FOOD TECHNOLOGY OPERATIONS

New Vistas

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PREFACE

Process can be defined as a set of activities or industrial operations that modify the properties of raw materials with the purpose of obtaining products to satisfy the needs of the society. Such modifications are directed to obtain products with greater acceptance of the market or with better possibilities of storage and transport.

The modern expectations related to sustainable food chain require technologies characterized by low energy intake and low raw material consumption but also at achieving maximal range, preserving high nutritional value, natural flavor and proper texture of products. It seems impossible to meet all such requirements. Anyway, food industry has to fulfill such expectations because of growing consumer health care awareness leading to very tough competition on the global market To open up new vistas, technologists have to be focused on minimal food processing, functional food creation and convenience food.

At the beginning of 21st century, minimal processing is defined as methods to preserve foods but also retain to a greater extent their nutritional quality and sensory characteristics by reducing the reliance on heating as the main preservative action. Another point of view is greatly focused on achieving sufficient shelf life during storage and distribution. The use of minimal techniques puts new demands on the supply chain from agricultural production to the point of consumption.

Athermal membrane processes permit not only concentration and separation of macro- and micro-molecules, but also give the possibility to improve product quality operating in ambient temperature thereby reducing thermal damage of the product, that is retaining colour, flavor/aroma and nutritive components of the product. One of the advantages of the membrane techniques is energy consumption lower than in case of other methods of concentration or separation. This is the main reason why membrane processing is emerging fast from among other operations available for food production.

Novel non-conventional technologies which are based on physical processes are highly acceptable by consumers. High hydrostatic pressure technique is one of such technologies. Nowadays, it is recognized not only in food preservation, such as pasteurization but also tenderization (meat products), texturation (fish proteins) and freeze-thawing technologies.

The fastest modified technique in texture development is extrusion, which is becoming a very sophisticated process. The main role of extrusion is conveying and shaping fluid forms of processed raw materials used for carbohydrates and protein processing. But now their functions may include conveying, mixing, shearing, separation, heating or cooling, shaping, co-extrusion, venting volatiles and moisture, flavor generation, encapsulation and sterilization. When minimal processing requirements are met, extrusion can be carried out at relatively low (pasta) or very high temperatures (extruded snacks).

Recently, osmotic dehydration process received more attention due to the consumer demand of minimally processed products. Osmotic drying has been generally credited with considerable energy savings, since moisture is removed by liquid diffusion process unlike the conventional drying which is controlled by vapor diffusion.

Energy may be saved thanks to enzymatic processes which also make it possible to utilize by-products. Biotechnological processes, as any other modern technology, help reduce

the need for raw materials and shorten manufacturing operations. They are directed to obtain high yield and purity of final products through process control.

It is the new understanding and development of novel powerful technologies that make it possible to describe future opportunities for research in basic food science. The opportunities involve increasing our understanding of molecular and cellular processes that create the quality of final products and also physiological processes affecting consumer perception of food.

It is our pleasure to present the monograph introducing the progress in physical methods in food technology including minimal processing techniques, novel applications of enzymes, inhibitors and functional additives in food technology as well as new trends in meat processing to the readers.

Wiesław Kopeć
Małgorzata Korzeniowska

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TRENDS IN MEAT PROCESSING

Introduction

Meat consumption is an important part of the human diet, for instance in Spain in 2006, meat and meat products represented around 21% of the total family budget dedicated to buying food, and in the same year meat consumption per inhabitant was 65.3 kg/year [MAPA, 2009], similar data can be found for different Western Countries as well. In the same way, in developing countries is expected an increase of meat consumption during the next years [Bruinsma, 2003].

The binomial between man and meat has a long history, and there are several theories that state that meat consumption was a driven force in human evolution. One of this theories is known as "man the hunter" [Ardrey, 1961; Washburn and Lancaster, 1968], and establish that increasing amounts of meat in the hominid diet lead to increasing levels of cooperation among the males in the hunt, which lead to brain expansion and the associated development of cognition, language and symbolic culture. This hypothesis was fuelled by the realisation that an increase in the apparent consumption of meat correlated with the increase in brain size seen in *Homo habilis* and *Homo erectus*. More recently, Aiello and Wheeler [1995] postulated the expensive tissue hypothesis, that correlates the expansion of brain with the intake of high quality diet (more energetic), meanly due to meat consumption, in hominids allowing a reduction of the gastrointestinal tract. In other words, the increased energetic demands of a relatively large brain are balanced by the reduced energy demands of a relatively small gastro-intestinal tract, and the super plus of energy required comes from high energetic diet

The relation between man and meat was really strong in the ancestral hominin diet, hunting was one of the main man activities during that period. As meat was not easy to obtain, its processing and preservation became a key point, and some of the oldest preservation methods such as drying and fermentation were applied to obtain more stable meat products starting the art of processing and meat preservation.

Profound environmental changes, in lifestyle (physical activity) and diet, that began with the discovery of agriculture and animal domestication, around 10 000 years ago, and more recently with advances in meat processing during Industrial Revolution, occurred too early on an evolutionary time scale for the human genome to adapt [Cordain et al., 2005]. According to these authors, this evolutionary discordance between environmental changes in contemporary Western populations and unchanged human genome, provoke the appearance of the so called chronic diseases of Western civilization that includes cardiovascular disease (CVD), which represents the leading cause of mortality in Western countries, followed by certain types of cancer related with nutritional factors, and obesity, bone health and osteoporosis and insulin sensitivity and diabetes [Vandendriessche, 2007]. Several studies have

related those diseases with meat or meat products consumption, for instance Norat et al. [2005] suggested a relation between colon cancer and the consumption of processed meat products. Moreover, based on epidemiological studies Biesalski [2005] and Chao et al., [2005] found a positive association between obesity and high saturated fat intake from animal products. Taking in account all comments state above, it seems clear that from the consumer point of view, health related issues are really a hot topic in meat processing, and actually it is one of the most important trends nowadays.

A part of health, consumers look for convenience meat products. Darian and Cohen [1995] defined this concept as any saving of time, physical energy or mental energy that occurs during one or more phases of the home food production chain, that comprises deciding what to eat, purchasing, preparation, consumption and clean up. Meat industry has responded to the convenience trend mostly in the development of ready-to-eat meals, where many of the products have a meat component [Grunert, 2006]. Another tendency towards meat avoidance has notice among young female consumers in Scandinavian countries [Larsson et al., 2002]. This tendency does not necessarily imply that they will vegetarians, but they simply prefer those products where meat origin is less prominent, for example to higher forms of processing. In that sense, convenience and meat avoidance have something in common in terms of product development, because both trends look for meat-based products with a high degree of processing, detached from their animal origin, and adapted to different motives and consumer segments [Grunert, 2006].

Consumer concerns about animal welfare and other aspects of meat production become more important, although it seems that these concerns are more and attitude than a purchase behaviour [Grunert, 2006].

Although health and convenience issues are important trends, a survey done by International Food Information Council in 2008 in USA market, pointed out that taste (87%) and price (74%) are the most important criteria influencing consumer's food and beverages purchases, followed by healthfulness (61%) and convenience (52%) [IFIC, 2009].

Besides these trends that attend the consumers' point of view, we need to consider as well, another trend coming from the industrial point of view, which is the reduction of cost production due to and increase of productivity that can be achieved by implantation of the meat processing technology.

In this paper different examples related to health and reduction of cost production will be detailed for the main four different meat products: whole muscle and ground products heat and non heat treated (cooked ham, luncheon meat, dry cured ham, and fermented sausages). Three of the following examples are referred to different developments done by the authors to obtain whole muscle meat products with reduced salt content, cooked sausages with a reduced and better dietetic fat composition, and a rapid method to produce dry-cured ham. Finally, some comments about a new drying-maturing process for dry fermented sausages QDS (Quick Dry Slice) will be done [Comaposada et al., 2004].

Whole muscle heat treated meat products

These products are obtained from whole meat primal cuts, as pork ham or shoulder or poultry breast, cured by means of a brine, which is spread inside the whole muscle with the help of vacuum tumbling, moulded and cooked. As these kind of products are made with

whole muscle cuts, and most of the fat is removed before brine injection, the overall fat content is very low. Sodium content in cooked meat products rises as big concern between consumers. As an average, in these products salt content could be around 2.3 and 3% [Desmond, 2006]. A part of this amount of sodium coming from salt, it is necessary to take in account in meat products that other additives used, such as sodium ascorbate, sodium nitrite and sodium lactate, together with other sodium salts, also contribute to rise Na+ content in the final product.

Salt intake exceeds the nutritional recommendations (<6g/person) in many industrialized countries. Excessive intake of sodium has been linked to hypertension and consequently to increased risk of stroke and premature death from cardiovascular diseases [Ruusunen and Poulane, 2005]. Salt reduction in meat products thus has adverse effects on water and fat binding, impairing overall texture and increasing cooking loss, and also on sensory quality, especially taste [Ruusunen et al., 2005]. The solubility of meat proteins is markedly enhanced when sodium chloride and/or other salts are used [Silkes et al., 2009]. These solubilised proteins form connections between particulate proteins and protein–lipid structures, and when subsequently heated, they denature and cause aggregation/binding of the whole product, resulting in meat gelation with good water retention [Gordon and Barbut, 1993]. Taking in to account these considerations, it seems clear that one of the more important trends for these kind of products is salt reduction.

Meat product manufacturers have marketed low-salt alternatives, or have progressively reduced salt content over the years, where the technological and microbiological considerations have made this possible. The most important drawback of this is that the consumers are not getting used to weaker perceived saltiness of low-salt meat [Ruusunen and Poulane, 2005]. Apart from lowering the level of salt added to products there are a number of approaches to reduce the sodium content in processing foods including the use of salt substitutes, in particular, potassium chloride (KCl) in combination with masking agents, the use of flavour enhancers with enhance the saltiness of products when used with salt and finally optimizing the physical form of salt that it becomes more functional and taste bioavailable [Desmond, 2006].

Alternatives processing techniques had been studied. These techniques have been directed towards the meat system itself and methods of enhancing the functionally of the meat system. One of these techniques is high hydrostatic pressure treatment. Pressure treatment could be interest to maintain or improve protein functionally where it is desired to reduce the NaCl content of processed meats [Cheftel and Culioli, 1997]. High pressure processing (HPP) has been used as a possible means of improving the functional properties of muscle proteins as it has been shown to increase the solubility of certain myofibrillar proteins [Macfarlane, 1974]; [Macfarlane and McKenzie, 1976] and also to increase binding between meat particles in patties following heat denaturation [Macfarlane et al., 1984]. Crehan et al. [2000] demonstrated that high pressure processing can be used to improve the functionally of frankfurters formulated with lower salt levels.

Corcuera et al. [unpublished results] realized a study of reducing salt levels of cooked turkey breast. This study evaluated the reducing of salt level in these products combined with high hydrostatic pressure treatment. Three levels of salt in final product were studied: 2,3% ([reference batch), 1,5% and 1%. Vacuum package raw turkey breasts were subjected to high pressure processing at 200 and 300 MPa for 3 min in industrial equipment (NC Hyperbaric. Burgos. Spain). After coarse grinding of meat, this was mixed with brine with the different

salt contents, vacuum tumbling and cooked after moulding. A separate batch not subjected to high pressure acted as a control for each salt level. The formulation of these products included also polyphosphates (0.45% in final product). Results showed no differences in cooked turkey breast product obtained by combination of high pressure treatment at 200 MPa level with a salt content of 1.5% in comparison to non pressure treated controls. Mandava et al. [1994] found similar results in emulsified sausage meat batter, where processing at 100-200 MPa increased the cooking yield and reinforced the texture of low salt and low phosphate frankfurter-type sausages, especially those with low fat and/or high added water contents.

Above 200 MPa, the effects became negative, possibly because of meat denaturation. This statement agree with Lee et al. [2007] who observed that HP treatments of purified myofibrillar proteins at pressures below 200 MPa might induce structural degradation, while HP treatments at pressures above 200 MPa would affect myosin as well as actin, thereby accelerating protein denaturation. Pressurization may cause denaturation and/or aggregation of meat proteins [Cheah and Ledward, 1996], which could limit heat gelation in meat batters showing a less hard texture [Carballo et al., 1997]. Future studies are needed to further get insights in the role that HHP could play in the production of different and healthier meat products.

Luncheon meat products

Luncheon meat products are referred to those products that are obtained by meat emulsions, stuffed in natural or artificial casings and cooked. As meat emulsions that they are, they show a high percentage of fat in their composition varying from 20–30% in commercial products. These kind of products are well liked among consumers, because they are easy to prepare, use to like children and are really palatable.

Over consumption of high-energy-dense foods, rich in saturated fat, together with the increased portion sizes, may contribute to positive energy balance and lead to increasing incidence and prevalence of overweight and obesity [ADA, 2005]. Obesity is an important risk factor for developing of some high prevalence of chronic diseases like CVD, diabetes type 2, cholesteremia, high blood pressure, and some types of cancer [Aranceta et al., 2003]. It is expected that in 2015 around 2300 million of people will be overweight and 700 million will be obese all around the World [WHO, 2005]. To prevent overweight and obesity the American Heart Association recommends the intake of fat-free and low-fat products, although individuals should aim to improve their whole diet, rather than focusing on a single nutrient or food [Lichtenstein, et al., 2006]. Currently, it is general recognize that total fat intake should be less that 30% of the total diet energy, <10% of this energy should come from saturated fat, and intake of *trans* fatty acid should be as low as possible. As fat is the main contributor to the overall energy level of processed meat products, it seems obvious that this has to be reduced to contribute to a healthier diet [Vandendriessche, 2008].

In our Department we have been working since long time ago in different strategies to reach the above mentioned objective. In that sense, a three steps strategy has been designed 1) reduction of 50% of fat in frankfurters, 2) fat reduction of 50%, and changing fat sources to obtain a more nutritionally balanced composition and 3) adding n3-fatty acids to previous developed sausages. Nevertheless, fat reduction can have important consequences in sausages sensory properties, such as texture (structure or hardness and mouth feel) and in flavour; it is well known that most of the volatile compounds are liposoluble.

Step 1: fat reduction

Fat reduction means that the reduced fat is replaced by other macronutrients. Fat replacers are ingredients that can be used to provide some or all of the functions of fat, yielding fewer calories than fat. Different types of fat replacers can be used depending of their nature, structure and functionality. Fat substitute is a synthetic compound designed to replace fat on a weight-by-weight basis, usually having a similar chemical structure to fat but resistant to hydrolysis by digestive enzymes, this is the case of SALATRIM (short and long chain acyltriglyceride molecules) (Benefat, Cultor Food Science, Inc, Ardsley, NY. USA) and olestra™ a sucrose polyester which is a mixture of hexa-, hepta- and octa- esters of sucrouse, esterified with long chain fatty acids (olean, Procter and Gamble, Cincinnati, OH. USA). Fat mimetic is a fat replacer based on carbohydrates, proteins or fat components used alone or in combination that requires a high water content to achieve its functionality and mimic one or more of the sensory and physical functions of fat in food [ADA, 2005]. In meat technology, fat can only be replaced by "structure water" that means that it is necessary to hold water in food matrix by adding mainly carbohydrates or proteins, that are able to form gels.

In a study of different frankfurters of the Spanish market in 1995, including some low-fat sausages, it was notice that consumers prefer sausages with an intermediate texture, and low fat frankfurters with high protein content [more meat added in formulation] were not accepted because they showed a harder texture, on the contrary cheap sausages with high water content were rejected because of the softer texture [Ordoñez, et al., 2001]. In the same study, it was possible to obtain sausages with a 50% of fat reduction, with a medium texture, accepted by consumers using a combination of proteins and karageenans together with carboxy methyl cellulose (CMC), or with pectin (Slendid, Hercules Inc, Copenhaguen, Denmark), with an increase in water content of 8% in comparison with standard sausages (20% fat).

Step 2: changing fat composition

Eskimos and Mediterranean paradox consists on that although in both places total dietary intake due to fat consumption is over the 30% recommended, people show a very low incidence in CVD. The explanation of this paradox is due to the different fat composition consumed by these types of population, because most of the fat they intake are mainly composed by polyunsaturated (PUFA) or monounsaturated (MUFA) fatty acids. It is clear now, that increasing low-density lipoprotein (LDL) cholesterol levels and tryglycerides, and reducing high-density lipoprotein (HDL) cholesterol, among other factors, increases the risk to developing coronary heart diseases (CHD) [Lichtenstein, et al., 2006]. The strongest dietary determinants of high LDL cholesterol concentrations are dietary saturated fatty acid (SFA) and *trans* fatty acid intakes [Lichtenstein, et al., 1999]. These data support the notion that fat quality is more important than fat quantity in regard to CVD risk [Cordain etal., 2005]. In that sense, a reduction in the% of energy dietary intake of saturated fat is desirable, together with an increase in MUFA and PUFA. It is generally accepted that around 15–20% of total dietary energy should come from MUFA and <5% of PUFA.

Taking in account all data mentioned above, one strategy to improve healthiness of luncheon meat products could be to develop tailor-made fat composition sausages, with a reduced fat content, which could be sensory acceptable by consumers. One approach to obtain this kind of products could be to employ meats as raw material coming from animals feed with different fat composition, to improve the fatty acid profile of carcass fat in pigs [Morgan

et al., 1992; Vanoeckel and Boucque, 1992]. Another approach is to use conventional raw material with low fat composition and modify the fat composition using different blends of vegetable oils.

The last one was the approach used by Broce [2003]. With the aim to use a meat with a lowest fat content and after analysing the basic composition of different types of meat, the conclusion was that chicken breast was the most suitable (average fat content around 1%). To obtain the most suitable fat composition, different vegetable oils were analysed and blended in different percentages till get a closer "ideal" fat composition according to dietetic standards. As an example, with a mixture of 60% olive oil and 40% of sunflower oil the following fat composition obtained was: 4.5% SFA; 20.5% MUFA and 10% PUFA. After different steps of processing optimization it was possible to obtain frankfurters with a slightly soft texture and with some olive oil flavour notes with the following nutritional characteristics: a reduction of 50% of fat intake per 100 g of product (10% fat content versus 20% of commercial sausages); a reduction in total energy intake of around 100 Kcal/g (from 253 Kcal/g to 147 Kcal/g) and more healthier fat profile UFA (unsaturated fatty acid)/SFA from 2.04 to 6.6 and better ratio of MUFA/PUFA from 2.6 to 3.7 [Broce, 2003].

Step 3: adding n-3 fatty acids

Another possibility to improve healthiness of luncheon meat products could be the addition or enrichment of omega-3 fatty acids (n-3 fatty acids). A number of studies have evaluated clinical benefits and acceptability of several n-3 enriched foods, including meat and meat products [Moghadasian, 2008]. Again, two possibilities raise here, first the implementation of n-3 fatty acids in animal carcasses by animal feed composition. In that sense, low level of dietary linseed oil (0.5%) is sufficient to markedly increase intramuscular n-3 fatty acid concentrations, including DHA levels in pigs [Rey et al., 2001]. Meat and other products [including sausages] from pigs that fed a linseed-rich diet had significantly higher levels of n-3 fatty acids as compared to controls [Sheard et al., 2000]. Second by adding directly n-3 fatty acids to meat products, this strategy is not completely new, as Park et al. [1989] used fish oil at a concentration of 50 g kg−1 in pork frankfurter-type sausages, but the products were unacceptable due to their fishy flavour. Nowadays, an improvement in fish oil extraction processes allows increasing efficiency and extraction ratios, but still oxidation is the key point to preserve n-3 enriched oils [Rubio-Rodriguez, et al., 2008].

Nowadays, different meat fat reduced products are available in the market, but consumer is not willing to accept significant reduction in sensory properties of the product, as flavour, taste and texture.

Dry-cured ham

Dry-cured meat products are well-known for their unique sensory characteristics. However, the traditional process is very time consuming. The traditional production of dry-cured ham lasts from 9 to 24 months, depending on the processing method and of the initial weight of the ham [Costa et al., 2008]. The process can be shortened especially by accelerating the drying period, which is the most time consuming. A shortening of the drying period would result in a reduction of the drying facilities, capital and labour, and would increase the profit

margin and the product competitiveness while reducing some safety concerns, such as mould growth, lipid oxidation and mite infestation [Arnau et al., 2007].

Actual market shares reveal a normal demand, that is, an inverse relationship between price and quantity demanded. Thus, around 90% of consumers have actually bought the cheapest category, while this proportion falls to 4.4% of actual consumers of the most expensive (available) category [Resano et al., 2009].

Corcuera et al [unpublished results], in order to unify the producers and consumers demands, made different trials to produce a ham which joined a good relationship between price and elaboration time. These characteristic were reached as a consequence of using a boned hams combined with the curing mixture in a tumbler. Hams after salting process were subjected to a mechanical constant pressure during 18 days at 3ºC in order to accelerate the process of dehydration, and to assure the final texture of the product (Mainalli. Girona. Spain). Following, hams were hung together in a drying room at 4ºC for 7 weeks (weight loss of 22%), at 8ºC for 2 weeks, at 12ºC for 2 weeks, at 16ºC for 2 weeks and T 20ºC for 2 weeks ([weight loss of 35%). Total producing time was situated at 120 days. The relative humidity ranged from 80 to 75%. Two batches were obtained: (1) without added flavor and (2) commercial flavor in a concentration of 0,8g per kg of ham. Physicochemical, texture and sensorial parameters were studied. Results were compared with normal ham elaborated in 240 days [Ordoñez, 2001].

Hams produced by accelerated drying process showed: (i) same values of humidity and activity water that the obtained values from hams which had been traditionally elaborated, (ii) higher values of pH, NNP content and salt content. [Ruiz-Ramirez et al., 2006] found out that hams with higher salt content and hams with higher initial pH showed lower proteolysis index (PI). The most important texture problems in the inner zones of dry-cured ham are excessive softness [Parolari et al., 1994] and pastiness [Arnau, 1991; Arnau et al., 1998; García-Garrido et al., 2000; García-Rey et al., 2004]. Softness is associated with proteolysis [Parolari et al., 1994; Virgili et al., 1995], which depends on moisture content, salt content and temperature.

From the sensory point of view, accelerated hams were softer than traditional ones, but this feature seems not to be a problem for consumers in general. No differences were found between commercial hams of the same quality.

A more detailed review about shorten the drying period of dry-cured meat products could be found in Arnau et al. [2007].

Dry-ferment meat products

These products are obtained by a coarse grinding of lean and pork belly or back fat, mixed with different ingredients and starter cultures, stuffed in natural or artificial casings and hung in a ripening room following a fermentation step of around 48h, depending on the sausage diameter at 20–25ºC and high RH (<90%) and ripening/maturation step at lower temperature (13–15ºC) and decreasing RH till reach 70%, and a weight loss of the product around 30–35%. From the healthier trend point of view, similar strategies as it has been discussed above for luncheon meat products have been developed for dry-fermented sausages. For instance, raw pork meat coming from pigs fed with modified fat diet and enriched with high oleic and high linoleic acids were used to make healthier dry-fermented sausages [Rubio

et al., 2007, 2008]. According with these authors, fermented sausages with high content of unsaturated fatty acids had similar sensory properties to those of conventional sausages, and even a comparable sensory stability. The second option is to modify fat composition in the manufacturing step adding some fat replacers or substitutes like vegetable oils, mainly olive oil, or fish oil extracts as it was suggested by Muguerza et al. [2004a; 2004b]. However, according to these authors the highest level of *n*-3 fatty acid seems to accelerate the oxidation process significantly.

A completely different trend in dry-fermented sausage is related with improving cost efficiency during production. It should be taken in account, that traditional processes are very time consuming varying from a couple of weeks till several months depending of the diameter of the sausage. Texture modifications, that imply and increase in hardness, start in the fermentation step, due to the decrease in pH by Lactic Acid Bacteria [LAB] metabolism that favours the release of water [González-Fernández, et al., 2006]. However, as it was state above, drying, during ripening/maturation, is the limiting step of the process in terms of time. To simplify the process and, with the clear aim to reduce ripening time, several approaches have been tested. Lu and Townsend [1973] applied freeze drying to the meat prior to fermentation to remove the highest amount of water to reduce drying time. Osmofood system [Sirami and Louthellier, 2002] proposed a drying system based on osmotic drying of minced meat layers. The osmo-convective drying of different kinds of meat was carried out in appropriate concentrated solution of sugar (environment 70%) and salt (5%) in water. The reduction in moisture content during osmotic dehydration process of 3 hours was 60%. Quality parameters like texture, moisture content, colour, hydration as well as rehydration ratios and microbiological loads were evaluated and found acceptance to a satisfactory level [ProsafeBeef, 2009].

In 1995, Chin, Keeton and Lacey, developed in laboratory scale a drying system under vacuum for pepperoni pieces, observing a drying time reduction of 30% (from 18 to 12 days) with acceptable sensory characteristics. More recently, Comaposada, Arnau, Gou and Monfort [2004] have proposed a new drying process for sliced meat products called QDS (Quick-Dry-Slice). In this system sausages are fermented to the desired pH, and are then frozen, sliced and dried in a continuous system that combines convective and vacuum drying, reducing the traditional drying system to 30 min [Arnau, et al., 2007]. This system is commercialized by the Spanish Company Metalquimia (Girona, Spain; www.metalquimia.com). Products obtained with this system have an slightly different flavour than traditional ones, but it improves during storage after packaging. From the microbiological point of view, the safety of the products made with this system is similar to the traditional ones [Arnau, et al., 2007]. From processing point of view, advantages of this new system are unquestionable, it allows to work in the "just in time" scheme, doing production much more flexible, drying facilities can be reduced with the save of space occupied now by big drying rooms, there is no possibility for growing moulds in the surface, and so on.

Conclusions

After many years and even centuries, meat processing is facing a very rapid change in its own conception. New trends that are forced by consumers and for industry demands are appearing. From the consumer point of view, elaboration of healthier meat products with

reduced salt and fat content and development of convenience meat products are the strongest ones. In that sense, modification of fat content and composition of animal carcasses through animal feed, and reduction, substitution, replacement of fat or enrichment with n-3 fatty acids during processing are the main strategies used to improve nutritional and functional properties of meat products. From technological point of view, reduction of production cost without compromising food safety and sensory quality and environmental issues are also a strong trend, especially in more traditional processes, which use to be very time and energy consuming. Meat scientists are doing a very hard work from the research point of view to support all these new challenges for meat sector.

References

Aiello L.C., Wheeler P., 1995. The expensive-tissue hypothesis: the brain and digestive system in human and primate evolution. Curr Anthropol., 36, 199–221.

Aranceta J., et al., 2003. Prevalencia de la obesidad en España: Resultados del estudio SEEDO 2000. Med Clin, 120, 608–612.

Ardrey R., 1961. African Genesis. Dell, New York.

Arnau J., 1991. Aportaciones a la calidad tecnológica del jamón curado elaborado por procesos acelerados. PhD Thesis, Universitat Autònoma de Barcelona, Facultat de Veterinària, Barcelona, Spain.

Arnau J., Guerrero L., Sarraga C. 1998. The effect of green ham pH and NaCl concentration on cathepsin activities and the sensory characteristics of dry-cured hams. J. Sci. Food Agric., 77, 387–392.

Biesalski H.K., 2005. Meat as a component of healthy diet- Are there any risks or benefits if meat is avoided in the diet? Meat Sci., 70, 509–524.

Broce K.T., 2003. Development of a new product: chicken frankfurters with nutritionally balanced fat composition. PhD Thesis, University of Burgos, Burgos, Spain.

Bruinsma J., 2003. World agriculture towards 2015/2030. An FAO perspective. London: Earthscan.

Carballo J., Fernández P., Carrascosa A.V., Solas M.T., Jimenez Colmenero F., 1997. Characteristics of low- and high-fat beef patties: Effects of high hydrostatic pressure. J. Food Protec., 60, 48–53.

Chao A., Thun M.J., Connell C.J., McCullough M.L., Jacobs E.J., Flanders W.D., et al., 2005. Meat consumption and risk of colorectal cancer. JAMA, 293, 172–182.

Cheah P.B., Ledward D.A., 1996. High pressure effects on lipid oxidation in minced pork. Meat Sci., 43, 123–134.

Cheftel J.C., Culioli J., 1977. Effects of high pressure on meat: A review. Meat Sci., 46, 211–236.

Chin K.B., Keeton J.T., Lacey R.E., 1995. Utilization of vacuum-drying for reduction of pepperoni drying time. Proceed., 41st ICoMST, 501-502, San Antonio, Texas, USA.

Comaposada J., Arnau J., Gou P., Monfort J.M., 2004. Accelerated method for drying and maturing sliced food products. Patent WO/2005/092109.

Cordain L., Eaton S.B, Sebastian A., Mann N., Lindeberg S., Watkins S.A., O'Keefe J.H., Brand-Miller J., 2005. Origins and evolution of the Western diet: health implications for the 21st century. Am. J. Clin Nutr, 81, 341–354.

Costa M.D.R., Bergamin-Filho W., Silveira E.T.F., De Felício P.E., 2008. Colour and texture profiles of boneless reestructured dry-cured hams compared to traditional hams. Scientia Agricola, 65, 169–173.

Crehan C.M., Troy D.J., Buckley D.J., 2000. Effects of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. Meat Sci., 55, 123–130.

Darian J.C., Cohen J., 1995. Segmenting by consumer time shortage. J. Consumer Marketing, 12, 32–44.

Desmond E., 2006. Reducing salt: A challenge for the meat industry. Meat Sci., 74, 188–196.

García-Garrido J., Quiles-Zafra R., Tapiador J., Luque de Castro M., 2000. Activity of cathepsin B, D, H and L in Spanish dry-cured ham of normal and defective texture. Meat Sci., 56, 1–6.
García-Rey R., García-Garrido J., Quiles-Zafra R., Tapiador J., Luque de Castro M., 2004. Relationship between pH before salting and dry-cured ham quality. Meat Sci., 67, 625–632.
González-Fernández C., Santos E.M., Rovira J., Jaime I., 2006. The effect of sugar concentration and starter culture on instrumental and sensory textural properties of chorizo-Spanish dry-cured sausage. Meat Sci., 74, 467–475.
Gordon A., Barbut S., 1993. Effect of chloride salts on protein extraction and interfacial protein film formation in meat batters. J. Sci. Food Agric., 58, 227–238.
Grunert K.G., 2006. Future trends and consumer lifestyle with regard to meat consumption. Meat Sci., 74, 149–160.
IFIC, International Food Information Council Foundation. Food and health survey: Consumer attitude towards food, nutrition and health. 2009, Available at <http//www.ific.org.
Lichtenstein A.H., Ausman L.M., Jalbert S.M., Schaefer E.J., 1999. Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. N. Engl. J. Med., 340, 1933–1940.
Lichtenstein A.H., et al., 2006. Diet and lifestyle recommendations Revision 2006: A scientific statement from the American Heart Association Nutrition Committee. available in: <http://www.circulationaha.org.
Macfarlane J.J., 1974. Pressure-induced solubilisation of meat proteins in saline solution. J. Food Sci., 39, 542–547.
Macfarlane J.J., McKenzie I.J., 1976. Pressure-induced solubilisation of myofibrillar proteins. J. Food Sci., 41, 1442–1446.
Macfarlane J.J., McKenzie I.J., Turner R.H., Jones P.N., 1984. Binding of comminuted meat: effect of high pressure. Meat Sci., 10, 307–320.
Mandava R., Fernández I., Juillerat M., 1994. Proceed. 40th Int. Congress of Meat Sci. Technol., S–VIB.11.
MAPA [Ministerio de Medio Rural y Marino]. El libro de la alimentación en España 2006. Available online at <http://www.mapa.es/alimentacion/
Moghadasian M.H., 2008. Advances in dietary enrichment with n-3 fatty acids. Crit. Rev. Food Sci. Nutr., 48, 402–410.
Morgan C.A., Noble R.C., Cocchi M., McCartney R., 1992. Manipulation of the fatty-acids composition of pig meat lipids by dietary means. J. Sci. Food Agric., 58, 357–368.
Muguerza E., Ansorena D., Astiasarán I., 2004a. Functional dry fermented sausages manufactured with high levels of *n*-3 fatty acids: nutritional benefits and evaluation of oxidation. J. Sci. Food Agric., 84, 1061–1068.
Muguerza E., Gimeno O., Ansorena D., Astiasarán I., 2004b. New formulations for healthier dry fermented sausages: a review. Trends Food Sci. Tech., 15, 452–457.
Norat T. et al., 2005. Meat, fish and colorectal cancer risk. The European prospective investigation into cancer and nutrition. J. Nat. Cancer Inst., 97, 906–916.
Ordoñez M., 2001. Influencia de la material prima sobre la textura del jamón curado. PhD Thesis. Universidad de Burgos, Facultad de Ciencias, Burgos, Spain.
Ordoñez M., Rovira J., Jaime I., 2001. The relationship between the composition and texture of conventional and low-fat frankfurters. Int. J. Food Sci. and Technol., 36, 749–758.
Park J., Rhee K.S., Keeton J.T., Rhee K.C., 1989. Properties of low-fat frankfurters containing monounsatured and omega-3 polyunsaturated oils. J. Food Sci., 54, 500–504.
Parolari G., Virgili R., Schivazappa C., 1994. Relationship between cathepsin B activity and compositional parameters in dry-cured hams of normal and defective texture. Meat Sci., 38, 117–122.
ProsafeBeef., 2009. EU 6th Framework Programme, available at: http://www.prosafebeef.eu.
Resano H., Sanjuán A.I., Albisu L.M., 2009. Consumers' acceptability and actual choice. An exploratory research on cured ham in Spain. Food Quality and Preference, 20, 391–398.

Rey A.I., Kerry J.P., Lynch P.B., López-Bote C.J., Buckley D.J., Morrissey P.A., 2001. Effect of dietary oils and α-tocopheryl acetate supplementation on lipid [TBARS] and cholesterol oxidation in cooked pork. J. Anim. Sci., 79, 1201–1208.

Rubio B., Martínez B., Dolores García-Cachán M., Rovira J., Jaime I., 2008. The effects of high pressure treatment and of storage periods on the quality of vacuum-packed "salchichón" made of raw material enriched in monounsaturated and polyunsaturated fatty acids. Innov. Food Sci. Emerging Technol., 8, 180–187.

Rubio B., Martínez B., Sánchez M.J., Dolores García-Cachán M., Rovira J., Jaime I., 2007. Study of the shelf life of a dry fermented sausage "salchichon" made from raw material enriched in monounsaturated and polyunsaturated fatty acids and stored under modified atmospheres. Meat Sci., 76, 128–137.

Rubio-Rodríguez N., de Diego S.M., Beltrán S., Jaime I., Sanz M.T., Rovira J., 2008. Supercritical fluid extraction of the omega-3 rich oil contained in hake [Merluccius capensis-Merluccius paradoxus] by-products: Study of the influence of process parameters on the extraction yield and oil quality. J. Supercritical Fluids, 4, 215–226.

Ruiz-Ramírez J., Arnau J., Serra X., Gou P., 2006. Effect of pH 24, NaCl content and proteolysis index on the relationship between water content and texture parameters in biceps femoris and semimembranosus muscles in dry-cured ham. Meat Sci., 72, 185–194.

Ruusunen M. Poulane E., 2005. Reducing sodium intake from meat products. Meat Sci., 70, 531–541.

Ruusunen M., Vainionpää J., Lyly M., Lähteenmäki L., Niemistö M., Ahvenainen R., Puolanne E., 2005. Reducing the sodium content in meat products: The effect of the formulation in low-sodium ground meat patties. Meat Sci., 69, 53–60.

Sheard P.R., Enser M., Wood J.D., Nute G.R., Gill B.P., Richardson R.I., 2000. Shelf life and quality of pork and pork products with raised n-3 PUFA. Meat Sci., 55, 213–221.

Silkes A.L., Tobin A.B., Tume R.K., 2009. Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. Innov. Food Sci. Emerging Technol., In Press, Corrected Proof, Available online 9 March 2009, ISSN 1466–8564, DOI: 10.1016/j.ifset2009.02.007.

Sirami J., Louthellier P., 2002. Method for continuous production of minced meat, dried and restored in thin slabs. Patent WO/2003/007722.

Vandendriessche F., 2007. Meat products in the past, today and in the future. Meat Sci., 78, 104–113.

Vanoeckel M.J., Boucque C.V., 1992. Omega-3-fatty-acids in pig nutrition: a review. Landbouwtijdschrift Revue del Agriculture, 45, 1177–1192.

Washburn S.L., Lancaster C.S., 1968. The evolution of hunting. in: Man theHunter [Lee, R.B. and DeVore, I., eds.]. Aldine, New York, 293–303.

WHO., 2005. <http://who.org.

2

CHANGES OF BEAVER MEAT QUALITY DURING MEAL PREPARATION

Introduction

In 2007 in the territory of Latvia have been around 72 000 beavers. Currently, there is a tendency to increase the number of beaver geometric. Beavers cause great distress for agriculture and forestry. Beaver is a vegetarian. It is therefore ruled out possible contamination with the classical forest animal diseases. It is very important to using beaver meat for meal preparation.

Historically, beaver meat had monks and noble guest's favourite ingredient. Some parts of Europe for the noble people for banquets special privilege of being declared a beaver tail. Beaver meat is high quality unless it is skilfully prepared. Meat culinary value affects the specific animal feed plants dominating [Base and Kills, 2003].

