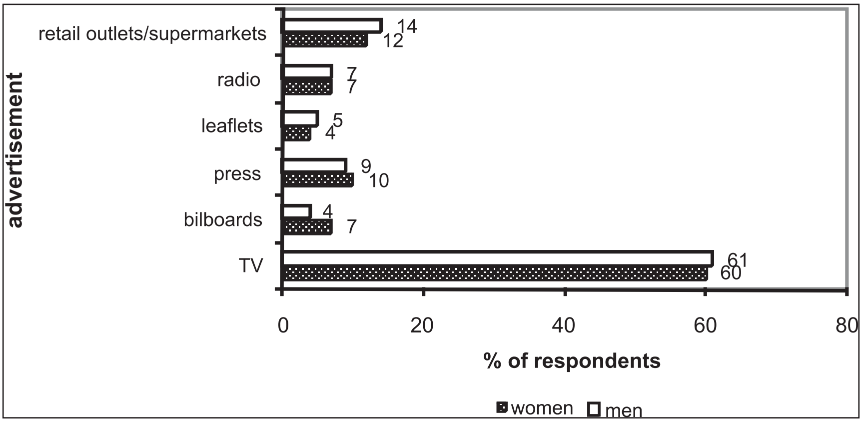

Fig. 3. Form of the advertisement of yoghurts remembered by the respondents

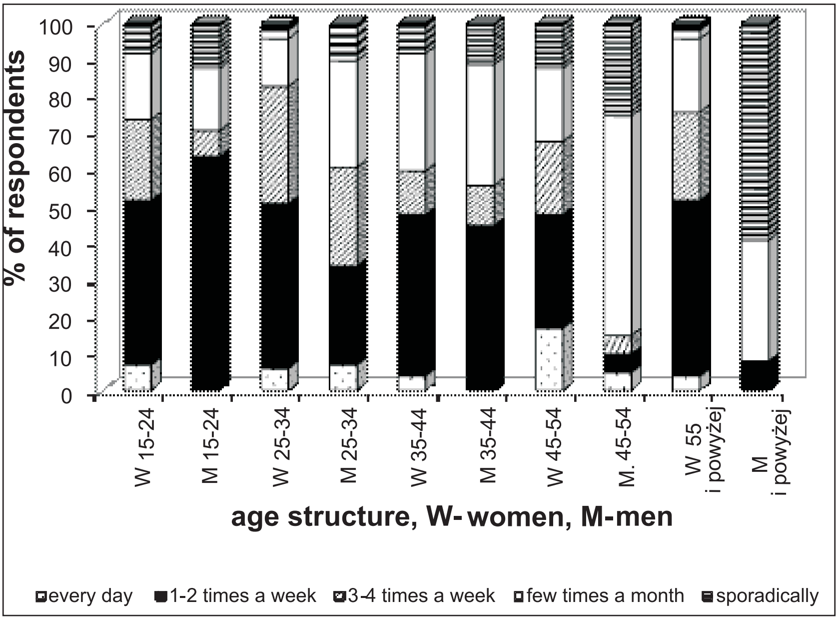

Fig. 4. Effect of age on the frequency of yoghurts purchase

In the entire surveyed population, a tendency was maintained for most often selected flavors of yoghurts. Irrespective of age, gender and education, the respondents indicated the following flavors: strawberry, peach, vanilla, blueberry and wild berries. In addition, it turned out that even amongst young respondents (15–24 years) No. distinct change occured in respect of the taste and preferences of yoghurt flavors, although it seems that these persons are more open to flavor novelties and more interested in new products. This is, however, not confirmed in the reality. It may be due to the fact that the consumer have some doubts as for flavor novelties, which is especially noticeable at age categories of: 45–54 years and over 55 years.

The most frequent reason of yoghurts purchase were their health-promoting and flavor values (Fig. 5). It should be noticed, however, that although consumers perceive advertisements and their contents with some distrust and distance, still they have an undisputable effect on the brand's recognizability, thus strengthening its position on the market, while the consumers usually chose the yoghurts of advertised brands.

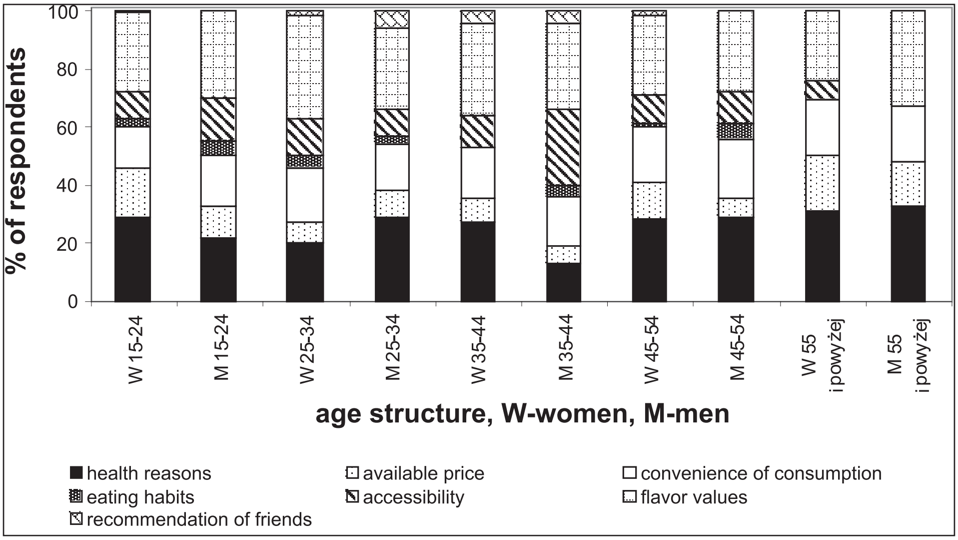

Fig. 5. Factors determining the purchase of yoghurts as affected by the age of the respondents

The conducted survey study covered 285 respondents with secondary and occupational education (57%) and 215 respondents with higher education (43%), amongst which the age categories of 15–24 years and 25–34 years included the highest percentage of respondents with higher education (44% and 75.5%, respectively), and age categories of 45–54 years and over 55 years – considerably less respondents with the higher educational status (26% and 13.5%, respectively). It is due to the fact that the first two age categories are represented in the prevailing part by students or graduates, and the study was conducted in the city of Olsztyn that offers education in a few higher schools. In turn, the age categories of 45–54 years and over 55 years are a generation raised in times when the higher schools were completed only by a small group of people owing to either financial or priority reasons.

Both the respondents with secondary education (66.3% of answers) and those with higher education (67.4% of answers) declared to purchase dairy products 3–4 times a week on average. Most frequently bought dairy products included: drinking milk, sour cream, hard cheese and yoghurt, for these are the products of an everyday diet.

Taking into account the frequency of yoghurts purchase, they were bought considerably more often by persons with higher education, irrespective of age. This is likely to result from the fact that they are more aware of the health-promoting value of yoghurts and their beneficial effects on human body. In analyzing choice factors at the purchase of yoghurts, it may be observed that the respondents with higher education were mostly driven by the health issues (27% of answers), followed by the flavor values (24% of answers). In the case of the consumers with secondary education, significant attention was paid to the flavor values (30%), followed by the health-promoting aspect (27%) as well as the price of yoghurt (13%). Yoghurt flavors the most frequently selected by the respondents with secondary education included: strawberry, vanilla and blueberry, whereas yoghurts with strawberry and forest fruits flavors as well as plain yoghurts were most often purchased by the respondents with higher education. Worthy of notice is the fact that such flavors as: stracciatella, tiramisu, marzipan and poppy, rafaello, coffee or nuts, were accepted with a greater approval and trust of the respondents with higher education, for they appear to be more open to market novelties and to more eagerly reach for new flavors.

In the case of both respondents with the secondary and those with higher education, the key choice factors at the purchase of yoghurts included, in the descending order: flavor, price and additives, e.g. pieces of fruits (Fig. 6). Those factors turned out to be independent of the age, gender and educational status of the respondents.