The quality and nutritive value of beaver meat depends on the quality of raw material and control of the production parameters of the technological process.

Beaver meat chemical composition, nutritional value and utility is dependent on muscle tissue, fat and connective tissue, the animal's sex, age and degree of fatness (Table 1). Energy value is higher in older, well-fed animals for meat because it contains more fat. Overall, the meat shall be considered as a source of protein and fat in humans.

Table 1

Beaver meat and domestic animals meat chemical composition [Baltes, 1998]

Meat	Liquid, [%]	Protein, [%]	Fat [%]			Mineral substances [mg]					
			Total	Saturated fatty acids	Unsaturated d fatty acids	Na	K	Ca	Mg	P	Fe
Beaver	70.5	22.1	5.8	44–49	48–53	67	356	4	22	214	6
Pork	51.6	14.6	33.0	33–49	48–64	58	285	7	24	164	1.7
Beef	67.7	18.9	12.4	46–60	43–52	65	325	9	22	188	2.7
Sheep	67.6	16.3	15.3	52–62	38–48	80	270	9	20	168	2

A comparison of beaver meat with the meat of domestic animals, we can conclude that water and protein in the flesh of the beaver is larger, but the fat content of less than domestic animal meat. Beaver meat is higher in nutritional value.

The quality of raw beaver meat is characterized by: chemical composition of meat; compliance with food safety criteria, it may not contain pathogenic and conditionally pathogenic microflora, heavy metals, pesticides, antibiotics that are harmful to human body; technological features; sensory features.

Quality deterioration in meat could be caused by several factors: external – storage temperature, relative air humidity, sanitary hygienic conditions in the catering enterprise; internal – connected with meat structure and its physical and chemical features, pH value, a_w, value of reduction oxidation potential E_h; processing factors; indirect factors which are of a great importance in the forming process of pathogenic microflora.

Materials and methods

The experiments were carried out in the Meal preparation laboratory, Scientific laboratory of Microbiological testing and Sensory evaluation laboratory of the Faculty of Food Technology Latvia University of Agriculture.

Microbiological analyses were performed within technological process in: the raw meat and final product. The samples were taken according to standard procedures (LVS ISO 3100 – 2:1988): raw meat – from LUA Hunting team hunted beavers, final product – from prepared meals. Fresh meat test pieces were taken in appliance with standard methods – 36 hours after hunting in maturing phase when meat had reached a condition fit for technological processing. Total number of bacteria was determined by using LVS ISO 4833:1991 standard method. Amino-ammonium nitrogen amount and pH value in the raw meat were determined by application of standard methods L. Antipova.

Sensory evaluation conducted using emotional techniques. Emotional method also known as methods for consumers, because they are mainly used to determine the market's view of consumer studies. Hedonic scale – the model used in the evaluation of 9 point hedonic scale, and evaluating the results of the analysis of variance test and Tukey test.

Results and discussion

Beaver meat mining process consists of the following basic operations:

- hunting of animals (with a hunting shotgun, firing the head to prevent damage to the animal's meat, or traps);
- bleeding immediately after the hunting;
- skinning and off, which starts from the head, trying to damage any of carcass surfaces and skin;
- internal organ removal (in order to avoid soiling of carcass and the occurrence of unpleasant odours, cutting a beaver glands musk);
- carcass cleaning (dry and wet) – dry cleaning separated tail, blood clot, bruising spot, but the wet cleaning the carcasses are washed with warm water beneath pressure to remove dirt. All the listed activities carried out by hunters, in strict compliance with the regulatory framework in the legislation defined hygiene.

Carcass immediately was transported to the Meal preparation laboratory and stored in the cold chamber 36 hours at the temperature +4±2°C.

The technological process of preparation of beaver meat meal includes the following critical control points: the preparation of raw material – disjointing of carcass; storage of disjointed meat before it is thermally processed; thermally processing and serving.

Using HACCP system principles, microbiological risk-cause analysis of the technological process of hot smoked pork ham production were performed, as a result of which the critical control points were determined.

Raw food preparation is one of the most important technological process steps in the microbiological risk formation. In order to evaluate the influence of storage time of fresh meat on product deterioration, the dynamics of the formation of amino-ammonium nitrogen was investigated.

The amount of amino-ammonium nitrogen in mg/100 g meat, which points to the amount of free amino- and carboxyl groups that have resulted from decomposition of proteins. It is up to 80 mg% for fresh meat, 80–130 mg% for meat with signs of deterioration and 130 mg% and more for spoiled meat (Figure 1).

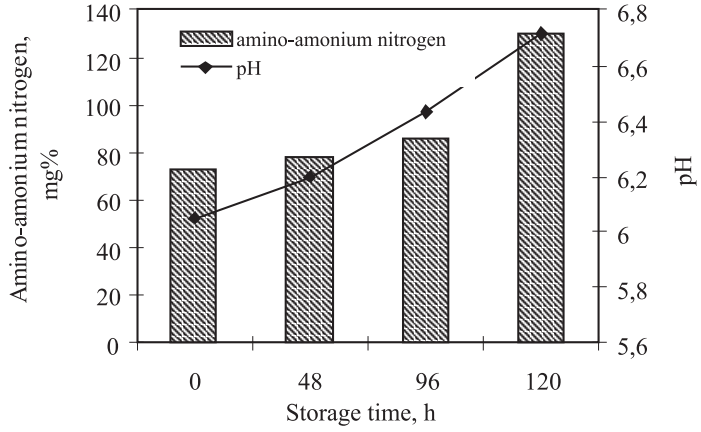


Fig. 1. Correlation between of amino-ammonium nitrogen and pH

The determination of indices was chosen in order to detect how the number of microorganisms changes in different time periods when meat and its products were stored in constant temperature conditions in compliance with regulations of the Republic of Latvia.

Total number of bacteria in 1 gram of product must not exceed $1*10^3$ CFU. After 120 hours (5 days) of storage, this value was exceeded in all of the chosen fresh meat test pieces (Figure 2).

The second technological process step in which microbiological contamination may possibly develop is the thermally processing. From beaver carcass were used non-thigh and scapular parts, boned leg parts and pieces of the fillets. Beaver meat has a distinct red colour and fat in a specific smell. To reduce the red colour and the smell of fat, meat can marinade 3–4 hours at the temperature +6±8°C. Roasting, braising and boiling are choosing as thermal processing methods.

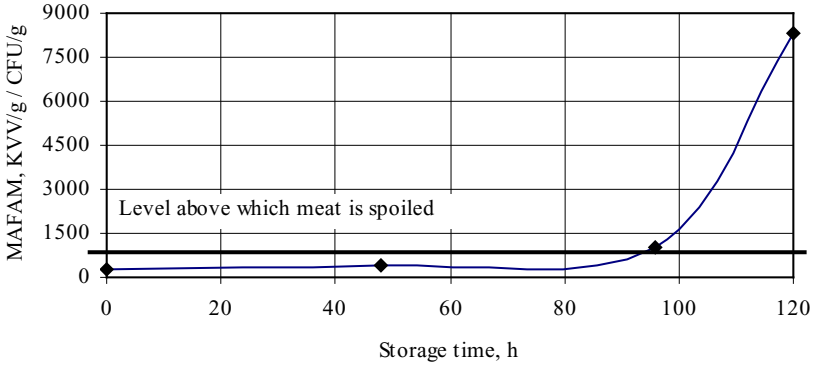


Fig. 2. Dynamic of total number of bacteria in storage time of fresh meat

The following temperature conditions during the meal preparation process and also in the production premises have to be strictly observed to avoid the changes in the quality of a product: in cutting premises the temperature shall not exceed +12°C; in meat forming premises +10°C and in the storage chamber prior to the thermal processing the temperature shall not exceed +2°C. The maximum permitted storage period of a marinade food before thermal processing is 2 hours.

An important factor for the development of microbiological contamination is the influence of individual technological operations. Microbiological testing of meat test pieces was performed after marinade and into the final product (Figure 3).

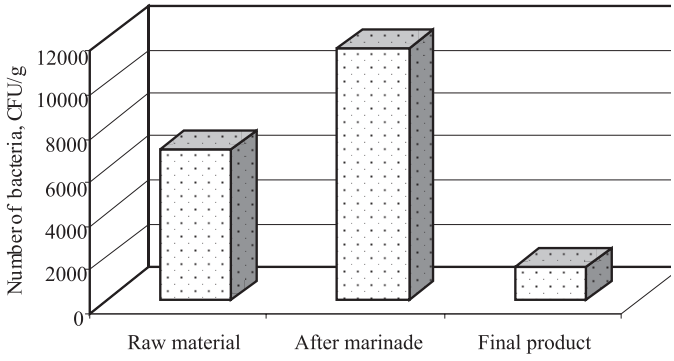


Fig. 3. Dynamic of bacteria number in technological process of cooked beaver meal

According to the microbiological testing results the total numbers of bacteria match the norms stated in legislative acts [Robinson et al., 2000; Sielaff, 1995].

Sensory evaluation took part in 26 trained panellists [Strautniece, 2004]. The sample was held encryptions using random three-digit combinations Roasted, braised and boiled beaver meat meals sensory evaluation results are given Table 2.

Table 2

Beaver meat meals hedonic evaluation results of variance analysis ($\alpha \leq 0.05$).

Variance source	SS	df	MS	F calc	F crit
Panellists	24.7	25	1.0	1.74	1.73
Samples	56.3	2	28.2	49.6	3.18
Error	28.4	50	0.6		
Total	109.4	77			

To determine whether the three evaluable samples have significant differences, then F_{calc} value should be compared with F_{crit} value. As shown F_{calc} is greater than F_{crit}, this means that the evaluable samples significant differences. The use of hedonic evaluation of boiling meat = 5.2, braising meat = 6.4, roasting meat = 7.3. Thus, the assessments for all evaluable samples are done in the use of hedonic scales range from 5 to 8, nor love, nor do not like to love. Evaluators liked best roasted beaver meat, and the lowest valuation is boiled beaver meat.

As the findings of microbiological testing and sensory evaluation results, beaver meat can be used for meal preparation. At the time of meal preparation the meat retains its quality and nutritional value.

Conclusions

The technological process of preparation of beaver meat meal includes the following critical control points: the preparation of raw material – disjointing of carcass; storage of disjointed meat before it is thermally processed; thermally processing and serving.

The following temperature conditions during the meal prepared process should be strictly observed:

- not more than +12°C in cutting premises,
- not more than +10°C in meat formation premises,
- not more than +2°C in the storage chamber before thermal processing.

Best beaver meat thermal processing is roasting.

References

Baltes W., 1998. Lebensmittelchemie. Riga, LU, 478 (in Latvian).

Base A., Kills K.R., 2003. Treatment and prescription. Riga, Jana seta, 174 (in Latvian).

Forsythe, S.J., Hayes, P.R., 1998. Chapter 3: Food Spoilage, in: Food Hygiene, Microbiology and HACCP. Forsythe, S.J., Hayes, P.R. eds. Maryland: Aspen Publishers, Inc. Gaithersburg, 86–149.

Roberts T.A., Baird-Parker A.C., Tompkin R.B., 1996. Microorganisms in foods. New York: V. Blackie Academic Profesional, 513.

Sielaff H., 1995. Fleischtechnologie. Hamburg: Behr's, 675.

Strautniece E., 2004. Sensory evaluation of food products. Jelgava, LLU PTF, 89 (in Latvian).

3

EFFECT OF DIFFERENT HEAT TREATMENT METHODS ON THE QUALITY PROPERTIES OF PORK

Introduction

Meat and meat products are basic components in the diets of developed countries. Their consumption is based largely on availability, price and tradition. Meat production is a very complex operation depending mainly on consumer's demand (which is usually based on price and income). The amount of meat consumed in different countries varies enormously with social, economic and political influences, religious beliefs and geographical differences [Millward, 1999; Jimenez-Colomero et al., 2001].

Meat is held in high esteem in most communities. It has prestige value, it is often regarded as the central food round which meals are planned, various types of meat are sometimes made the basis of festive and celebratory occasions, and from the popular as well as the scientific point of view, it is regarded as a food of high nutritive value. Meat and meat products can make an important contribution to nutrient intakes in the diet They provide a number of essential nutrients, including long-chain n-3 PUFA, iron, zinc, selenium, essential amino acids and vitamin B6. Also is a concentrated source of protein which is not only of high biological value but its amino acid composition complements that of cereal and other vegetable proteins. The reason why meat is an important source for some micronutrients is due to the fact that meat is either the only source or provides a substantially higher bioavailability of some micronutrients.

In industrialized countries there have been changes over the years in the relative amounts of different types of meat consumed (beef, pork, lamb, poultry) depending partly on price and influenced by fashion, advertising, etc. In nowadays one of the most important factors is that meat and meat products need to be perceived as healthier [Bender, 1992].

The eating satisfaction of meat in consumer's feeling is due to a combination of overall acceptability, tenderness, juiciness and flavour [Koohmaraie et al., 2002]. The process of heat treatment has a large effect on those properties. The meat preservation by thermal treatment is most popular method also for preparing and makes it more palatable. Physico-chemical processes going on in the meat tissue during heating cause significant changes in its technological and quality properties [Palka, 2003].

The objective of this research was to compare the effect of used heat treatment methods on some quality properties of pork.

Materials and methods

The experimental material included ham muscles removed from chilled half-carcasses class E of fatteners born from sows of Polish Landrace breed matted with a cross-breed boar (pietrain x duroc).

Material under investigation was divided into two parts (about 370 g each one) and put into baking tin (sample A) or into special nylon baking foil (sample B). Hams thermal treatment was carried out in electric roaster in 170°C for the period of 1.5 h until they were light brown (until the inside temperature reached 90°C). In order to characterize technological efficient and quality of roasted hams, in samples were determined some of physicochemical, textural and sensory properties. The estimation of the physicochemical properties of ham muscles under investigation included a measurement of colour indices: L*, a* and b* using a Minolta Chromameter CR 200, values of saturation (C*) and shade (H*) using method described by Hunter and Harold [1987], pH value according to PN-ISO 2917:2001, yield of the roasting process was estimated by ratio: weight after heat treatment to weight of raw meat. Basic chemical components were analyzed according to following methods: dry matter – PN-ISO 1442:2000, protein content – by Kjeldahl's method, using Kieltec™ 2300, free fat content – PN-ISO 1444:2000. Tenderness expressed by shear force was measured using the Zwick/Roell type Z010 machine in meat samples which were cut into cuboids 15 mm long with a 100 mm^2 cross-section area (the muscle fibres in samples were running perpendicular to the cutting plane). Sensory evaluation after heat treatment process was performed by a sensory panel. The sensory panel included seven assessors (only one training session before testing). The sensory attributes judged by the panellists were overall acceptability, odour, colour, flavour and tenderness. The panel assessed the sensory descriptors on a 5-point Tilgner's scale of acceptance [Baryłko-Piekielna, 1975]. Isolation of volatile compounds was performed by SPME method. The 50/30 μm cabowax/divinylobenzene fiber was purchased from Supelco Inc. Sorption was conducted from 5g roasted meat homogenate (ratio with water 1:1), kept at 80°C by a water bath for 60 min. After that, the SPME fiber was exposed in the upper space for 15 min., and then introduced to the GC–MS injector for desorption and analysis. GC–MS analysis was performed on an Agilent 6890 N gas chromatograph coupled with a mass spectrometer (Agilent Technologies, USA). The carrier gas was helium in 1 ml/min. The separation was on a HP-5 MS 60 m x 250 μm x 0.25 μm column (Agilent Technologies,USA). The initial oven temperature was at 40°C, and ramped to 240°C at 4 °C /min.

The experiment was carried out for four muscle samples in each of the four series. The data were analyzed statistically, using STATISTICA 7.1 software. Three-way analysis of variance was used to test the effect of used heat treatment methods on the variables examined. Significant differences between the mean values were determined using Duncan's test ($\alpha = 0.05$).

Results and discussion

Thermal losses, defined as liquid and soluble compounds of meat running out from meat samples during heat treatment, were responsible for weight losses in experimental material. The results obtained in this study (Tab. 1) showed that the method of heat treatment had not

influence on yield of the roasting process. The weight losses were estimated on level of about 42%, but the differences between samples under investigation were insignificant. What can be connected with both the same time of process and time of reached the require temperature inside samples.

Tabela 1

Physical traits of hams after heat treatment

Parameter	Sample A	Sample B	NIR	F	α
Yield [%]	57.59[a]	57.83[a]	0.599	0.279	0.599
pH	6.0[a]	6.1[b]	0.0009	11.23	0.001
Shear force [N/cm^2]	47.73[a]	43.82[b]	0.001	10.92	0.001

Different letters (a,b,c) following numbers in the same row indicate significant differences between means (P<0.05)

The effect of weight losses in meat samples after heat treatment process was observed in basic chemical composition (Tab. 2). After Pospiech et al. [1995] the highest changes will be noted in water and protein content. Results of experiment proved the significantly influence of the method of roasting process on protein and fat content. However no significant differences were recorded for dry matter content. Experimental material was characterized by high protein content, but the highest amount of this compound was measured in hams roasted in special nylon baking foil (samples B, 37.09%). Applied special baking foil caused lower losses of protein (by 0.46%) and also caused better dietetic value of roasted meat. In samples roasted with special foil the content of fat was measured on level 3.24% and was lower than in samples roasted without it (3.68% in samples A). The lower fat content in samples of hams roasted in nylon baking foil is probably related with releasing more amount of this component to meat juice during process of heat treatment.

Tabela 2

Chemical components of hams after heat treatment

	Parameter		
	Water content [%]	Protein content [%]	Fat content [%]
Sample A	59.37[a]	36.63[a]	3.68[b]
Sample B	59.67[a]	37.09[b]	3.24[a]
NIR	0.153	0.015	0.00048
F	1.69	5.63	11.08
α	0.195	0.018	0.001

Different letters (a,b,c) following numbers in the same column indicate significant differences between means (P<0.05)

The estimation of the reaction (Tab. 1) of roasted hams demonstrated significant diversity in pH value. The lowest value was observed in ham samples roasted in special nylon foil (6.1). Samples A were characterized by lower pH value (6.0).

Analysis of tenderness (Tab. 1) expressed by shear force and measured in meat samples which were cut into cuboids 15 mm long with a 100 mm^2 cross-section area proved that the method of roasting process had an impact on this parameter. With higher shear force will increase the difficulty to bite meat sample for consumer, what indicate to it high coriaceus.

The force necessary to cut samples roasted in baking tin was higher than those measured for samples roasted in special nylon foil (47.73 and 43.82 N/cm^2, respectively). It is probably due to more water evaporation from meat roasted in baking tin. Samples B were baking in nylon foil and so they were stewing in meat juice and probably for that reason they were more tenderness. Consumers prefer more tenderness meat what was confirmed in sensory evaluation for this variable (Tab. 4).

Table 3 presents the results of colour traits analyses of roasted hams. It was observed that the method of roasting process had significant effect on CIE L*, a*, b* values. The colour measured on the cross-section of roasts was more light (L*: 64.89 sample A and 64.00 sample B) than those measured on topping (43.42 and 49.70, respectively). Roasts prepared with using a special nylon foil were lighter than those roasted without it. It was related with direct touch topping of meat samples with atmosphere of dry air and more intensity process of non-enzymatic browning reaction. Also humidity had a strong impact on the rate and degree of Maillard reaction course. Higher moisture of medium where browning reaction take place limited this reaction [Bejerholm and Aaslyng, 2003], what was confirmed in colour values of samples B. Those roasts were also more redness and yellow on the cross-section than those roasted in baking tin but on topping they were less redness. The saturation was affected by thermal processing in a similar manner to yellowness.

Tabela 3

Colour properties of hams after heat treatment

Parameter	topping			Cross-section		
	Sample A	Sample B	NIR	Sample A	Sample B	NIR
L*	43.42^a	49.70^b	0.00	64.89^a	64.00^a	0.071
a*	8.02^b	6.48^a	0.00	0.96^a	1.41^b	0.0001
b*	15.24^a	17.94^b	0.0006	15.91^a	16.38^b	0.0002
Saturation (C*)	17.22	19.07	–	15.94	16.44	–
Shade (H*)	0.03	0.05	–	0.30	0.21	–

Different letters (a,b,c) following numbers in the same row indicate significant differences between means (P<0.05)

Table 4 shows some parameters of sensory evaluation for hams after heat treatment. The method of thermal treatment had a significant influence on judged sensory attributes. The highest results of overall acceptability, odour and colour of roasts under investigation were obtained for samples A (4.3; 4.1; 4.4 points). Samples roasted in baking foil were characterized by lower score (4.0; 4.0; 4.2 points). Differences of tenderness for the investigated samples were statistically significant. The highest notes for this attribute were noticed in samples B. There were statistically insignificant differences between results of flavour estimation. The results of these analyses were in agreement with the earlier observation of Aaslyng et al. [2007], where most of consumers prefer meat samples more juiciness and tenderness with characteristic aroma.

The odour of the roasted pork and other meats after heat treatment is mainly derived thermally, and the most important mechanisms responsible for the volatile formation are lipid degradation and Maillard reactions [Jianchun et al., 2008]. From Table 5, it could be seen that 24 volatile compounds were found in experimental roasts, including hydrocarbons, aldehydes, ketones, alcohols, heterocycles, phenols, furanes. In ham samples under investigation were identified almost the same amount of volatile substances affecting meat flavour. In hams

after heat treatment in baking tin were signified more volatile compounds formed in process of lipid degradation, i.e. hydrocarbons, aldehydes and alcohols. Researches conducted by Elmore et al. [2000] confirmed formation of toluene, butanal, pentanal, heksanal, benzaldehyde, acetone and aliphatic alcohols in meat samples after heat treatment. The higher amount of in samples A is probably related to non-restricted access topping of roasts to oxygen and so on the more intensity oxidation process. In samples roasted in baking foil were indicated more compounds formed in browning reaction, i.e furanes, pyridines and pyrazines what was consisted with observation of other authors [Garcia-Esteben et al., 2004; Jianchun et al., 2008; Mottram, 1998; Soncin et al., 2007].

Tabela 4

Sensory evaluation of hams after heat treatment

Parameter	Overall acceptability	Odour	Colour	Flavour	Tenderness
Sample A	4.3[b]	4.1[b]	4.4[b]	4.1[a]	3.9[a]
Sample B	4.0[a]	4.0[a]	4.2[a]	4.1[a]	4.0[b]
NIR	0.00	0.024	0.001	0.274	0.011
F	29.18	5.118	11.0	1.20	6.494
α	0.00	0.024	0.001	0.274	0.011

Different letters (a,b,c) following numbers in the same column indicate significant differences between means ($P<0.05$)

Tabela 5

Volatiles identified in GC–MS from the roasted pork

Compounds	Sample A	Sample B
Hydrocarbons:	▪ o-izopropylenotoluene ▪ 1-methylo-4-(1-methyloethylo)benzene ▪ 1,2-dimethoksy-4-(2-prophyleno) benzene	▪ cyclopentan ethylene ▪ 1,2-dimethoksy-4-(2-prophyleno)benzene ▪ 4-ethylo-1,2-dimethylobenzene ▪ 1-ethylo-1,2-dimethylobenzene
Aldehydes:	▪ butanal ▪ pentanal ▪ heksanal ▪ benzaldehyde	▪ heksanal ▪ benzaldehyde ▪ oxime 4-methylobenzaldehyde
Ketones:		▪ acetone
Alcohols:	▪ 1-octanol ▪ 1-nonen-3-ol ▪ 2-nonen-1-ol	
Phenols:	▪ 2-methoxy-3-(2-propyleno)phenol ▪ 2-methoxy-4-(1-propyleno)phenol ▪ 3-allylo-6-methoxyphenol	▪ 2-methoxy-4-(1-propyleno)phenol
Furanes:		▪ 2-penthylofurane ▪ 2,5-dihydroxyfurane
Heterocycles:	▪ pyridine	▪ 4-ethylopyridine ▪ pyrazine 1-tetrazol-5-yl
Others:	▪ acetic acid ▪ butyrolactone	▪ butyrolactone

Conclusions

The results of the performed investigations provided information about differences occurring between samples of ham after two different heat treatment methods. The obtained results pointed to advantageous dietetic and sensory properties of hams roasted with special foil. The results of sensory evaluation confirmed that method of heat treatment have a strong impact on quality traits. Samples roasted in baking tin were characterized by more favorable overall acceptability and colour (4.3 and 4.4 points) but samples roasted in baking foil were more tenderness (4.0 points). The analysis of volatile components also revealed the influence of used methods on flavor of roasted hams. Meat samples after heat treatment in nylon foil were more aromatic than those roasted in baking tin.

References

Aaslyng M.D., Bejerholm C., Ertbjerg P., Bertram H.C., Andersen H.J., 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Qual. Pref., 14, 277–288.

Baryłko-Pikielna N., 1975. Zarys analizy sensorycznej żywności. WNT Warszawa.

Bejerholm C., Aaslyng M.D., 2003. The influence of cooking technique and core temperature on result of a sensory analysis of pork – depending on the raw meat quality. Food Qual. Pref., 15, 19–30.

Elmore J.S., Mottram D.S., Hierro E., 2000. Two fibre solid-phase microextraction combined with gas chromatography-mass spectrometry for the analysis of volatile aroma compounds in cooked pork. J. Chromatography A, 905, 233–240.

Garcia-Esteban M., Ansorena D., Astiasaran I., Ruiz J., 2004. Study of the effect of different fibre coatings and extraction conditions on dry cured ham volatile compounds extracted by solid phase microextraction (SPME). Talanta, 64, 458–466.

Hunter R.S., Harold R.W., 1987. The measurement of appearance. 2ed Ed. Wiley J.&Sons. New York.

Jianchun X., Baoguo S., Fuping Z., Shuaibin W., 2008. Volatile flavor constituents in roasted pork of Mini-pig. Food Chem., 109, 506–514.

Jimenez-Colomero F., Carballo S., Cofrades S., 2001. Healthier meat and meat products: their role as functional foods. Meat Sci., 59, 5–13.

Koohmaraie M., Kent M.P., Shackleford S.D., Veiseth E., Wheeler T.L., 2002. Meat tenderness and muscle growth: Is there any relationship. Meat Sci., 62, 345–352.

Millward D.J., 1999. The nutritional value of plant-based diets in relation to human amino acid requirements. Proceed. Nutrition Society, 53, 289-295.

Mottram D.S., 1998. Flavour formation in meat and meat products: a review. Food Chem., 62, 415–424.

Palka K., 2003. The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. Meat Sci., 64, 191–198.

Pospiech E., Domagała A., Kałuża E., Stefańska D., 1995. Wpływ ogrzewania na rozgotowalność kolagenu. Rocz. Nauk. AR w Poznaniu, Technologia Żywności, 19, 87–92.

Soncin S., Chies L.M., Cantoni C., Bondi P.A., 2007. Preliminary study of the volatile fraction in the raw meat of pork, duck and goose. J. Food Comp. Anal., 20, 436–439.

4

THE EVALUATION OF RHEOLOGICAL AND SENSORY PROPERTIES OF HAMBURGERS IN RELATION TO PACKAGING AND STORAGE CONDITIONS, AS WELL AS HEATING PROCEDURE

Hamburger production is one of the way of utilisation of less valuable meat cuts, fats and edible by-products obtained during trimming the basic elements of the animal's carcasses, including poultry and rabbits [Feiner, 2006; Prokopp et al., 2008; Souza Tavares et al., 2007]. Usually, this kind of raw material contains high amount of connective tissue, thus the crucial quality properties of its final products are rheological and sensory characteristics [Chan-Ho Lee et al., 2005; Ozkan et al., 2004]. Those quality traits are also important due to hamburgers production is based mainly on beef meat, which quality is generally characterised by tenderness [Hildrum et al., 2002; Hwang et al., 2002; Palka, 2003; Pospiech et al., 2003; Purslow, 2004; Tornberg, 1996]. Therefore, in the quality evaluation of hamburgers texture and sensory traits are the most crucial [Kyung-Sook Park et al., 2004; Erdogdu et al., 2005]. Probably specific consumer preferences are dependent on the way of consumption e.g. with bread or as a full meal. Food texture, which is connected also with sensory properties, is influenced among others by packaging technology and way of thermal treatment before consumption [Kyung-Sook Park et al., 2004; Erdogdu et al., 2005].

The objective of the study was the evaluation of rheological and sensory properties of hamburgers in relation to packaging method, storage conditions and thermal treatment before consumption.

Materials and methods

Materials used in the experiment were hamburgers formulated in industrial conditions with beef meat according to the commercial formula and technology. Production conditions were previously described by Szmańko et al. [2009].

Variation factors used in the experiment were as follows:

- Packaging method:
  - vacuum packaging using laminated bags PA20/PE80 (90% of air evacuation was applied),
  - packaging under inert gasses (MAP: 80% N_2, 20% CO_2) using laminated bags with two layers: MULTISEVEN 80 HS TOP (upper layer) and HIGH GLOSS FP (bottom layer).
- storage conditions:
  - chilling storage (+3°C±1°C),

  - storage at the temperature close to cryoscopic point (-3°C±°C), cryoscopic temperature for hamburgers was -2,33°C.
- Hamburgers were stored for 0, 7, 14, 21, 28 days.
- After storage hamburgers were thermally treated in order to prepare for consumption using:
  - heating in microwave oven SHARP, GRILL MICROWAVE OVEN type R-6R71, λ 2450 MHz, 1,2 kW (1). Hamburgers were thermally treated for 2 min 10 sec.
  - heating in steam at the temp. 100°C (2) for 6 min.,
  - baking in an electric oven at the temp. 170°C (3) for 7 min.

All samples were heated up to temp. 70°C was reached in the geometrical centre of the product. The temperature after removing from the oven increased to 73°C ±1°C. Processing conditions were established in preliminary experiment.

Hamburgers were subjected to rheological analysis, especially texture (cutting force and strength needed for product crosscut or disruption), performed on Stevens – QTS 25 equipment. Machine calibration was done in range from 0 N to 50 N. Spindle-shaped head on the round end with the 6 mm diameter (rheological parameters analysis) as well as the knife in the shape of inverted V (cutting force measurements) were moving during the tests 50 mm/min. Samples for rheological analysis were cut from the centre part of the product including surface area (8 mm thick and 1 cm width, cross section area 0.8 cm^2), analysis was based on the measurement of the material resistance on moving in spindle-shaped head up to 6 mm of depth (texture) or total cut of the material (cutting force). All analyses were carried out on hamburgers samples (10 replication in each group) with the uniform temperature of 20°C.

Sensory analysis was performed on heated hamburgers according to PN-ISO 6658 1998 using 5-grades method for intensity and acceptability of selected traits.

The results collected in the study were statistically analysed using STATGRAPHICS ver. 5.0 at the probability level $p>0.05$

Results and disscussion

The effects of packaging method on rheological properties of hamburgers were analysed using penetrometric tests and as well sensory analysis. Higher values of cutting force and strength needed for product crosscut were analysed in vacuum packed hamburgers (Tab. 2). Whereas hamburgers packed under MAP were characterized by the highest values of hardness and strength needed for sample disruption. Packaging under modified atmosphere caused higher degree of squeezing of the hamburgers in bags, what resulted in more uniform consistency of the product. As a consequence higher force was required for crosscutting of the hamburger. Simultaneously, closer and more compact location of the product in bags under vacuum during storage tend to higher moister content and soften of the surface, which effected in lower penetrometric parameters values. Adverse relations were observed for hamburgers packed under MAP, which were exposed to drying of the surface, thus higher rheological parameters values, especially for hardness, were noticed.

Lower storage temperature (-3°C) effected in higher cutting force and hardness values, as well as a clear tendency to higher strength needed for sample disruption (Tab. 3). These mechanical effects could be caused by higher cohesiveness of analysed material stored at lower temperature.

Storage of the hamburgers for 14 days resulted in strong tendency in increasing the cutting force and strength needed for sample crosscut values, as well as hardness and strength needed for sample disruption (Tab. 1). The values of analysed penetrometic properties increased several times, what was probably caused by drying the surface of the hamburgers packed under MAP or higher cohesiveness of the material packed under vacuum.

Table 1

The influence of the storage time on the rheological parameters of beef hamburgers, n = 120

Indicator		Storage period [days]				
		0	7	14	21	28
Cutting force [G]	X	875.8^{b*}	650.8^{a}	1156.6^{c}	877.1^{b}	1137.5^{c}
	sd	17.47	15.67	14.71	15.54	16.40
Strength needed for sample Crosscutting [g x s]	X	11537^{b}	8240^{a}	11446^{b}	8842^{a}	13007^{c}
	sd	667	543	631	580	651
Hardness [G]	X	110.7^{a}	91.3^{a}	423.2^{d}	352.4^{c}	470.6^{e}
	sd	6.6	5.4	6.1	6.4	4.3
Strength needed for sample disruption [g x s]	X	812.2^{a}	701.9^{a}	3221.4^{c}	2470.5^{b}	3221.5^{c}
	sd	92.5	50.1	34.6	80.1	102.2

*a, b,c – Means within a row do not differ significantly (P>0.05) if they have a common letter or if they have no letters

Table 2

The influence of packaging method on the rheological parameters of beef hamburgers, n = 300

Indicator		Packaging method	
		vacuum	MAP
Cutting force [G]	X	1003.4^{b}	875.8^{a}
	sd	17.1	21.4
Strength needed for sample crosscutting [g x s]	X	11727^{b}	9502^{a}
	sd	428	310
Hardness [G]	X	270.4^{a}	308.9^{b}
	sd	4.2	6.8
Strength needed for sample disruption [g x s]	X	1817^{a}	2313^{b}
	sd	121	142

Table 3

The influence of storage temperature on the rheological parameters of beef hamburgers, n = 300

Indicator		Storage temperature [°C]	
		+3	-3
Cutting force [G]	X	920,1^{a}	959,0^{b}
	sd	11,2	15,4
Strength needed for sample crosscutting [g x s]	X	10655	10574
	sd	428	514
Hardness [G]	X	276,3^{a}	303,1^{b}
	sd	4,2	3,8
Strength needed for sample disruption [g x s]	X	2057	2073
	sd	121	110

The results of the study revealed characteristic effects of applied heating methods on rheological properties of beef hamburgers (Tab. 4). The highest values of rheological parameters were analysed in microwaved hamburgers. Probably, the shortest time of heating used in this method caused the lowest level of thermohydrolysis of connective tissue, what resulted in higher resistance of hamburgers during rheological measurements. Preparation of hamburgers by steaming or baking had similar effects on rheological parameters of the material, however the reasons of these changes were probably different. During steaming (method 2) collagen was intensively thermohydrolised, what resulted in lower values of analysed rheological parameters. Whereas, during baking (method 3), because of longer time of heating and higher temperature, apart from the extent of collagen thermohydrolysis, lipids releasing from cell structures could be a reason of better rheological properties of the hamburgers. Our results were different from those presented by Lee et al. [2005], whose got higher hardness of hamburgers treated with conventional thermal methods.

Table 4

The influence of heating method on the rheological parameters of beef hamburgers, n = 120

Indicator		Heating method		
		microwaving	steaming	baking
Cutting force [G]	X	987.8^{b}	916.0^{a}	914.9^{a}
	sd	13.5	15.3	12.7
Strength needed for sample crosscutting [g x s]	X	10696	10260	10887
	sd	524	320	317
Hardness [G]	X	298.0^{b}	291.0^{ab}	279.9^{a}
	sd	5.1	6.4	7.8
Strength needed for sample disruption [g x s]	X	2206	2059	1930
	sd	149	127	130

MAP packaging technology resulted in a tendency to higher stores of intensity and desirability of colour evaluated by sensory analysis (Tab. 6). Probably vacuum packed hamburgers were exposed to gluing and thus wetting the surface, which consequently could result in a partial colour change. Storage of the product at lower temperature (of about 6°C) resulted in a higher grades for colour intensity and desirability, which according to the rule of van't Hoff, could be connected with lower changes in haem colourants.

According to many authors low temperatures should be used either during food production or during storage of final products [Feiner, 2006; Przybył et al., 1998].

Results of our study showed that storage of beef hamburgers for 28 days did not influence the sensory analysis of colour intensity and desirability (Tab. 5). Heating method, within all experimental factors used in the study, had the greatest impact on the hamburgers colour. The most positive effects were observed for microwaving (Tab. 7). Lighter colour of the hamburgers, which was not desired effect, was observed after steaming.

Odour intensity was another feature in sensory analysis of beef hamburgers. Positive effects on odour intensity were observed when the hamburgers were vacuum packed. However, type of the applied packaging method was not influenced odour desirability of the product. Similarly there was no significant differences in both analysed odour indicators between hamburgers stored at different temperatures. However, tendency to higher notes was

observed for the hamburgers stored at -3°C. Odour intensity was not influence by the time of storage, but within different time storage sequences tendency to lowering scores of analysed indicator was observed (Tab. 5). The heating method had varying effects on evaluated odour parameters. Baked hamburgers were characterized by tendency to higher scores in sensory odour analysis. Steaming resulted in lower odour desirability in relation to other heating method. It can be explained that volitaile compounds were removed from the hamburgers by hot steam.

Packaging of beef hamburgers under MAP had a positive effects on the product flavor intensity. However, higher flavor desirability was observed for vacuum packed products. The hamburgers stored at lower temperature were characterised by tendency to higher scores of flavour intensity and desirability. Storage of the products at the temperature +3°C resulted in lower scores, which was caused by higher extension of undesirable changes, such as oxidative and hydrolytic processes in lipids. Flavour intensity decreased during storage (Tab. 5), however flavour desirability was not influence by the time of storage. The highest changes in the hamburgers flavour intensity were observed when microwaves were applied during heating. There was no differences in flavour desirability in relation to heating methods. Ozkan et al. [2004] reported higher sensory stores for hamburgers heated with conventional methods.

Table 5

Sensory properties of the hamburgers in relation to storage period [points], n = 72

Sensory properties		Storage period [days]				
		0	7	14	21	28
Colour – intensity	X	4.2	4.4	4.4	4.7	4.3
	sd	0.3	0.2	0.1	0.2	0.1
Colour – acceptability	X	4.3	4.5	4.3	4.3	4.8
	sd	0.2	0.1	0.08	0.07	0.12
odour – intensity	X	4.3	4.4	4.4	4.3	4.9
	sd	0.2	0.1	0.1	0.3	0.3
odour – accptability	X	4.3^{a}	4.5^{b}	4.2	4.2^{a}	4.2^{a}
	sd	0.1	0.1	0.1	0.17	0.20
flavour – intensity	X	5.0^{d}	4.5^{c}	4.4^{b}	4.0^{a}	4.3^{b}
	sd	0.1	0.18	0.21	0.15	0.12
flavour – acceptability	X	4.9	4.5	4.9	5.0	4.6
	sd	0.5	0.01	0.02	0.01	0.02
Juiciness	X	4.2^{a}	4.4^{b}	4.4^{b}	4.2^{a}	4.3^{a}
	sd	0.05	1.37	0.98	1.63	1.84
Texture	X	4.9^{b}	4.4^{a}	4.2^{a}	4.2^{a}	4.2^{a}
	sd	0.01	0.01	0.02	0.01	0.02
Saltiness – intensity	X	5.0^{b}	4.7^{a}	4.7^{a}	4.7^{a}	4.8^{ab}
	sd	0.03	0.07	0.08	0.13	0.24
Saltiness – acceptability	X	5.0^{b}	4.3^{a}	4.6^{ab}	4.7^{ab}	4.8^{ab}
	sd	0.26	0.01	0.02	0.01	0.02
General sensory analysis	X	4.5	4.5	4.3	4.3	4.5
	sd	0.2	0.3	0.1	0.3	0.1

Different methods of packaging as well as storage temperature did not affect juiciness of beef hamburgers (Tab. 5). Storage of the hamburgers resulted in increasing juiciness in relation to control sample. Heating method significantly influenced the hamburgers juiciness. As was predicted, the highest juiciness was observed for steamed hamburgers.

Lower values of the cutting force and strength needed for product disruption analysed for vacuum packed hamburgers were positively correlated with tendency to higher scores of sensory evaluated texture. However, data collected during rheological analysis of the hamburgers for the cutting force and strength needed for product crosscut were not correlated with the results of sensory analysis. It can be stated that relationship exist between penetrometric analysis and sensory evaluated texture of the hamburgers, whereas no relationship was observed between sensory analysis and mechanical properties of the product measured by cutting force and strength needed for product crosscut. There was no correlation between sensory evaluated texture and rheological parameters of the hamburgers stored at different temperature. However, beef hamburgers stored at -3°C were characterised by tendency to higher scores of sensory evaluated texture, whereas mechanical analysis revealed higher values of rheological parameters. Similarly to rheological analysis the hamburgers stored for 14 days got lower sensory scores for texture parameter, which was connected with higher hardness caused probably by surface drying. Differential values obtained for rheological parameters of the thermally treated hamburgers were not correlated with sensory evaluated texture.

Table 6

Sensory properties of the hamburgers in relation to packaging method and storage temperature [points], n = 180

Sensory properties		Packaging method		Storage temp. [°C]	
		vacuum	MAP	+3	-3
Colour – intensity	X	4.3	4.5	4.3	4.5
	sd	0.2	0.2	0.1	0.1
Colour – acceptability	X	4.3	4.6	4.3	4.6
	sd	0.2	0.15	0.08	0.07
odour - intensity	X	4.6	4.3	4.5	4.4
	sd	0.2	0.2	0.1	0.3
odour – accptability	X	4.3[a]	4.3	4.2	4.4
	sd	0.1	0.04	0.11	0.17
flavour – intensity	X	4.4[a]	4.5[b]	4.4	4.5
	sd	0.03	0.18	0.21	0.03
flavour – acceptability	X	5.0	4.7	4.6	5.0
	sd	0.3	0.01	0.03	0.01
Juiciness	X	4.3	4.3	4.3	4.5
	sd	0.03	0.3	0.13	0.03
Texture	X	4.6[b]	4.2 [a]	4.3	4.5
	sd	0.13	0.01	0.03	0.01
Saltiness – intensity	X	4.8	4.8	4.7	4.8
	sd	0.03	0.3	0.03	0.1
Saltiness – acceptability	X	4.5	4.3	4.3	4.5
	sd	0.16	0.01	0.16	0.01
General sensory analysis	X	4.5	4.3	4.2	4.3
	sd	0.2	0.3	0.19	0.03

Table 7

Sensory properties of the hamburgers in relation to heating method [points], n = 120

Sensory properties		Heating method		
		microwaving	steaming	baking
Colour – intensity	X	4.8[b]	4.0[a]	4.4[ab]
	sd	0.2	0.1	0.2
Colour – acceptability	X	4.6	4.3	4.4
	sd	0.19	0.08	0.16
odour – intensity	X	4.4	4.4	4.5
	sd	0.2	0.1	0.2
odour – accptability	X	4.5[b]	3.9[a]	4.5[b]
	sd	0.04	0.2	0.1
flavour – intensity	X	4.5[b]	4.4[a]	4.4[a]
	sd	0.04	0.12	0.10
flavour – acceptability	X	4.8	4.9	4.8
	sd	0.3	0.2	0.2
Juiciness	X	4.2[a]	4.7[c]	4.0[a]
	sd	0.2	0.1	0.1
Texture	X	4.4	4.4	4.4
	sd	0.16		
Saltiness – intensity	X	4.8[b]	4.7[a]	4.9[b]
	sd	0.03	0.2	0.1
Saltiness – acceptability	X	4.2[a]	4.8[b]	4.2[b]
	sd	0.02	0.1	0.2
General sensory analysis	X	4.5	4.5	4.5
	sd	0.2	0.1	0.2

Packaging method, storage temperature as well as time of the storage did not affect intensity and desirability of the hamburgers saltiness. In relation to control samples stored hamburgers were characterised by lower intensity and desirability of saltiness. The lowest saltiness intensity was analysed for steamed hamburgers, which was probably caused by the partial removal of NaCl by hot steam. This resulted also in the highest saltiness desirability.

Sensory analysis of overall acceptance of beef hamburgers revealed that there was a tendency to higher scores for vacuum packed products stored at -3°C. Overall acceptance of the hamburgers was not affected by heating method as well time of the storage. An average value of overall acceptance equalled 4.5 points. Advers results were reported by Ozkan et al. (2004), whose found the highest overall acceptability for hamburgers treated with conventional thermal methods.

Conclusions

The use of vacuum packaging method for beef hamburgers resulted in higher cutting force and strength needed for sample crosscut values. Hamburgers packed under MAP were characterised by higher hardness and strength needed for sample disruption values. There

was clear tendency in higher acceptability of taste and flavor intensity of the vacuum packed hamburgers stored at the temperature close to cryoscopic point (-3°C). Sensory analysis of the hamburgers texture was correlated with penetrometric parameters, whereas no relationship existed between sensory analysis and cutting force values. Strong tendencies to higher scores in sensory evaluation were observed for vacuum packed beef hamburgers stored at the temperature close to cryoscopic point (-3°C).

References

Lee C-H., Jeong J-Y., Lee E-S., Choi J-H., Choi Y-S., Kim J-M., Kim C-J., 2005. Effects of cooking method on physicochemical characteristics and qualities of hamburger patties. Korean J. Food Sci. Anim. Resour., 25, 149–155.

Erdogdu F., Zorilla S.E., Singh R.P., 2005. Effects of different objective functions on optimal decision variables: a study using modified complex method to optimize hamburger cooking. LWT–Food Sci. Technol., 38, 111–118.

Feiner G., 2006. Meat products handbook. CRC Press, New York.

Hildrum K.I., Nielsen B.N., Wahlgren M., 2002. Improving the tenderness of hot boned beef muscles. Fleischwirtsch. Int., 1, 42–48.

Hwang I.H., Gee A., Polkinghhorne R., Thompson J., 2002. The effect of different pelvic hanging techniques on meat quality in beef. Proceed. 48th ICoMST, Rome 1, 220–221.

Park K-S., Kim J-G., Lee J-W., Oh S-H., Lee Y-S., Kim J-H., Kim J-H., Kim W-G., Byun M-W., 2004. Effects of combined treatment of gamma irradiation and addition of rosemary extract powder on ready-to-eat hamburger steaks: 2. Improvement in quality. J. Korean Society of Food Sci. Nutr., 33, 694–699.

Ozkan N., Ho I., Farid M,. 2004.Combined ohmic and plate heating of hamburger patties: quality of cooked patties. J. Food Eng., 63, 141–145.

Palka K., 2003. The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. Meat Sci., 64, 191–198.

PN-ISO 6658.,1998. Analiza sensoryczna. Metodologia. Wytyczne ogólne.

Pospiech E., Grześ B., Łyczyński A., Borzuta K., Szlata M., Mikołajczyk B., 2003. Muscle proteins and their changes in the process of meat tenderization. Anim. Sci. Papers and Raports, 21, 133–151.

Purslow P., 2004. Intramuscular connective tissue and its role in meat quality. Meat Sci., 70, 435–447.

Prokopp Hautrive T., Ruffo de Oliveira V., Roque Dutra da Silva A., Nascimento Terra N., Bastianello Campagnol P.C., 2008. Physicochemical and sensorial analyses of ostrich hamburger. Ciencia e Tecnologia de Alimentos, 28, 95–101.

Przybył H., Cwiertniewski K., Egierski K., 1998. Keeping quality of vacuum packaged hamburgers. Chłodnictwo, 33, 44–46.

Souza Tavares R., Gomes da Cruz A., Silva de Oliveira T., Braga A., Almeida dos Reis F., Carvalho da Hora I. Costa Teixeira R., Ferreira E., 2007. Processing and sensorial acceptance of rabbit hamburger. Ciencia e Tecnologia de Alimentos, 27, 633–636.

Szmańko T., Korzeniowska M., Wierzbicka E., Kawałko R., 2009. Cechy technologiczne hamburgerów w zależności od warunków pakowania i przechowywania oraz sposobu ogrzewania. In press.

Tornberg E., 1996. Biophysical aspects of meat tenderness. Meat Sci., 43, 175–191.

5

TECHNOLOGICAL PROPERTIES OF HAMBURGERS IN RELATION TO PACKAGING AND STORAGE CONDITIONS, AS WELL AS HEATING PROCEDURE

Introduction

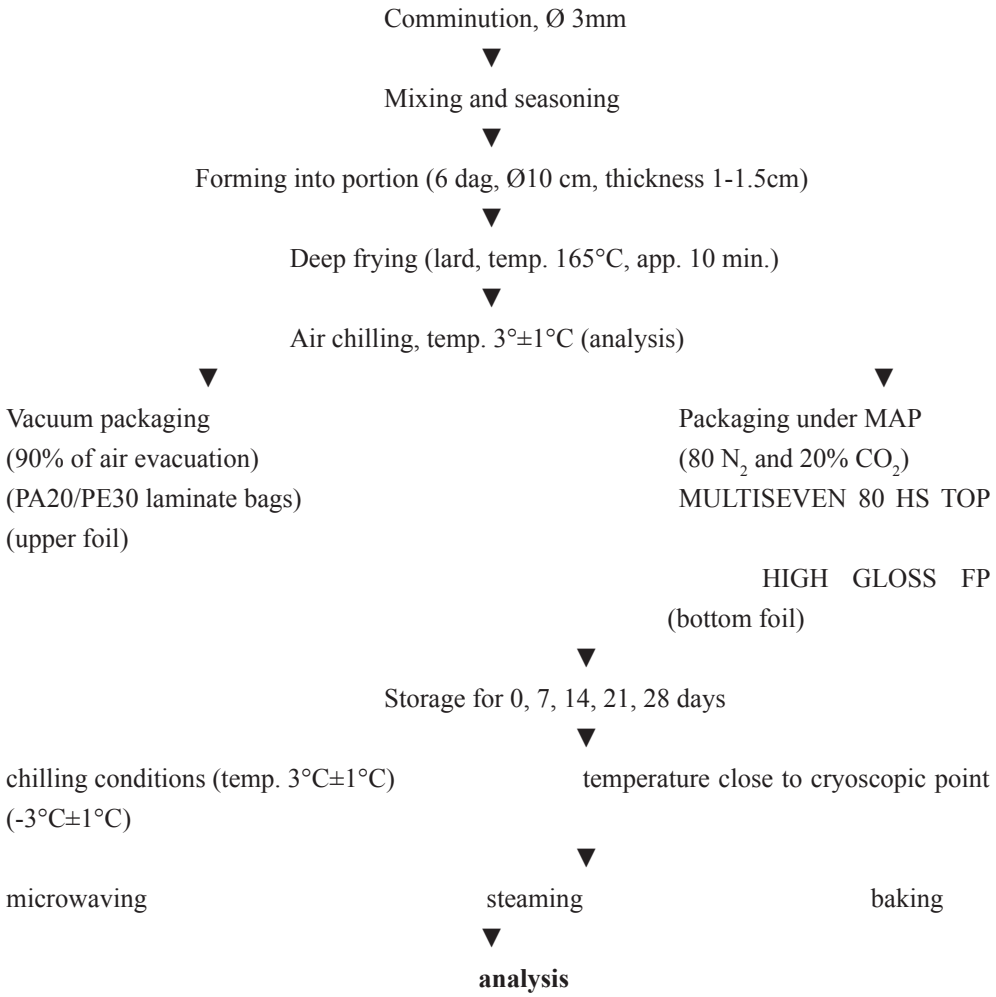
The demand for convenient food will grow continuously [Richards and Padilla, 2009]. The growing competition on the market will effect in selling only the test products, the ones that meet high expectations of consumers [Prokopp et al.,2008; Souza et al., 2007]. The conditions of production, preservation, storage, as well as preparation for consumption should correspond with high quality foods like traditional one [Han, 2005; Schellekens, 1996]. Among the important properties of culinary products the most crucial is possibility to apply different thermal treatment method. Moreover, comfortable, a very well matched packaging, both for individual as well as aggregated food products, is important factors in quality evaluation [Robertson, 1993; Han, 2005; Chan-Ho Lee et al., 2005; Ozokan et al., 2004].

Hamburgers are without any doubt the most popular meat products belong to convenient group. Despite the fact that they are produced for more than 250 years, due to the development of new technologies, facilities, as well as implementation of new raw materials, semi-products and some changes in the idea of food and nutrition sciences, technology of hamburgers has to be still improved [Earle et al., 2007; Komatsu, 2001; Mater, 2002]. Raw material composition of the hamburger formula is the parameter modified relatively often. Nowadays, they are produced from all types of meat [Prokopp et al., 2008; Souza et al., 2007]. The key issue in the hamburger production is the selection of appropriate packaging materials and method, which should be convenient and quality protective [Komatsu, 2001; Mater, 2002]. The other crucial aspects in hamburger formulation is an optimalisation of storage conditions as well as heating method [Chan-Ho Lee et al., 2005; Ozokan et al., 2004].

The objective of the study was to evaluate the technological properties of beef hamburgers in relation to packaging method, storage conditions and heating procedure.

Materials and methods

Materials used in the study were beef hamburgers manufactured in industrial conditions according to the commercial formula and technology. The hamburgers were formulated from beef (class III and IV), salt and crumbs according to the scheme:



The experimental material came from 3 separated technological productions carried out in two-weeks intervals. Chilled hamburgers were packed in 10 pieces using:

- vacuum packaging with laminated bags PA20/PE80 (90% of air evacuation was applied),
- packaging under inert gasses (MAP: 80% N_2, 20% CO_2) using laminated bags with two layers: MULTISEVEN 80 HS TOP (upper layer) and HIGH GLOSS FP (bottom layer).

Then packaged hamburgers were stored at two different storage conditions for 0, 7, 14, 21, 28 days:

- chilling storage (+3°C±1°C),
- storage at the temperature close to cryoscopic point (-3°C±°C), cryoscopic temperature for hamburgers was -2,33°C.

After storage hamburgers were thermally treated in order to prepare for consumption using:

- heating in microwave oven SHARP, GRILL MICROWAVE OVEN type R-6R71, λ 2450 MHz, 1,2 kW (1). Hamburgers were thermally treated for 2 min 10 sec.

– heating in steam at the temp. 100°C (2) for 6 min.,
– baking in an electric oven at the temp. 170°C (3) for 7 min.

All samples were heated up to temp. 70°C was reached in the geometrical centre of the product. The temperature after removing from the oven increased to 73°C ±1°C. Processing conditions were established in preliminary experiment.

Analyses of technological parameters of the hamburgers performer in the study were as followed: protein (PN-75/A-04018) and NaCl (PN-73/A-82112) contents, pH (using Microcomputer CP-551), water holding capacity (WHC) by Grau-Hamm with the modification of Szmańko [Szmańko, 1986], colour parameters (L^*, a^*, b^* using CR-200b reflectometer Minolta), changes in the hamburgers dimensions after heating (the hamburgers surface and thickness) expressed as a difference in specific dimension before and after heating. Moreover, deformation degree [%] (height of the highest point of the product) and heating shrinkage [%] as a difference in weight before and after thermal treatment.

All data collected in the experiment were statistically analysed using STATGRAPHICS ver. 5.0 at the probability level $p>0.05$.

Results and disscussion

The results of the study showed that experimental factors such as storage temperature, packaging and heating methods did not affect protein content in the hamburgers. An average protein content in experimental material was about 14%±0.1, which represented high degree of repetitiveness of raw materials. This was not comparable with the results reported by Lee et al. [2005], whose showed lower protein content in microwaved comparing to baked hamburgers.

Salt (NaCl) content in the experimental hamburgers was dependent only on heating method. The lowest salt content (3.99%) was analysed in steamed hamburgers, whereas the highest (4.42%) in baked products. It can be explained by hot steam extraction of NaCl from the hamburgers surface.

Changes in hamburgers shape during heating is one of the most important factors taking into account in the analysis of technological process correctness [Lee et al., 2005]. Storage under MAP resulted in bigger area of heated beef hamburgers in comparison to vacuum packed products (Tab. 1). Packaging method did not influence thickness and deformation degree of thermally treated hamburgers. However, it was noticeable that weight loss analysed for heated hamburgers was almost of about 1.5% lower when stored under MAP.

Storage temperature applied in the study (+3°C, -3°C) did not affect technological properties of beef hamburgers i.e. changes in the hamburgers area, thickness, deformation degree as well as weight loss after heating (Tab. 2).

The most significant impact on technological properties of the hamburgers was observed for different heating method (Tab. 3). The highest increase in the hamburgers area was caused by microwaving. It was probably the effect of simultaneous heating of the whole mass of the product. Increase in the volume of air trapped in micropores of stuffing resulted in rising of all dimensions of the hamburgers. Lower changes in the hamburgers area were observed when heating in hot steam or hot air conditions. In baked hamburgers, characterised by the lowest changes in the area, crusty layer on the surface was observed, which protected the products shape against changes.

Table 1

The effect of packaging method on technological properties of beef hamburgers after thermal treatment, n = 300

Indicator			Packaging method	
			vacuum	MAP
Changes in the hamburger area [%]		X	7.34[a*]	10.17[b]
		sd	0.44	0.40
Changes in the hamburger thickness [%]		X	3.19	3.17
		sd	0.17	0.15
Percentage of deformated hamburgers [%]		X	26.90	23.11
		sd	2.15	1.90
Weight loss [%]		X	5.63[b]	4.15[a]
		sd	0.29	0.21
L*	After storage	X	54.53[b]	53.16[a]
		sd	0.27	0.20
	After storage and heating	X	44.32	45.55
		sd	0.98	0.87
a*	After storage	X	8.45	9.03
		sd	0.27	0.20
	After storage and heating	X	11.64[b]	11.35[a]
		sd	1.12	0.91
b*	After storage	X	20.04	20.23
		sd	0.09	0.07
	After storage and heating	X	20.06	19.93
		sd	0.12	0.07
pH	After storage and heating	X	6.0[a]	6.1[b]
		Sd	0.01	0.02
WHC [%]	After storage and heating	X	79.48	79.50
		sd	0.21	0.18

[*]a, b,c – Means within a row do not differ significantly (P>0.05) if they have a common letter or if they have no letters

The highest increase in the hamburgers thickness was observed for steamed products, which was the consequence of hot steam operations on the whole product. Due to intensive heating increase in volume of trapped air as well as thermohydrolysis of collagen occurred. Microwaved hamburgers were characterized by the lowest degree of shape deformation among all analysed thermal treatments (Tab. 3). Our results were in a good agreement with those reported by Lee et al. [2005], whose found that microwaving caused the lowest deformation of the hamburgers.

Heating method had significant influence on weight loss of beef hamburgers. The highest weight loss after heating was observed for microwaved hamburgers, which was connected with intensive and uniform heating in the whole mass of the product. High level of weight loss during baking was the result of fat rendering. Steamed hamburgers were characterized by more than 2-fold lower weight loss due to collagen thermohydrolysis and high moisture environment during processing.

Table 2

The effect of storage temperature on technological properties of beef hamburgers after thermal treatment, n = 300

Indicator			Storage temperature [°C]	
			+3	-3
Changes in the hamburger area [%]		X	9.04	8.47
		sd	0.44	0.41
Changes in the hamburger thickness [%]		X	3.07	3.29
		sd	0.19	0.14
Percentage of deformated hamburgers [%]		X	25.09	24.92
		sd	2.15	1.81
Weight loss [%]		X	5.21	4.57
		sd	0.29	0.20
L^*	After storage	X	54.87^b	52.82^a
		sd	0.27	0.21
	After storage and heating	X	45.39	44.48
		sd	0.98	0.96
a^*	After storage	X	8.51	8.97
		sd	0.27	0.21
	After storage and heating	X	11.64	11.40
		sd	0.12	0.15
b^*	After storage	X	19.97^a	20.30^b
		sd	0.09	0.07
	After storage and heating	X	20.03	19.96
		sd	0.12	0.10
pH	After storage and heating	X	6.1^b	6.0^a
		sd	0.01	0.01
WHC [%]	After storage and heating	X	78.46^a	80.53^b
		sd	0.21	0.18

As far as extending the period of beef hamburgers storage systematic tendency to increase their thickness after heating was noticed. Those changes were probably caused by higher susceptability to swelling the outer layer of the burgers, which was soften in higher degree during storage in comparison to those stored for shorter time. Applied storage periods did not effect the degree of deformation of experimental material (Tab. 4).

An average reaction (pH) of the hamburgers was about 6.0±0.01. Packaging and heating methods as well as storage temperature and time did not influence pH of beef burgers.

There was no significant impact of packaging method and storage temperature on water holding capacity (WHC) of the hamburgers. However, stored burgers were characterized by lower WHC than control samples. As was predicted heating method effected the ability to hold water by the hamburgers. The lowest WHC was analysed for steamed burgers, whereas the highest for the products heated in microwave oven. Lower water holding capacity of steamed hamburgers was correlated with significant decrease in NaCl concentration, due to

this substance improves the ability to hold water by meat and its products [Szmańko, 1998], as well as their durability [Przybył et al., 1989].

Table 3

The effect of heating method on technological properties of beef hamburgers, n = 120

Idicator			Heating method		
			microwaving	steaming	baking
Changes in the hamburger area [%]		X	10.83^{c}	9.12^{b}	6.32^{a}
		sd	0.54	0.38	0.27
Changes in the hamburger thickness [%]		X	3.08^{a}	3.86^{b}	2.60^{a}
		sd	0.24	0.20	0.17
Percentage of deformated hamburgers [%]		X	22.07	26.76	26.19
		sd	2.63	2.40	1.80
Weight loss [%]		X	6.48^{c}	2.86^{a}	5.34^{b}
		sd	0.36	0.27	0.30
L^{*}	After storage	X	53.84^{B**}	53.84^{B}	53.84^{B}
		sd	0.33	0.26	0.34
	After storage and heating	X	46.16^{bA}	45.87^{abA}	42.77^{aA}
			1.21	0.91	1.10
a^{*}	After storage	X	9.07^{A}	9.07^{A}	9.07^{A}
		sd	0.33	0.18	0.31
	After storage and heating	X	11.42^{aB}	11.23^{aB}	11.89^{bB}
		sd	0.14	0.11	0.15
b^{*}	After storage	X	20.13	20.13	20.13
		sd	0.11	0.14	0.06
	After storage and heating	X	19.85^{a*}	20.35^{b}	19.78^{a}
		sd	0.15	0.11	0.15
pH	After storage and heating	X	6.05	6.05	6.05
		sd	0.01	0.01	0.02
WHC [%]	After storage and heating	X	81.18^{c}	77.37^{a}	79.92^{b}
		sd	0.25	2.16	1.27

*a, b,c – Means within a row do not differ significantly (P>0.05) if they have a common letter or if they have no letters.

***A,B,C – Means within a column do not differ significantly (P>0.05) if they have a common letter or if they have no letters

The method of packaging of the hamburgers had only slight effect on colour parameters of stored and thermally treated hamburers (Tab. 1). Vacuum packed products were characterised by fighter colour after storage, as well as heating resulted in higher intensity of red colour compounds. There was no influence of packaging method and thermal treatment on values of b^{*} colour parameter either in stored or heated hamburgers. Products stored at the temperature close to cryoscopic point (-3°C) were characterised by darker colour with tendency to higher red and yellow parameters values in reflectance spectrum (Tab. 2). This tendency was also observed for L^{*} parameters after thermal treatment.

Due to positive effects of the temperature close to cryoscopic point on physicochemical properties and durability of the hamburgers it is applied in selected production operations [Feiner, 2006].

Table 4

The influence of storage period on technological properties of beef hamburgers analysed after heating, n = 120

Indicator		Storage period [days]				
		0	7	14	21	28
Changes in the hamburger area [%]	X	8.11^{a}	8.44^{ab}	9.06^{ab}	10.19^{b}	7.97^{a}
	sd	0.70	0.67	0.71	0.54	0.40
Changes in the hamburger thickness [%]	X	1.87^{a}	3.35^{b}	3.01^{b}	3.05^{b}	4.62^{c}
	sd	0.31	0.15	0.08	0.07	0.12
Percentage of deformated hamburgers [%]	X	24.60	25.05	25.00	25.10	25.25
	sd	2.1	1.8	1.6	1.9	2.0
L	X	42.63^{a}	50.49^{b}	44.32^{a}	42.94^{a}	44.30^{a}
	sd	1.56	1.40	1.11	1.43	1.30
a^{*}	X	11.00^{b}	13.65^{c}	10.97^{ab}	10.47^{a}	11.47^{b}
	sd	0.18	0.10	0.11	0.17	0.20
b^{*}	X	16.40^{a}	19.39^{c}	16.90^{a}	18.79^{b}	18.48^{b}
	sd	0.2	0.18	0.21	0.15	0.12
pH	X	6.0^{a}	6.2^{b}	6.0^{a}	6.0^{a}	6.0^{a}
	sd	0.01	0.01	0.02	0.01	0.02
WHC [%]	X	81.43^{c}	79.75^{b}	78.05^{a}	78.45^{a}	79.77^{b}
	sd	1.33	1.37	0.98	1.63	1.84

Beef hamburgers baked after storage was darker than control samples, as well as than microwaved products (Tab. 3). This was also expressed by higher values of a* colour parameter (redness) obtained for baked hamburgers in relation to products heated using other methods. Applied heating methods did not influence the intensity of yellow colour in reflectance spectrum. Storage period had inconclusive effects on the hamburgers color (Tab. 4). There were only slight differences in colour parameters values within all storage periods, which were probably caused by small differences in raw materials compositions like darker colour of beef or changes during technological process. Moreover, Sayago-Ayerdi et al. [2009] explained changes in L^{*}, a*, b* values by oxidation processes occurred in hamburgers.

Conclusions

The hamburgers stored under MAP were characterised by lower weight loss comparing to vacuum packed products. Lighter colour with lower red shade contribution was observed in vacuum packed hamburgers. Products stored at the temperature -3°C, as well as baked one was characterised by darker colour with higher contribution of red shade. Steaming of the hamburgers resulted in almost two fold lower weight loss in comparison to other heating methods applied in the study.

References

Lee C-H., Jeong J-Y., Lee E-S., Choi J-H., Choi Y-S., Kim J-M., Kim C-J., 2005. Effects of cooking method on physicochemical characteristics and qualities of hamburger patties. Korean J. Food Sci. Anim. Resour., 25, 149–155.

Earle M., Earle R., Anderson A., 2007. Opracowanie produktów spożywczych. WNT. Warszawa.

Feiner G. 2006. Meat products handbook. CRC Press, New York.

Han J.H., 2005. Innovations in food packaging. Elsevier Academic Press. London.

Komatsu S., 2001.Method of making plate-like frozen raw hamburger-like matter packaged by film. United States Patent. US 6 254 911 B1.

Mater Y., 2002. Pack, especially for packaging of hamburgers, made from a prefolded glued blank. French Patent Application. FR 2 824 810 A1.

Ozkan N., Ho I., Farid M., 2004.Combined ohmic and plate heating of hamburger patties: quality of cooked patties. J. Food Eng., 63, 141–145.

PN -73/A-82112.,1973 Mięso i przetwory mięsne. Oznaczanie zawartości soli kuchennej.

PN-75/A04018., 1975. Produkty rolniczo-żywnościowe. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko.

Hautrive P.T., de Oliveira R.V., da Silva R.D.A., Terra N.N., Campagnol B.P.C., 2008. Physicochemical and sensorial analyses of ostrich hamburger. Ciencia e Tecnologia de Alimentos, 28, 95–101.

Przybył H., Cwiertniewski K., Egierski K., 1998. Keeping quality of vacuum packaged hamburgers. Chłodnictwo, 33, 44–46.

Richards T., Padilla L., 2009. Promotion and fast food demand. Am. J. Agric. Economics, 91, 168–183.

Robertson G.L., 1993. Food packaging. Marcel Dekker, Inc. New York. Basel. Hong Kong.

Sayago-Ayerdi S.G., Brenes A., Goni I. 2008. Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. LWT- Food Sci. Technol., 42, 971–976.

Schellekens M., 1996. New research issues in sous-vide cooking. Trends in Food Sci. Technol., 7, 256–262.

Tavares S.R., da Cruz G.A., de Oliveira S.T., Braga A., dos Reis A.F., da Hora C.I., Teixeira C.R., Ferreira E., 2007. Processing and sensorial acceptance of rabbit hamburger. Ciencia e Tecnologia de Alimentos.27, 633–636.

Szmańko T., 1986. Urządzenie do pomiaru zdolności utrzymywania wody. Prawo Ochronne nr 40767. Biuletyn Urzędu Patentowego RP.36.

Szmańko T., 1998. Ocena efektywności przechowywania wędzonek w temperaturze bliskiej krioskopowej oraz w stanie zamrożonym. Zesz. Nauk AR Wroc. 334, Rozpr. 154 1–124.

6

EFFECT OF THERMAL PROCESSING AND VEGETABLE OIL ADDITION ON FUNCTIONAL PROPERTIES OF MODEL MEAT PRODUCTS

Introduction

Thermal processing affects production costs, weight losses and sensory attributes and thus has a general effect on the quality of processed meats.

Cooking has a large effect on textural properties of meat. This method of thermal processing is defined as the heating of meat to a sufficiently high temperature to denature proteins. Temperature and duration of cooking process have a large effect on physical properties and eating quality of meat and meat products. As the meat is usually cooked before being eaten, it is important to understand the physical changes of meat texture during heating. The initial increased in meat toughness in temperature range between 40 and 60°C, could be explained by heat denaturation of myofibrillar proteins, especially myosin. The rise in meat toughness from 65 to 80°C could be attributed to the denaturation of intramuscular collagen. High pressure treatment at different temperatures will induce different effects on meat texture since linkages stabilising the secondary, tertiary and quaternary structures of a protein respond differently to heat and pressure. The properties of processed meat products such as tenderness, juiciness and perception of flavors during mastication are based on functional properties of proteins [Ibanoglu, 2005, Tornberg, 2005, Verbeken et al., 2005].

In order to improve the functional properties of meat and meat products, manufacturers are currently using a wide range of vegetable additives, such as vegetable oil.

The objective of this research was to assess the effect of thermal processing and addition of vegetable oil on the functional properties (cooking loss, physical properties), textural profiles (hardness, springiness, cohesiveness, gumminess and chewiness) and sensory characteristics of model meat products.

Materials and methods

The samples of pork meat *(m. longissimus lumborum)* was obtained from Meat Plant "Edward i Grzegorz Dworeccy" placed in Golejowo (Poland). The ingredients used in the production of model meat products included: vegetable oil (OLVIT Sp. z o.o., Gdańsk, Poland), emulsifier 3302-EY21d (Palsgaard, Danmark), curing salt (containing 99.5% sodium chloride and 0.5% sodium nitrite), polyphosphates Hamina-S (ZHU Żuk-Pol, Wro-

cław, Poland), sodium isoascorbinate (ZHU Żuk-Pol, Wrocław, Poland), product "Glutalin" (Prowana "Raps", Sp. z o.o., Poland), sugar (Sugar Factory "Góra Śląska" S.A., Poland).

Table 1

Levels of variables

Content of vegetable oil in final products	Thermal processing		
	75°C	85°C	95°C
0%	A	D	G
3%	B	E	H
5%	C	F	I

Before processing, the meat was portioned and its pH (pH 5.78) was measured. The meat samples were injected with brine solutions to 20% of the raw meat weight. Prior to injection f oil emulsion containing 50% of vegetable oil, 3% emulsifier and 47% water was prepared. . To prepare this emulsion oil and water was heated near to 65°C. Next the ingredients of emulsion were prepared by mixing for 30s using a BÜCHI "MIXER B – 400" (9000 rev/min). The oil emulsion was added to brine solution in the amount of 0, 30 and 50% in order to deliver 0, 3 or 5% of vegetable oil in final products (Tab. 1). After injection, the meat samples were weighed and massaged (PEK-MONT M203). The meat products were massaged for 8h at 4°C (4 rev/min). After massaging, the model meat products were heated in scalding vat (PEK-MONT MRA500) to a final internal temperature of 75, 85 or 95°C. Afterwards the samples were cooled to 14°C in an ice bath and stored overnight at 3°C.

The following properties were determined in final products: pH values (using Microcomputer CP – 551), dry matter content (by thermal drying method in 105°C according to PN-ISO 1442:2000). The modified Grau-Hamm procedure [1957] was used to measure WHC (Water Holding Capacity) of the model meat products, and was expressed as the ratio of moisture retained in the sample to the initial moisture content. Cooking loss was estimated by the equation: weight before cooking minus weight after cooking/weight before cooking x 100. Textural characteristics of meat products were analysed according to the texture profile analysis (TPA 25 test) using a STEVENS-QTS texture analyser. The samples were compressed twice to 25% of their original height at a constant cross – head speed of 60 mm/min. The TPA test parameters: hardness (peak force on first compression [N]), cohesiveness (ratio of the active work done under the second force – displacement curve to that done under the first compression curve [-]), springiness (distance the sample recovered after the first compression [mm]), gumminess (hardness x cohesiveness [N]), chewiness (hardness x cohesiveness x springiness [N x mm]) were computed [Pietrasik, 2003]. Sensory evaluation was carried out using ten points scale of intensity and desirability (from 1 point as very slight to 10 points results as very much desirable). All processed meat products were sensory evaluated by 7 panellists. The fallowing sensory parameters were investigated: colour, aroma, flavour, saltiness, juiciness and fragility. For parameters 4, 5, 6, and 7 both intensity and desirability scale were used.

The data were analysed statistically, using STATISTICA 6.0 software. Response surface methodology (RSM) was used to study the simultaneous effect of the experimental of thermal processing and addition vegetable oil. Significant differences between the mean values were determined using Duncan's test ($\alpha = 0.05$).

Results and discussion

The results of the thermal processing on physical properties of model meat products are presented in Table 2. The pH value of the meat products under investigation ranged from 6.03 to 6.25. The temperature of thermal processing was variable that most influence the pH value. Vegetable oil level had no effect on these properties of the products.

The results obtained in the study showed that the temperature of thermal processing and vegetable oil level was responsible for dry matter content. The measurements of dry matter content in meat products showed that the highest content was observed in model meat products with 5% vegetable oil level and heated at 95°C (sample I, 34.38%) and the lowest in sample A and B (27.21 and 27.81%, respectively).