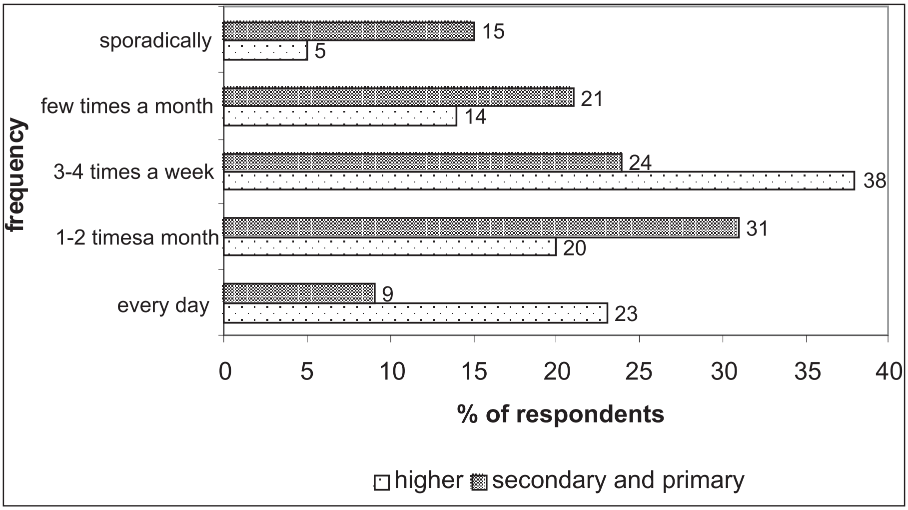

Fig. 6. The effect of education on the frequency of yoghurts purchase

Answers provided by the respondents prompted us to a conclusion that when making a decision on the purchase of a given yoghurt, a consumer is driven by the so-called "first impression". In that case, the "first impression" involves such characteristics as flavor values, health-promoting values, size of a unit package and accessibility. In analyzing the factors that affect a decision on the purchase, it is difficult to name the aggregated characteristics. It has been confirmed in a study by Doroszewicz, who has called them "the first impression" and has been treating them as inherent properties of a product noticed by a customer that determine its quality [Doroszewicz, 2008].

Conclusions

A survey research enables the prediction of purchase preferences and behaviors of consumers in a long time perspective. It additionally allows to determine requirements of a consumer in respect of products, through the identification of factors increasing consumer's satisfaction. Based on the survey study conducted to examine preferences and expectations of consumers in respect of yoghurts available on the market, it may be observed that they are a product whose consumption diminished proportionally to the age of respondents, which results from a greater awareness of the younger generation of the health-promoting properties of yoghurts and from their greater care over a healthy lifestyle.

The analysis of factors determining the purchase of yoghurts demonstrated that, irrespective of gender and age, the respondents were mainly driven by their flavor values, followed by health-promoting properties. It should additionally be emphasized that price was also of great significance to the respondents.

Again, irrespective of age, gender and education, yoghurt flavors most often chosen by the respondents include: strawberry, blueberry, vanilla and forest fruits. It enables concluding that the consumer are still distrustful of flavor novelties appearing on the market and reach for them rather reluctantly, remaining loyal to the proven traditional flavors, thus eliminating the risk of wrong choice.

The conducted survey demonstrated a tendency maintaining in the entire surveyed population in respect of yoghurt packaging properties, namely: the Polish consumers pay little attention to the detrimental effect of the package on the environment, which may be noticed even amongst young persons who should not underestimate the environmental aspect but rather treated it as of key priority.

Advertisements that affect the awareness of the consumers to the greatest extent include a TV commercial and the tasting at supermarkets. Nevertheless, they appear little convincing means of providing information, for the respondents feel rather distrustful of producers declarations. The advertisements affect mainly the recognizability of promoted brands and, consequently, their choice, but not the decision on purchase itself.

In view of an ever increasing and stronger competition of other companies, a questionnaire survey enables gathering valuable data determining the effective functioning on the market of food products. It additionally facilitates the determination of the factors being of the outmost significance to a consumer while making a decision on the purchase, for satisfied customers ensure the success of each company.

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CHAPTER 5

FOOD QUALITY IN ASPECT OF MICROBIOLOGY

1

MICROBIOLOGICAL QUALITY IN THE SELECTED INSTANT SOUP CONCENTRATES

Introduction

One of the basic characteristics of food quality is its microbiological state. For health reasons, because of the risk of food poisoning and infection, microbiological quality is of fundamental importance for health safety of food [Czapski, 1995; Kołożyn-Krajewska, 2003; Kołożyn-Krajewska, 1993].

Food concentrates are food products protected against spoilage through suitable technical manipulations and particularly thermal processes (thickening, drying) leading to their dehydration. Food concentrates are classified as convenience food, with extended shelf life [Słowiński & Remiszewski, 1996].

The microbiological contamination of foodstuffs is dependent among other things on: the quality of materials, type of packaging, cleanliness of equipment, apparatus and rooms, personal hygiene of staff, frequency and effectiveness of washing processes and disinfection, and presence of preservatives. Food products, both materials and processed products, constitute a favourable environment for microorganisms – bacteria, yeast, moulds – and they may be an ideal habitat for some viruses [Windyga, 1996].

Nowadays a consumer not only seeks ‘convenient’ and ‘fast’ prepared products but also demands ‘healthy’ natural food containing natural dyes, flavours, seasoning and moreover products with lowered content of various ingredients detrimental for health [Słowiński & Remiszewski, 1996].

Food acquisition and processing is connected with occurrence and development of micro-organisms. The quality of an end-product and especially its sensory properties, nutritional value and shelf life depend on to what extent the processed material was attacked by putrefactive micro-organisms, how far they could develop during the processing and the number of micro-organisms in the final product [Kołożyn-Krajewska, 2003].

Materials used in processing play an essential role in creating quality features of products. Their quality in turn depends on the level of agricultural and environmental conditions. The processing and preservation technologies used play a comparable role. These are the key stages in determining quality, which is why their control by creating appropriate systems is so important. Implementing them is tantamount to departure from the assessment of the end-product in favour of providing conditions for producing food of expected quality and safe for the customer [Kołożyn-Krajewska, 1993].

The frequency of convenience food intake including soup concentrates is increasing from year to year, as confirmed by surveys carried out by the authors [Adamczyk, 2006; Krełowska-Kułas, 2005; Kowalczuk, 2004].

The health quality of convenience food is determined not only by nutritional and sensory qualities but also by good microbiological quality [Stobińska et al., 2000].

The aim of the study was to determine the level of microbiological contamination of selected instant soup concentrates of four domestic producers.

Materials and methods

The area of research included instant soup concentrates of four selected domestic producers, designated A, B, C and D. Material for laboratory research was purchased in a retail outlet directly after the process of production was finished. Identical batches of a product and expiry date were selected for every kind of soup. The subject of the research was the six following instant soups: 1) chicken soup with noodles, 2) red borscht, 3) Polish rye soup with croutons, 4) tomato soup with noodles, 5) mushroom cream soup with croutons, 6) boletus cream soup with croutons.

The microbiological analysis included the following determinations:

1) total number of microorganisms in 1 g by plate method which included preparation of culture on plates by pour plate method and incubation of plates under oxygen conditions at a temperature of 30°C for 72 hours [PN-EN ISO 4833:2004/Ap1:2005].
2) presence of E. coli bacteria in 0.01 g which included preparation of culture on plates with the specific selective medium and then incubation at a temperature of 30, 35 and 37°C for 24 hours [PN-ISO 4831:2007].
3) detection of Salmonella bacteria in 25 g, which took place in 4 stages: pre-enrichment in non-selective liquid medium at a temperature of 37°C for 18 hours, then selective enrichment in liquid media at a temperature of 37°C for 24 hours and passage on agar culture media [PN-EN ISO 6579:2003/A1:2007],
4) amount of moulds and yeast in 1 g by plate method where there was culture of the examined sample with the selective medium poured on and then incubation of plates under oxygen conditions at a temperature of 25°C for 3–5 days [PN-ISO 7954:1999],
5) presence of anaerobic sulphite reducing bacteria in 0.01 g, where samples were heated at a temperature of 80–83°C for 10 minutes, culture of sample and pouring on sulphite iron agar medium then incubation at a temperature of 37°C for 1–4 days [PN-A-86014-12:1993].

Results and discussion

An important criterion for the quality of food is its microbiological state, which determines the stability of sensory qualities and product shelf life. The appropriate microbiological purity of food is one of the most important factors guaranteeing food safety. The microbiological quality of soup concentrates depends on the level of contamination of ingredients used while producing them (seasoning and dried vegetables), the content of water in a product and hygiene conditions during the production process. The presence of pathogenic bacteria may be the reason for the occurrence of diseases and food poisoning [Burbianka et al., 1983; Sikorski, 2002; Wieczorek, 2003; Windyga & Ścieżyńska, 2005].

The content of water in examined soup concentrates varied within the limits 2.6 to 7.6%. The Polish Norm does not give any requirements about water content in food concentrates. The examined soup concentrates were characterized by the appropriate water content as applicable to loose products, i.e. under 10% [Świderski, 1999].

In examined soup concentrates the total number of micro-organisms in 1 g was determined (Tab. 1). Red borscht of the producer (B) contained the smallest total number of micro-organisms – 4.5x10^1 CFU/g (Tab. 1). In two soups, i.e. tomato soup with noodles and Polish rye soup with croutons of producer (D), the content of the total number of micro-organisms was at a high level (1.2x10^4 CFU/g) in comparison to other instant soup producers.