The water holding capacity ranged from 63.56 to 70.82%. The highest WHC (sample G and I) was observed in meat samples heated at 95°C and the lowest in sample E, one of the samples with the highest pH value.

Cooking loss results are the most important test for the meat industry to predict the behaviour of the products during cooking due to non-meat ingredients or other factors. In the present study cooking losses were not appreciably affected by vegetable oil addition. The amount of cooking loss was 6.1% for temperature of thermal processing 75°C and reached 24.4% with increasing the temperature to 95°C. Thermal losses were mainly due to water and fat loss. These losses depend on the mass transfer process during thermal treatment, which in turn is influenced by the cooking procedure (i.e. final cooking temperature) and the composition of meat systems (i.e. moisture, fat and protein content). The amount of loss is probably related to the composition of muscle, denaturation of proteins by the ionic strength of the extracellular fluid. Denaturation is the change of protein structure during cooking which brings a decrease in diameter and thickness of the protein and so a less juicy and tougher cut [Joo et al., 1999, Westphalen et al., 2005].

Table 2

The effect of thermal processing and vegetable oil level on physical properties of model meat products

Variable	Parameters			
	pH	Dry matter [%]	WHC [%]	Cooking loss [%]
A	6.08^{a}	27.21^{a}	$64.69^{a.b}$	6.1^{a}
B	6.05^{a}	27.83^{a}	$65.16^{a.b}$	$7.4^{a.b}$
C	6.03^{a}	$29.6^{b.c}$	$68.34^{a.b.c}$	6.1^{a}
D	6.16^{b}	31.06^{c}	$64.8^{a.b}$	12.4^{b}
E	6.24^{c}	$29.25^{a.b}$	63.56^{a}	$9.3^{a.b}$
F	6.23^{c}	$33.47^{c.d}$	$69.85^{b.c}$	14.6^{b}
G	6.14^{b}	29.33^{b}	72.34^{c}	23.1^{c}
H	6.18^{b}	$32.02^{c.d}$	$69.65^{b.c}$	20.4^{c}
I	6.25^{c}	34.38^{d}	70.82^{c}	24.4^{c}

a,b,c,d – mean values denoted by various letters and placed in the columns are statistically different at $p \leq 0.05$

Textural properties of model meat products affected by temperature of thermal processing and vegetable oil levels are shown in Figure 1–5.

The hardness values (Fig. 1) measured for model products were higher for temperature of thermal processing 75°C (about 75N). While increasing the temperature to 95°C resulted in lower hardness values (about 50N). The addition of vegetable oil had not influence on hardness.

Cohesiveness was affected by thermal processing and vegetable oil levels in a similar manner to hardness (Fig. 2).

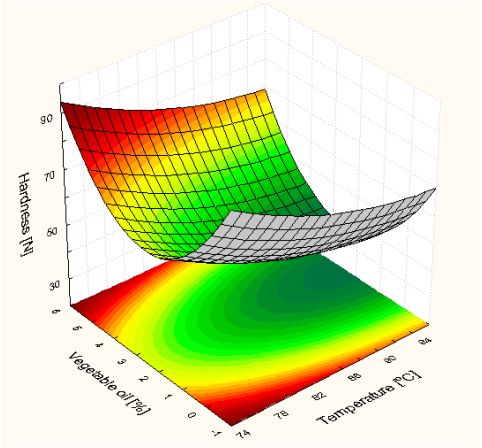


Fig. 1. Effect of thermal process and vegetable oil level on hardness meat products

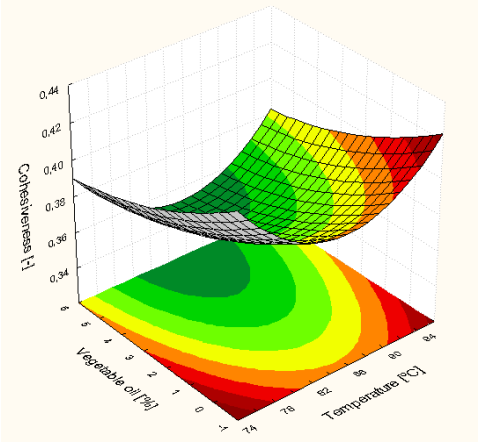


Fig. 2. Effect of thermal process and vegetable oil level on cohesiveness meat products

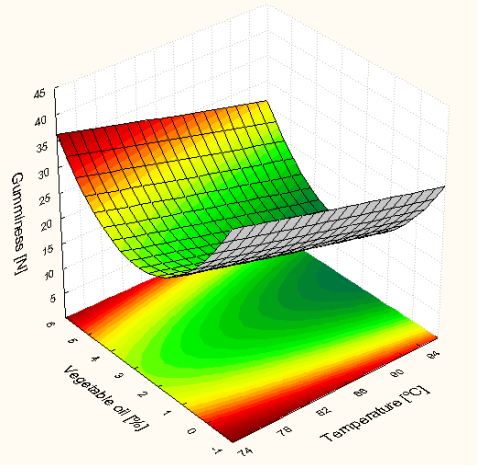


Fig. 3. Effect of thermal process and vegetable oil level on gumminess meat products

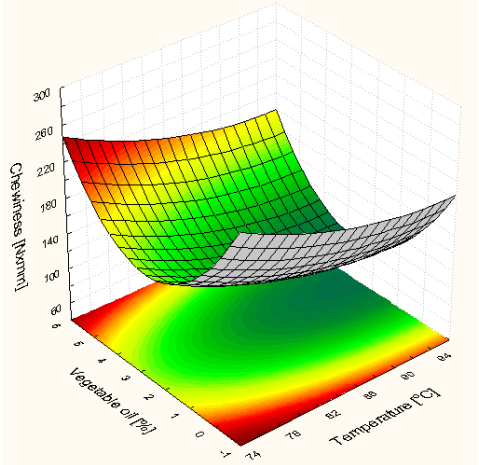


Fig. 4. Effect of thermal process and vegetable oil level on chewiness meat products

In Figure 3 and 4 the TPA test results for gumminess and chewiness are shown. The temperature was a factor that influences these textural attributes of model meat products.

The gumminess values were higher for samples heated in temperature 75 and 85°C comparing to those heated at 95°C. The presence of vegetable oil in meat products resulted in a decrease in their gumminess and chewiness. The behaviour of chewiness was similar to the gumminess.

The results obtained in the study show that the springiness depended on the thermal processing and vegetable oil level (Fig. 5). The springiness values were lower with increasing the temperature to 95°C. Addition of vegetable oil to the meat products resulted in a decrease in their springiness.

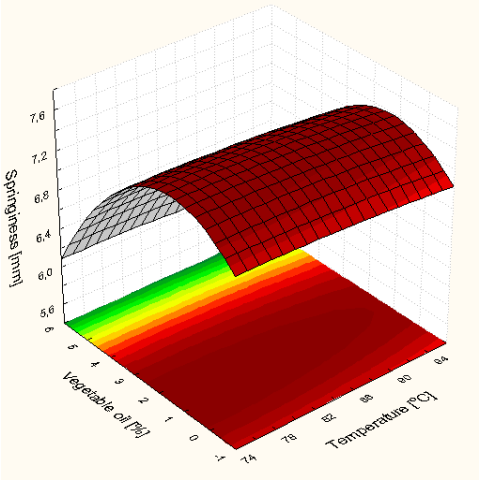


Fig. 5 Effect of thermal process and vegetable oil level on springiness meat products.

Table 3 shows some parameters of sensory evaluation of the model meat products. The temperature of thermal process had a strong impact on textural properties and eating quality of meat. Alongside with raising the temperature there were observed the increase of acceptability model meat products. The higher internal temperature was perceived to maximize the palatability characteristics (colour, aroma, flavour) of cooked meat.

Table 3

Sensory analysis of meat products (points: 1 – minimum, 10 – maximum)

Variable	Parameters					
	Colour	Aroma	Flavour	Saltiness	Fragility	Juiciness
A	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}
B	5.2^{a}	5.4^{a}	5.4^{a}	6.0^{b}	7.0^{b}	6.4^{b}
C	6.6^{b}	5.8^{a}	6.6^{b}	6.0^{b}	6.4^{b}	6.8^{b}
D	6.2^{b}	6.4^{b}	6.8^{b}	5.6^{b}	$7.8^{b.c}$	5.4^{a}
E	6.2^{b}	6.8^{b}	$7.0^{b.c}$	6.6^{c}	$7.6^{b.c}$	7.2^{c}
F	$6.8^{b.c}$	6.8^{b}	$7.0^{b.c}$	6.6^{c}	$7.6^{b.c}$	7.2^{c}
G	7.0^{c}	7.8^{c}	6.8^{b}	6.0^{b}	7.2^{b}	$7.0^{b.c}$
H	$6.6^{b.c}$	$7.4^{b.c}$	7.2^{c}	6.8^{c}	8.0^{c}	$7.0^{b.c}$
I	6.2^{b}	$7.0^{b.c}$	6.6^{b}	6.6^{c}	7.0^{b}	5.6^{a}

a,b,c – mean values denoted by various letters and placed in the columns are statistically significantly different at $p \leq 0.05$

There were observed similar results for desirability of colour. The highest results of intensity and desirability of colour were obtained for sample G (7.0 points). Samples heated at 75°C were characterized by lower score.

Differences of aroma intensity for the investigated samples were statistically significant. The highest notes for intensity and desirability of aroma were noticed also in sample "G" (7.8 points).

Meat products under investigation were characterized great inconstancy also in flavour assessment. The highest scores of flavour evaluation were obtained for sample H and the lowest for reference sample A and sample B.

There were statistically significant differences between results of saltiness, fragility and juiciness of meat products. Average value 8.0 points (sample H) for fragility desirability of investigated products was one of the highest evaluated sensory parameters.

The most of model meat products were classified above 6.0 points for intensity of saltiness.

Average value of juiciness was 7.0 points. Samples heated at 85°C with addition oil emulsion to brine solution obtained the highest notes for saltiness (7.2 points). There was no effect of oil content in the processed meat products on their juiciness.

Conclusions

The results of this research indicate that increased cooking temperature results in lower WHC and cook loss and higher pH and dry matter. Addition of vegetable oil was not effecting in improving quality parameters of final products. There were observed changes in textural profile which were affected by increased temperature. Addition of vegetable oil did not improve significantly sensory quality and textural properties of meat products under investigation.

References

Ibanoglu E., 2005. Effect of hydrocolloids on the thermal denaturation of protein. Food Chem., 90, 621–626.

Joo S.T., Kauffman R.G., Kim B.C., Park G.B., 1999. The relationship of sarcoplasmic and myofibrillar protein solubility too colour and water-holding capacity in porcine longissimus muscle. Meat Sci., 52, 291–297.

Pietrasik Z., 2003. Binding and textural properties of beef gels processed with κ-carrageenan, egg albumin and microbial transglutaminase. Meat Sci., 63, 317–324.

Tornberg E., 2005. Effects of heat on meat proteins – implications on structure and quality of meat products. Meat Sci., 70, 493–508.

Verbeken D., Neirinck N., Van Der Meeren P., Dewettinck K., 2005. Influence of κ-carrageenan on the thermal gelation of salt-soluble meat proteins. Meat Sci., 70, 161–166.

Westphalen A.D., Briggs J.L., Lonergan S.M., 2005. Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. Meat Sci., 70, 293–299.

7

VISCOELASTIC BEHAVIOR OF PROTEIN-CARRAGEENAN BLENDS

Introduction

Carrageenans (CGN) are a family of linear sulphated polysaccharides extracted from red seaweeds, possesing different structure depending on seaweed species. In general, carrageenan is an alternating polymer of β (1-3) – D – galactose – 4 sulfate and 3, 6 – anhydro – D – galactopyranose units (Van de Velde et al., 2001). Depending on quantity of sulfate groups and their linked position, carrageenans differ in their gelation properties. Three main CGN types are: kappa- (κ, gelling), iota- (ι, gelling), and lambda-CGN (λ, nongelling).

Proteins and polysaccharides are biopolymers present together in many food systems and both macromolecules contribute to the structure, texture and stability of foods (DeFreitas et al., 1997; Roesch et al., 2004; Nunes et al., 2006; Verbeken et al., 2006). Behaviour of those kinds of blends Rheological and structural properties of these blends vary with pH, ionic strength, temperature and polymer concentration (Tolstoguzov, 1991).

Carrageenans are thermally reversible hydrocolloids and this is the main reason for their functionality in meat system. Carrageenan dissolves throughout meat during thermal processing and gels on cooling. Those polysaccharides induce changes in thermal properties of meat protein, indicating protein/carbohydrate interaction (DeFreitas et al., 1997). Carrageenan increases water-holding capacity of meat emulsions by holding water in interstitial spaces of the gel network better than chemical substances with proteins. The ability to interact with myofibrillar protein is a result of their anionic nature (Montero and Perez Mateos M., 2002).

Interactions between polysaccharides and muscle proteins occurring in biological systems play a role in determining the functional properties of these systems. The interactions are also important in determining meat products binding and textural properties and have been subject of many investigations [Bernal et al., 1987; Trius and Sebranek, 1996; DeFreitas et al., 1997; Montero et al., 2000). However, the gelation of myofibrillar proteins in combination with carrageenan on the heat induced gelation properties of muscle proteins/carrageenan complexes is not fully understood. A better understanding of the gelation properties of proteins and carrageenan and their interactions in meat systems would contribute to improved utilization and processing qualities of these ingredients in meat products.

The objective of this study was to investigate the effects of varying concentration of carrageenan and myofibrillar proteins on rheological properties of these complexes during heating and cooling.

Materials and methods

Pork meat (*longissimus dorsi* muscle) was obtained from carcasses 48 h *post mortem*. Kappa carrageenan (WG-2000) Amco Poland. Potassium chloride (KCl), sodium chloride (NaCl), disodium phosphate (Na_2HPO_4), potassium dihydrophosphate (KH_2PO_4), ethylenediaminetetraacetic acid (EDTA) and sodium azide (NaN_3) were used to prepare two extraction buffers.

Myofibrillar Protein Extraction

Longissimus dorsi muscle was trimmed from visible fat and connective tissue and ground through a 3 mm plate (Grinder W-82AN, Spomasz). Ground muscle was washed with distilled water (ratio 1:3, v/v, 4 ^{0}C) to remove sarcoplasmic protein and homogenized (Buchi Mixer B – 400) with first extraction buffer containing: 0,1 M KCl, 0,066 M Na_2HPO_4, 0,066 M KH_2PO_4, 1 mM NaN_3, 1 mM EDTA, pH 7,0 (4 ^{0}C) in the ratio of 1 to 4 (v/v). Homogenate was centrifuged at 2000 x g for 10 minutes (Sigma 6K15) and the myofibrillar pellet was then re-suspended in the phosphate buffer, filtered, centrifuged. The procedure was repeated three times. The final pellet of the myofibrils was suspended in 4 volumes of extraction buffer containing: 1 mM NaCl, 1 mM NaN_3. Suspension of protein solution was adjusted to pH = 6,0 with 0,1 M HCl and centrifuged at 9000 x g for 10 minutes. Protein concentration was determined by nitrogen analyzer (Kjeltec 2300 Foss Tecator). Nitrogen was converted to protein via multiplying by 6,25.

Sample Preparation

Myofibrillar protein suspension was diluted to suitable concentration using 2% NaCl solution and mixed by mechanical stirrer at 300 rotate/min for 30 min with polysaccharide powder. The levels of proteins and CGN are shown in Table 1.

After preparation, carageenan – myofibrillar protein blends were kept in 4°C for one hour before subjecting to rheological analysis.

Table 1

Experimental design and variants coding

Variants coding		C		
		0%	0,5%	1%
P	2%	P2/C0	P2/C0,5	P2/C1
	3%	P3/C0	P3/C0,5	P3/C1
	4%	P4/C0	P4/C0,5	P4/C1

P – myofibrillar protein concentration [%]
C – carrageenan concentration [%]

Dynamic Rheological Properties

Dynamic rheological properties of protein/carrageenan mixtures were measured by using a Rheotest 4.1 rheometer (RHEOTEST Messgeräte Medingen GmbH), which was

equipped with a 40 mm parallel plate measuring system. The gap between the parallel plates was set at 1 mm. The sample was heated at a rate of 2°C/min from 16 to 52°C and then cooled back to 16°C, using a programmable temperature-controlled water bath (LKB Bromma 2219 Multitemp II). Oscillatory measurements were made at a fixed frequency of 1 Hz and at constant strain amplitude of 0.1 mNm. For all the tests, the storage modulus (G′), loss modulus (G″), tangent phase angle (tan δ = G″/ G′) during heating (from 16 to 52 C) and cooling (from 52 to 16 C) processes of experimental samples were measured.

Data Analysis. Response surface methodology (RSM) was used to investigate the simultaneous effect of the two experimental factors. This test allows for the calculation of the coefficient estimates of quadratic equation. Polynomials were fitted to the experimental data as follows:

$$Y = \beta 0 + \beta 1x1 + \beta 2x2 + \beta 11x11 + \beta 22x22 + \beta 12x12$$

where Y is the estimated response, β_0, β_1, β_2, β_{11}, β_{22}, β12 are constant and regression coefficients of the model; and x_1, x_2 are levels of independent variables, i.e., the concentrations of protein and carrageenan, respectively.

Statistical analysis was performed using the Software STATISTICA (Version 7.1, Statsoft, Inc.). The significance of the equation parameters for each response variable was assessed by F test $P \leq 0.05$.

Results and discussion

Heat-induced rheological changes in protein/carrageenan mixtures are displayed in Figure 1–3. The storage modulus (G´) of 2 and 3% protein samples without addition of carrageenan remained practically unchanged during heating process.

Reduced gel forming ability of MP may be due to the fact that the protein concentration during dynamic rheological testing may also have been insufficient for gelation to occur.

The storage modulus (G′, elastic response) of 4% protein samples started to increase from the beginning of heating and increased steadily until it reached the first peak at 40°C. Later in the heating cycle the G′ values of the MP decreased sharply and reached a minimum at the end of heating process.

Analysis of variance of phase angle calculated at 42°C showed a significant reduction of phase angle with increase concentration of carrageenan during heating process. The effect of increased protein concentration was dependant of carrageenan level. There was a slight increase in phase angle but only in in samples without carrageenan addition.

The changes in G′ with heating are typical of muscle protein and similar to that observed by Xiong [1993] for chicken myofibrillar proteins. The initial increase in G′ probably resulted from myofibrillar protein structure changes which increased the fluid viscoelasticity without initiating cross-links or aggregates of proteins [Xiong & Blanchard, 1994b].

The decline of G′ between after reaching a maximum value at 40°C has previously been observed for beef myosin [Egelandsdal et al., 1986], chicken myofibrillar proteins [Xiong and Blanchard, 1994a; 1994b] and porcine myofibriallar proteins [Westphalen et al., 2005]. Egelandsdal et al. [1986] suggested that the decrease of G′ was attributed to denaturation of light meromyosin, leading to increased filamental "fluidity". Xiong and Blanchard [1994b]

suggested that some event may have led to the disruption of some protein networks that had already been formed, resulting in a diminished G′.

The lowering of G′ may have resulted from the disruption of hydrogen bonds which supported the αhelix structure with subsequent unfolding of the α-helical structure. This suggests that the unfolding process, which resulted in a double helix-to-single coil transformation of myosin (mainly myosin rods) in NAM, was responsible for weakening the elastic component of the NAM in this temperature range [Wang et al., 2009].

When carrageenan was mixed with MP prior to dynamic rheological testing, the responses for storage and loss modulus were very different and very high values were observed compared to samples without CGN.

Elastic G' and viscous modulus G" of protein gels increased upon adding the carrageenan during the heating (Fig. 1–3) and cooling processes (Table 2, Fig. 10–15). First changes in myofibrillar protein structure were observed shortly after reaching a temperature of 35^0C and maximum peak were showed at temperature around 40^oC in G' and G" changes (Fig. 1–3) during heating [Xiong, 1997].

This indicated that there was a network structure formed in the CGN added sample prior to rheological testing. The earlier onset of gelation for MP/CGN samples when compared to those without its addition suggests that MP/CGN mixture protein has a lower temperature requirement for producing an elastic gel network structure.

The effect of cooling on the viscoelastic parameters of the protein-polysaccharide complex mixtures can also be seen in Fig. 10–15. At the end of the cooling process (Fig. 11) gel network at 34°C was probably created [Ould and Turgeon, 2000; Verbeken et al., 2004].

In the cooling process, viscoelastic value of parameters G' and G" increased, but G' increased faster than G" and a cross over these curves for samples with 0,5% carrageenan concentration was observed between 35 and 33°C (Fig. 10–12). The crossover temperature of G' and G" can be considered as an indicator of the gel point of MP/CGN mixtures.

For 0.5% CGN mixtures with 2% of proteins, crossover of G' (elastic response) with G" happened at 35ºC and when 4% MP was present this point was lowered to 33ºC (Fig. 10, 14).

The aggregation, indicated by the increase in the storage and loss modulus, and the decrease in phase angle, occurred at 35–30°C for samples with 0,5% concentration of carrageenan and about 40°C for samples with 1% polysaccharide, which is in agreement with the previous studies on k-carrageenan gelation [Hermansson et al., 1991; Kohyama et al., 1996].

A crossover temperature for these two modulus was not observed for samples with 1% CGN level within the range of temperature the observations were made. These results indicate that increased concentration of CGN facilitated the formation of a rigid gel structure and its addition was beneficial for producing a more viscoelastic and cohesive gel.

To quantify the effect of protein and carrageenan concentration on the viscoelastic properties of the resulting mixtures G' and G" values from the above temperature range (transition temperature) were used for RSM analysis of the effects of CGN and MP levels.

Analysis of variance indicated that the regression models developed for phase angle, G' and G" were all significant ($p<0.01$) (Table 2). Significant linear and quadratic effects were observed for CGN concentration on G', G" and phase angle during a cooling phase at 33, 34 and 35^oC (Fig. 16–23). Generally, regardless of protein content, an increse of carrageenan

concentration resulted in significant increase of storage and loss modulus and reduction of phase angle.

CGN level also contributed to the regression model for G' and G" and exhibited an influence on these characteristics of gels through positive quadratic. Up to a point, the higher CGN concentration, the more springy and elastic were the final gels; however, the quadratic term reflected the presence of a minimum predicted for the gels at 0.2% CGN level.

Table 2

Regression coefficients and analysis of variance of the regression models for protein and carrageenan

Rheological parameters		Coefficients						R^2
		T[°C]	MP	MPxMP	CGN	CGNx-CGN	MPx-CGN	
Heating	δ	35	8,35	-0,72	17,98	3,79	-4,50	0,869
		42	-26,67	5,33*	52,24*	-41,74*	-9,78*	0,984
		47	-44,72	7,87	26,78	-30,41	-6,07	0,952
	G’	35	3,18	-1,51	3,48	-1,73	4,66	0,802
		39	-11,84	1,79	-27,51	13,07	7,06	0,863
		45	-13,17	1,93	-43,75	30,04	9,65	0,932
	G”	35	-3,37	0,52	-6,56	3,03	1,69	0,839
		39	-2,63	0,40	-8,30	4,91	2,07	0,926
		45	-5,82	0,91	-11,49	9,32	2,27	0,914
Cooling	δ	34	-15,85	2,59	33,98	-72,67*	2,26	0,909
		40	7,76	-1,63	4,26	-26,27	-1,82	0,931
		45	6,17	-1,23	12,96	-22,17	-4,92	0,979
	G’	33	39,21	-6,21	-173,08*	413,20*	-11,00	0,992
		34	20,71	-3,31	-162,85*	355,98*	-5,00	0,999
		35	-31,45	5,84	-64,85	258,81*	-21,50	0,997
	G”	33	-12,65	2,10	-25,60	46,40*	1,01	0,987
		34	-11,33	1,96	-11,63	37,01*	-2,24	0,977
		35	-9,53	1,60	-13,94	31,32*	-0,58	0,987

Significant ($p<0.05$) linear, quadratic or interactive effects are marked by star "*".
R^2 – determination coefficient for the calculated models
MP – muscle protein [%]
CGN – carrageenan [%]

Effect of protein concentration on storage modulus G′, loss modulus G″ and phase angle during heating phase.

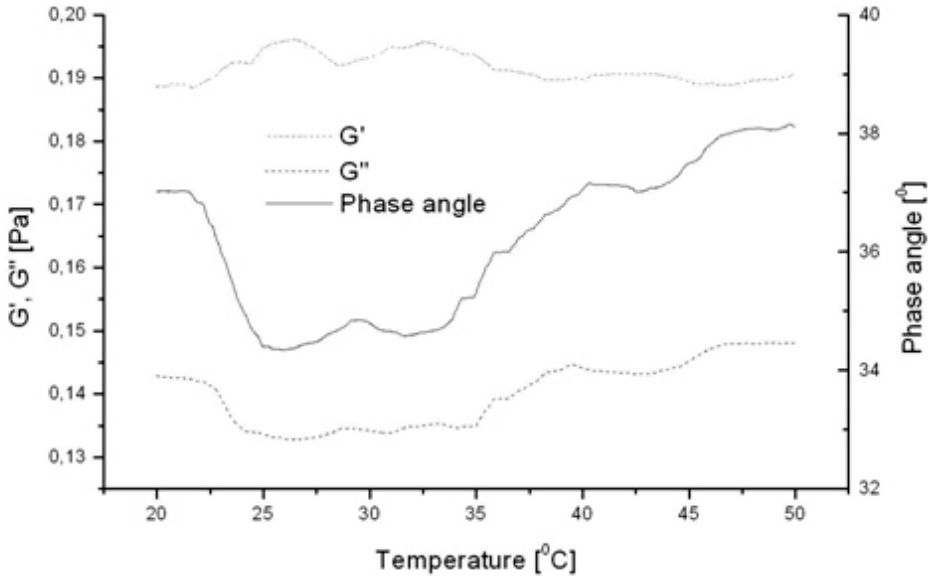


Fig. 1. Heating curves of protein 2%

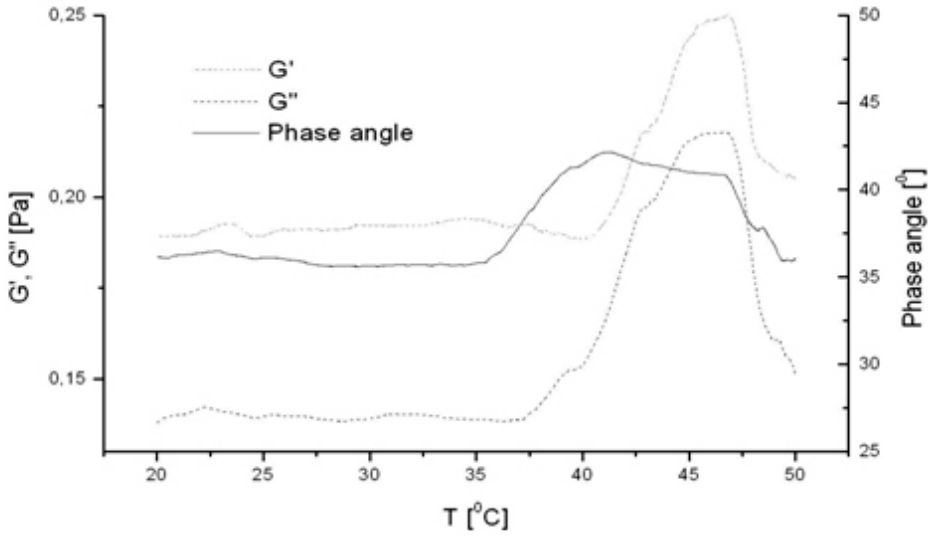


Fig. 2. Heating curves of protein 2% and carragenan 0,5%

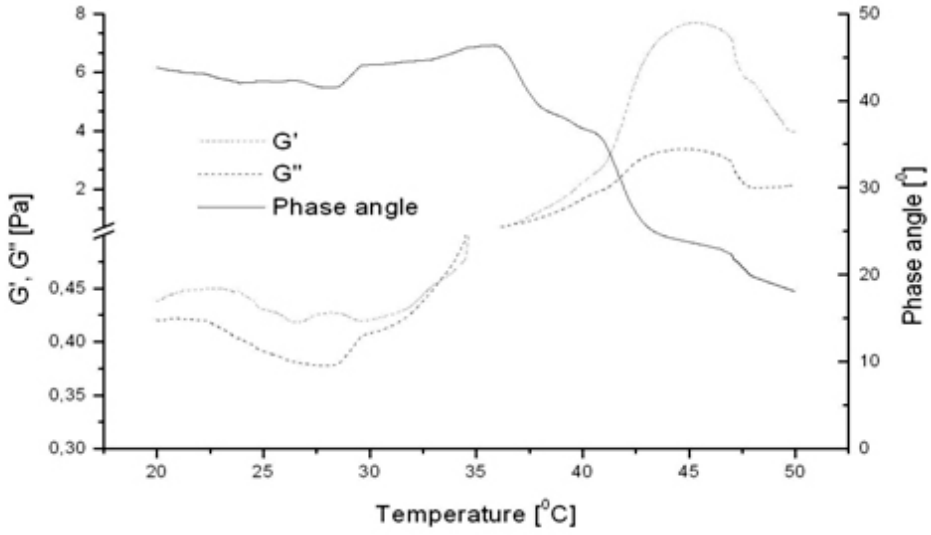


Fig. 3. Heating curves of protein 2% amd carrageenan 1%

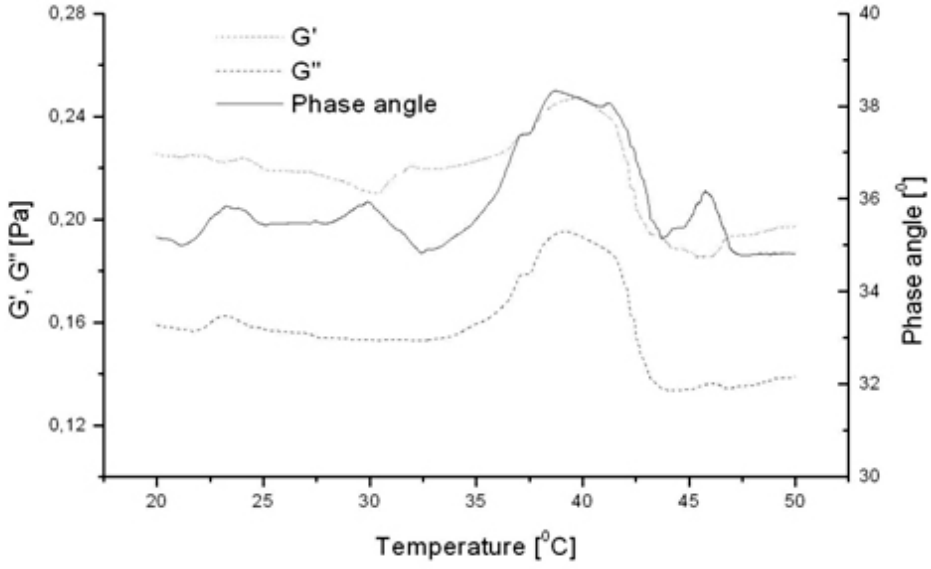


Fig. 4. Heating curves of protein 3%

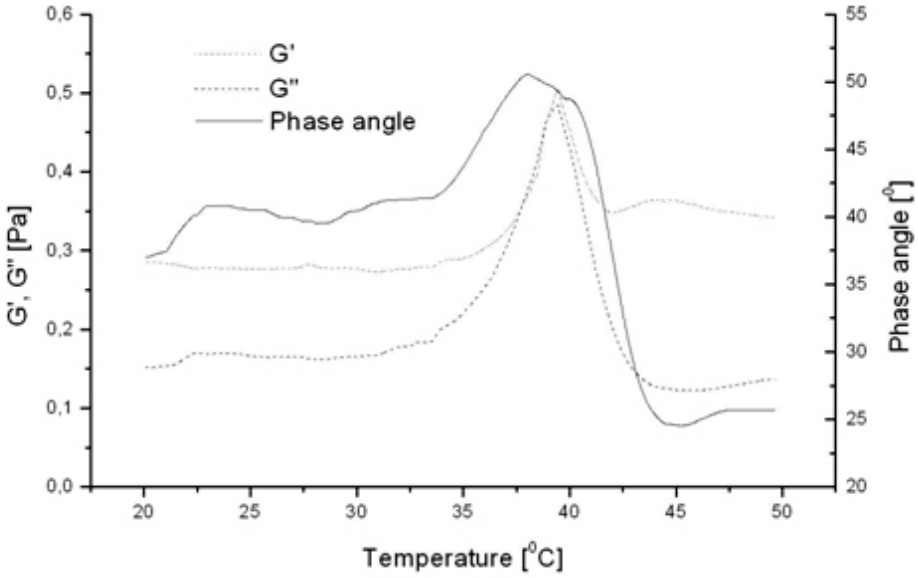


Fig. 5. Heating curves of protein 3% and carrageenan 0,5%

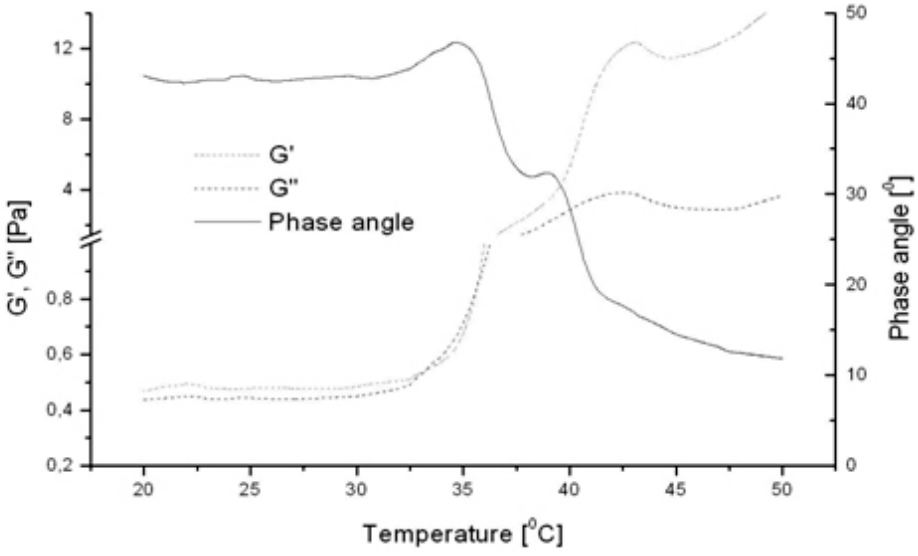


Fig. 6. Heating curves of protein 3% and carrageenan 1%

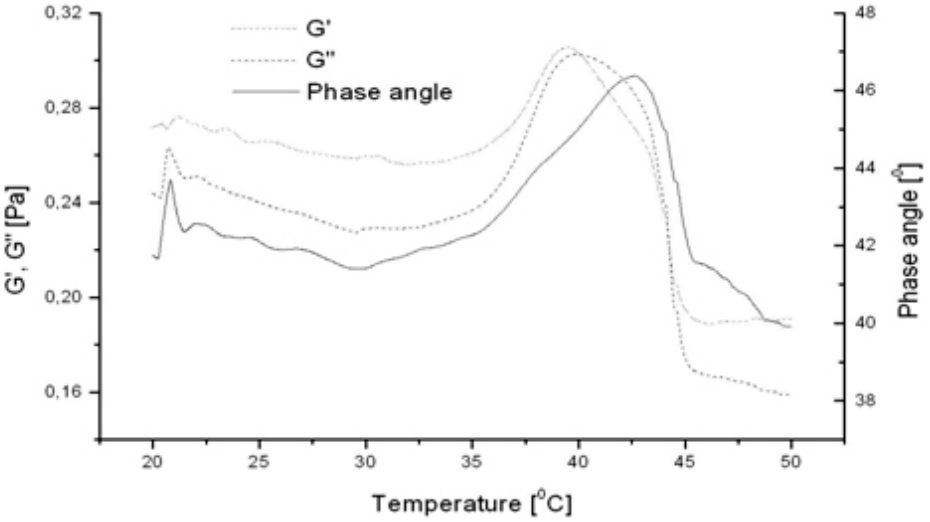


Fig. 7. Heating curves of protein 4%

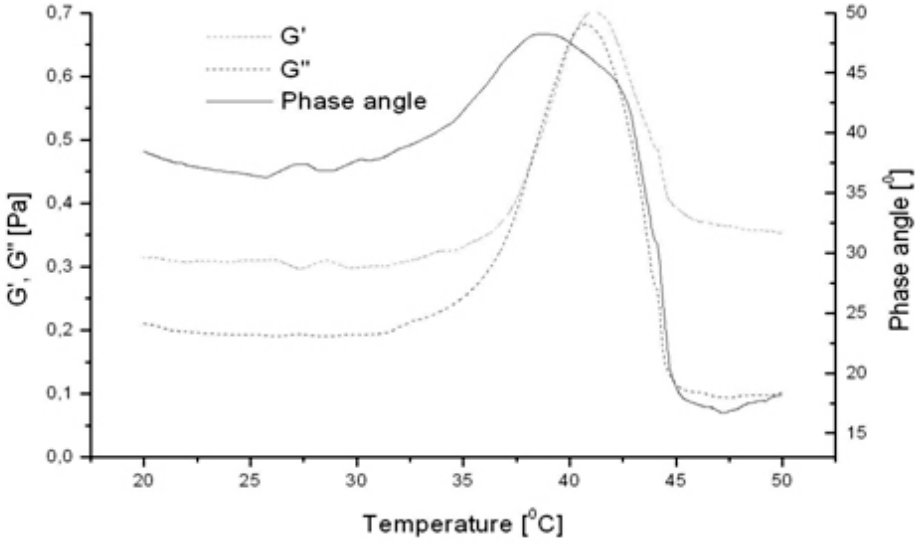


Fig. 8. Heating curves of protein 4% and carrageenan 0,5%

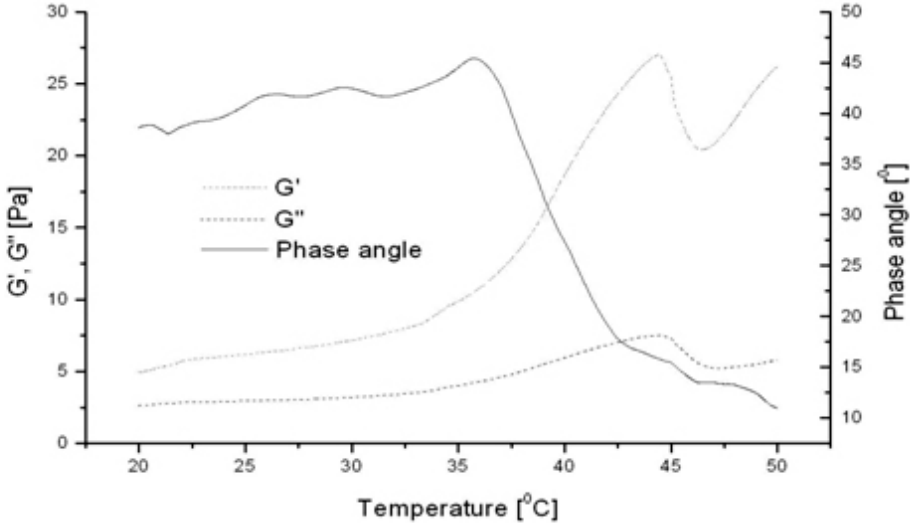


Fig. 9. Heating curves of protein 4% and carrageenan 1%

Effect of protein concentration on storage modulus G′, loss modulus G″ and phase angle during cooling phase.