Table 1

Total number of microorganisms, amount of moulds and yeast in the examined instant soup concentrates [CFU/g]

Name of soup	Producer code	Total number of microorganisms [CFU/g]	Amount of moulds [CFU/g]	Amount of yeast [CFU/g]
Polish rye soup with croutons	A	8.5 x 10^3	1.4 x 10^2	less than 10
	B	1.0 x 10^3	3.2 x 10^2	less than 10
	C	2.3 x 10^2	less than 10	less than 10
	D	1.2 x 10^4	4.5 x 10^2	less than 10
Red borscht	A	2.7 x 10^3	1.6 x 10^2	less than 10
	B	4.5 x 10^1	2.5 x 10^1	less than 10
	C	5.2 x 10^2	1.0 x 10^1	less than 10
	D	5.0 x 10^1	0.5 x 10^1	less than 10
Tomato soup with noodles	A	4.9 x 10^2	2.5 x 10^1	less than 10
	B	3.6 x 10^2	less than 10	less than 10
	C	1.8 x 10^3	less than 10	less than 10
	D	1.2 x 10^4	8.5 x 10^1	5.5 x 10^1
Boletus cream soup with croutons	A	2.6 x 10^5	5.5 x 10^1	less than 10
Chicken soup with noodles	A	1.5 x 10^3	less than 10	less than 10
	B	1.4 x 10^2	less than 10	less than 10
Mushroom cream soup with croutons	A	4.2 x 10^2	1.0 x 10^1	less than 10
	B	1.9 x 10^2	less than 10	less than 10
	D	1.6 x 10^3	6.0 x 10^1	less than 10

The Polish Norm PN-A-94050:1996 provides microbiological requirements for the total number of microorganisms in instant soup concentrates, for which 1 g of the examined product may not contain more than 1x10^5 CFU/g. Among the examined soups only boletus cream soup with croutons of producer (A) did not meet the requirements of the norm since the total number of microorganisms in 1 g amounted to 2.5x10^5 CFU/g.

Convenience food containing an excessive number of microorganisms reveals hygiene neglect during the production process, inappropriate selection of materials or a flawed technical process [Windyga, 1996]. The soup concentrates examined by Wójcik-Stopczyńska et al. were more contaminated by microorganisms. The authors reported an excessive number of micro-organisms in the following soups: white borscht (1.7x10^5 CFU/g), mushroom cream soup (3.30x10^5 CFU/g), tomato soup (1.6x10^5 CFU/g) and Polish rye soup (1.07x10^5 CFU/g) [Wójcik-Stopczyńska et al., 2002].

The greatest number of moulds was found in Polish rye soup of producer (D) – 4.5x10^2 CFU/g (Tab. 1). In the other soups the number of moulds was as follows: in tomato soup with noodles (A – 2.5x10^1 CFU/g, D – 8.5x10^1 CFU/g), in Polish rye soup with croutons (A – 1.4x10^2 CFU/g, B – 3.2x10^2 CFU/g), in mushroom cream soup with croutons (A – 1.0x10^1 CFU/g, D – 6.0x10^1 CFU/g), in boletus cream soup with croutons (A – 5.5x 10^1 CFU/g) and in red borscht (A – 1.6x10^2 CFU/g, B – 2.5x10^1 CFU/g, C – 1.0x10^1 CFU/g, D – 0.5x10^1 CFU/g). A smaller number of moulds than 10 CFU in 1 g of the examined product was recorded in 6 soups: chicken soup with noodles (A, B), tomato soup with noodles (B, C), mushroom cream soup with croutons (B) and Polish rye soup with croutons (C).

The determined number of moulds in the examined instant soups of domestic producers was in compliance with the requirements of the Polish Norm. None of the examined soups exceeded the permissible level of moulds (1x10^3 CFU/g) provided for by the norm. The Sanitary and Epidemiological Station in Poznań carried out an inspection of 16 packages out of 20 thousand unit packages of dinner concentrate batches and stated that the concentrates were in general of good quality but 4% of the samples revealed the presence of moulds [Kępińska, 2000]. The research carried out by Wójcik-Stopczyńska et al. displayed microbiological contamination of instant soup concentrates as well [Wójcik – Stopczyńska et al., 2002].

The greatest amount of yeast was determined in tomato soup with noodles (D) (5.5x10^1 CFU/g). The amount of yeast was smaller than 10 CFU in 1 g of the examined product in the other soups (Tab. 1). The Polish Norm and the ordinance do not give any requirements about the amount of yeast in instant soup concentrates. According to literature data the maximum content of yeast in dinner concentrates is 1x10^3 CFU/g [Trojanowska *et al*., 1996]. As can be seen from Table 1 the amount of yeast in the examined soup concentrates did not exceed the required content.

The results of the microbiological analysis of *E. coli* bacteria, *Salmonella* bacteria and spores of anaerobic sulphite reducing bacteria are given in Table 2.

The presence of E. coli bacteria was not recorded in 17 out of 18 examined instant soups (Table 2). The one exception was boletus cream soup with croutons of producer (A), in which *E. coli* bacteria were present in 0.01 g. In accordance with the requirements of the norm PN-A-94050:1996 E. coli bacteria may not be present in 0.01 g of the examined product. Boletus cream soup (A) did not meet the requirements of the Polish Norm.

Table 2

E. coli bacteria, *Salmonella* bacteria and spores of anaerobic sulphite reducing bacteria in the examined instant soup concentrates [CFU/g]

Name of soup	Producer code	*E. coli* bacteria [CFU/g]	*Salmonella* bacteria [CFU/g]	Anaerobic sulphite reducing bacteria [CFU/g]
Polish rye soup with croutons	A	not present	not present	not present
	B	not present	not present	present
	C	not present	not present	not present
	D	not present	not present	present
Red borscht	A	not present	not present	not present
	B	not present	not present	not present
	C	not present	not present	not present
	D	not present	not present	not present
Tomato soup with noodles	A	not present	not present	not present
	B	not present	not present	not present
	C	not present	not present	present
	D	not present	not present	present
Boletus cream soup with croutons	A	present	not present	not present
Chicken soup with noodles	A	not present	not present	not present
	B	not present	not present	not present
Mushroom cream soup with croutons	A	not present	not present	not present
	B	not present	not present	not present
	D	not present	not present	not present

The presence of *Salmonella* bacteria was not found in the examined instant soups of four producers (Tab. 2). *Salmonella* bacteria may not be present in 25 g of the examined product in compliance with the requirements of the Polish Norm for instant soup concentrates. All examined soups met requirements provided for by the Polish Norm.

Windyga et al. examined the occurrence of *Salmonella* pathogenic bacteria in different types of food products on the market including in samples of dinner concentrates. In the examined dinner concentrates they did not discover the presence of Salmonella bacteria either [Windyga et al., 2002]. The research conducted by Wójcik-Stopczyńska et al. also proved that in none of the examined samples was the presence of pathogenic bacteria found, which confirms the health safety of the assessed concentrates [Wójcik-Stopczyńska et al., 2002].

The last of the determinations concerning the microbiological quality of the examined soup concentrates was the presence of spores of anaerobic sulphite reducing bacteria in 0.01 g (Tab. 2). In four examined soups there were spores of anaerobic bacteria present in 0.01 g, namely, in Polish rye soup with croutons (B, D), and in tomato soup with noodles (C, D). The Polish Norm provides requirements for spores of anaerobic sulphite reducing bacteria, which may not be present in 0.01 g of a product. Polish rye soup with croutons (B, D) and tomato soup with noodles (C, D) did not meet the requirements of the norm since the number of spores of anaerobic sulphite reducing bacteria was excessive. The remaining soups met the requirements of the norm.

The presence of spores is essential as they can survive at a high temperature when dissolving concentrates in boiling water [Wójcik-Stopczyńska et al., 2002].

Reducing water availability in the environment as a result of heat treatment (thickening, drying) stops the development of most microorganisms, which effectively allows the shelf life of convenience food to be prolonged. Low water activity food may contain osmotolerant microorganisms such as: moulds, yeast and bacterial spores. The source of increased microbiological contamination of dinner concentrates may be flavour substances such as dried vegetables, cereals and herbs [Burbianka et al., 1983; Stobińska et al., 2000].

As can be seen from research, seasoning and dried vegetables are not free from microbiological contamination. Added to dishes or used in the production process, they may be dangerous for health. Seasoning contamination is varied; the number of microorganisms may fluctuate from 10^3 to 10^7 cells/g. The occurrence of rod-shaped *Bacillus cereus* and *Clostridium perfingens* in seasoning is particularly dangerous. The number of spores of these bacteria can be from 10^3 to 10^7 in 1 gram. The seasoning contamination caused by moulds fluctuates from several to a dozen or so thousand in 1 gram. Seasoning can also contain mycotoxins including aflatoxins, e.g. black pepper, red pepper and chili [Burbianka et al., 1983; Kołożyn-Krajewska, 1993].