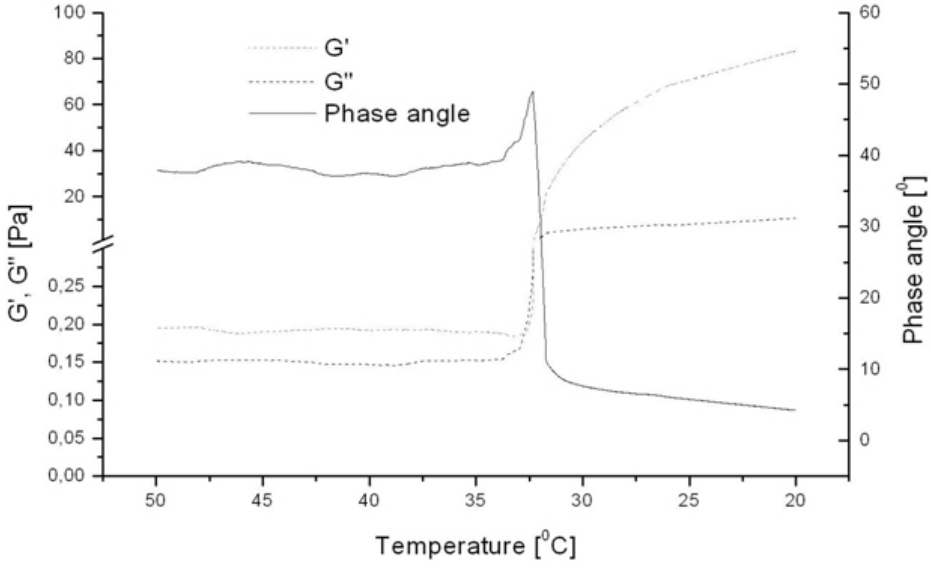


Fig. 10. Cooling curves of protein 2% and carrageenan 0,5%

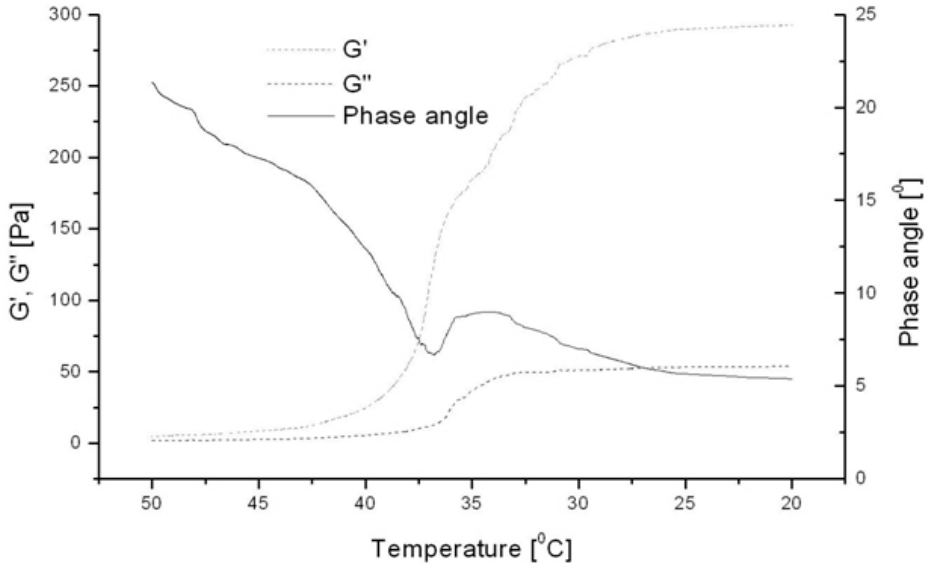


Fig. 11. Cooling curves of protein 2% and carrageenan 1%

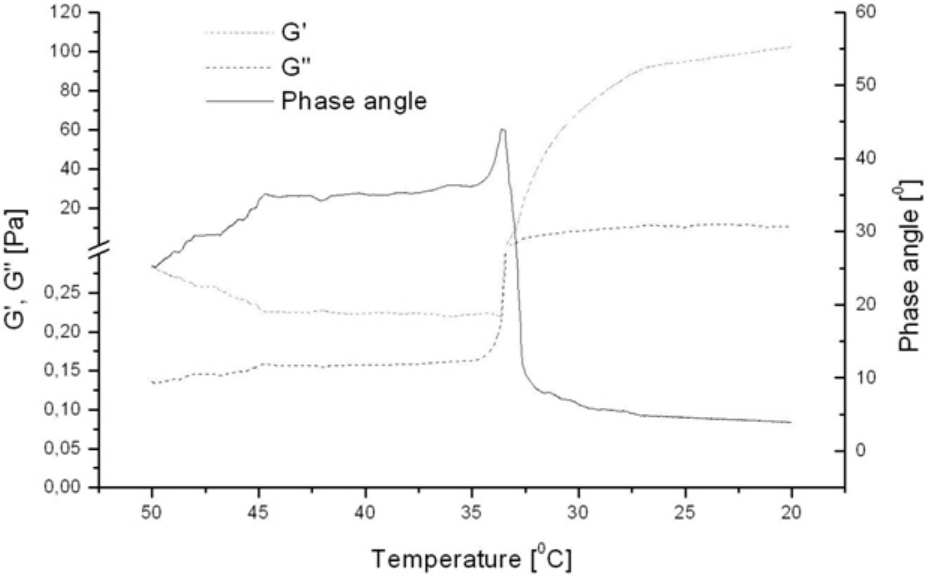


Fig. 12. Cooling curves of protein 3% and carrageenan 0,5%

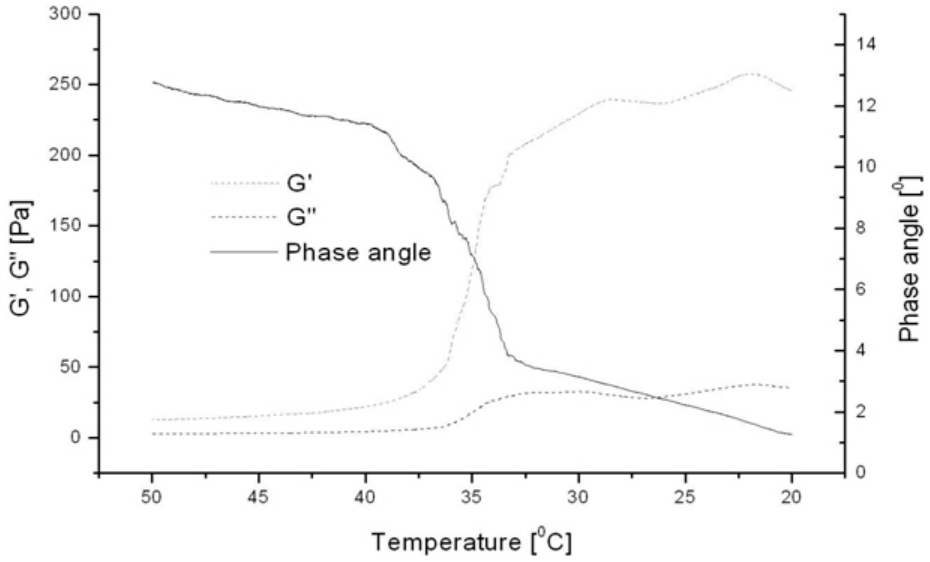


Fig. 13. Cooling curves of protein 3% and carrageenan 1%

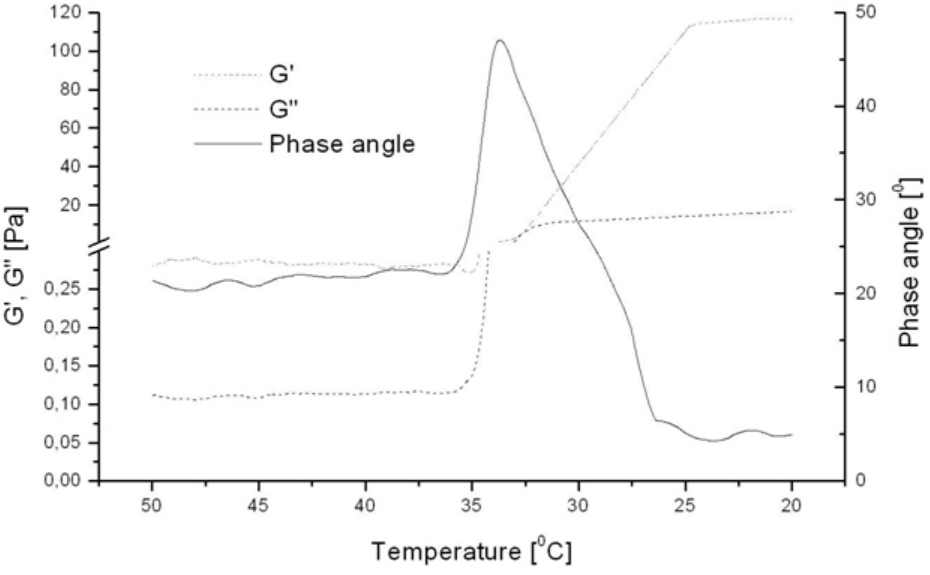


Fig. 14. Cooling curves of protein 4% and carrageenan 0,5%

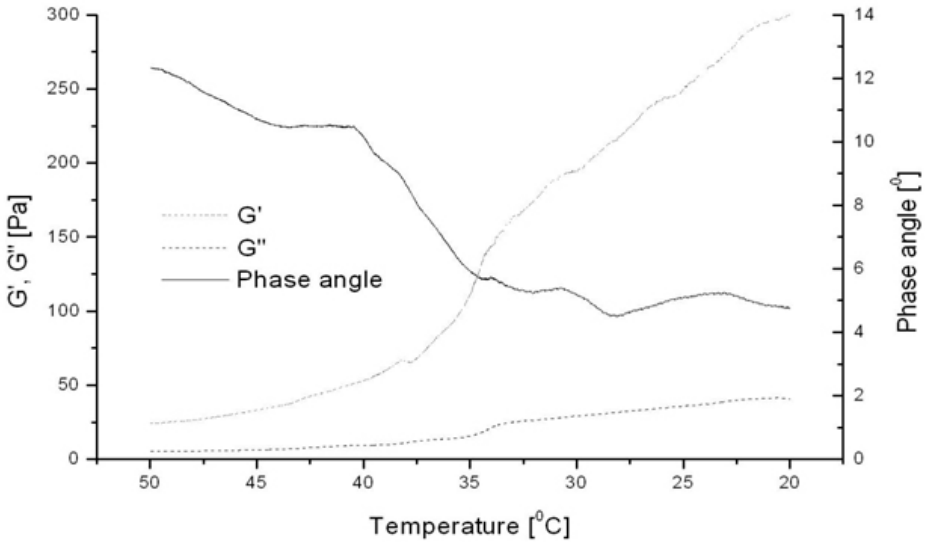


Fig. 15. Cooling curves of protein 4% and carrageenan 1%

Response surface for δ, G', G" of MP/CGN complexes.

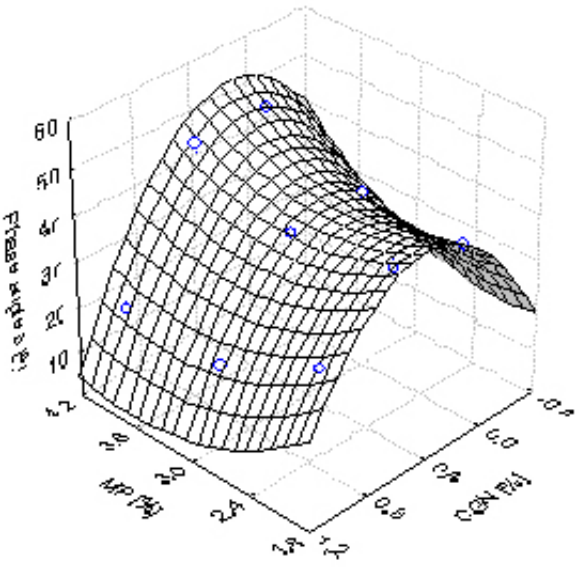


Fig. 16. During heating process at 42°C
Phase angele δ[°]

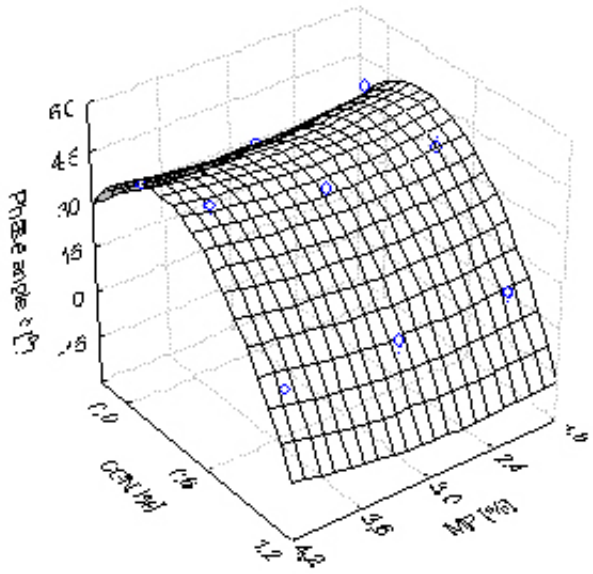


Fig. 17. During cooling process at 34°C
Phase angele δ[°]

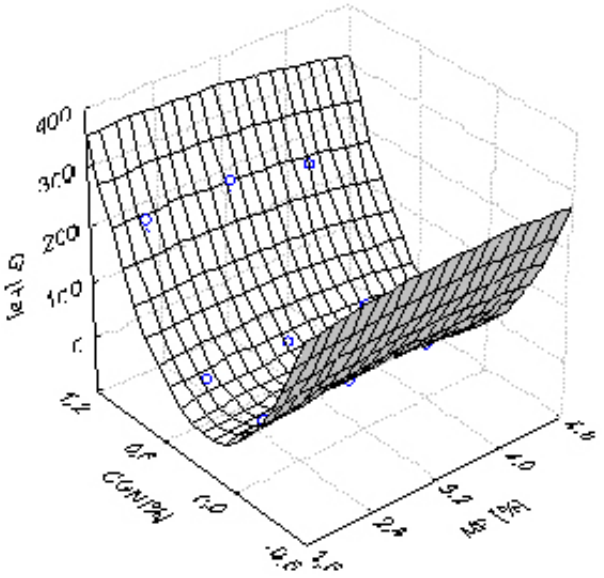


Fig. 18. During cooling process at 33°C
Storage modulus G'[Pa]

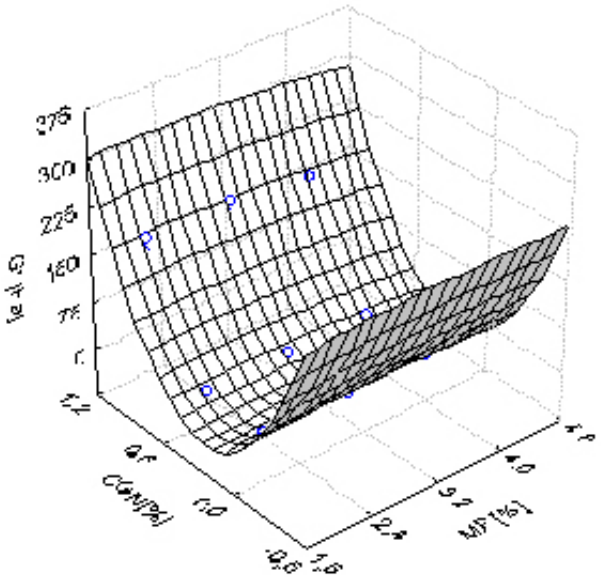


Fig. 19. During cooling process at 34°C
Storage modulus G'[Pa]

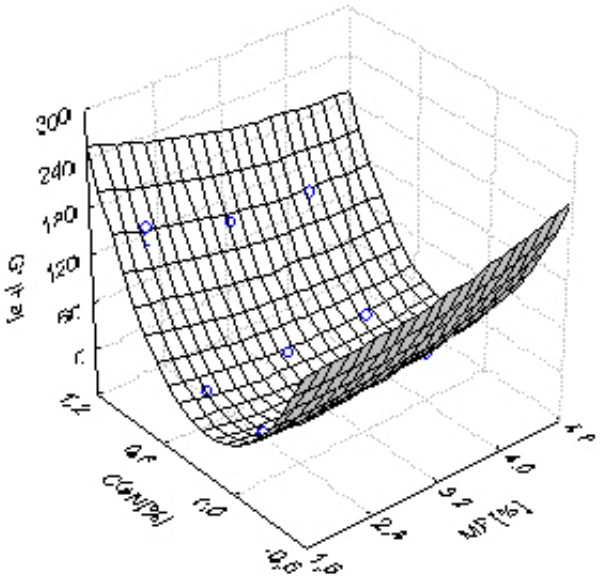


Fig. 20. During cooling process at 35°C
Storage modulus G'[Pa]

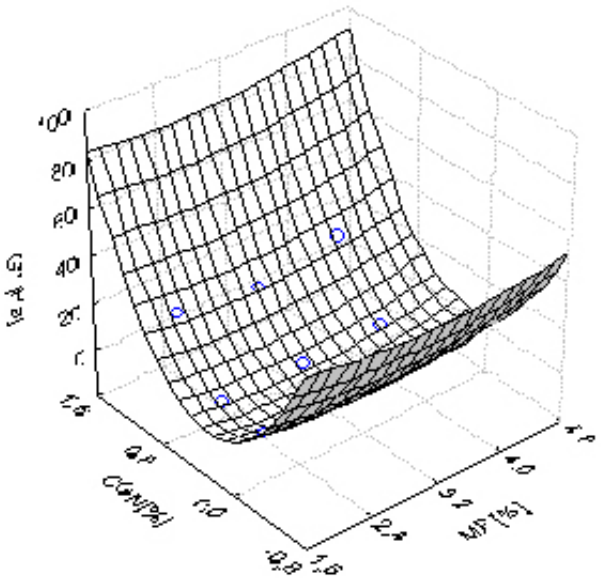


Fig. 21. During cooling process at 33°C
Loss modulus G"[Pa]

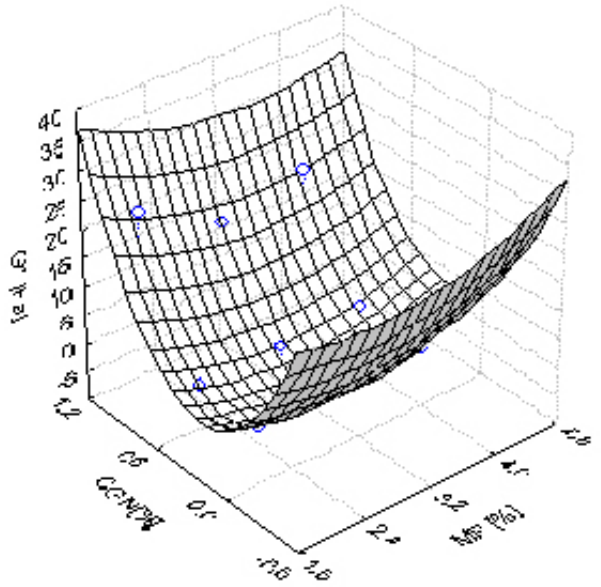


Fig. 22. During cooling process at 34°C
Loss modulus G"[Pa]

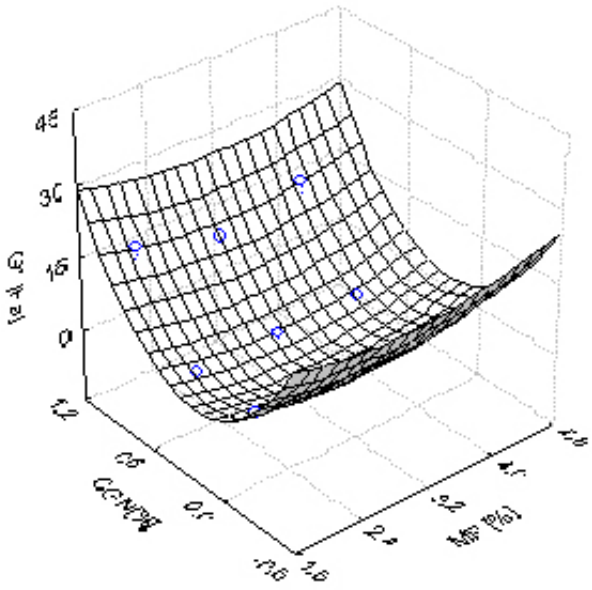


Fig. 23. During cooling process at 35°C
Loss modulus G"[Pa]

Conclusions

Of the two biopolymers studied, only carrageenan concentration significantly affected the rheological properties of complexes with myofibrillar proteins, which resulted in greater rigidity of the protein matrix and would contribute to higher gel strength. Protein concentration had little effect on viscoelastic properties of protein-CGN complexes during heating and was its effect was negligible upon cooling compared to CGN.

References

Barbut S., Mittal G.S., 1992. Use of carrageenans and xanthan gum in reduced fat breakfast sausages. LWT, 25(6), 509–513.

DeFreitas Z., Sebranek J.G., Olson D.G., Capr J.M., 1997a. Carrageenan effects on salt soluble meat proteins in model systems. J. Food Sci., 62, 540–543.

DeFreitas Z., Sebranek J.G., Olson D.G., Capr J.M., 1997b. Carrageenan effects on thermal stability of meat proteins. J. Food Sci., 62, 544–547.

Egelandsdal B., Fretheim K., Samejima K., 1986. Dynamic rheological measurements on heat induced myosin gels: effect of ionic strength, protein concentration and addition of adenosine triphosphate or pyrophosphate. J. Sci. Food Agric., 37, 915–926.

Hermansson A.,M, Ericksson E., Jordansson E.,1991. Effect of potasium, sodium, and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. Carbohydrate polymers, 16, 297–320.

Hsu S.Y., Chung H.Y., 2001. Effects of kappa-carrageenan, salt, phosphates and fat on qualities of low fat emulsified meatballs. J. Food Eng., 47, 115–121.

Kohyama K., Sano Y., Nishinari K., 1996. A mixed system composed of different molecular weights konjac glucommannan and κ -carrageenan. II Molecural weight dependence of viscoelasticity and thermal properties. Food Hydrocolloids, 10, 229–238.

Montero P., Perez Mateos M., 2002. Effect of Na^{+}, K^{+}, Ca^{+2} on gels formed from fish mince containing carrageenan or alginate. Food Hydrocolloids, 16, 375–385.

Nunes M.C., Raymundo A., Sousa I,. 2006. Rheological behaviour and microstructure of pea protein/ κ-carrageenan/starch gels with different setting conditions. Food Hydrocolloids, 20, 106–113.

Ould Eleya M., Turgeon S., 2000. Rheology of k-carrageenan and b-lactoglobulin mixed gels. Food Hydrocolloids, 14, 29–40.

Roesch R., Cox., Happek U., Corredig M., 2004. κ-carrageenan and β-lactoglobulin interactions visualized by atomic microscopy. Food Hydrocolloids, 18, 429–439.

Tolstoguzov V.B., 1991. Functional properties of food proteins and role protein-polysaccharide interaction. Food Hydrocolloids, 4, 429–468.

Van de Velde F., Peppelman H.A., Rollema H.S., Tromp R. H,. 2001. On the structure of κ/ι – hybrid carrageenans. Carbohydrate Research, 331, 271–283.

Verbeken D., Thas O., Dewettinek K., 2004. Textural properties of gelled dairy desserts containing κ-carrageenan and starch. Food Hydrocolloids, 18, 817–823.

Verbeken D., Thas O., Thas O., Dewettinek K., 2006. Interactions between κ-carrageenan, milk proteins and modified starch in sterilized dairy desserts. Int. Dairy J., 16, 482–488.

Wang H., Pato M., Pietrasik Z., Shand P.J., 2009. Thermally-induced gelation properties of natural actomyosin extracted from normal and PSE pork Longissimus muscle. Food Chem., 113, 21–27.

Westphalen A.D., Briggs J.L., Lonergan S.M., 2005. Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. Meat Sci., 70, 293–299.

Xiong Y.L., 1997. Structure – function relationships of muscle proteins. Food proteins and their applications, Marcel Dekker, Inc, New York, 359–392.

Xiong Y.L., 1993. A comparison of the rheological characteristic of different fractions of chicken myofibrillar proteins. J. Food Biochem., 17, 217–227.

Xiong Y.L., Blanchard S.P., 1994a. Dynamic gelling properties of myofibrillar protein from skeletal muscles of different chicken parts. J. Agric. Food Chem., 42, 670–674.

Xiong Y.L., Blanchard S.P., 1994b. Myofibrillar protein gelation: viscoelastic changes related to heating procedures. J. Food Sci., 59, 734–738.

8

MODIFICATION OF CHEMICAL COMPOSITION OF POULTRY BROTH USING MEMBRANE TECHNIQUE

Introduction

Poultry broths being concentrates of meat extracts obtained after heat treatment of poultry meat or from protein hydrolysates prepared with an enzymatic or chemical (acid/basic) processes. Enzymes most often applied for protein hydrolysis are pancreatin, papain and deaminases. Hydrolysates consist up to 28% amino acids and 21% peptides depending on raw material and process conditions [Flaczyk, 1997]. Raw materials used for broths preparation are breast frames, bone residues after mechanical deboning and/or whole carcasses but lower quality e.g. frozen or from laying hens. Chemical composition of the broth is strongly affected by time of cooking and/or kind of acidulants used for partially hydrolyzed poultry meat extracts [Masood and Chen, 1995, 1996]. Hydrolysates express the ability to fat stabilization, foaming ability, emulsifying capacity and/or gelling properties when proteolysis was limited. Being the product of the processing of lower quality raw materials e.g. poultry frames, skin, wings, etc. are rich in gelatin. It can effect functional properties like viscosity, gelling but also flavor of the final products. As the effect of proteolysis also some bitter components e.g. peptides, are created. Another problem is energy value and fat content which in case of poultry broths could be as low as possible. To meet mentioned requirements new technological possibilities to improve chemical composition of the extract should be searched.

The aim of the study was an attempt to modify the chemical content of chicken concentrated hydrolysate to lowering hydroxyproline and fat content using membrane microfiltration.

Materials and methods

Material for the study were two batches of poultry extract produced by Regis Ltd. (Krakow, Poland). from chicken raw materials using an enzymatic method. The model study was carried out on the extracts dissolved with water at the ratio 1 to 4. The obtained suspension was filtered through ceramic (Al_2O_3) membrane at pores 3-5 μm, porosity 35%, membrane diameter 20 mm, membrane area 0.06 m^2 and the trans membrane pressure 0.05 MPa. The dynamic of the filtration process was monitored and shown as the relation between filtrate volume and normalized filtrate flux, e.g. as a ratio of J (t) permeate flux in time t to J (0) permeate flux at the beginning of the process. During the process samples after filtration of 20, 40, 60, 80% of feed were taken and dry matter, total nitrogen, protein, fat and hydroxyproline

contents also in feed and retentate were analysed. Total nitrogen was determined using Kjeldhal method [PN-75/A04018], protein content with biuret method [Gornall et al., 1949], dry matter using drying method [PN-A-79011-3:1998], fat with Soxhlet extraction method PN-A-79011-4:1998 and hydroxyproline according to PN-ISO 3496:2000. The balance of the process was done taking into account contents of each component in membrane feed, filtrates and retentate. The results (each batch was replicated three times) were statistically analysed using analysis of variance and compared by Duncan test applying Statistica 8.0 at $p>0.05$

Results and discussion

In the experiment diluted (1:4) chicken broth was filtered on microfiltration station at 0.05 MPa trans-membrane pressure. During filtration process an increase in filtrate volume was noted for evaluation the progress of microfiltration. On the basis of these results normalized filtrate flux vs. degree of filtration was drawn (Fig. 1) and first stage of the process, so called running phase (about 20% filtrate volume), could be evaluated. During this time permeate flux decreased due to formation of surface layer (fouling) up to setting the process conditions in stationery phase (increased in filtrate volume from 20 to 80%). According to Rautenbach [1996] continuous flux of filtrate increased along with decreasing of membrane feed concentration and flowing intensity. Moreover, cited author stated that filtrate constituents detained on membrane have to be returned to membrane feed stream, which was called the formation of surface reversible layer.

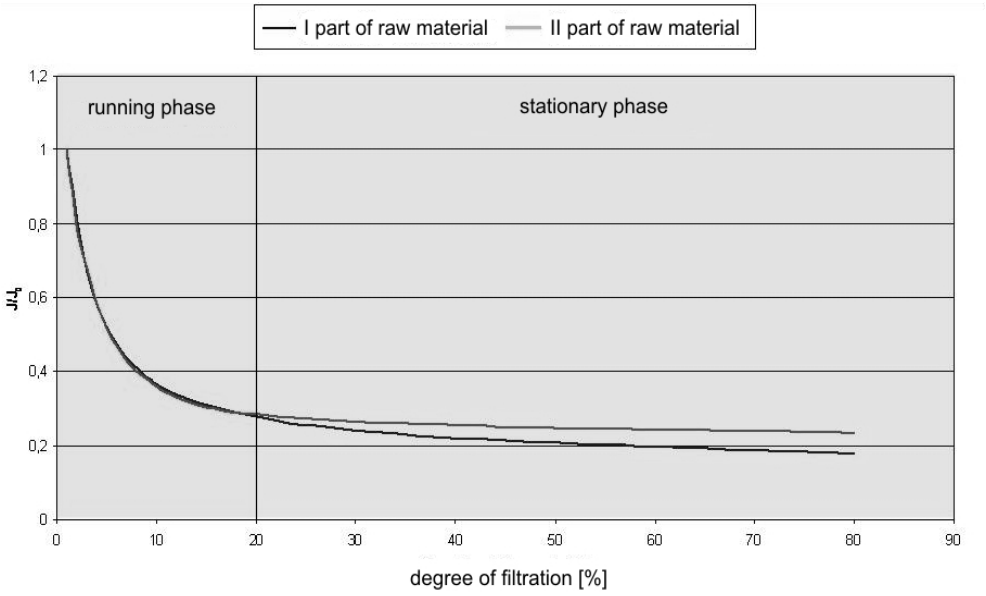


Fig. 1. Typical normalized filtrate flux for two batches of raw material

Protein content in examined fractions (Fig. 2) showed only that part of nitrogen compounds which are in real colloidial state (not in suspension). Statistical analysis of collected results revealed significant differences ($p>0.05$) in protein conentration between different batch of raw material and filtrates. Feed was characterised by protein content of 47 mg/cm^3. The lowest protein content was analysed in $F_{20\%}$ filtrate. Significantly higher protein content was observed for further filtrates ranged from 38 mg/cm^3 to 40 mg/cm^3 for $F_{40\%}$ and $F_{80\%}$, respectively (Fig. 2). The highest protein concentration was analyzed in concentrate (63 mg/cm^3). Similar relation was observed for second batch, where protein concentration in membrane feed was about 38 mg/cm^3. In filtrates protein content increased from 10 mg/cm^3 in $F_{20\%}$ filtrate to 29 mg/cm^3 in $F_{80\%}$ filtrate. The highest content of protein was analyzed in concentrate (about 54 mg/cm^3). The results of the study showed that microfiltration process caused mainly transfer of protein substances from dissolved chicken broth to filtrates. Protein together with other constituents were detained on the surface of membrane and they formed polarization layer, which under pressure was returned to membrane feed stream and increased the final protein content in concentrate. Obtained concentrates were characterized by at about 40% higher protein content in relation to the feed. Microfiltration process balance (feed equals 100%) showed that the highest protein recovery was observed in filtrate (64.5%), whilst in concentrate was much lower i.e. only at about 25%. Protein loss during microfiltration process was about 10% (Fig. 3).

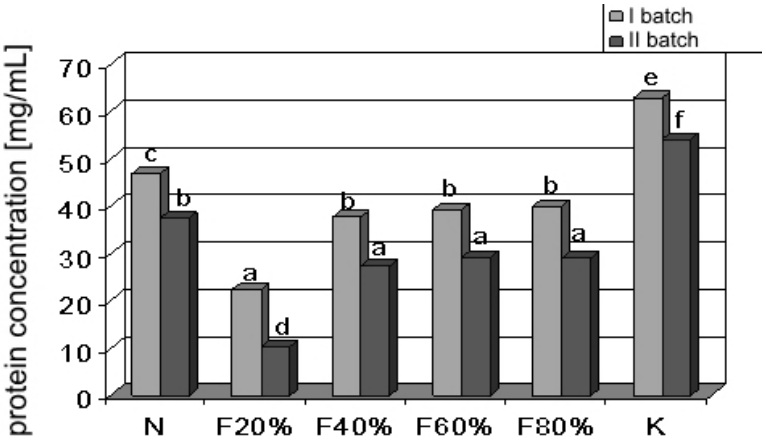


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Fig. 2. Protein concentration [mg/cm^3]

Total nitrogen content analysed in permeates was not significantly dependent on raw material used in the study (batch no 1 and 2) and ranged from 7.8 to 8.2% (Fig. 4). The lowest nitrogen content was analysed for $F_{20\%}$ filtrates (2.4–2.9%). Following, during microfiltration process the significant increase in nitrogen concentration in next portion of filtrates was observed. $F_{40\%}$ filtrate was characterised by at about 6.0% nitrogen content, whereas in $F_{60\%}$ and $F_{80\%}$ filtrates significantly higher level of nitrogen was analysed (from 6.7 to 7.5%). The highest concentration of nitrogen was evaluated in concentrates (up to 10%), what represented

about 21% more substance than in membrane feed. Because $F_{20\%}$ fraction consisted of 20% of the feed volume the share of total nitrogen in relation to nitrogen in feed was only 4.8%. The basic assumptions of the chicken broth production were established by Niewiarowicz et al. [1960], whose published also a standard chemical composition of chicken meat concentrates, including nitrogen content. The results of our study were similar to this model. Moreover, raw material composition affects the total nitrogen content in the broth. For example Masood and Chen [1995] obtained the highest total nitrogen content in extracts made from bone residue after mechanical deboning. Also in case of acid hydrolysates kind of acidulant determinate nitrogen content, the highest values were observed when phosphoric acid was applied.

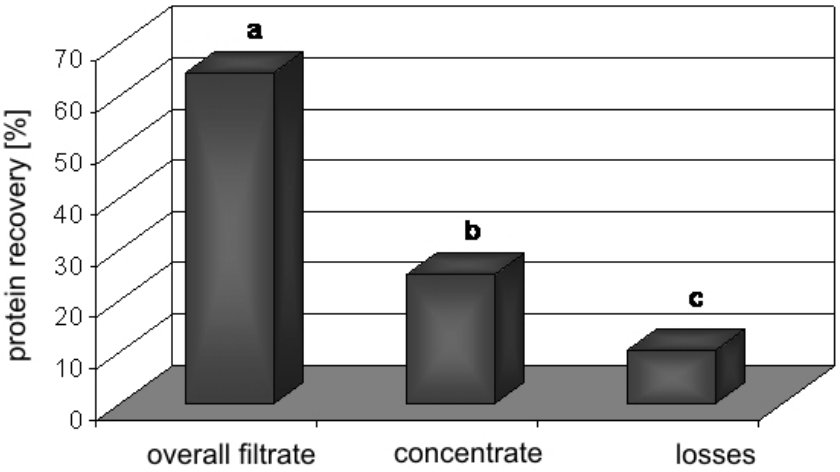

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Fig. 3. Recovery of proteins [%] depending on examined fraction

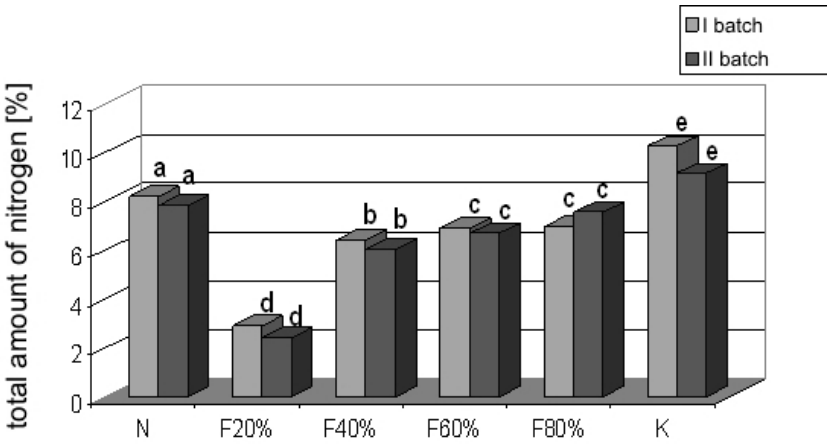

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Fig. 4. Total nitrogen concentration in studied fractions [%]

Microfiltration process balance revealed that the highest recovery of nitrogen constituents was observed in filtrate (57%). Nitrogen recovery in concentrate was on the level of 25%, whereas nitrogen loss during process was closed to 19%.

Statistical analysis of the results of dry matter content showed that there is no significant differences between specific batch of raw material. The highest dry matter content was analysed in membrane feed and final retentates, the average value ranged from 10.8 to 13.0% (Fig. 5). The lowest value of dry matter was observed for $F_{20\%}$ filtrates (about 75% lower dry matter content than the feed), what can be explained by the dilution occurred at the onset of process. In other filtrate fractions dry matter concentration varied from 8.65% in $F_{40\%}$ to 9.51% in $F_{80\%}$. It means that microfiltration membrane was not specific for dry matter components separation.

Retentates obtained in the study after microfiltration process were characteried by average dry matter content of 12%, while in undiluted broth dry matter consisted of 44–52%. This obtained concentrates should be further processed by evaporation and drying to prolong their durability, especially from microbiological point of view. As published by Janicki et al. [2006] dry matter content in final dried commercial chicken concentrates was ranged from 91.2 to 94.2%.

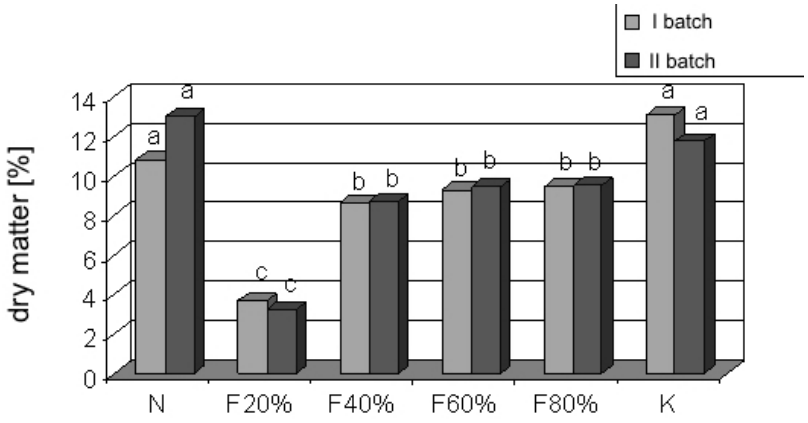

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Fig. 5. Dry matter content [%]

Analysis of microfiltration process balance i.e. recovery and loss of dry matter, revealed that the highest recovery of dry matter was observed in filtrate (56%), whilst dry matter recovery in concentrate and process loss (in both about 22%) were much lower.

There were no statistically significant differences in fat content between all analysed fractions of chicken broth (raw material, filtrates, concentrate) in both experimental batches. The average fat concentration in chicken broth ranged from 1.0 to 2.3% (Fig. 6). No differences in fat content between membrane feed, filtrates and concentrate can be explained by the small selectivity of the membrane used, which was no effective for lipids particles separation. However, the lack of significant differences in fat content between specific samples, the inte-

resting trend was observed. The highest fat content was analysed in $F_{20\%}$ filtrate despite high dilution factor. This filtrate was characterized by almost 45% higher level of fat in relation to membrane feed. As the consequence fat in $F_{20\%}$ permeate consisted of 57.4% (average value) in dry matter, while in feed (diluted broth) only 11.2%. Fat concentration decreased in further filtrate fractions. The lowest fat concentration was found in concentrate (1.3%).

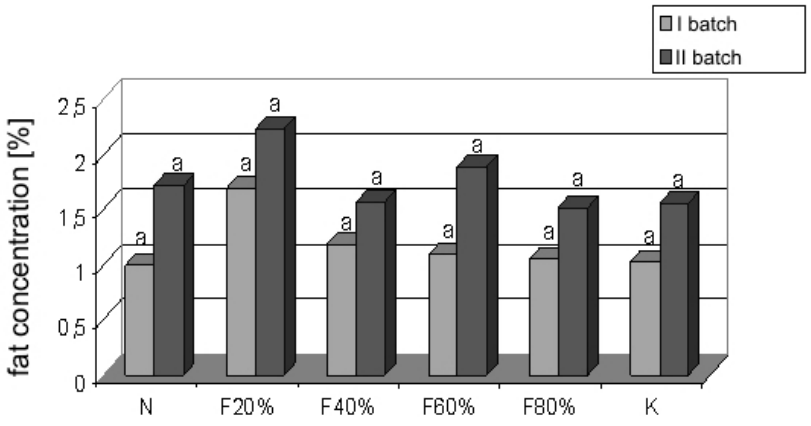

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Fig. 6. Fat concentration [%]

Microfiltration process analysis of recovery and loss showed that fat balance is significantly influenced by specific filtrate fraction. The highest fat recovery was observed, similarly to other chemical substances, in filtrate (82% of fat substances including 30% in $F_{20\%}$). Whereas, concentrate consisted with only 18% of total fat from feed.

Data [Anonim, 2005] showed that extracts prepared at home from fresh chicken carcass contained from 10 to 20% fat, whereas broth prepared from commercially purchased concentrates had only 3– 4% fat. According to Niewiarowicz et al. [1960] standard chicken broth could consist even up to 27% fat. Differences occurred in fat content in broths depend on manufacturing method, when hydrolysis method is used low fat contribution is observed.

Mulifactorial analysis of variance revealed that hydroxyproline content showing on gelatin level was significantly dependent on raw material characteristics (batch) as well as on the specific filtrate fraction. (Fig. 7). First experimental batch was characterised by much higher hydroxyproline content than the next one. Hydroxyproline content increased along with microfiltration process despite the amino acid concentration in membrane feed.

The lowest hydroxyproline concentration was analysed in $F_{20\%}$ filtrate (0.06 and 0.12% for I and II batch, respectively). Continuous microfiltration process resulted in increasing level of hydroxyproline. $F_{40\%}$, $F_{60\%}$ and $F_{80\%}$ filtrates contained from 0.18 to 0.26% hydroxyproline, which represented about 76% of analysed amino acid in relation to the feed. The highest hydroxyproline level was analysed in concentrates, 0.51% for I batch and 0.35% for II batch and it was almost 50% higher content of hydroxyproline in comparison to the feed.

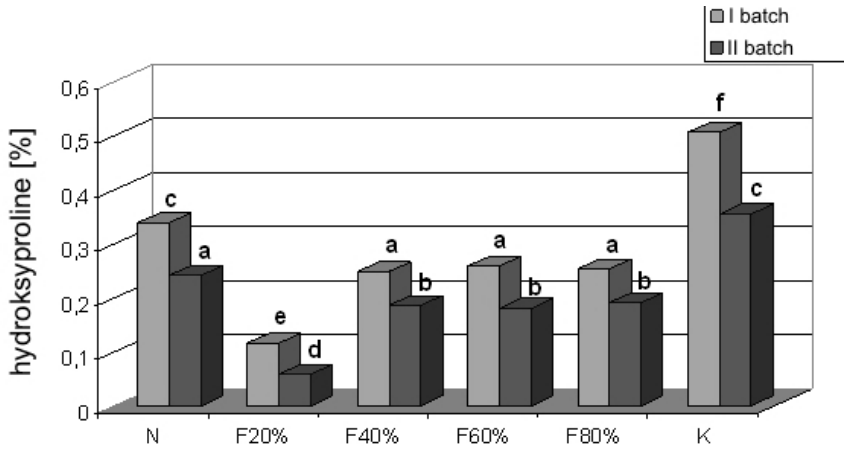

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Fig. 7. Hydroxyproline content [%]

Knowledge about hydroxyproline content in chicken broth is still limited. It is obvious that gelatin content in poultry meat extracts increases in time of heat treatment of raw materials. After three hours of the process significant increase in gelatin content in relation to 1 or 2 hours process was observed [Masood and Chen, 1995]. Analysis of microfiltration process balance in own studies revealed that hydroxyproline content differed during process (Fig. 8). Similarly to other constituents, the highest amount hydroxyproline was analyzed in filtrate (about 51.8%,) and concentrate (about 30% higher level of the quantity determined in membrane feed). The amino acid loss during microfiltration was 18.4% (Fig. 8).

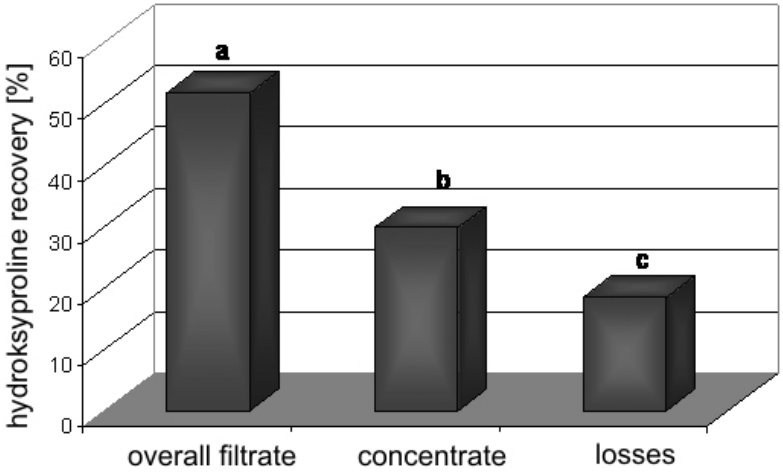

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Fig. 8. Hydroxyproline recovery [%] during filtration

Conclusions

It can be stated on the basis of obtained results that microfiltration process was not effective for modification of chicken broth composition if was conducted up to filtrate all added to feed water (80%) because of high looses of broth constituents. But if process was performed up to filtrate 20% of added water lowering of fat content in chicken broth can be obtained. In opposite to that hydroxyproline content in broth (showing share of gelatine in total protein) after microfiltration process was not lowered in both operation e.g. carried out up to filtrate of 20 or 80% of added water.

References

Anonim, 2005. Consumer test of stock cubes. Świat Konsumenta, 48, 1–3.

Flaczyk E., 1997. Zalety technologiczne i żywieniowe hydrolizatów białkowych. Część I. Przem. Spoż., 3, 6–9.

Gornall A.G., Bardawill C.J. David M.M., 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177, 751–766.

Masood M., Chen T.C., 1996. Calcium, phosphorus and nitrogenous compound contents of chicken broth from broiler breast frames as affected by various acidulants. J. Food Process. Preserv., 20, 165–173.

Masood M., Chen T.C., 1995. Gelatin, calcium and phosphorus contents of chicken broth from broler deboning by-products. J. Food Process. Preserv., 19, 165-173.

Niewiarowicz A., Wcisło H., Borys A., 1960. Elementy produkcji koncentratu rosołu drobiowego. Przem. Spoż., 3(19), 134–138.

PN-75/A04018. 1975. Produkty rolniczo-żywnościowe. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko.

PN-A-79011-4:1998. Koncentraty spożywcze. Metody badań. Oznaczanie zawartości tłuszczu.

PN-A-79011-3:1998. Koncentraty spożywcze. Metody badań. Oznaczanie zawartości wody.

PN-ISO 3496:2000. Mięso i przetwory mięsne. Oznaczanie zawartości hydroksyproliny.

Rautenbach R., 1996. Procesy membranowe. WNT, Warszawa.

9

MODIFICATION OF SENSORY PROPERTIES OF POULTRY BROTH USING MEMBRANE TECHNIQUE

Introduction

The volatile fraction created after heating of chicken meat consist more than 300 substances and its composition is responsible for creation of specific flavor [Gasser and Grosch, 1990]. The main groups of components are aldehydes (pentanal, hexanal, nonanal, 2-(E)-4-(E)-decadienal, 2-undecenal), ketones (3-hydroxy-2-butanone and acetophenone), alcohols (2-ethyl-1-hexanol) and nitrogen or sulphur containing compounds (2,4-dimethyl-2-oxazoline-4-methanol, 2-methyl-3-furantiol) as well as lactones (2-dodecalactone) [Gasser and Grosch, 1990, Wettasinghe et al., 2001]. The aldehydes like hexanal, pentanal, octanal and nonanal are products of oxidation of poultry fat rich in linolic acid [Gandemer, 2002] and are indicators of off flavor (WOF) development in cooked or processed foods [Brunton et al., 2000]. In case of hydrolysates obtained with acidic or enzymatic methods high bitterness and saltiness are negative indicators of the final poultry broth. There is a lot of technological possibilities to depress bitter taste of broths and hydrolysates. Most often plastification reactions and membrane techniques are applied.

The aim of the study was an attempt to modify the composition of flavor/volatile fraction of chicken stock made on the base of hydrolysates using membrane microfiltration.

Materials and methods

The study was carried out on poultry broth manufactured by Regis Ltd (Krakow, Poland). Stock was produced from chicken raw materials using an enzymatic method. Two different lots of production was used in the study. The model study was carried out on the broth dissolved with water at the ratio 1 to 4. The obtained suspension was filtered through ceramic (Al_2O_3) membrane at pores 3–5 μm, porosity 35%, membrane diameter 20 mm, active membrane area 0.06 m^2 and the trans membrane pressure 0.05 MPa. During the microfiltration process fraction after 20% of the total volume of the feed was filtered was collected. Sensory analysis of taste and odour profiling of membrane feed, filtrate $F_{20\%}$ and final concentrate was conducted followed by identification of volatile compounds. Sensory traits (taste, flavour, overall appearance) of chicken broth was analysed using 5-grade scale of intensity, where 1 point represented the lowest and 5- the highest level of intensity. Moreover, in taste profiling: sweet, bitter, salty, metallic, meaty and brothy tastes were analysed, similarly to odour profiling. Volatile compounds of chicken broth and its fraction collected during microfil-

tration were analysed using SPME chromatography (GC/MS). For chromatographic analysis samples of experimental material were heated up to 50ºC, then polydimethylosiloxane (PDMS) fibre (85 μm) was inserted in order to exposition on volatile aldehydes for 30 min. Main analysis was performed in GC/MS chromatograph (Agilent). Collected results were then statistically analysed by Duncan test analysis of variance using Statistica 8.0 at $p>0.05$

Results and discussion

Sensory analysis play an important role in consumer science but also in food industry and gastronomy. Continuous control of sensory properties of foodstuffs affects the production cycle and is one of the essential tool in quality improvement by monitoring and controlling [Byrne et al., 2002]. Sensory properties of food products are critical for consumer selection and they influence market demand for specific food items. Nowadays, well educated consumers require a good quality products, which forces food producers to strict control of their foods and also enhance their value, including sensory properties [Fanatico et al., 2007, Lawlor et al., 2003, Armstrong and McIlveen, 2000].

Figure 1 show in overall the taste profiles of membrane feed, filtrates and concentrate. On the basis on collected data bitter was the most distinctive in taste analysis of membrane feed, i.e. diluted chicken broth. Panelists pointed out also brothy, metallic and sweet tastes. Unexpectedly, chicken extract was characterized by the lowest intensity of salty and meaty tastes. On the contrary to membrane feed, in filtrates collected during microfiltration salty was the most perceptible taste. Lower intensity of bitter and metallic tastes was evaluated in filtrates. Among all analysed tastes brothy and sweet were the least detectible. In concentrate analysis brothy taste was the most intensive, as was previously mentioned, whereas meaty taste was the least perceptible.

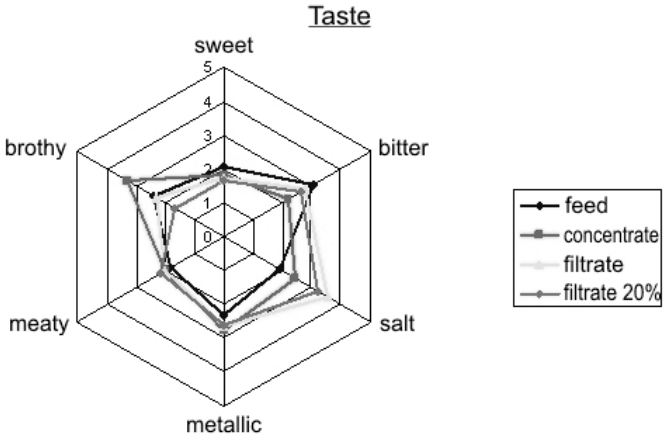

Fig. 1. Sensory taste profiling in feed (diluted chicken broth), filtrates and concentrate

Statistical analysis of collected results revealed significant differences between the intensity of salty ($0.01< P \leq 0.05$) and brothy ($P \leq 0{,}001$) taste in relation to filtrate fractions. Other tastes were not effected by the stage of microfiltration process.

The intensity of sweet taste was similar in all analysed samples and was scored at about 2 points (Fig. 1). However, some tendency in lowering of the intensity of analysed taste was observed. Sweet taste was the most perceptible in the feed (chicken broth) (2.05 points), whereas concentrate was characterised by slightly lower intensity of this taste (1.8 points).

The intensity of bitter taste in chicken broth was evaluated between 2 and 3 points (Fig. 2) and was more perceptible than sweet taste. Similarly to sweet taste, there were no statistical differences in bitter taste intensity between all analysed samples. However, distinct trend in lowering the bitter taste intensity along with microfiltration process was observed. The highest bitterness was analysed in membrane feed (3.05 points), whilst the lowest in concentrate (2.2 points). It was a positive aspect of microfiltration process of chicken broth due to bitter taste is one of the main undesirable factor in sensory analysis of this type of products.

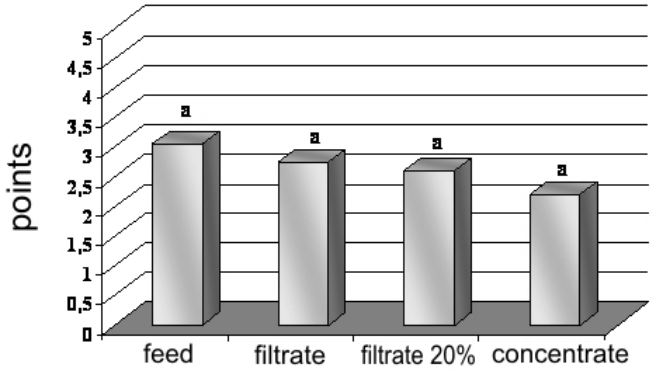

Fig. 2. Sensory evaluated intensity of bitter taste in chicken broth along with microfiltration process

Analysis of salty taste in chicken broth under microfiltration process showed that the highest intensity of salty taste was evaluated in filtrates, already after filtered 20% of total volume of the feed (Fig. 3). Significantly lower saltiness was ascertained in raw chicken broth, which was used as a membrane feed (1.9 points). At the end of the microfiltration process the intensity of salty taste in concentrate was higher than in membrane feed but at the same time lower than in filtrates (3.45 points).

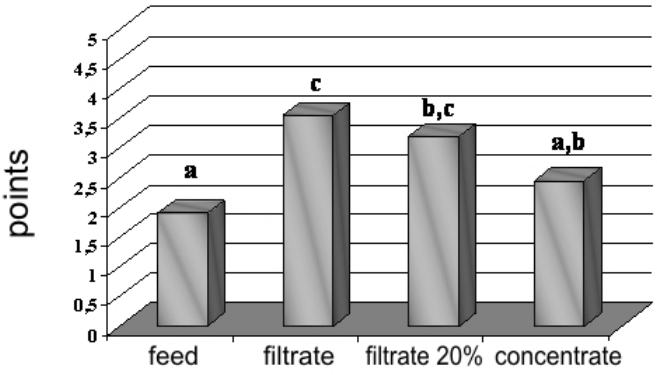

Fig. 3. Sensory evaluated intensity of salty taste in chicken broth along with microfiltration process

In the study on microfiltration of chicken broth metallic taste was also evaluated (Fig. 1) as an important factor in total flavour of the product. Any significant differences were observed in metallic taste intensity within all analysed samples.

Meaty taste is consider as new separate category, which apart from previously described four basic tastes, can be used in product tastiness evaluation. Meaty taste (in contrast to generally perceptible tastes and odour, so called flavour, which are dependent on volatile compounds) is created by the action of sodium glutamate (MSG) and inozyne monophosphate (IMP) in contact with taste buds [Fitz and O'Cuinn, 2006]. The intensity of meaty taste in chicken broth was scored on the low level, the average value 1.8 points (Fig. 1). Slightly higher perceptibility of meat taste was analysed in filtrate after filtration of 20% volume of raw material and in final concentrate (2.0 – 2.15 points). However, no significant differences were revealed in meaty taste within this group of products.

Brothy taste is the most desirable in meat extracts and chicken broths due to its silmilarity to the natural meat products and meals. The results of the study showed that the lowest intensity of brothy taste was analysed in the filtrate after 20% filtration of total volume of membrane feed. As was expected the most intensive brothy taste was noticed in concentrate (3.3 points) and it was significantly higher than in all other samples including raw chicken extract (Fig. 4). It can be concluded that brothy taste is connected with bigger particles of the broth not filtered through applied microceramic membrane.

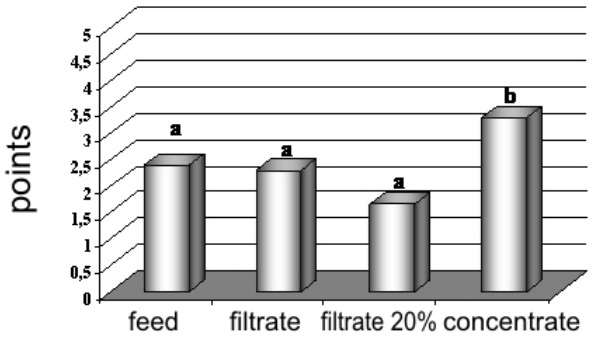

Fig. 4. Sensory evaluated intensity of brothy taste in chicken broth along with microfiltration process

Second sensory parameter analysed in this study was odour, due to it significantly counterpart in overall flavour, which determine the consumer acceptability of the product. Three the most important for meat extracts types of odour were evaluated i.e. meaty, brothy and rancid. Overall acceptability of odour intensity was also judged (Fig. 5).

Statistical analysis of collected results revealed that there were no differences in odour parameters evaluation between experimental material, except for meaty odour in filtrate ($0.01<P\leq0.05$).

The highest intensity of meaty odour (3.0 points) was evaluated in concentrate (Fig. 6), whereas filtrate was characterized by the lowest perceptibility of this smell. In membrane feed the intensity of meaty odour was on the average level (2.45 points).

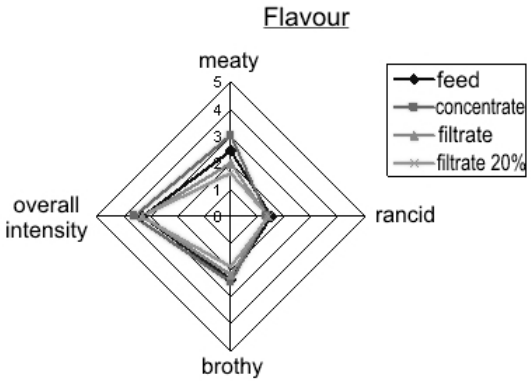

Fig. 5. Sensory analysis of odour parameters in membrane feed, filtrate and concentrate

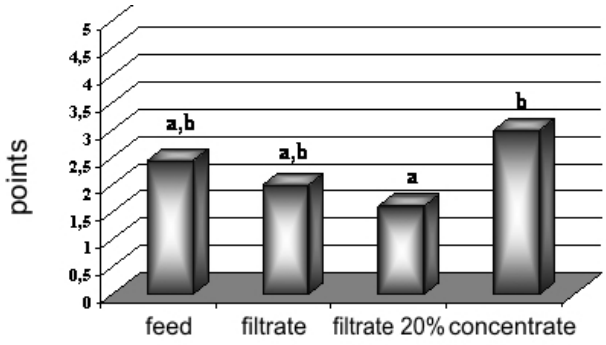

Fig. 6. The average scores of meaty odour in chicken broth, filtrate and concentrate

Rancid odour is highly unacceptable in food products because it is connected with degenerative changes in lipids and is one of the warmed over flavor (WOF) factor [Byrne et al., 2002]. Rancid odour was percept in all analysed samples but on quite low level (1.35 – 1.5 points, for concentrate and filtrate respectively), which showed the proper quality (freshness) of the raw material (Fig. 5). Also no significant differences in brothy odour were revealed between membrane feed, concentrate and filtrate fractions collected during microfiltration process (Fig. 5).

The highest intensity of odour was analysed in concentrate fraction (2.45 points), and the lowest in filtrate (Fig. 5). However, there were no significant differences in odour intensity between all analysed samples.

SPME analysis of volatile compounds

The results of the chromatographic analysis of volatile compounds in chicken broth, filtrate and concentrate obtained during microfiltration are shown on Fig. 7 and 8.

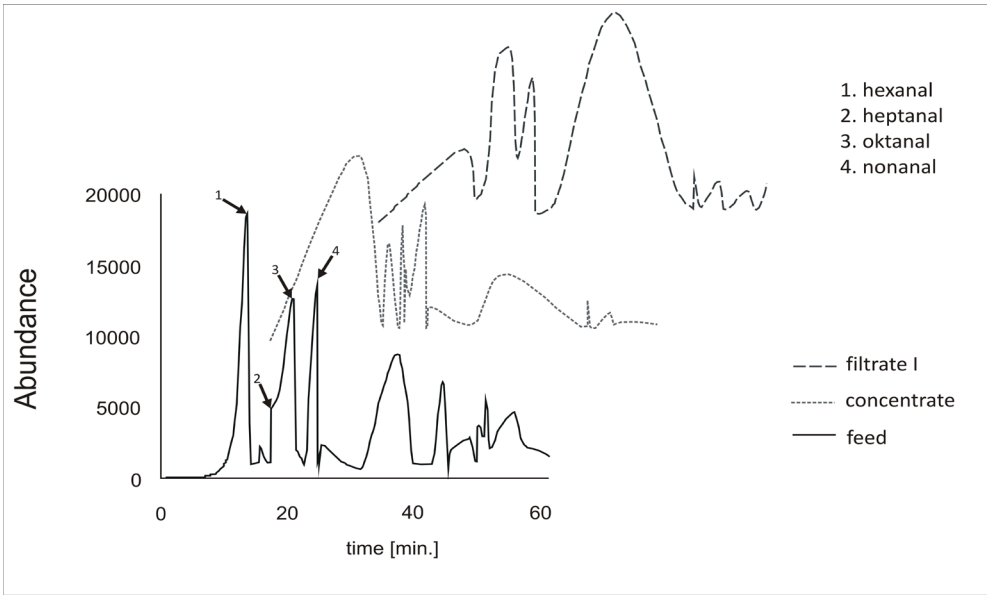

Fig. 7. Chromatogram GC/MS of chicken broth fractions

It was noticeable that in starting phase of the microfiltration process the highest amount of octanal was transferred from the feed into filtrate. Final concentrate was also characterised by higher concentration of this compound in comparison to raw chicken broth. It could be the effect of membrane fouling or membrane blocking, which resulted in lower effectiveness of separation process. Microfiltration of chicken broth solution only slightly increased nonanal and heptanal level in the concentrate, comparison to the feed. Concentrate was also characterized by the highest content of hexanal (24%) in relation to raw chicken broth (19%) and filtrate (7%). Because hexanal is mainly responsible for rancid flavor it should be stated that microfiltration did not cause lowering of its content in retentate.

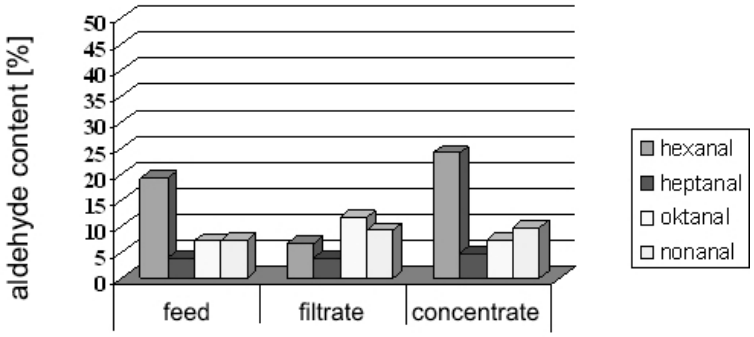

Fig. 8. Percentage content of selected volatile aldehydes in chicken broth, filtrate and concentrate

As was previously mentioned WOF (warmed over flavor) is mainly characterised by undesirable rancid and metallic taste and odour. Hexanal is of the most important aldehydes effected in food products undesirable flavour [Pignoli et al., 2009, Pettersen et al., 2004, Fernando et al., 2003]. Similar lipid oxidation compounds are formed during thermal treatment such as frying, grilling or boiling. Brunton et al. [2000] reported that hexanal is among others the most crucial product of lipid oxidation. Analysis of this compound in meat and meat products is often applied in order to measure lipid oxidation and also the occurrence of warmed over flavour in boiled foodstuffs.

Chromatographic analysis of volatile compounds after microfiltration of chicken broth confirmed the results of sensory analysis. High content of hexanal was shown both in feed and concentrates but perception of rancid odour by sensory panel in all examined fractions was almost the same. On the other hand, high concentration of hexanal in concentrate fraction could be the reason of higher intensity of metallic taste. Hexanal, heptanal, octanal and nonanal are products of antioxidation of linolic acid and are indicators of WOF [Brunton et al., 2000, Gasser and Grosch, 1990]. Especially high level of hexanal was found in volatile extract from cooked poultry muscles [Wettasinghe et al., 2001] and the increase of pentanal content parallely to TBARS rise during cold storage was stated [Brunton et al., 2000]. It means that all aldehydes being products of autoxidation processes affects WOF in poultry broths.

Conclusions

Microfiltration process positively influenced the taste of chicken broth. Final concentrate was characterised by high intensity of meaty and brothy taste, and low intensity of bitter taste. Filtration improved also odour profile of final concentrate, i.e. higher intensity of meaty odour were evaluated.

References

Armstrong G.A., McIlveen H., 2000. Effects of prolonged storage on the sensory quality and consumer acceptance of sous vide meat-based recipe dishes. Food Quality Preference, 11, 377–385.

Brunton N.P., Cronin D.A., Monahan F.J., Durcan R., 2000. A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. Food Chem., 68, 339–345.

Byrne D.V., Bredie W.L.P., Mottram D.S., Martens M., 2002. Sensory and chemical investigations on the effect of oven cooking on warmed-over flavor development in chicken meat. Meat Sci., 61, 127–139.

Fanatico A.C., Pillai P.B., Emmert J.L., Gbur E.E., Meullenet J.F., Owens C.M., 2007. Sensory attributes of slow- and fast-growing chicken genotypes raised indoors or with outdoor access. Poultry Sci., 86, 2441–2449.

Fernando L.N., Berg E.P., Grün I.U., 2003. Quantitation of hexanal by automated SPME for studying dietary influences on the oxidation of pork. J. Food Comp. Anal., 16, 179–188.

Fitz G.R.J., O'Cuinn G., 2006. Enzymatic debittering of food protein hydrolysates. Biotechnol. Adv., 24, 234–237.

Gandemer G., 2002. Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. Meat Sci. 62, 309–321.

Gasser U., Grosch W., 1990. Primary odorants of chicken broth: a comparative study with meat broths from cow and ox. Z. Lebensm. Unters. Forsch., 190, 3–8.

Lawlor J.B., Sheehan E.M., Delahunty C.M., Kerry J.P., Morrissey P.A., 2003. Sensory characteristics and consumer preference for cooked chicken breasts from organic, corn-fed, free-range and conventionally reared animals. Int. J. Poultry Sci., 2, 409–416.

Pettersen M.K., Mielnik M.B., Eie T., Skrede G., Nilsson A., 2004. Lipid oxidation in frozen, mechanically deboned turkey meat as affected by packaging parameters and storage conditions. Poultry Sci., 83, 1240–1248.

Pignoli G., Bou R., Rodriguez- Estrada M.T. Decker E.A., 2009. Suitability of saturated aldehydes as lipid oxidation markers in washed turkey meat. Meat Sci., in press.

Wettasinghe M., Vasanthan T., Temelli F. Swallow K., 2001. Volatile flavour composition of cooked by-product blends of chicken, beef and pork: a quantitative GC–MS investigation Food Res. Int., 34, 149–158.

10

APPLICATION OF MEMBRANE FILTRATION TO RECEIVE SOLUTIONS ENRICHED WITH CYSTATIN FROM EGG WHITE

Introduction

Egg white fractionation process regarded mainly recovery of lysozyme, which is found in quite high amount in this material [Chiang et al., 1993; Leśnierowski and Kijowski, 2000]. However, nowadays increasingly interest is observed in cystatin (cysteine proteinases inhibitor) isolation from egg white solutions [Kopeć et al., 1996, 2002].

Hen's egg white cystatin is in 44% homologue to human cystatin C and also is very similar in activity mechanism [Jankowski et al., 2001; Konopska et al., 2007]. Cystatin inhibits the activity of cysteine proteases such as cathepsins B, H and L [Turk et al., 2002]. Cystatins are divided into three families: 1 – stefins, 2 – cystatins and 3 – kininogens. This classification is based on proteins aminoacids sequencing and also the mechanism of activity. Cystatin was for the first time isolated from hens egg white by Barrett [1980] and classified on the basis of aminoacids sequencing to 2 family of cystatins. Cystatins have received much attention in the last two decades due to their potential in regulating the function of cysteine proteinases [Henskens et al., 1996]. However, this classification is based only on animal cystatins, because many studies have been conducted to clarify the physiological and clinical significance of cystatins in animal tissues and products. Contrary to the general recognition of proteinase inhibitors as antinutrient factors, beneficial effects of these inhibitors have been reported after long-term feeding [Kennedy, 1993]. Since most proteases in muscles are sulphydryl proteinases (e.g. cathepsins B, H, L, M, S and N), involvement of cystatins in the function and malfunction of muscles is highly probable. The potential of cystatins broad application in medical treatments has been reported in the literature, and includes antimicrobial [Bjorck et al., 1989, Wesierska et al., 2004, Trziszka et al., 2004], antiviral [Ebina and Tsukada, 1991] and insecticidal effects [Koiwa et al., 1998], the prevention of cerebral haemorrhage [Abrahamson et al., 1986] and the control of cancer cell metastasis [Saleh et al., 2005, 2006]. Purified cystatin preparations can be utilized in pharmaceutical applications, mainly in gingival diseases such as paradontosis. Moreover, there are some possibilities for cystatin preparation application in clinical conditions, such as intravenous infusion due to cystatin ability to slowing down a cancerogenic changes in vivo. The inhibitor can be also used as an indicator in the diagnosis of cancer cells.

Membrane techniques are widely used, among others, in many food processing. Microfiltration (MF) and ultrafiltration (UF) are the most popular techniques. Microfiltration is

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generally used for initial purification and clarification of different solutions, whilst ultrafiltration for separations of the solutions containing particles differ of one order of magnitude [Rautenbach, 1996]. UF is applied in egg industry for partial water removing before egg white spray drying. It resulted in reduction of final production costs almost twice. Membrane techniques are now widely used in egg industry for egg white fractionating in order to obtained biologically active substances [Chiang et al., 1993].

Isolation and purification methods of cystatin from the egg white can not decrease its activity in order to use inhibitor as biomedical or nutraceutical preparations. But also low content of cystatin in egg white has to be taken into account, thus the most efficient method for inhibitor isolation is affinity chromatography using resins with immobilized papain.

Existing applications of immobilized enzymes are expensive and difficult to transform into industry scale production. Moreover, there are some difficulties in carrying out chromatographic processes due to high natural viscosity of egg white, which decreases the final yield of the process. Therefore, solutions obtained after membrane filtration, which are characterised by lower viscosity than egg white, are good alternative for further chromatographic techniques application.