As far as dried vegetables are concerned, the researchers stated that dried carrot, parsnip and celeriac are substantially more contaminated than dried onion and garlic. This probably results from the production of antibiotic substances by these vegetables. Ground seasoning is more contaminated (mainly by *E. coli* bacteria) than one that is not ground. The microbiological contamination of dried vegetables can be considerably reduced by keeping the optimal hygiene conditions in the whole production process, keeping to the minimum time between blanching and drying, and by appropriate drying (preferably by hot air) [Kołożyn-Krajewska, 2003].

To sum up, most of the analysed instant soup concentrates are characterized by a good state of microbiological purity. The level of microbiological contamination in the examined instant soup concentrates was variable and specific for the given assortment. The obtained results of the research on soup concentrates indicate the necessity of close microbiological control of production processes of half-finished products by paying special attention to appropriate washing and cleansing materials, especially plant ones – vegetables and mushrooms, which can be strongly contaminated by spore-forming microflora and mould spores. The production of soup concentrates generally involves mixing ready components, which can be an additional source of microbiological contamination. These include seasoning, croutons and instant noodles.

Conclusions

1. As a result of the conducted microbiological analysis, the presence of *Salmonella* bacteria was not detected in any of the examined instant soups.

2. The amount of mould and yeast was within the limit provided for by the Polish Norm.

3. The research results indicate that the acceptable limit of the total number of microorganisms and *E. coli* bacteria in boletus cream soup with croutons (A) was exceeded.

4. The presence of spores of anaerobic bacteria in tomato soup with noodles (C, D) and Polish rye soup with croutons (B, D) was detected.

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2

MICROBIOLOGICAL QUALITY OF RAW MATERIALS FOR ETHANOL PRODUCTION

Introduction

Due to recent developments in the liquid biofuel market, lower quality materials which are useless for consumption, processing or animal feeding have manifested themselves as possible material for ethanol production. Even grain that is cracked or immature or affected by diseases can still be very useful in ethanol production. The application of these crude materials may reduce production costs as they can be purchased from agricultural or industrial enterprises at lower prices. It also eliminates bad grain from trade, which has its own positive economic and environmental implications.

Still, ethanol production from grain with lower quality that is otherwise useless in consumption and animal-feeding has its own associated difficulties. First, the material may be contaminated by mycotoxins and bacterial microflora. Second, the crude materials do not meet standards or quality requirements needed for maintaining the repeatability of the technological process.

These factors may adversely affect the production process, its productivity and quality of final products. That is why the application or recycling of these materials for bioethanol production must be analysed with regard to its profitability [Bothas et al., 1992, Gulami et al., 1996, Krishnan et al., 2000, Lipski 2003] .

The aim of this experiment was to examine the influence of the microbial quality of the tested materials on the process and results of ethanol fermentation using distillery yeast.

Materials and methods

the material used was maize grain of varying quality: maize KB 1903 – good quality grain, from the Plant Breeding Experimental Station NASIONA KOBIERZYC Sp. z o.o., maize A – grain with a lower technological value obtained from a distillery and maize B – grain with the lowest technological value, with evidence of mould infection. Grain KB was a control sample.

Distillery yeast *Saccharomyces cerevisiae* Etanol Red obtained from the Lesaffre Company was chosen as the biological material.

In the examined grain, we estimated the dry mass (using the drier method) and starch content (using the Evers-Grossfeld method). The results obtained served to calculate the productivity of fermentation.

Grain mycological analysis was carried out at the Chair of *Phytopathology* – Department of *Plant* Protection at the Wrocław University of Environmental and Life Sciences according to the method by de Tempe [1970]. From each examined sample, 100 seeds were randomly selected. All isolated fungi were determined using the available monographs: Raper et al., 1965, 1968, Ellis, 1971, Nelson et al., 1983.

Fermentation tests were carried out using the batch fermentation method in starch mash, prepared from maize of varying quality: KB, A, B. Maize mash with 20% concentration was prepared using the BUS method in the ZL1 masher. The starch was gelatinized and then liquized and saccharificated using the commercial enzymatic substances Termamyl 120L and San Ultra L, using doses recommended by the manufacturer [Solarek, 2004]. The process of fermentation was performed at 30ºC using the distillery yeast *Saccharomyces cerevisia* Etanol Red. The inoculum of yeast was prepared through the proliferation on the YM medium.

During fermentation the ethanol content [g/L] was determined using the HPLC method. Based on the obtained results practical yield of fermentation was compared to the theoretical one; the yield of ethanol from starch [g ethanol/1 g starch], from the dry mass of the material [g ethanol/1 g dry mass], and from the unit of the material’s mass [g ethanol/1 g material].

Results

The characteristics of the maize material with regard to the starch content and dry mass are presented in Table 1. Starch content in the material ranged between 65 and 68%. Dry mass ranged from 87 to 92%.

Table1

Characteristic of raw material.

Raw material	Starch content [%]	dry mass content [%]
Maize grain KB 1903	68,5	92,52
Maize grain A	67,50	92,28
Maize grain B	64,75	87,26

The microbial analysis of the maize grain with lower technological value was performed at the laboratory of Phytopathology Deparment at the Wroclaw University of Environmental and Life Sciences. The obtained results are presented in Tables 2 and 3.

In the disinfected and non-disinfected maize A, mainly fungi from genera *Penicillium, Rhizopus, Fusarium* and *Nigrospora* were isolated*,* whereas mainly fungi *Penicillium* and *Rhizopus* were isolated from the non-disinfected grain (about 21 and 20% of all the isolated fungi, respectively). Fungi *Fusarium* and *Nigrospora* were isolated from the disinfected grain. Their content was 18% (*Fusarium*) and 17% (*Nigrospora oryzae*). The mean quantity of the isolated microorganisms (from non-disinfected and disinfected grain) was 65 c.f.u. (colony forming units).

Table 2

Fungi isolated from maize grain A

	Non-disinfected grain			**Disinfected grain**		
	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3
Alternaria alternata (Fr.) Keissl.	0	0	0	0	0	1
Aspergillus flavus	5	3	2	0	0	0
Aspergillus niger	0	0	0	0	5	0
Cladosporium cladosporioides	0	0	0	0	1	0
Epicoccum purpurascens	0	0	1	0	0	0
Fusarium avenaceum (Corda ex Fr.) Sacc.	1	0	0	0	0	0
Fusarium oxysporum Schlecht.	2	1	3	6	6	0
*Mucor hiemalis*Wehmer	2	4	1	0	0	0
Nigrospora oryzae	3	3	2	5	6	6
Penicillium notatum Westling	7	4	19	0	0	0
Penicillium purpurogenum	1	1	5	1	2	1
Penicillium vermiculatum	1	0	0	0	0	0
Penicillium sp.	0	1	2	0	4	4
Rhizopus arrhizus Fischer	12	11	16	0	0	0
Rhizopus glaucus	0	0	0	0	5	0
Trichoderma aureoviridae	4	5	1	0	0	1
Trichoderma harzianum Rifai	8	4	0	0	0	6
Total amount	**46**	**37**	**52**	**12**	**29**	**19**

Table 3

Fungi isolated from maize grain B

Fungi species	**Non-disinfected grain**			**Disinfected grain**		
	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3
Acremoniella atra (Corda) Sacc.	3	0	0	0	0	0
Alternaria alternata (Fr.) Keissl.	0	0	0	2	0	0
Fusarium avenaceum (Corda ex Fr.) Sacc.	6	3	4	6	4	8
Fusarium culmorum (W.G.Smith) Sacc.	4	6	9	11	16	8
Fusarium equiseti (Corda) Sacc.	0	2	2	0	1	1
Fusarium oxysporum Schlecht.	44	52	45	44	44	43
Mucor hiemalis Wehmer	3	2	4	1	1	0
Paecilomyces farinosus (Dicksen ex Fr.) Brown & Smith	0	6	0	0	0	0
Papulaspora polyspora Hotson	1	0	0	0	0	0
Penicillium notatum Westling	60	60	56	11	8	6
Rhizopus arrhizus Fischer	16	2	6	0	0	0
Sordaria fimicola(Roberge) Griffiths and Seaver	0	0	0	0	2	2
Trichoderma harzianum Rifai	2	8	5	3	4	5
Trichoderma koningii Oud.	0	1	0	0	0	0
Trichoderma polysporum (Link ex Pers.) Rifai	2	0	0	0	0	0
Trichothecium roseum Link ex Fr.	0	0	3	0	0	0
Total amount	141	142	134	78	80	73

From the non-disinfected and disinfected maize grain B, we isolated mainly fungi from the genera *Penicillium* and *Fusarium*. In the non-disinfected grain, fungi *Penicillium* were 37% of all the isolates, and *Fusarium* 23%. In the disinfected grain, *Penicillium* constituted only 4% of all the isolated fungi, and *Fusarium* 24%. The mean quantity of the isolated microorganisms (from both types of grain) was 216 c.f.u.

The microbial analysis of maize grain with lower technological value showed that maize grain B had three times the number of microorganisms than grain A.

Maize material of varying quality served to prepare fermentation media. Table 4 presents the process of ethanol fermentation with regard to the produced ethanol during the first, second and third day of the process.

Table 4

The amount of ethanol produced during corn mash ethanol fermentation

Raw material	**Ethanol[g/l]**		
	24 h	**48 h**	**72 h**
grain KB 1903	**34,26**	**60,18**	**62,04**
grain A	**29,11**	**56,99**	**60,25**
grain B	**27,12**	**44,10**	**52,97**

The results obtained show that the greatest amount of the produced ethanol was observed for fermentation using a good quality medium (maize KB 1903). However, fermentation of media prepared from grain affected by a smaller number of fungi (maize A) did not result in any significant decrease in the amount of the produced ethyl alcohol.

The difference between the amount of ethanol produced by fermentation using a good quality medium (KB 1903) and the one using maize A was about 1.79 g/l. In the case of grain affected mainly by fungi *Penicillium* and *Fusarium* (material with the lowest quality – maize grain B), the amount of ethanol produced was much lower than for the good quality grain KB1903, by about 9 g/l.

Yield parameters based on the obtained quantities of ethanol are presented in Table 5. The theoretical yield of ethanol per 1 g starch is 0.568 g.

Data presented in Table 5 indicates that the quality of material used for growth medium influenced the yield of fermentation. The highest practical yield was observed for the medium based on grain KB1903 (good quality material) – at 80%. The application of materials with much lower technological value resulted in a decreased yield.

Table 5

The yield of ethanol after fermentation of corn mashes of different microbiological value Table 1

Raw material	**Yield of ethanol [Y]**			
	Y g ethanol/ g starch	**Y g etanol/ g s.m.**	**Y g etanol/ g raw material**	**Y practical**
grain KB 1903	**0,453**	**0,335**	**0,310**	**79,73**
grain A	**0,446**	**0,326**	**0,301**	**78,57**
grain B	**0,409**	**0,303**	**0,265**	**72,01**

In the case of fermentation on a medium based on grain with the lowest quality (maize grain B), we observed a 8% decrease in practical yield. However, using a medium based on maize grain A (from distillery), the decrease in yield was not as significant.

In samples in which growth medium was prepared from a good quality material (grain KB1903), the yield of the process was 0.45 g ethanol/1 g starch. Observed yields were the lowest when using media with maize grain B (affected mainly by fungi *Penicillium* and *Fusarium*): 0.41 g ethanol/ g starch. A similar effect on material quality was observed for the remaining parameters of productivity.

Discussion

Maize grain is one of the most effective high-energy materials for bioethanol production [Borktun, 2003, Gulami et al., 1996; Krishnan et al., 2000; Latif and Rajoka, 2001; Lee and Yoon, 2000; Lipski 2003, Lipski 2003, Soto et al., 2005, Shigetchi et al., 2004]. So far, reports have compared the usefulness of maize in ethanol production to other distillery materials, traditionally used in Poland, such as rye [Kłosowski et al., 2001, Siliwanowicz, 2003], and less conventional materials, such as amarantus [Dobrzeniecka et al., 1996]. The efficiency of the energy-saving method of cool maize mashing has been examined [Shigetchi et al., 2004; Stecka et al., 1996], and also the usefulness of marinating as a method of preserving maize used for ethanol production [Zielińska, 2003].

However, little attention has been paid to the issue of material quality, especially microbial quality. The atmospheric conditions of Poland result in a grain moisture levels of 40%. During storage, this grain is characterized by high enzymatic activity, intense respiration and starch decomposition into simple sugars and easily soluble proteins – a good growth medium for various microorganisms.

In fresh and well stored grain, fungi are not so frequent. Field fungi from the genera *Alternaria, Clodosporium, Fusarium* tend to attack grain during maturation and before harvest and are the most prevalent species. In optimum storage conditions (humidity below 18%), they usually die out after a few weeks. After a harvest, they are substituted by storage fungi, resistant to drier conditions (13–18%), for example from genera *Aspergillus* and *Penicillium*. They are capable of surviving many months of storage, especially Aspergillus niger. The intensity of their growth in 20ºC is not high, and changes in the infected material are visible only after 6 to 12 months [Kluczek, Kojder, 2000]. A high microbial activity may completely spoil the material and lead to the production of mycotoxins (aphlatoxins, ochratoxins and zearalenon) [Newsome, 2006; Stolarska, 2004]. The presence of fungus metabolites and the loss of nutrients may negatively affect the technological process and the quality of final products [Bothast,1992].

Conclusions

The analyzed materials were different with regard to their infection with moulds. Maize grain A was infected by *Penicyllium notatum, Rizopus arrhizus, Fusarium oxysporum,* *Nigrospora oryze, Trichoderma harzianum and Aspergillus flavus,* at 65 c.f.u.. (colony forming units)

The quantity of fungi isolated from maize grain B was 3–4 times higher than in the grain A. This grain was infected by *Aspergillus flavus, Penicyllium notatum, Rizopus arrhizus, Fusarium oxysporum, Fusarium culmorum*, *Fusarium avenaceum* and *Trichoderma harzianum*. Maize grain KB was a control sample.

It was observed that the microbial quality of material used in this study influenced the effects of ethanol fermentation. The lowest practical yield of ethanol compared to the theoretical one was observed for fermentation of mash prepared from maize grain B, affected by the greatest number of fungi. In this case, yeast needed more time for adaptation in the growth medium and was more sensitive to fermentation conditions.

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3

DETECTION OF *LISTERIA MONOCYTOGENES* AND *SALMONELLA* SPP. IN POWDERED MILK: A COLLABORATIVE STUDY

Introduction

Proficiency testing is an important element of quality assurance in a laboratory. The reliability and accuracy of the collaborative studies results are widely recognized. Participation in collaborative studies enable to obtain independent quality assessment, which is an important factor necessary for laboratory accreditation. The results of interlaboratory trials are the proof of reliability of the results obtained in the laboratory [Ludwicki, 1995; ISO/IEC Guide 43-1:1997; Feldsine et al., 2003; Rola, 2005]. Department of Hygiene of Food of Animal Origin of the National Veterinary Research Institute – State Research Institute in Pulawy as the reference laboratory regularly organizes collaborative studies for the Veterinary Inspections. This interlaboratory studies have been undertaken to assess the ability of the Veterinary Inspection laboratories in detection of two pathogens – *Listeria monocytogenes* and *Salmonella* spp. A total of 23 laboratories representing Veterinary Inspection and the organizer participated in this trial [Anonymous, 2004b].

L. monocytogenes is an oportunistic pathogen, that causes listeriosis in human and animals. Human infections are often associated with consumption of dairy products such as raw and pasteurized milk, cheeses and ice-cream. The ability of growing in low temperatures enable multiplication of this pathogen in refrigerated foods. Identifiaction of *Listeria* genus involving culture methods is based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties [EN ISO 11290-1:1999; Gasanov, 2005].

Salmonella spp. is one of the most common and widely spread foodborne pathogen. Salmonellosis in humans is generally caused by the consumption of food of animal origin, mainly meat, poultry, eggs and milk. The basic procedures used in the routine detection of *Salmonella* in foods are similar, involving primary enrichement, selective enrichment and isolation on plates. Differences exist in the specified media and incubation conditions [Beckers, 1985; Feldsine, 2003].

According to the new microbiological criteria, there is an obligation of analysing milk products for the presence of *L. monocytogenes* and *Salmonella* spp. The reference methods for identification of these bacterias are EN ISO 11290 – 1:1999 and EN ISO 6579:2003, respectively [Comission Regulation (EC), 2005].