The objective of the study was the evaluation of the possibility of membrane techniques application for receiving solutions enriched with cystatin from hen's egg white.

Materials and methods

The study was carried out on fresh eggs obtained from Lohmann Brown hens at 30–45 weeks of age kept in battery system (Gajewski Egg Production Plant, Gruszczyce, Poland). Layers were fed with standard fodder with an addition of commercial premixes. Eggs were collected between 3 to 5 days after laying and kept in chilling conditions until analyses. Eggs content was separated on white and yolk. Obtained egg white was filtered due to chalase, shell membranes and shells remaining removal. Then egg white was filtered through the Schott's funnel with 1 mm pores in order to remove impurities and make the solution more homogeneous. Following egg white was diluted in ratio 1:4 v/v with 150 mM NaCl and pH was adjusted to 8.5. Prepared solution was then input into microfiltration module (MF) using *cross flow* technique on polypropylene(PP) tubes filter. Nominal size of the membrane pores was 0.2 – 0.6 μm and active working area of the membrane was about 0.24 m^2. For one passage 10.000 ml of membrane feed was input into filtration membrane module using peristaltic pomp with elastic rotor (FIP). Transmembrane pressure (TMP) was adjusted to 0.05 MPa using regulatory inverter of feeding pomp. The membrane specification is presented in table 1. During the filtration process the volume of permeate was continuously observed and expressed as normalized filtrate flux J/J_0, where J [cm^3xs/cm^2] is filtrate flux depending on permeate volume and J_0 [cm^3xs/cm^2] initial filtrate flux rate.

Protein content was determined using biuret method [Gornall et al., 1949]. Cystatin activity against papain was analysed according to method reported by Siewinski [1991]. The analysis is based on colorimetric method, in which amount of BANA (hydrochloride Na-benzoyl-DL-arginyl-B-naftylamid) hydrolysis products is measured as a result of papain (cysteine proteinase) activity. One unit of inhibitory activity correspond with one unit of enzymatic activity of papain, that is the quantity of enzyme which is able to hydrolyse 1.0 mM of substrate per minute in standard conditions (37°C).

Table 1

Microfiltration membrane characteristics

Microfiltration	
Material	Polypropylene PP
Membrane area [m^2]	0.24
Pores size: nominal maximal	 0.2 μm ≤0.6 μm

Results and disscussion

The microfiltration membranes used in the study enabled initial fractionation of proteins present in egg white solutions, as well as lowered the structural viscosity of the raw material. During microfiltration the normalized filtrate flux was lowering due to fouling process on the membrane (Fig. 1). Membrane fouling referred to the irreversible alteration in membrane properties is caused by specific interactions feed stream components and membrane. Fouling can occur by several forms in particular deposition of denatured or agglomerated proteins at the surface of the membrane, or adsorption of proteins inside the pore structure of the membranes [Saxena et al., 2009]. The fundamental mechanisms involved in membrane fouling by protein suspensions may be grouped according to Saxena et al. [2009] as follows: the formation of a gel layer due to concentration polarization, adsorption of species on the membrane surface and inside the, pore structure, deposition and pore blocking after the formation of protein cake.

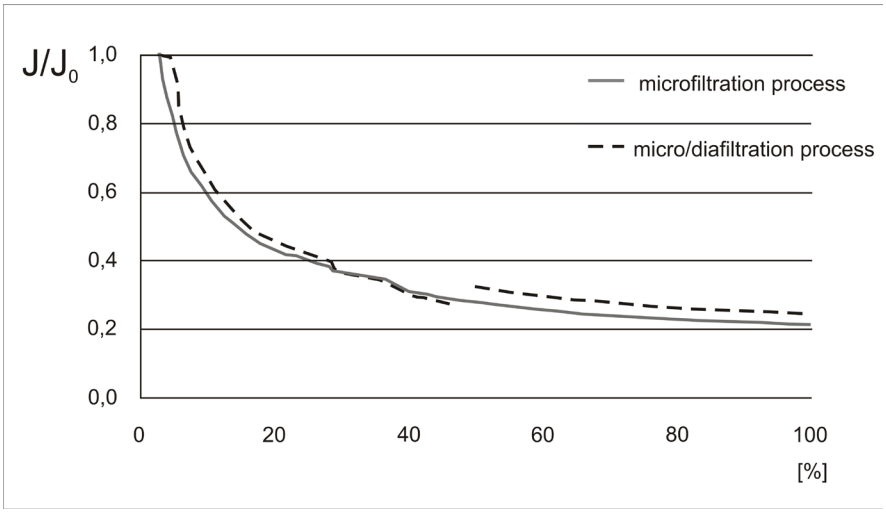


Fig. 1. Normalized filtrate flux in microfiltration and micro/diafiltration processes

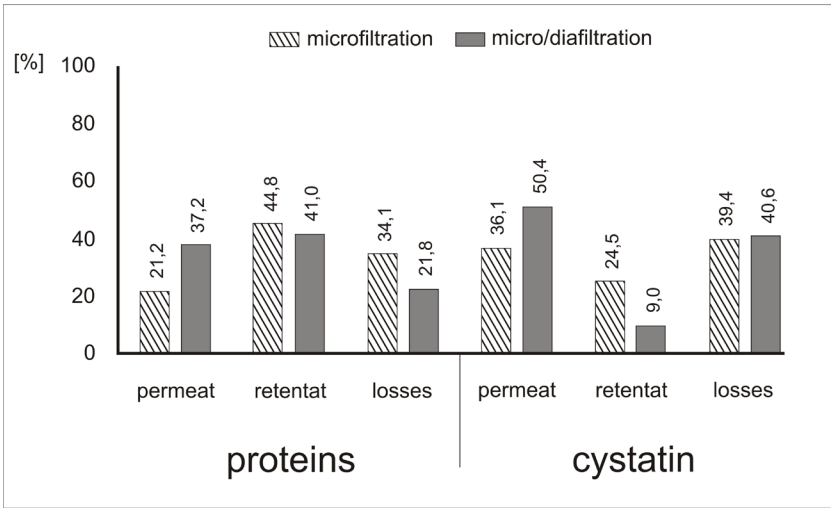


Fig. 2. Recovery of protein and active cystatin after microfiltration (MF) and micro/diafiltration (MFD) processes

Fouling connected with growth of the filtration cake resulted in our own studies in blocking of membrane pores as well as changing of the membrane selectivity towards egg white protein molecules. Such changes enabled the increase of low molecular weight proteins concentration in collected filtrate fractions.

Filtrate fractions collected during microfiltration were characterised by about 21% of total protein content in which ovoalbumin (44 kDa) dominated, and as well almost twice higher content of active cystatin (Fig. 2). Only few investigations have been devoted to protein interactions with microfiltration membranes and they have been performed mainly with albumins with molecular weight over 40 kDa like bovine serum albumin (67 kDa) and very seldom with low molecular weight proteins luke lysozyme [Yeu et al., 2009]. Hlavacek and Bouchet [1993] studied dead-end filtration of bovine serum albumin solution by microfiltration membranes and quantified fouling by means of the constant filtration rate equation. However, the different roles of adsorption, gel formation and the deposition of aggregates were not clear. Fouling of microfiltration membranes were also investigated by measuring the pressure drops across two membranes fed in series by a constant rate pump, which enables a distinction to be made between surface fouling and internal fouling of the membrane. In the case of the microfiltration of bovine serum albumin solutions the type of pump and the operating temperature influenced membrane fouling affecting protein denaturation and adsorption with the intensity depending on type of fouling [Xu-Jiang et al., 1995].

Local breakage of permeate flux was observed when 50% of membrane feed was filtered. Because of that fact simultaneous diafiltration process was also carried out, which enabled decrease in protein loss in the final balance of the filtration process. Diafiltration was performer by incorporation to membrane feed additional portion of the solvent (150 mM NaCl solution), when 50% of membrane feed was already filtered in quantity equaled the volume of filtrates. This operations increased the permeate flux volume (Fig. 1), as well as increased the transmission of active inihibtor to permeate fraction. Simultaneous diafiltration process enabled 16% of protein recovery in filtrates along with decreasing in protein loss

(12%) in overall balance of the process (Fig. 2). Percentage range of active cystatin recovery was counted as the quotient of collected filtrate fraction volume, protein content and specific cystatin activity in relation to this factor calculated for membrane feed. The value of percentage range of active cystatin after simultaneous diafiltration process was 50%. Purification degree of cystatin enriched preparations was 2.17, and was expressed as increase in specific inhibitory activity of cystatin in filtrates in relation to raw material.

Xu-Jiang et al. [1995] studied both dead-end and cross-flow microfiltration of bovine serum albumin solutions at a constant flowrate and showed that physico-chemical conditions influenced the pressure drop during processes. But the structure of cake was different at dead-end in comparison to cross-flow techniques. According to Xu-Jiang et al. [1995] protein cake created in cross-flow process is partially open, filamentous and quite permeable, enables filtration of low molecular weight proteins. It was the reason in our own studies of permeate enrichment in cystatin.

Conclusions

Application of microfiltration membrane techniques enabled fractionation of egg white solutions, as the initial step for further isolation and purification of cystatin.

Microfiltration of egg white resulted in up to 36% of active cystatin recovery and up to 50% when combined micro/diafiltration processes was conducted Microfiltration process enabled to obtain almost twice higher specific activity of cystatin in relation to the inhibitory activity in raw material.

References

Abrahmson M., Barrett A.J., Salvesen G., Grubb A., 1986. Isolation of six cysteine proteinase inhibitors from human urine. J. Biol. Chem., 261, 11282–11289.

Barrett A.J., 1980. Fluorimetric assays for cathepsin B and cathepsin H with methylcoumarylamide substrates. Biochem. J., 187, 909–912.

Bjorck L., Akesson P., Bohus M., Trojnar J., Abrahamson M., Olafsson I., Grubb A., 1989. Bacterial growth blocked by a synthetic peptide based on the structure of human proteinase inhibitor. Nature, 337, 387–388.

Chiang B.H., Su C.K., Tsai G.J., Tsai G.T., 1993. Egg white lysozyme purification by ultrafiltration ona affinity chromatography. J. Food Sci., 58, 303 – 306.

Ebina T., Tsukada K., 1991. Protease inhibitors prevent the development of human rotavirus-induced diarrhea in sucking mice. Microbiol. Immunol., 35, 583–588.

Gornall A.G., Bardawill C.J. David M.M., 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177, 751–766.

Henskens Y.M.C., Veerman E.C.J., Amerongen A.V.N., 1996. Cystatins in health and disease. Biol. Chem., 377, 71–86.

Hlavacek M., Vouchet F., 1993. Constant flowrate blocking laws and an example of their application to dead-end microfiltration of protein solutions. J. Membrane Sci., 82, 285–295.

Jankowski R., Kozak M., Janowska E., Grzonka Z., Grubb A., Abrahamson M., Jaskólski M., 2001. Human cystatin C, an amyloidogenic protein, dimerizes trough three-dimensional domain swapping. Nat. Struct. Biol., 8, 316–320.

Kennedy A.R., 1993. Overview: Anticarcinogenic activity of protease inhibitors, in: Protease inhibitors as cancer chemopreventive agents. Troll W., Kennedy A.R., Eds. Plenum Press, New York, 9–64.

Koiwa H., Shade R.F., Zhu-Salzman K., Subramanian L., Murdock L.L., Nielsen S.S., Bressan R.A., Hasegawa P.M., 1998. Phage display selection can differentiate insecticide activity of soybean cystatins. Plant J., 14, 371–379.

Konopska B., Gburek J., Gołąb K., Warwas M., 2007. Characterization of chicken cystatin binding to rat renal brush-border membranes. Comp. Biochem. Physiol., 146, 482–488.

Kopeć W., Trziszka T., Chrzanowska J., 1996. The metod of direct isolation of lysozyme from egg white using chromatography and ultrafiltration. Preceed XX World's Poultry Cong. WPSA, New Dehli, India, 3, 37–41.

Kopeć W., Korzeniowska M., Trziszka T., 2002. An attempt to industrial process of simultaneous isolation of lysozyme and cystatin from egg white. Proceed. 11th Europ. Poultry Conf WPSA, Bremen, 1–8. Abstract: Arch. Geflügelk., 2002, 66, 55–56.

Leśnierowski G., Kijowski J., 2000. Otrzymywanie lizozymu z białka jaja kurzego metodą bezpośredniej ultrafiltracji. Żywność. Nauka. Technologia. Jakość, 23, 59–69.

Rautenbach R., 1996. Procesy membranowe. WNT, Warszawa.

Saleh Y., Wnukiewicz J., Andrzejak R., Trziszka T., Siewinski M., Ziółkowski P., Kopeć W., 2006. Cathepsin B and cysteine protease inhibitors in human tongue cancer: Correlation with tumor staging and in vitro inhibition of cathepsin B by chicken cystatin. J. Cancer Mol., 2, 67–72.

Saleh Y., Sebzda T., Warwas M., Kopeć W., Ziółkowski J., Siewinski M., 2005. Expression of cystatin C in clinical human colorectal cancer tissues. J. Exp. Ther. Oncol., 5, 49–53.

Saxena A., Tripathi B.P., Kumar M., Shahi V.K., 2009. Membrane-based techniques for the separation and purification of proteins: An overview. Adv. Coll. Inter. Sci., 145, 1-22.

Siewiński M., 1991. Metod of purification of thiol proteinase inhibitors from human urine. Cancer Biochem. Biophys., 12, 33–44.

Trziszka T., Saleh Y., Kopeć W., Siewiński M., Wesierska E., 2004. Effect of hen's age on the level of cystatin in the chicken egg white. Int. J. Poultry Sci., 3, 471–477.

Turk B., Turk D., Salvesen G.S., 2002. Regulating cysteine protease activity: essential role of protease inhibitors as guardians and regulators. Curr. Pharm. Des., 8, 1623–1637.

Wesierska E., Saleh Y., Trziszka T., Kopec W., Siewinski M., Korzekwa K., 2005. Antimicrobial activity of chicken egg white cystatin. World J Microbiol. Biotechnol., 21, 59–64.

Xu-Jiang Y., Dodds J., Leclerc D., 1995. Cake characteristics in crossflow and dead-end microfiltration. Filtr. Sep., 9, 795–798.

Yeu S., Lunn J.D., Rangel H.M., Shantz D.F., 2009. The effect of surface modifications on protein microfiltration properties of Anopore™ membranes. J. Memb. Sci., 327, 108–117.

11

EGG-WHITE CYSTATIN ISOLATION FROM PROTEINACEOUS AGENTS OBTAINED BY ETHANOL EXTRACTION

Introduction

It is well known that eggs are more than an excellent source of dietary nutrients. Many extensive studies identifying and characterizing the biologically active components of eggs have been carried out [Mine and Kovacs-Nolan, 2004; Trziszka et al., 2006; Gołąb and Warwas, 2005]. The potentials of some of the biologically active substances have already been determined, including those of lysozyme, ovomucin, avidin, IgY, and cystatin. They have been applied in the prevention and treatment of various medical conditions [Trziszka et al., 2006; Gołąb and Warwas, 2005].

Cystatins are small proteins found in the body fluids and tissues of animals and plant seeds [Kato et al., 2002]. They are members of one evolutionary superfamily consisting of three distinct subfamilies of closely related proteins: stefins, cystatins, and kininogens [Węsierska et al, 2005]. These proteins are reversible, tight-binding, competitive inhibitors of papain, ficin, and cathepsins B, H, L, S, and K [Turk et al, 2002; Kato et al., 2002; Sokołowska et al., 2007]. Chicken egg-white cystatin is a single-chain nonglycosylated 12.7-kDa protein [Trziszka et al., 2006]. This protein, consisting of 116 amino-acid residues, exists in two isoelectric forms differing by the occurrence of phosphorylation on serine 80 [Sokołowska et al, 2007]. Cystatin, as a substance with a high inhibitory activity against cysteine proteases, is a factor which inhibits the development of cancer cells [Węsierska et al., 2005; Trziszka et al., 2006]. These enzymes play an important role in cancer invasion and metastasis by the degradation of the extracellular matrix, which is an essential step in metastasis [Saleh et al., 2003]. Cystatin also exhibits antimicrobial activity towards Gram-positive and Gram-negative bacteria [Węsierska et al., 2005; Sokołowska et al., 2007]. Recently, research on cystatin as a factor in fighting AIDS and paradontosis has been intensified [Węsierska et al., 2005].

The aim of this study was to develop a simple method of isolating cystatin from the proteinaceous agent obtained by the ethanol extraction of egg white.

Materials and methods

Biological Material

The eggs were laid by hens of the "zielononóżka kuropatwiana" line. The fresh whole eggs were broken and the albumen was separated from the yolk. The albumen was precipitated by the method of Sokołowska et al. [2007]. The resulting supernatant was the starting material for the investigations.

Purification of cystatin from supernatant obtained by ethanol precipitation of egg white

Affinity chromatography. The supernatant was evaporated under reduced pressure at 30°C to remove ethanol. Then it was subjected to affinity chromatography on carboxymethyl-papain linked to an acrylic resin equilibrated with acetic acid (pH 4.0). The supernatant was applied and after 12 h of incubation at 2°C the column was packed and washed with 0.1% BRIJ reagent with 0.5 M NaCl. Proteins bound to carboxymethyl-papain-acryl were eluted with 50 mM Na_3PO_4 containing 0.5 M NaCl (pH 11.5) and the active fractions were pooled.

Cation exchange chromatography. The eluted fraction was applied to an Amberlite IRC 50 column. After 40 min of incubation at 2°C, the unabsorbed material was separated and Amberlite IRC 50 was washed (30 min) twice with bi-distilled water. Proteins bound to the Amberlite IRC 50 were eluted with 1% NaCl and then with 6.5% NaCl.

Protein assay. Protein content was determined either by the bicinchonic acid method [Smith et al., 1985] or spectrophotometrically according to Whitaker and Granum [1980].

Inhibitory activity against papain. The inhibitory activity was measured spectrophotometrically using the substrate N-α-benzoyl-DL-Arg-β-naphtylamide (BANA) [Barrett, 1977]. Papain (5 μg) was preincubated for 5 min at 37°C in 200 mM phosphate buffer (pH 8.0) containing 2 mM EDTA and 10 mM cysteine to activate the enzymes. Then the suitable amount of inhibitor was added to a final volume of reaction solution of 1 ml. After 10 min of incubation, the enzyme activity was measured by the addition of BANA (1.5×10^{-4} M). After 20 min of incubation, the reaction was stopped with 1 ml of 1% DMBA (7,12-dimethylbenzanthracene) in 50% v/v acetic acid and the released naphtylamine was measured at 450 nm. One unit of inhibitory activity against papain corresponded to the amount of enzyme which hydrolysed 1.0 mM substrate per minute under standard conditions (37°C).

Trypsin inhibitory activity. Trypsin was allowed to complex with a suitable amount of inhibitor. The residual enzyme activity was measured spectrophotometrically using the substrate N-α-benzoyl-DL-arginine-p-nitroanilide (BApNA) [Broadway, 1997]. The chromatographed materials were incubated with an appropriate amount of inhibitor in a buffer containing Ca^{2+} at 37°C. The ability of the sample to inhibit trypsin activity was analysed by the lowering of the absorbance at 412 nm compared with the control without inhibitor. One unit of trypsin inhibitory activity corresponded to the μg amount of inhibitor which inhibited 1 μg of trypsin.

Lysozyme activity. Lysozyme activity was analysed spectrophotometrically according to Weisner [1984] with some modification. The method is based on the measurement of changes in the absorbance of a suspension of *Micrococcus lysodeicticus* bacteria during reaction of the enzyme with the bacterial cell. Measurements were made at a stable temperature of 25°C and a wavelength of 450 nm every 60 sec for 6 minutes of the reaction. One unit of enzyme activity was defined as the amount of lysozyme which yielded a decrease in absorbance at 450 nm (A_{450}) by 0.01 per min.

SDS-PAGE electrophoresis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [1970] using 15% (w/v) polyacrylamide gel in the presence of SDS. Electrophoresis was performed under reducing conditions and egg-white cystatin (13.4 kDa), ovomucoid (29 kDa), ovalbumin (45 kDa), and ovotransferrin (66 kDa) were used as standards for molecular weight calculations. The gel was stained using 0.1% Coomasie Brilliant Blue R-250 for 6 h. After that the gel was distained with a solution containing 40% methanol in 10% acetic acid.

Results

Purification of cystatin from supernatant obtained by ethanol precipitation of chicken egg white

The albumen was precipitated by the method of Sokołowska et al. [2007] described in Materials and Methods. The resulting supernatant exhibited specific activity against papain (4.42 μ/mg), trypsin inhibitory activity (0.54 μ/mg), as well as lysozyme activity(1434.0 μ/mg) (Tab. 1). The specific activity of the preparation against papain was not increased compared with the starting material.

Affinity chromatography

The majority of the non-inhibitory proteins were removed from the proteinaceous agent by affinity chromatography on carboxymethyl-papain linked to an acrylic resin (Tab. 1). The starting material was applied to the resin and after 12 h of incubation at 2°C the column was packed. The unabsorbed proteins were collected. Then the carboxymethyl-papain acrylic resin was washed with 0.1% BRIJ reagent containing 0.5 M NaCl. The specifically bound proteins were eluted with 50 mM Na_3PO_4 containing 0.5 M NaCl (pH 11.5) (Fig. 1). The inhibitory activity against papain, inhibitory trypsin activity, and lysozyme activity of the bound and unbound fractions were measured. The fractions with nos. 45 to 120, which exhibited considerable specific activity for cystatin, flowed together. This procedure resulted in a threefold increase in inhibitory activity against papain. Inhibitory trypsin activity was not noted for the eluted protein solution. All of the trypsin inhibitory activity obtained by the ethanol precipitation method (99%) was in the fractions washed with 0.1% BRIJ reagent containing 0.5 M NaCl (data not shown), whereas lysozyme activity was 159.0 μ/mg for the eluted material. The efficiency of purification was 37%. The composition of proteins bound to carboxymethyl-papain-acryl was analysed by SDS-PAGE electrophoresis (Fig. 2). The results showed two quantitatively predominant proteins with molecular masses of 13.4 kDa and ca. 50 kDa. Furthermore, the electropherogram showed the presence a protein with a molecular mass of 14.4 kDa.

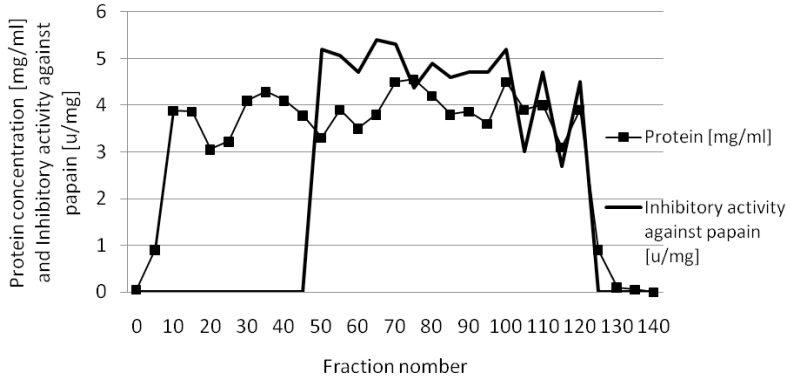


Fig. 1. Elution profile of affinity chromatography of the proteinaceous agent. The carboxymethyl papain linked to the acrylic resin with bound proteins was packed into the column (33 x 4 cm) and washed with 0.1% BRIJ reagent containing 0.5 M NaCl. The proteins were eluted with 50 mM Na_3PO_4 containing 0.5 M NaCl (pH 11.5). Conditions: 2°C, constant pressure of 0.05 mPa. Five-ml fractions were collected. The protein content was determined by the bicinchonic acid method

Cation exchange chromatography

Lysozyme was separated from cystatin by cation-exchange chromatography on an Amberlite IRC 50 column. This resin effectively absorbed only lysozyme (98.7 μ/mg), while it had no effect on cystatin. As shown in Table 1, the specific inhibitory activity against papain increased from 13.3 to 16.6 μ/mg. The efficiency of this procedure was 32.2%.

Table 1

Purification protocol of cystatin from egg white

Purification step	Total protein [mg]	Total inhibitory activity against papain [U]	Total trypsin inhibitory activity [U]	Total lysozyme activity [U]	Specific activity [u/mg]*	Purification factor*	Yield [%]*
Homogenate	26,160	31,382	17,700	38,066,640	4.42	1.0	100
Ethanol protein precipitation	15,660	29,040	8550	22,456,440	4.4	0.9	92.0
Affinity chromatographyproteins bound to the carboxymethyl-papain-acrylic resin	828.4	11,600	Not detected	131,716	13.3	3.0	37.0
Cation-exchange chromatography on Amberlite IRC 50 resin unabsorbed fractions	609.5	10,117.7	Not detected	60,158	16.6	3.8	32.2

*Specific activity, purification factor, and efficiency related to proteins exhibiting specific cystatin activity (inhibitory activity against papain) only

Fig. 2. SDS-PAGE analysis of the proteins bound to carboxymethyl-papain-acryl. Lane 1 – protein standard (13.4 kDa, 29 kDa, 45 kDa, and 66 kDa), lane 2 – proteins bound to carboxymethyl-papain-acryl (20 μg of protein/lane was applied).

Discussion

Cystatin has potential possibilities for extensive medical applications, which include antimicrobial, antiviral, antifungal, and insecticidal effects, the prevention of cerebral haemorrhage, and the control of cancer cell metastasis. However, the most important problem in utilising cystatins in medicine is their high cost. The purpose of this study was to propose a simple isolation method of cystatin from proteinaceous agent obtained by ethanol extraction of chicken egg white.

Many egg-white cystatin isolation methods have already been developed. Anastasi et al. [1983] proposed a purification method which included precipitation of ovomucin, affinity chromatography on carboxymethylated papain-sepharose, and chromatofocusing. Trziszka et al. [2004] described a six-step method to purify cystatin: ovomucin precipitation, affinity chromatography on a papain-Sepharose, gel filtration on Sephadex G-100, DEAE, and reverse-phase chromatography. Another method includes preheating egg white at 62°C (pH 12.0) followed by liquid chromatography on CM-Sepharose and a Mono-S cation-exchange column [Réhault, 2007].

The first step of our cystatin purification procedure was an ethanol precipitation method previously proposed by Sokołowska et al. [2007]. This rapid and inexpensive one-step precipitation of egg white leading to the proteinaceous agent involves mainly cystatin, lysozyme, and trypsin inhibitors. This bifunctional preparation, exhibiting both antimicrobial and antiproteinase activities, can be a good candidate as a natural preservative prolonging food storage [Sokołowska et al., 2007]. Cystatin in unpurified form can be used in the food as well as the cosmetic industry, whereas medicine needs very highly purified preparations.

The supernatant obtained in the previous step (after ethanol extraction), which demonstrated 4.4 μ/mg specific activity against papain, was further purified by affinity chromatography on immobilized carboxymethyl-papain linked to an acrylic resin. The specific activity against papain of the eluted protein was 3.0 times higher than that of the starting material. The efficiency of cystatin isolation amounted to 37%. The eluted proteins still demonstrated lysozyme activity, but lost their trypsin inhibitory activity, indicating that affinity chromatography effectively separated trypsin inhibitors from cystatin. However, SDS electrophoresis of the proteins bound to carboxymethyl-papain-acryl revealed the presence of predominant bands with molecular weights of ca. 13 kDa, which certainly corresponds to cystatin, and ca. 49 kDa, corresponding to the trypsin inhibitor ovoinhibitor. Our results are very similar to those obtained by Trziszka et al. [2004], who attempted the purification of cystatin from egg white and finally also obtained a preparation consisting of two proteins with molecular masses of 40 and 13 kDa. The problem with separating these two proteins probably comes from their very similar biochemical properties. The specific activity for the fraction with a molecular mass of 13 kDa obtained by Trziszka et al. [2004] was 4.13 mEu/ml. The efficiency of this procedure led to a 25.8-fold increase in specific cystatin activity, also with high efficiency (62.5%) [Trziszka et al., 2004].

The lysozyme from the proteins bound to carboxymethyl-papain-acryl was removed by cation exchange chromatography on Amberlite IRC 50 resin. This preparation resulted in a 3.8-fold rise in cystatin-specific activity compared with the starting material. The specific cystatin activity of the final preparation was 16.6 μ/mg.

References

Anastasi A., Brown M.A., Kembhavi A.A., Nicklin M.J., Sayers C.A., Sunter D.C., Barret A.J., 1983. Cystatin, a protein inhibitor of cysteine proteinases. Improved purification from egg white, characterization and detection in chicken serum. Biochem. J., 211, 129–138.

Barrett A.J., 1977. Cathepsin B and other thiol proteinases. In: Proteinases in Mammalian Cells and Tissues (ed. A.J. Barrett). Amsterdam: Elsevier/North-Holland Biomedical Press, Inc., 181–208.

Broadway R.M., 1997. Dietary regulation of serine proteinases that are resistant to serine proteinases inhibitors. J. Insect Physiol., 43, 855–874.

Gołąb K., Warwas M., 2005. Białka jaja kurzego – właściwości biochemiczne i zastosowania. Adv. Clin. Exp. Med., 14, 5, 1001–1010.

Kato T., Imatani T., Minaguchi K., Saitoh E., Okuda K., 2002. Salivary cystatins induce interleukin-6 expression via cell surface molecules in human gingival fibroblasts. Molecular Immunol., 39, 423–430.

Laemmli U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage 4. Nature (London), 227, 680–685.

Réhault S., 2007. Antiproteases. Bioactive egg compounds. Huopalahti, R., López-Fandiño R., Anton, M., Schade, R. (Eds.) Springer-Verlag Berlin, 85–98.

Saleh Y., Siewiński M., Kielan W., Ziółkowski P., Gryboś M., Rybka J., 2003. Regulation of cathepsin B and L expression in vitro in gastric cancer tissues by egg cystatin. J. Exp. Therapeut. Oncol., 3, 319–324.

Smith P.K., Khron R.I., Hermanson G.T., Mallia A.K., Gartner F.H., Provenzano M.D., Fujimoto E.K., Goeke N.M., Olson B.J., Klenk D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem., 150, 76–85.

Sokołowska A., Kubiak A., Trziszka T., Polanowski A., 2007. Rapid one step separation of proteinaceous agents of antimicrobial activity from hen's egg white. Pol. J. Food Nutr. Sci., 57, 499–502.

Trziszka T., Saleh Y., Kopeć W., Siewiński M., Węsierska E., 2004. Effects on hen's age on the level on cystatin in the chicken egg white. Int. J. Poultry Sci., 3, 7, 471–477.

Trziszka T., Polanowski A., Saleh Y., Kopeć W., 2006. Egg white isolation and its medical application. http://www.animalscience.com/uploads/additionalfiles/wpsaverona.htm

Turk B., Turk D., Salvesen G.S., 2002. Regulating cysteine protease activity: essential role of proteinase inhibitors as guardians and regulators. Curr. Pharm. Des., 8, 1623–1637.

Weisner B., 1984. Lysozyme (Muramidase). in: Methods in Enzymatic Analysis, 4 (ed. H.U Berbmeyer), Verlag Chemie, Weiheim, 189–195.

Węsierska E., Saleh Y., Trziszka T., Kopeć W., Siewiński M., Korzekwa K., 2005. Antimicrobial activity of chicken egg white cystatin. World J. Microb. Biot., 21, 59–64.

Whitaker J.R., Granum P.E., 1980. An absolute method for protein determination based on difference in absorbance at 235 and 280. Anal. Biochem., 109, 156–159.

12

SUBSTRATE SPECIFICITY OF TRANSGLUTAMINASE. INFLUENCE OF TRANSGLUTAMINASE ON MILK WHEY PROTEINS CROSS-LINKING

Introduction

Substrate specificity of transglutaminase. Enzymes play an important role in the food producing of both traditional and novel products. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. But the traditional products like yoghurt and fermented beverages are performed by endogenous enzymes that occur naturally in the plant and animal tissues or in the microorganism's cells. The idea of isolated, exogenous enzymes adding to improve existing reactions or to initiate new reactions, dates from the start since second part of past century in the USA led to the development of enzymes for the leather industry and started the commercial production of papain for use in the beer industry. Now there are many enzymes for food processing available that originate from different sources. Majority of enzymes applied in food industry are hydrolases such as glycosidases, and in part proteases used for the meat tenderizing. The new direction is the use of enzymes as a tool for modificaton of protein structure. Microbial transglutaminase is used for this aim. It produces the both inter- and intra-molecular isopeptide cross-linking bonds in the proteins. We investigate its substrate specificity to attempt to develop the combined products consisting of the proteins from different sources.

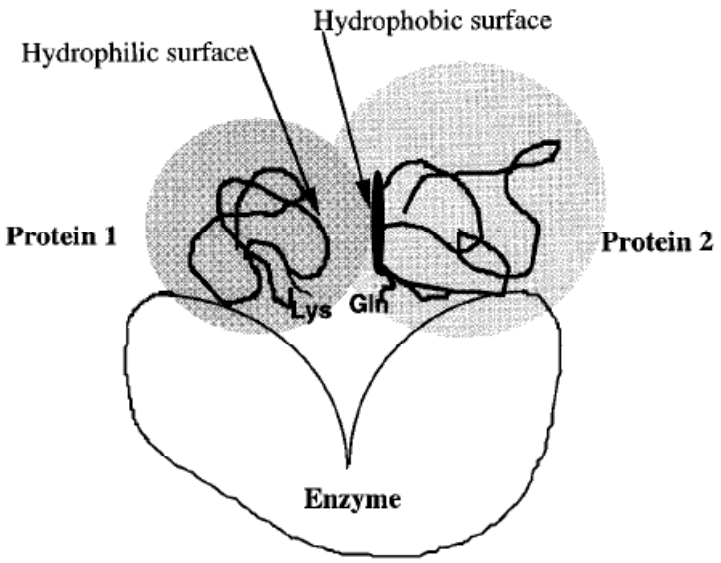
Transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13; TGase) catalyzes acyl-transfer reactions introducing covalent cross-linkages between proteins, creating high molecular weight polymers.

Generally, protein substrates of TG are classified into four groups: (1) Gln-Lys-type, in which both Gln and Lys residues are available for crosslinking; (2) Gln-type, in which only the Gln residue is available for reaction; (3) Lys-type, in which only Lys residues are available; and (4) a nonreactive type, in which both Glu and Lys residues are unavailable for reaction [Ikura et al., 1984]. This classification is mainly based on the accessibility of Lys and Gln residues located on the protein's surface. According to the above classification, a mixture of two Gln-Lys-type substrate proteins or a mixture of Gln-type and Lys-type substrate proteins should be able to form heteroconjugates in TG-catalyzed reaction. However, in addition to the availability of Lys and Gln residues, another factor that could potentially affects cross-linking of two different macromolecular protein substrates by TG is the thermodynamic compatibility of mixing of the protein substrates at the enzyme's active site.

The thermodynamic compatibility is related to the nature and the intensity of interaction between two macromolecules as they approach each other. As stated by Flory [1986], two

polymers are mutually compatible with one another only if their free energy of interaction is favorable, i.e., negative. In a majority of cases, the interaction energy, ΔG_{ij}, where the subscripts *i* and *j* refer to polymers 1 and 2, respectively, is usually positive, and thus incompatibility of dissimilar polymers is the rule and compatibility is the exception [Flory, 1986]. Proteins belonging to different classes have been shown to exhibit limited thermodynamic compatibility, and an aqueous mixture of binary protein solution separates into two distinct phases. In proteins, limited thermodynamic incompatibility arises as a result of interaction between polar and apolar surfaces. Because of this mutual repulsion between polar and apolar surfaces, two dissimilar proteins will approach each other only up to a distance at which the free energy of the interaction is zero. Beyond this closest distance of approach, a positive free energy of interaction will develop. Because of this mutual repulsion, i.e., limited thermodynamic compatibility, each protein molecule will possess an excluded volume which cannot be accessed by other proteins. This excluded volume is the sum of those arising from spatial requirements and from the positive free energy of polar-nonpolar repulsive interactions. Because of this excluded volume effect, thermodynamically incompatible proteins may not be able to mix at the active site of TGase, as shown in the next Scheme.

Scheme 1



The inability to come close together at the active site and form a transient enzyme-substrate complex may preclude formation of heterologous dimers and polymers.

In paper [Han, X.-Q. and Damodaran, S., 1996] is provided evidence to show that heteroconjugation does not occur in some mixtures of Gln-Lys-type substrate proteins and formation of heteroconjugates between two proteins may fundamentally depend on their thermodynamic compatibility of mixing at the enzyme's active site. In proteins, limited thermodynamic incompatibility arises as a result of interaction between polar and apolar surfaces [Han, X.-Q. and Damodaran, S., 1996].