Aim

The aim of this study was to evaluate the ability of Laboratories of Veterinary Inspection to detect L. monocytogenes and Salmonella spp. in milk products

Materials and methods

Design of the study. In this study powdered milk artificially contaminated with *L. monocytogenes* IW 26 strain (ATCC 19111) and *Salmonella* Typhimurium IW 41 strain (ATCC 14028), respectively has been used. Suspensions containing 100 cfu and 100 000 cfu of *L. monocytogenes* and *S.* Typhimurium were liophilized separetly. These inoculum were added to the matrice by the participants. Powdered milk that we used as a matrice was purchased in a local store and was analysed for the presence of *Listeria monocytogenes* and *Salmonella* spp. according to EN ISO 11290-1:1999 and PN-93/A-86034/11. The results of both tests were negative. The participants received vials containing low and high level (100 cfu and 100 000 cfu) of *L. monocytogenes* in blind duplicated, low and high level (100 cfu and 100 000 cfu) of *Salmonella* Typhimurium also in blind duplicates, uninoculated control samples and the matrix.

Test portion distribution. The parcels with samples were dispatched overnight to the participants. Each collaborating laboratory have received 6 samples for the detection of *L. monocytogenes* and 5 samples for the detection of *Salmonella* spp., the instruction how to deal with the material and the worksheets.

Analysis of foods. The laboratories were advised to analyze the samples within one week. Before starting, the participants had to dissolve 20 g of powdered milk in 250 ml of water. This amount of matrice was enough for analysing 12 samples. Directly before performing the analyse the participants were obligated to add 2 ml of prepared milk to the liophilized bacterial pellets and add it to the rest of milk to receive in total 11 samples containig 25 ml of test material. The samples were analyzed according to the procedures used in the laboratories.

The participants have completed the worksheets with the obtained results and sent the documentation to the National Veterinary Research Institute for review and tabulation of the results. On the basis of this records the coordinator has prepared the report, that was sent to all of the participating laboratories.

Results and discussion

Detection of *L. monocytogenes* was performed by 19 laboratories according to EN ISO 11290 – 1:1999, 3 laboratories according to ISO 10560:1999 and one laboratory used Oxoid Listeria Rapid Test. In the first method there is pre-enrichement in half-Fraser broth, enrichment in Fraser broth and isolation on agar Oxford and Palcam. In the procedure according to ISO 10560:1999 there are two steps: enrichement in Leb (Listeria enrichment broth) and isolation on Agar Oxford [EN ISO 11290-1:1999, ISO10560:1999]. The Oxoid Listeria Rapid Test performed by one laboratory uses two enrichment steps – in Fraser broth and in buffered

Listeria enrichment broth, followed by an immunoassay using a test device. The *Listeria* device contains monoclonal antibodies specific to the B flagella antigen that is present in the *Listeria* species (exept *Listeria grayi*). Two laboratories did not detect *L. monocytogenes* in samples with low level of contamination and four laboratories detect *L. monocytogenes* in blind samples. The results obtained by the rest laboratories were satisfactory. The results in *L. monocytogenes* presence/absence are shown in Table 1.

Table 1

Results – detection of *L. monocytogenes*

Lab. code	Sample number						Method used
	1	2	3	4	5	6	
	Low level	Low level	High level	High level	Level "zero"	Level "zero"	
1	–	–	+	+	–	–	PN-EN ISO 11290-1:1999
2	+	+	+	+	–	+	PN-EN ISO 11290-1:1999
3	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
4	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
5	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
6	+	+	+	+	–	–	PN ISO 10560:1999
7	+	+	+	na*	–	–	PN ISO 10560:1999
8	+	+	+	+	+	+	PN-EN ISO 11290-1:1999
9	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
10	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
11	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
12	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
13	+	+	+	na*	–	–	PN-EN ISO 11290-1:1999
14	+	na*	+	+	–	+	No. data
15	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
16	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
17	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
18	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
19	–	–	+	+	–	–	Oxoid Listeria Rapid Test
20	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
21	+	+	+	+	–	–	PN-EN ISO 11290-1:1999
22	+	+	+	+	–	–	PN ISO 10560:1999
23	+	na*	+	na*	–	–	PN-EN ISO 11290-1:1999
24	+	+	+	+	–	–	PN-EN ISO 11290-1:1999

*na –not analyzed

The detection of *Salmonella* spp. was performed in 20 laboratories using EN ISO 6579:2003 and in 3 laboratories using PN 93/A-86034/11. In the procedure used by most of the laboratories, there is pre-enrichment in buffered peptone water (BWP), enrichment in Rappaport-Vasiliadis soya peptone broth (RVS) and in Muller-Kauffmann tetrathionate broth with novobiocin (MKKTn) and isolation on selective agars – XLD and the second medium to be chosen by the laboratory. The procedure according to PN-93/A-86034/11 is very similar to the procedure described above but it uses Rappaport-Vasiliadis peptone broth (RV) and selenite cystine broth (SC) for the enrichment step and brilliant green agar (BGA) and the second agar is to be chosen for the isolation step [EN ISO 6579:2003, PN-93/A-86034/11]. One laboratory did not give any information concerning used method. Two laboratories detected *Salmonella* spp. in blind samples. The results obtained by the other laboratories were satisfactory. The results for *Salmonella* spp. presence/absence are shown in Table 2. Nineteen out of 24 participating laboratories reached the expected results in both analysed pathogens and they have proved their ability to detect *L. monocytogenes* and *Salmonella* spp. in milk products. Four laboratories have to improve their ability in the detection of *L. monocytogenes* and two laboratories should improve their ability to detect both pathogens [Anonymous, 2004].

Table 2

Results – detection of *Salmonella* spp.

Lab. code	Sample number					Method used
	7	8	9	10	11	
	Low level	Low level	High level	High level	Level "zero"	
1	+	+	+	+	–	PN-EN ISO 6579:2003
2	+	+	+	+	+	PN-93/A-86034/11:1993
3	+	+	+	+	–	PN-EN ISO 6579:2003
4	+	+	+	+	–	PN-EN ISO 6579:2003
5	+	+	+	+	–	PN-EN ISO 6579:2003
6	+	+	+	+	–	PN-EN ISO 6579:2003
7	+	+	+	+	–	PN-EN ISO 6579:2003
8	+	+	+	+	+	PN-EN ISO 6579:2003
9	+	+	+	+	–	PN-EN ISO 6579:2003
10	+	na*	+	+	–	PN-EN ISO 6579:2003
11	+	+	+	+	–	PN-EN ISO 6579:2003
12	+	+	+	+	–	PN-EN ISO 6579:2003
13	+	na*	+	+	–	PN-EN ISO 6579:2003
14	+	+	+	+	–	No. data
15	+	+	+	+	–	PN-EN ISO 6579:2003
16	+	+	+	+	–	PN-EN ISO 6579:2003
17	+	+	+	+	–	PN-EN ISO 6579:2003
18	+	+	+	+	–	PN-EN ISO 6579:2003
19	+	+	+	+	–	PN-93/A-86034/11
20	+	+	+	na*	–	PN-EN ISO 6579:2003
21	+	+	+	+	–	PN-EN ISO 6579:2003
22	+	+	+	+	–	PN-EN ISO 6579:2003
23	+	na*	+	na*	–	PN-93/A-86034/11
24	+	+	+	+	–	PN-EN ISO 6579:2003

*na –not analyzed

The participants obtained 91.6% of positive results after analysing samples containing low level of *L. monocytogenes* and 100% accordance in samples containing high level of this pathogen. They have also obtained 100% accordance in analysing samples containig both levels of *Salmonella* Typhimurium. The accordance of uninoculated control samples was 83% for *L. monocytogenes* and 87.5% for *Salmonella* spp. Another organiser – Gdansk Water Foundation have prepared interlaboratory studies in 2003, 2004 and 2005 in the detection of *L. monocytogenes* and *Salmonella* spp. In their studies the percent of positive results among positive samples in the detection of *L. monocytogenes* was 94.83%, 97.22% and 90.0%, respectively and the accordance of the negative results was 94.44% in 2004 and 96.7% in 2005. In the samples analyzed for the presence of *Salmonella* spp. in 2003 they have received 96.15% of accordance among positive samples and in 2004 and 2005 the participated laboratories have obtained 100% accordance both in positive and negative samples [Anonymous, 2003; Anonymous 2004a; Anonymous, 2004b; Anonymous, 2005].

Conclusion

The interlaboratory comparisons described above proved the ability of Veterinary Inspection Laboratories to detect *L.monocytogenes* and *Salmonella* spp in milk products.

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4

THE EFFECT OF HERBICIDES ON WINTER WHEAT GRAIN HEALTH

Introduction

At present there are some European legislative acts controlling food contamination, taking into account micotoxins. Commission Regulation (EC) No. 466/2001 defines maximum acceptable level of aflatoxins in cereal and buckwheat grain. Afterwards some other regulations were introduced due to increasing requirements for food safety. Those regulations were, e.g. Commission Regulation (EC) No. 472/2002 setting maximum acceptable level of ochratoxin A, Commision Regulation (EC) No. 683/2004, defining aflatoxins and ochratoxin A in foods for infants and young children, Commision Regulation (EC) No. 856/2005, defining maximum acceptable level of *Fusarium* toxins.

Those acts also indicate the cause of micotoxins contamination – fungi. At the same time they say, that with current scientific and technical knowledge it is impossible to prevent from moulds development, and Commission Regulation (EC) No. 466/2001 clearly says, that "efforts to improve production, harvesting and storage methods in order to reduce the development of moulds should be encouraged". Commission Regulation (EC) 2006/583 indicates general rules of preventing from Fusarium toxins in cereals and cereal products. They are based on good agriculture, harvest and storage practices.

Fungi harmfulness can be considered in the aspect of grain quality. According to Narkiewicz-Jodko [1998], changes in grain colour and smell can indicate damaging fungi activity, but then it is too late to reverse changes decreasing grain quality. Thus evaluating fungi composition allows determining weather conditions during grain ripening and harvest. Leonard and Bushnell [2005] show many examples of decreasing of wheat and barley grain quality caused by *Fusarium* genus. Some of them decrease milling properties of wheat grain and baking properties of wheat flour, as well as worsening of barley malt, wort and beer.

Weed control is of a great importance in disease control, as weeds compete with crops for water, nutrients and light. That competition leads to worsening growing conditions and can increase disease susceptibility. For example, in Canada all species of grass are ergot (Claviceps purpurea) host. Meanwhile Stagonospora nodorum and Rhizoctonia secalis have wide range of hosts among cereals and grass. S. nodorum can infect e.g. couch grass Agropyron repens, Lolium perenne and Poa annua [Tylkowska et al., 2007].

Materials and methods

The materials were grain of four winter wheat varieties (Smuga, Zawisza, Kobiera and Nadobna) from crops treated with herbicides. The herbicides used in experiment were Glean

75 WG (chlorsulfuron) at dose 0,25 g/ha, Kantor 050 SC (florasulam) at dose 0,1 l/ha, Atlantis 04 WG (iodsulfuron-methyl-sodium, netsulfuron-methyl) + Atpolan 80 EC (paraffin oil) at dose 0,4 g/ha + 1,0 l/ha and Axial 100 EC (pinoxaden) + Chwastox Extra 300 SL (MCPA) at dose 0,3 l/ha + 3,0 l/ha. Whole material came from Regional Station of Plant Protection Institute in Trzebnica.

In separate years of experiments there were different weather conditions. In season 2005/2006 January and February were frosty (-6,4 i -2,4°C) and July was hot (23,1°C). Rainfalls were weak, less than 25 mm a month and only in December they were higher (76,4 mm). In season 2006/2007 winter was warm and summer was temperate. Temperatures in December and January were 4,5 and 4,6°C respectively, in February it was 2°C lower and in June and July it did not exceed 19,1°C. Except from very dry April (1,9 mm), rainfalls were quite high, from 29,8 mm in December to 75,8 mm in June, and in July it was 164 mm. In season 2007/2008 temperature distribution was similar to previous one, but winter and spring was cooler about 1°C, and the lowest temperature (1,0°C) was noticed in December. Rainfalls were from 20 to 30 mm, but in November, January, April and July they were doubled and reached 55–70 mm.

Fungi composition examination was executed according to de Tempe [1970].

Results

Fungi occurrence on grain surface and in its deeper tissues was evaluated. Generally, grain surface was more infected. More species were isolated, and they also were more numerous. Although in every year of experiment fungi species compositions were little different, some species occurred permanently. They were saprotrophic Alternaria alternata (Fr.) Keissl. and Epicoccum nigrum Link, as well as pathogenic Fusarium spp. The Fusarium genus was represented by F. poae (Peck) Wollenw., and depending on wheat variety and weather conditions during plant vegetation also F. avenaceum (Fr.) Sacc., F. culmorum (W.G. Sm.) Sacc., F. graminearum Schwabe and F. solani (Mart.) Sacc. were noticed. In the last year of experiment small quantities of Bipolaris sorokiniana (Sacc.) Shoem. were isolated.

Numbers of total fungi and most common species (*A. alternata*, *E. nigrum* and *Fusarium* spp.) were statistically estimated (Tab. 1, 2). It came out that quantities of total fungi numbers depended on chemical weed control and weather conditions. The same factors affected the number of *A. alternata*, but the number of *E. nigrum* on grain surface depended only on weather conditions, while in case of deeper tissues also wheat variety and chemical weeds control influenced the number of that species. Meanwhile genus *Fusarium* quantity on grain surface depended on weather conditions, and in case of deeper tissues also wheat variety changed the number of *Fusarium* spp.

Herbicide application significantly decreased the number of total fungi and *A. alternata* both on grain surface and in its deeper tissues, but in case of non-disinfected grain reduction of *A. alternata* number was slight. *E. nigrum* occurrence depended on chemical weed control only in case of grain deeper tissues. All preparations used in experiment decreased the number of that species, and the most effective was combination of Atlantis 04 WG and Atpolan 80 EC.

Table 1

Most numerous fungi occurring on non-disinfected winter wheat grain depending on wheat variety, herbicides and year of harvest

Factor / Fungi		Total	*Alternaria alternata*	*Epicoccum nigrum*	*Fusarium* spp.
Variety	Smuga	113 a	63 a	17 a	16 a
	Zawisza	117 a	63 a	17 a	17 a
	Kobiera	111 a	62 a	15 a	16 a
	Nadobna	101 a	63 a	16 a	11 b
Herbicide	Glean 75 WG	104 b	62 b	16 a	15 a
	Kantor 050 SC	104 b	62 b	14 a	12 a
	Atlantis 04 WG + Atpolan 80 EC	106 b	62 b	16 a	16 a
	Axial 100 EC+ Chwastox Extra 300 SL	110 b	63 b	17 a	14 a
	Control	128 a	66 a	18 a	19 a
Year of harvest	2006	93 b	58 c	9 b	10 b
	2007	120 a	64 b	21 a	22 a
	2008	119 a	67 a	19 a	13 b

a, b, c – homogenous groups according to Duncan's test at p = 0,95

Table 2

Most numerous fungi occurring on disinfected winter wheat grain depending on wheat variety, herbicides and year of harvest

Factor / Fungi		Total	*Alternaria alternata*	*Epicoccum nigrum*	*Fusarium* spp.
Variety	Smuga	85 a	48 a	13 a	12 a
	Zawisza	79 a	47 a	13 a	10 a
	Kobiera	83 a	49 a	11 ab	13 a
	Nadobna	76 a	49 a	9 b	8 a
Herbicide	Glean 75 WG	78 b	48 b	12 b	11 a
	Kantor 050 SC	79 b	46 b	13 ab	9 a
	Atlantis 04 WG + Atpolan 80 EC	78 b	48 b	8 c	11 a
	Axial 100 EC+ Chwastox Extra 300 SL	72 b	47 b	11 b	9 a
	Control	96 a	54 a	15 a	13 a
Year of harvest	2006	61 c	44 b	3 b	6 b
	2007	96 a	51 a	17 a	19 a
	2008	86 b	51 a	15 a	8 b

a, b, c – homogenous groups according to Duncan's test at p = 0,95

Nadobna var. was less infected by *Fusarium* spp. than other varieties. Also deeper tissues of grain of that variety were less infected by that genus; however the difference was not statistically significant. But *E. nigrum* occurrence in deeper tissues of Nadobna grain was significantly smaller than in case of other varieties.

Depending on element of grain, the effect of weather conditions on number of fungi was different. Fungi occurring on grain surface were most numerous in the 2nd and 3rd year of experiment, and after disinfection the number of fungi in the last year was not as high as a year earlier. On grain surface *A. alternata* was most numerous in least year of experiment, and least numerous in the 1st season. Meanwhile in deeper grain tissues that species was most numerous in last 2 years of experiment. Apart from element of grain, the number of *E. nigrum* was the highest in the same seasons, while *Fusarium* spp. was most numerous only in the 2nd year.

Discussion

Most scientists agree that herbicide application, apart from winter wheat variety and year of harvest, decreases numbers of total fungi occurring on grain [Narkiewicz-Jodko et al., 1999, 2005, 2007]. However, Lemańczyk and Jaskulski [2006] noticed that herbicide treatment increased infection of winter wheat leaves by brown rust (*Puccinia recondita*), speckled leaf blotch (*Stagonospora tritici, Stagonospora nodorum*), and in case of winter triticale it increased the occurrence of brown rust (*P. recondita*) and other speckled blotch. Those authors suggest that lack of chemical weed control caused cornfield thinning, and lowering its humidity, which was unpropitious to fungi growth. They also pointed out that weeds can be a barrier for spores spread.

According to Boligłowa and Lepiarczyk [2004], Narkiewicz-Jodko et al. [1999, 2005, 2007] wheat varieties differ in susceptibility to infection. Besides weather conditions significantly affect wheat pathogens growth and in case of susceptible varieties they can increase infection [Jaczewska-Kalicka 2002, Narkiewicz-Jodko et al., 2005, 2007].

In a 3-year experiment every year different pathogen group was dominant on separate plant parts. From winter wheat heads, apart from fungicide treatment, species of *Alternaria*, *Cladosporium* and *Epicoccum* genus, as well as pathogen *Blumeria graminis* and *Stagonospora nodorum* were isolated. *Fusarium* genus was less common. What more, every year share of mentioned fungi was different [Jaczewska-Kalicka, 2002]. Also Narkiewicz-Jodko et al. [2005] noticed some differences between fungi occurrence in following years of experiment. In one year pathogenic genus *Fusarium* was remarkably numerous and was represented by *F. poae*. Simultaneously, the same year grain quality was worsened, which could be connected right with fungi occurrence. Whereas in other years *Fusarium* spp. rate was lower, and a dominant species was saprotrophic *Alternaria alternata*. Narkiewicz-Jodko et al. [2007] in the 2nd year of their experiment gained the highest fungi number and the most often isolated species were *Alternaria alternata*, *Epicoccum nigrum*, *Fusarium* spp.: *F. poae*, *F. culmorum*, *F. graminearum*, *F. avenaceum*. In own studies evaluated species and total fungi were most numerous in the 2nd year, in which winter was warm and wet and in July rainfalls were very high with compare to other seasons.

Similarly to own studies, Narkiewicz-Jodko et al. [1999, 2007] noticed that on disinfected grain there were less fungi isolates than on non-disinfected one. Among isolated species *Alternaria alternata*, *Epicoccum purpurascens* (currently *Epicoccum nigrum*) and pathogenic *Fusarium* genus, represented by *F. avenaceum* oraz *F. culmorum*, were most numerous. According to Tylkowska et al. [2007], surface seeds disinfection, mainly with 1% solution of sodium hypochlorite, is to remove fast growing saprotrotrophs, which can limit pathogens growth. But there is also a risk of removing pathogens from grain surface.

In own studies, fungi composition was similar to those mentioned above. Also variably impact of weather conditions and genetic factor on fungi number was reported. It is required to look closer at the dominant species to predict their impact on grain health.

Alternaria alternata is a dominant species occurring on grain surface and in its deeper tissues. Its occurrence on cereals amounts to 20–90%. This species occurs on grain mostly right after the harvest, and its presence on grain stored for a longer time (3 years) indicates good grain quality and proper storage conditions. A. alternata is considered not to worsen both seeding value, as well as grain quality features [Narkiewicz-Jodko 1998, Tylkowska et al., 2007].

Fusarium head blight (FHB) is a serious problem all over the world. It is a cause of decrease of yield quantity and quality. Losses connected with micotoxin occurrence, even at the low infection, are unevaluated. Consumption of grain infected by *Fusarium* genus can lead to micotoxicosis people and animals [Carter et al., 2000, Góral, 2006, Browne, 2007, Tunali et al., 2006, Gosman et al., 2007]. This is why *Fusarium* damage of wheat is concentrated mainly on food safety, however some cases of worsening of wheat and barley technological value are known [Narkiewicz-Jodko, 1998, Leonard and Bushnell, 2005].

At least 17 fungi species can cause FHB (Browne 2007). Mainly they are fungi of *Fusarium* genus: *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. sambucinum* var. *coeruleum*, *F. crookwellense* and *F. sporotrichoides* [Tunali et al., 2006]. Those species can infect heads at different time. When infecting during waxy or full ripeness, grain quality features are not worsened and fungus occurs mainly on a fruit-seed coat, while blossoms and infected grain become red, however not always red colouring indicates the *Fusarium* genus occurrence. The most severe is infection during anthesis or milk ripeness and the weather is wet and warm, because whole grain is infected then. *F. graminearum* is the main cause of FHB in warm climate, also in the USA, while *F. culmorum* is a dominant in temperate climate, and *F. avenaceum* – in cool areas. In a cool marine climate North-West Europe, FHB is caused mostly by *F. culmorum*, *F. poae* and *Microdochium majus* (syn. *M. nivale* var. *majus)* [Narkiewicz--Jodko 1998, Tunali et al., 2006, Browne, 2007].

In Poland FHB is caused mainly by *F. culmorum*, *F. graminearum* and *F. avenaceum*, and percentage rate of those species in pathogenic complex is variable and depending on weather conditions. However, *F. avenaceum* is less pathogenic than 2 other species. *F. avenaceum* and other species, e.g. *F. poae* or *F. sporotrichoides* are less pathogenic, but they are quite common in wheat crop. In recent years in Poland meaning of *F. graminearum* as an FHB cause increases, which is connected with the increase of mean temperature in spring and Summer and extension of maize crops, which crop residues are main inoculums of that fungus. However, wheat varieties susceptibility to FHB caused by *F. culmorum* is connected with genotype, thus at average weather conditions not every wheat variety will be diseased and their grain will be free from micotoxins [Góral, 2006]. In present work the most numerous species of *Fusarium* genus was *F. poae*, which confirms its significance in wheat.

Wheat varieties differ in FHB susceptibility, which can be affected by environmental conditions. FHB expansion depends on favourable weather conditions from anthesis to kernel evolution. Infection occurs in spring after warm and wet winter, and higher temperature are conducive to fungi growth and their severity [Tunali et al., 2006, Gosman et al., 2007], which also was observed in present studies.

In own studies, in the last year of experiment, some colonies of *Bipolaris sorokiniana* (Sacc.) Shoem. were isolated. It is quite unusual effect in winter wheat for at least 2 reasons.

First of all, *B. sorokiniana* is a pathogen of warm climate, and in cool areas it should not cause significant losses [de Tempe, 1964 according to Wiewióra, 2006]. It can grow in a wide temperature range, from 4 to 36°C, with optimum at 24–28°C. On the other hand, climate changes in Europe lead to increase yield losses caused by *B. sorokiniana*, and economical importance of that species is so huge, that e.g. in Norway seed dressing is recommended when barley kernels infection exceeds 10% [Wiewióra, 2006]. Also Duveiller and AltamiraNo. [2000] think that *B. sorokiniana* is present all over the world, but in terms high humidity and temperature and unbalanced soil fertility it is particularly aggressive. At higher latitudes, e.g. a Canadian prairie, parts of Australia and southern Brazil that species is a dominant among fungi causing roots rot.

Besides *B. sorokiniana* can cause great losses in spring cereal crops, especially barley, but it can also be observed in wheat and grass, which can be regarded as its hosts. Yield losses caused by *B. sorokiniana* can be varied and sometimes they can make wheat production unprofitable. Severity of that fungus consists in causing root rot, seedlings rot, spot blotch, black point. It is considered that leaf spot blotch caused by *B. sorokiniana* is one of the most important leaf diseases restraining wheat production in areas of warm climate and non-typical growth conditions [Duveiller and Altamirano, 2000, Wiewióra, 2006].

Infected kernels are one of the main sources of *B. sorokiniana*. Both sowing material and soil can be inoculums and the infection arises from mycelium located between husk and fruit-seed coat, as well as from conidia occurring on grain surface. Infection frequency depends on inoculums quantity occurring in plants surrounding during anthesis and kernel forming and weather conditions, because high humidity is conducive to leaf and kernels infection. Despite that, the main factor determining *B. sorokiniana* severity is varieties susceptibility [Duveiller and Altamirano, 2000, Wiewióra, 2006]. But as it was said before, grass can also be a host of *B. sorokiniana*, thus chemical weed control can contribute to reduction its occurrence.

In own studies occurrence of *B. sorokiniana* in last year of experiment can be explained by quite warm winter and spring and the highest rainfalls in that period during whole experiment. Actually, infection was low – only few colonies in each sample, but despite that grain from untreated crops was more infected than other samples. Probably higher weeds occurrence would increase that effect.

Conclusions

1. Herbicide application reduced the number of fungi, probably due to removing potential inoculums source, which can be weeds. The decrease in fungi number was connected with reduction of *Alternaria alternata* population, and in case of deeper tissues of grain – also *Epicoccum nigrum*.
2. The effect of genetic factor on susceptibility to diseases was weak. Fungi occurrence on Smuga var. grain depended on herbicide application.

3. Impact of weather conditions on fungi occurrence on grain was strong. Frosty winter, dry spring and hot and dry summer decreased fungi occurrence, while high rainfalls before harvest increased the number of pathogenic *Fusarium* genus. Warm and wet winter and spring could increase the number of *Bipolaris sorokiniana*, which is uncommon in winter wheat.

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