Table 1

Summary of the properties of the proteins used and their ability or inability to form heterologous dimers and polymers with each other [Han and Damodaran, 1996]

Protein	Type of substrate	Cross-linking with						Thermodynamic compatibility with					
		CN	suCN	LA	LG	BSA	OV	CN	suCN	LA	LG	BSA	OV
β-casein (CN)	Gln-Lys	+	+	+/-	-	+	-	+	+	+/-	-	-	-
Succinylated β-casein (suCN)	Gln	+	-	+/-	+			+	+	+/-	+		
α-lactalbumin (LA)	Gln-Lys	+/-	+	+		+	-	+/-	+	+	+	+/-	
β-lactoglobulin (LG)	Gln-Lys	-	+	+	+		-	-	+		+		-
BSA	Gln or Lys	-	+	+	-	-	-	-	+	+	-	+	
Ovalbumin (OV)	Gln or Lys	-	+	-	-	-	-	-	+/-	-	-	-	

In Table 1 is summarized the properties of the protein substrates used and their ability/ inability to form heterologous dimmers and polymers with each other. Dissimilar proteins such as caseins and albumins cannot form heterologous dimers. This is primarily because of the inability of these proteins to overlap one another at the enzyme's catalytic sites. The thermodynamic incompatibility of mixing arises because of repulsive interactions between the hydrophobic and hydrophilic proteins. On the other hand, proteins of similar kind, i.e., globular proteins such as albumins and globulins, can form dimers and polymers in the TGase reaction because of their thermodynamic compatibility of mixing. In addition to thermodynamic compatibility, the structural state and disposition of lysyl and glutamine residues in proteins also seem to affect formation of heterologous polymers. Thus, although ovalbumin and α-lactalbumin are compatible proteins, they do not form heterologous polymers because both lysyl and glutamine residues of ovalbumin are unavailable for the TGase reaction.

Milk whey utilization problem

Large amounts of wastewaters emerge from milk processing in dairies, which are one of the largest sources of industrial effluents. Thus, wastewater and organic residues produced from dairy production have the potential to be converted into economic gain. The whey constitutes a major ecological burden to be disposed of as a waste material because the biological oxygen demand (BOD) of whey is very high at 40.000 mg per kg. Utilization of this nutrient rich byproduct, which would otherwise go as a waste is predominantly favored due not only to the economic opportunities because of the nutrients but also from the disposal point of view.

Milk whey proteins (lactalbumin, lactoglobulin and immunoglobulin) have the highest splitting speed among whole proteins. The concentration of amino acids and peptides in

blood rise steeply in the space of an hour after eating milk whey proteins food. Herewith, stomach acid-forming function keeps up so that excludes violation its work and gas generation. Milk whey protein assimilation is entirely high.

Whey proteins amino acid content most allied with man muscular tissue amino acid content. Milk whey proteins excel all others animal and vegetable origin proteins in matter of essential amino acids and branched-chain amino acids content. As well as, about 14% milk whey proteins are in the form of hydrolyzate (amino acids, di-, tri- and polipeptides) which are digestion initiators and take part in synthesis of majority vital ferments and hormones. Milk whey proteins also sensible reduce cholesterol level in the blood.

68.5% cheese whey drain in waste water in Russia [Sviridenko et al., 2008] although several ways recovery wastes of dairy industry now exist. The development new binding methods for whey proteins permits solve up-to-date scientific and economic problem – milk whey utilization.

Material and methods

Milk components and sour culture. Pasteurised milk (protein 2.5%, w/w) was obtained from a local dairy. The sour culture *L. acidophilum* was own made.Enzyme. Ajinomoto Activa GS as source of TGase was used without further purification.

Fermented milk processing conditions.Fermentation of pasteurized milk (2.5% fat) was carried out. Collagen or TGase; *L. acidophilum* was brought in heated milk, mixed for 15 min. Temperature was kept up 37–40°C, fermentation time 4–5 hours (milk clot formation, 65–85°T). Fermented samples was centrifuged at 113 x g for 1 hour.

Kinetic measurements. Determination of fermentation speed was carried out by Terner method. Protein concentration was determined by biuret and Lowry methods. Rheological measurements were carried out at viscometer VZ-246 with inner diameter 4 mm. The extent of TGase-induced cross-linking was estimated by SDS–PAGE using 12.5% acrylamide gel. Gels were run at 300 V, stained using 0.2% (w/v) Coomassie Brilliant Blue R250 in a 4:1:5 mixture of methanol, acetic acid and deionised water and destained in a 25:7.5:67.5 mixture of methanol, acetic acid and deionised water. Gel permeation chromatography was performed on Sephadex G-75 1.4x50 cm column. Eluent was 0.15 M NaCl. Detection was realized at 230 nm wavelength. Elution speed was 33 ml/h.

Results and discussion

First of all it was necessary to investigate the influence of nonenzymatic protein added to milk fermented.

It was established that collagen addition up to 2.5% w/w increases the speed of milk fermentation (Fig. 1). Collagen addition more than 2.5% is not effective because reaction mixture is saturated with collagen. It is possible that collagen is the carrier for *L. acidophilum* ferment microflora fastened there. Such immobilization of *L. acidophilum* perhaps promotes its sour effectiveness by analogy with activity increasing of immobilized ferments or microorganisms cells. However, this suggestion requires more detail investigation.

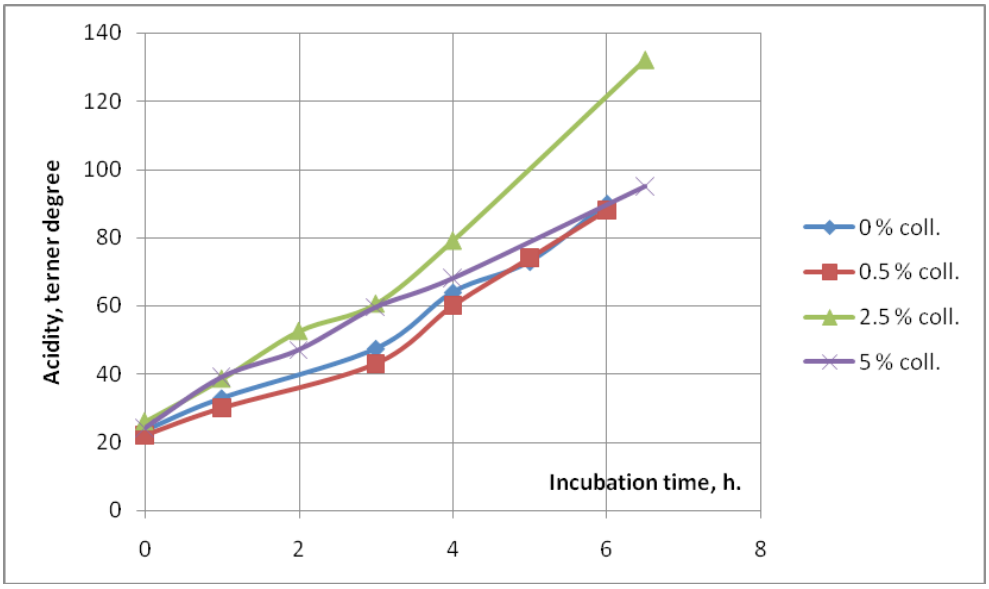


Fig. 1. Pasteurised milk incubation with collagen

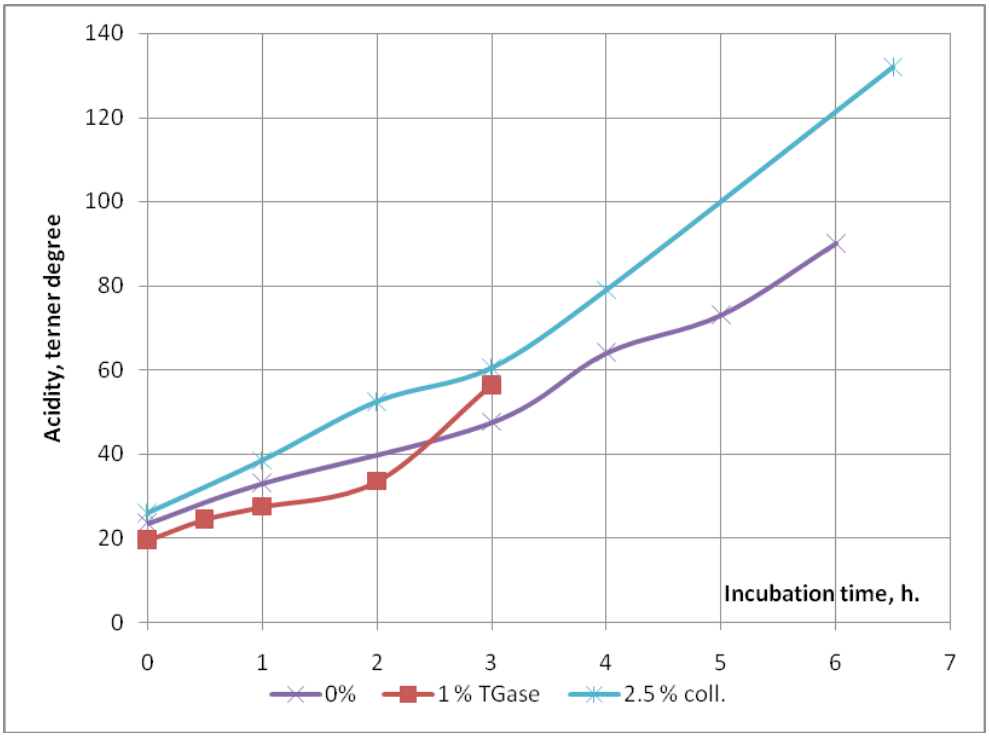


Fig. 2. Milk fermentation speed depended on protein added

TGase addition does not considerably increase the fermentation speed (Fig. 2). That fact coordinated with paper by Bönisch et al. (2007). A too intense level of cross-linking could possibly reduce the availability of low molecular mass peptides for lactic acid bacteria nutrition and slow down their growth (Færgemand et al., 1999].

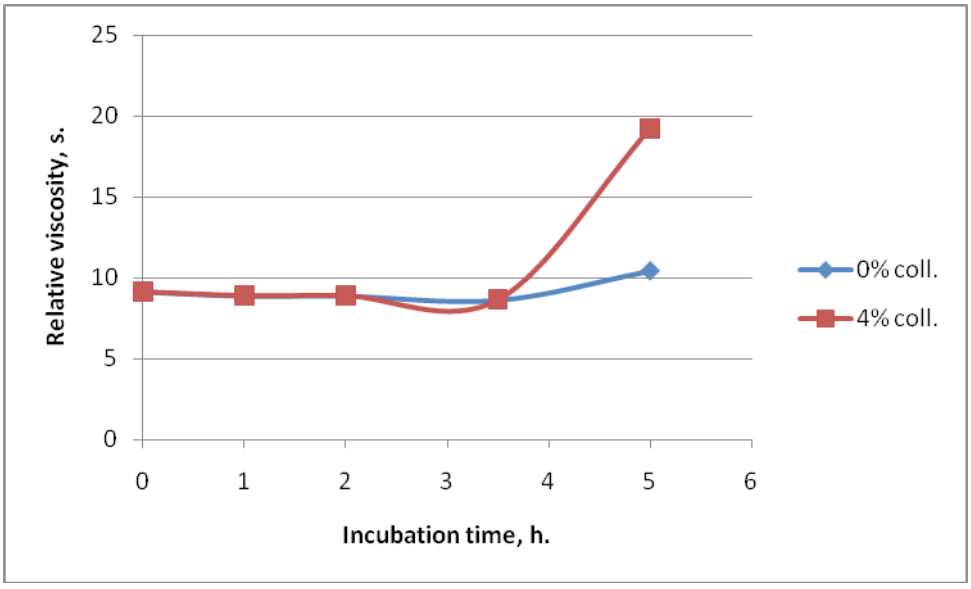


Fig. 3. Relative viscosity change during incubation

Relative viscosity was determined (Fig. 3). Collagen addition induced growth in viscosity in 5 hours of incubation from 10 – 11 sec. to 18 – 19 sec. More strong clot is formed.

Milk product structure after fermentation represented in Fig. 4. Separated whey percentage was determined (Fig. 5).

Fig. 4. Fermented product structure: a) 0% TGase; b) 1% TG with milk whey moving off; c) 1% TGase (whey remaining).

Product with 1% TGase has had less smooth texture than control sample, coarse-grained and lumpy (Fig. 4). As stated by De Jong et al. [2001], grain effect rises with increasing extent of cross-linking. Grain effect and separated whey volume increase with acidity growth. Milk incubation with TGase lead to modification casein micelles which become more stable to dissociation that explains granulation [De Jong et al., 2001].

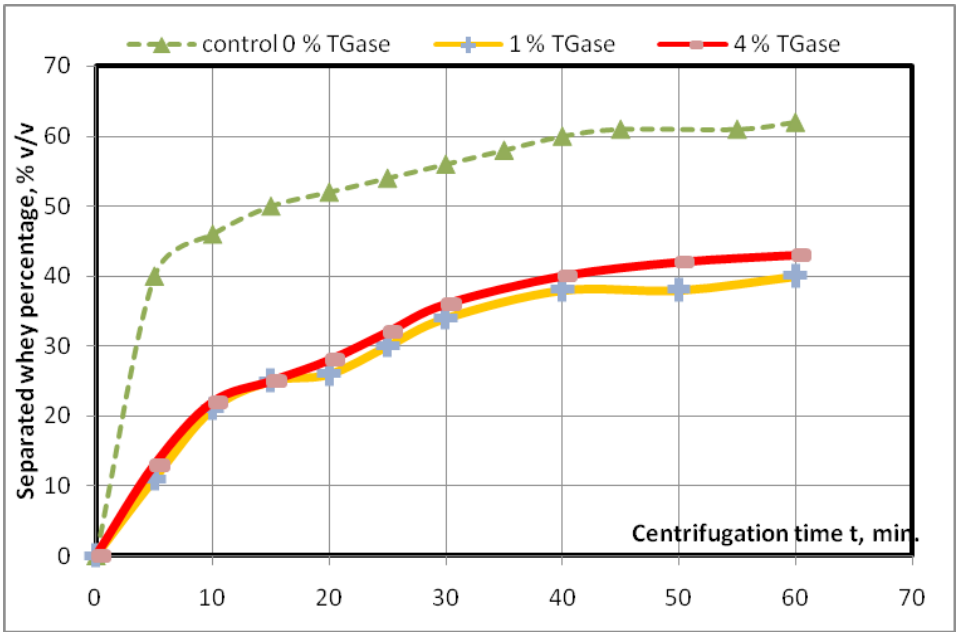


Fig. 5. Separated whey in fermented samples [%]

Separated whey percentage decreases if use TGase (Fig. 5). Therefore, clot firmness increases. Intra-micellar cross-linking transforms the casein micelles from an association colloid into micro-gel particles, and thus leads to altered properties of casein micelles; e.g., cross-linked casein micelles are considerably more stable to a wide range of dissociating agents than native micelles [O'Sullivan et al, 2002a; Smiddy et al., 2006]. Furthermore, cross-linking also alters the colloidal stability of casein micelles; the stability to heat-induced coagulation is higher for crosslinked micelles than for native micelles [O'Sullivan et al., 2001; O'Sullivan et al., 2002b] Caseins and whey proteins cross-linking promotes micelles stabilization. Simultaneously pores in protein network becomes significantly smaller improving moisture retaining and stability. 1% TGase concentration preferably use in comparison with 4% TGase because of better protein binding. Supersaturation reaction medium with TGase takes place if 4% ferment was used.

There was determination (Table 2) of separated whey volume after centrifugation, protein content in whey by biuret method and absolute protein content in whey as multiplication of protein content in whey and absolute protein content in whey (C_{abs} = V x C). It was decreases in all stated magnitudes with TGase.

Table 2

Separated whey volume and absolute protein content in whey (samples number = 5)

TGase concentration [% w/w]	Separated whey volume V [l/l sample]	Protein content in whey C [g/l]	Absolute protein content in whey $C_{abs,}$ [g/l sample]
0%	0.59±0,04	38.25±5.86	22.73±5.14
1%	0.49±0,07**	34.10±3.94**	16.38±2.83*

*distinctions are valid at a relevance level α = 0.05

** distinctions are valid at a relevance level α = 0.10

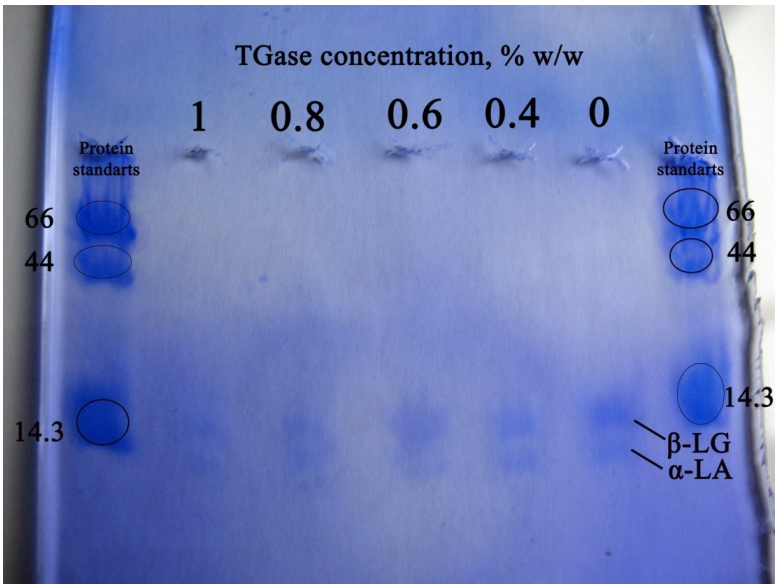


Fig. 6. SDS-PAGE results of milk whey samples

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. There are bands of α-lactalbumin and β-lactoglobulin represented on the fig. 6. Bands intensity decreases with increasing TGase concentration from 0 to 1% w/w that confirms cross-linking effect.

Gel-permeation chromatography on Sephadex G-75 was used for protein conjugates molecular mass determination (Fig. 7.) There are 3 peaks correspondended to protein aggregates with molecular masses 79.4–89.1; 21.4–30.2; 3.5 kDa (Fig. 7, from right to the left). Peaks intensity decreases with increasing TGase concentration from 0 to 1% w/w that also confirms cross-linking effect.

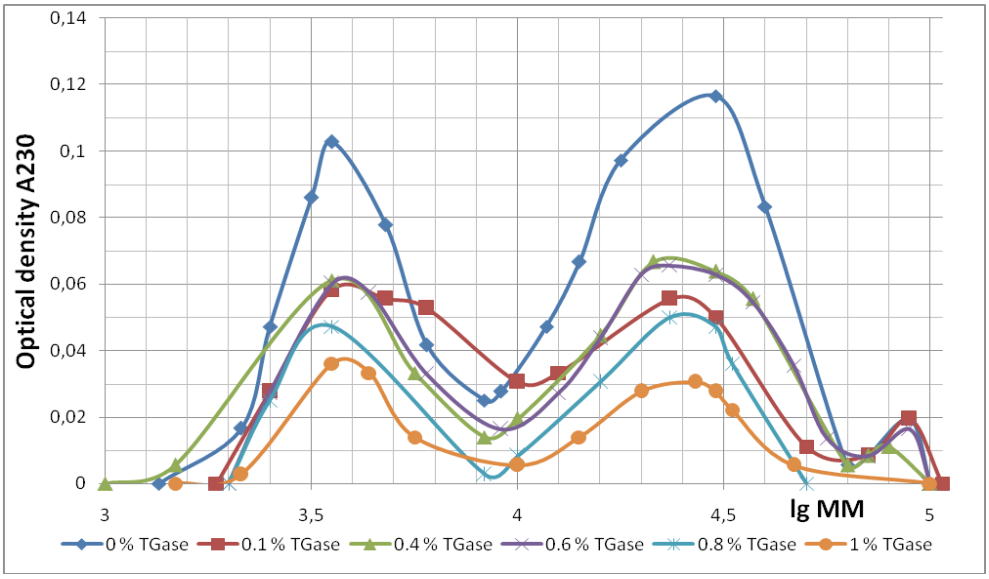


Fig. 7. Gel-permeation chromatography results of milk whey samples

Among the dairy proteins, the casein fraction represents a favourable substrate for TGase [Bonisch et al., 2006; Kurth and Rogers, 1984], mainly due to the highly accessible and flexible open chain structure [Fox, 2003; Walstra et al., 1999]. Whey proteins in their native globular structure are, by comparison, less prone to the cross-linking reaction, mainly due to the stabilization of the globular conformation by disulphide bonds limiting the susceptibility of the cross-linking sites [Færgemand et al., 1997]. However, the cross-linking of whey proteins can be improved by prior denaturation either by heat treatment [De Jong and Koppelman, 2002; Sharma et al., 2001] or by addition of reducing agents such as dithiotreitol (DTT) [Aboumahmoud and Savello, 1990; Traoré and Meunier, 1992], cysteine or sodium sulphite [De Jong and Koppelman, 2002; Færgemand et al., 1997]. The reducing agent GSH, an isotripeptide (γ-Lglutamyl-L-cysteinyl-L-glycine) consisting of cysteine, glycine and glutamic acid can split protein disulphide bonds enhancing accessibility of protein molecules to cross-linking:

$$\begin{aligned} &\text{protein} - \text{S} - \text{S} - \text{protein} + \text{GSH} \\ &\quad \rightarrow \text{protein} - \text{SH} + \text{protein} - \text{S} - \text{S} - \text{G}. \end{aligned}$$

Active site of TGase is in Cys-64, sulfur containing amino acid. It is possible GSH can activates TGase molecule.

Milk whey samples incubation with TGase and GSH was investigated (Fig. 8).

Protein concentration decreases with incubation time (Fig. 8). Control sample was treated without GSH. Protein concentration remained constant in control sample. Thus GSH positively affects cross-linking.

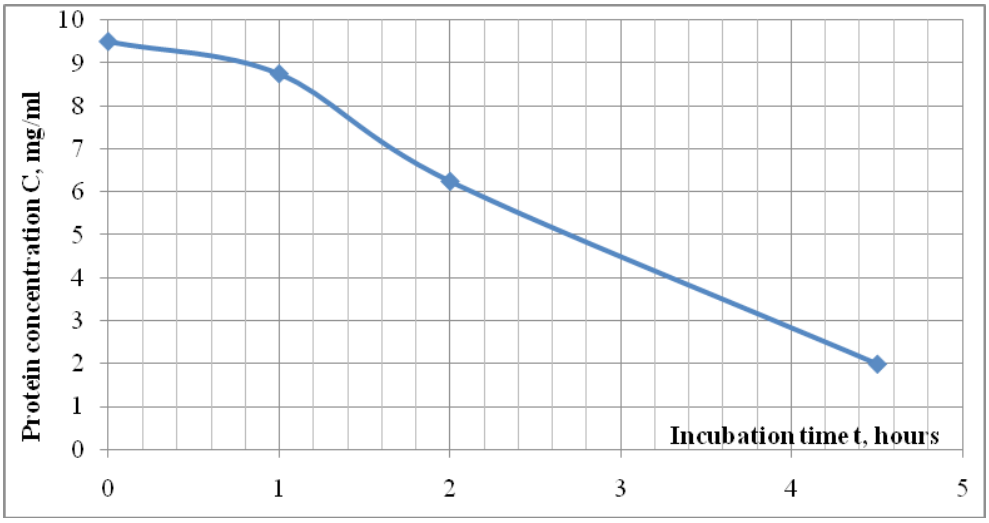


Fig. 8. Milk whey samples incubation with TGase and GSH

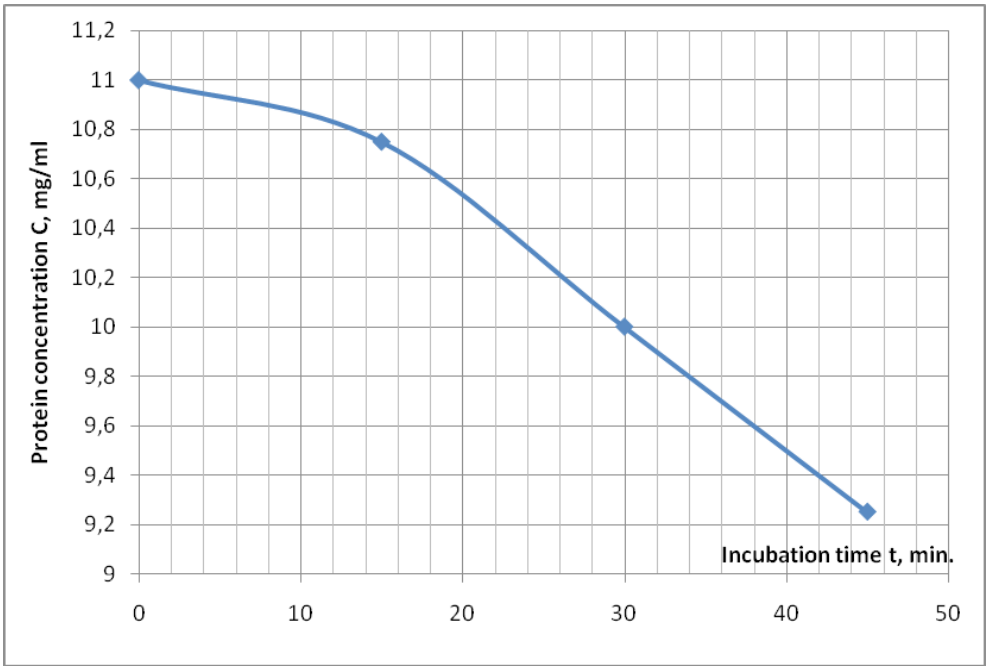


Fig. 9. Incubation of heat treated milk whey samples with TGase

Heat treatment (95°C, 5 min) was also used to improve cross-linking. Protein concentration decreases with incubation time points to cross-linking was processed well.

Gelatin was exposed to TGase cross-linking (Fig. 10). TGase concentration was 1% w/w.

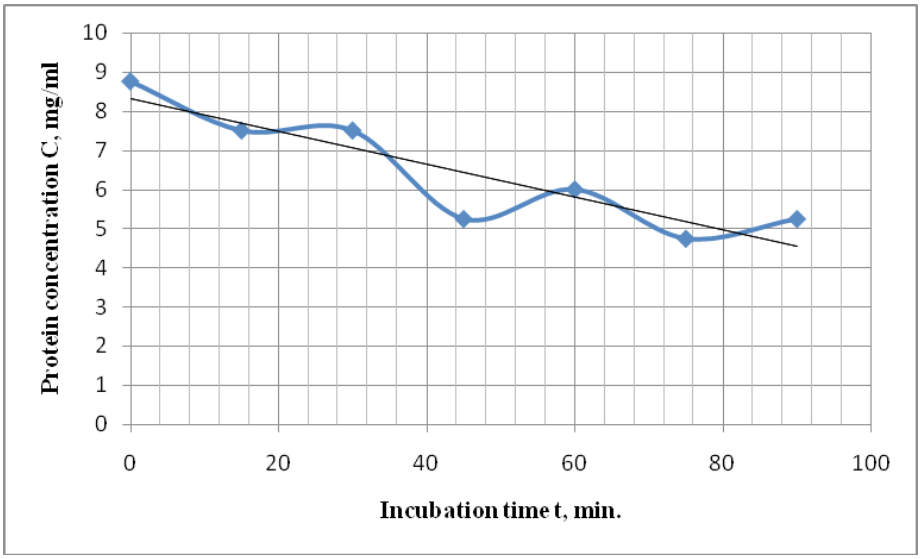


Fig. 10. Cross-linking of gelatin

There was decreasing in gelatin concentration during 90 min. fermentation. Cross--linking proceeded well.

Conclusions

Nonenzymatic protein addition can accelerates milk fermentation and leaves valuable milk whey protein in clot. This encourages solving milk whey utilization problem.

Substrate specificity of TGase was investigated. Substrates were chosen milk whey proteins and gelatin. Milk whey proteins cross-linking was enhanced by GSH addition and by heat treatment.

The use of TGase allows to decrease protein concentration in milk whey up to 11%, to decrease separated whey volume up to 17% and to decrease absolute protein content in milk whey up to 28% comparably with control sample (Tab. 2). Obtained data indicate practicability of TGase use for solving milk whey utilization problem.

Development and production brand new products excessive nutrition value are most quick, economically acceptable and scientifically based way of decision the problem of human rational nutrition. Creation fortified with vitamins and other biologically active substances of milk products is one of ways for decision of providing people with full-rate well-balanced nutrition. Milk is an excellent basis for creation of products possessed complex raw composition with functional properties. So, the aim of further investigation will be fermentative cross-linking milk proteins with other proteins, especially of vegetable origin. The use of vegetable proteins has advantages such as enrichment of low-grade stock with proteins, products texture improving, reduction of production cost, manufacture of dietetic and functional nutrition with addition dietary fibers. In summary, integration milk and vegetable proteins is object of future interest.

References

Aboumahmoud R., Savello P., 1990. Crosslinking of whey protein by transglutaminase. J. Dairy Sci., 73, 256–263.

Bönisch M.P., Huss M., Weitl K., Kulozik U., 2007. Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. Int. Dairy J., 17, 1360–1371.

Bönisch M.P., Tolkach A., Kulozik U., 2006. Inactivation of an indigenous transglutaminase inhibitor in milk serum by means of UHT-treatment and membrane separation techniques. Int. Dairy J., 16, 669–678.

De Jong G.A.H., Koppelman S.J., 2002. Transglutaminase catalyzed reactions: Impact on food applications. J. Food Sci., 67, 2798–2806.

De Jong G.A.H., Wijngaards G., Boumans H., Koppelman S.J., Hessing, M., 2001. Purification and substrate specificity of transglutaminases from blood and streptoverticillium mobaraense. J. Agric. Food Chem., 49, 3389–3393.

Færgemand M., Otte J., Qvist K.B., 1997. Enzymatic cross-linking of whey proteins by a Ca^{2+}-independent microbial transglutaminase from Streptomyces lydicus. Food Hydrocoll., 11, 19–25.

Færgemand M., Sørensen M. V., Jørgensen U., Budolfsen G., Qvist K.B., 1999. Transglutaminase: Effect on instrumental and sensory texture of set style yoghurt. Milchwiss., 54, 563–566.

Flory P.J,. 1986. Principles of Polymer Chemistry, 13th ed.; Cornell University Press: Ithaca, NY, 555.

Fox P.F., 2003. Milk proteins: General and historical aspects. In 3rd edP. F. Fox, & P. L. H. McSweeney (Eds.), Advanced dairy chemistry, Vol. 1: Proteins, pp. 1–48. New York, NY, USA: Kluwer Academic/Plenum Publishers.

Han X.Q., Damodaran S., 1996. Thermodynamic compatibility of substrate proteins affects their crosslinking by transglutaminase. J. Agric. Food Chem., 44, 1211–1217.

Ikura K., Goto M., Hoshikawa M., Sasaki R., Chiba H., 1984. Use of transglutaminase: reversible blocking of amino groups in substrate proteins for a high yield of specific products. Agric. Biol. Chem., 48, 2347–2354.

Kurth L., Rogers P.J., 1984. Transglutaminase catalyzed crosslinking of myosin to soya protein, casein and gluten. J. Food Sci., 49, 573–576.

O'Sullivan M.M., Kelly A.L., Fox, P.F., 2002a. Influence of transglutaminase treatment on some physico-chemical properties of milk. J. Dairy Res., 69, 433–442.

O'Sullivan M.M., Kelly A.L., Fox P.F., 2002b. Effect of transglutaminase on the heat stability of milk: A possible mechanism. J. Dairy Sci., 85, 1–7.

O'Sullivan M.M., Lorenzen P.C., O'Connell J.E., Kelly A.L., Schlimme E., Fox P.F., 2001. Influence of transglutaminase on the heat stability of milk. J. Dairy Sci., 84, 1331–1334.

Sharma R., Lorenzen P.C., Qvist K.B., 2001. Influence of transglutaminase treatment of skim milk on the formation of ε-(γ-glutamyl)lysine and the susceptibility of individual proteins towards crosslinking. Int. Dairy J., 11, 785–793.

Smiddy M.A., Martin J.E.G.H., Kelly A.L., De Kruif C.G., Huppertz T., 2006. Stability of casein micelles cross-linked by transglutaminase. J. Dairy Sci., 89, 1906–1914.

Sviridenko Y.Y., Kravchenko E.F., Yakovleva O.A., 2008. Organization efficient use of milk whey and localized cleanup waste water – the important factor of branch ecologization. Digest of Russian conference «Theoretical and practical aspects of foodstuff ecologization», Uglich, Russia, 252–257.

Traoré F., Meunier J.C., 1992. Cross-linking activity of placental FXIIIa on whey proteins and caseins. J. Agric. Food Chem., 40, 399–402.

Walstra P., Geurts T.J., Noomen A., Jellema A., Van Boekel M.A.J.S., 1999. Dairy technology–Principles of milk properties and processes. New York, NY, USA: Marcel Dekker.

13

EFFECT OF *LACTOBACILLUS CASEI* ON FATTY ACIDS IN MODELS OF RIPENING CHEESE SLURRY

Introduction

The fat of cheese plays several roles in cheese quality. It affects the cheese texture and it is a source of aromatic compounds that influence the cheese flavor development [De Wit et al., 2005]. At the same time, milk fat contains ingredients with pro – healthy properties, for example rumenic acid (C18:2 *cis* – 9, *trans* – 11). This fatty acid is a conjugated linoleic acid isomer (CLA) which belongs to a group of isomers of linoleic acid differ in conjugated bound position and configuration (*cis / trans*). CLA exhibits inhibitory activity against cancer cell development, it reduces the level of cholesterol and triacyloglicerole in blood, as well as inhibits development of osteoporosis, diabetes and arteriosclerosis. Also the butyric acid or isopentadecanoic acid posses pro – healthy characteristic [Wahle et al., 2004; Żegarska, 2005]. Lactic acid bacteria posses ability of carrying out the transformation of unsaturated fatty acids to CLA, what was confirmed both in culture medium with the addition of free linoleic acid, alternatively in milk about natural composition of fat or with external lipid source [Lin et al., 1999; Lin et al, 2002; Xu et al., 2004]. But in scientific literature, there is still a little information treating about the influence of lactic acid bacteria (LAB) on lipid components of fermented milk products. It is also consider, that LAB is group of microorganisms with weak lipolytic proprieties, although such an activity depends on strain [Meyers et al., 1996].

The aim of presented work was to determine the changes in composition of lipid fraction during ripening of cheeses models under influence of metabolic activity of *Lactobacillus casei* DN – 114001.

Materials and methods

Strains. Probiotic strains included in this study were *Lactobacillus casei* DN –14001 (isolated from Actimel, Danone). *Lactococcus lactis subsp.* lactis R – 603 (DVS, Chr. Hansen) was utilised as a starter culture for cheese manufacture. Material. The modified procedure of model cheese preparation proposed by Crespo et al. (2004) was use. Two type of model cheese products were made. The control cheese was prepared only with starter cultures of the fermentation process – *Lactococcus lactis* subsp. lactis (R – 603). Other chesses were made as a combination of R – 603 with *Lactibacillus casei* DN – 114001. Model cheeses were produced in aseptic conditions. Ingredients for model cheese production – 158.0 g of UHT cream (30), 157.0 g of skim milk powder, NaCl (4.0 g) and sodium citrate (1.75 g) were

weighed to Schott's glass bottles (1 L capacity). Then, 250 mL of sterile water was added. After warming to 31°C temperature in a water bath, the cheese mass was inoculated with bacterial cultures to achieve 10^7–10^8 cfu / g of model. The model cheese masses were thermostated at 31°C for additional 40 min. Then, rennet was added (1:13000, Marzyme, Chr. Hansen). After curd formation (about 40 min) the curd was cut and scalded (41°C / 45 min). So prepared model cheeses were subjected to ripening for 8 weeks at a temperature of 14°C. The final mass of the model cheese was approx. 580 g. Samples for microbiological and physicochemical analyses were taken beginning with the time 0, which means after cheese production and after 4 and 8 weeks of ripening. Samples intended for fatty acid methyl ester (FAME) analyses were kept at a temperature of – 21°C until investigation. Survival assay. Lactococcus count was determined using 10 – fold serial dilutions and M17 – agar of pH 7.2 (Merck). Aerobic incubation was carried out at 30°C ± 1°C for 72 h. Lactobacillus count was determined using 10 – fold serial dilutions and MRS – agar, pH 5.4. The gas packages for anaerobic cultivation were used (Anaerocult, Merck). Anaerobic incubation was carried out at 42°C for 72 h. Microbiological analyses were performed in duplicate for each cheese. Results were given as log c.f.u. / g of the model cheese. Chemical analysis. For fatty acid analysis in glyceride fraction the fat was extracted according to Folch method [1957]. The sample (1.0 g) was mixed with 12 cm^3 of chloroform : methanol (2:1 v/v) mixture and 0.5 cm^3 of internal standard was added (triglyceride of heneicosanoic acid – TAG C21:0, ≥99%, Nu – Chek – Prep. USA, 5 mg / cm^3), as also saturated KCl solution. After vortexing, the chloroform phase was collected and the solvent was evaporated under nitrogen. The fat was dissolved in 3 cm^3 of hexane and 0.1 cm^3 of KOH in methanol (2 M) was added before mixing. Transesterification of fatty acids to fatty acid methyl esters (FAME) was conducted at 37 °C during 30 min. FAME were analysed using gas chromatography coupled with mass spectrometry (GC / MS – QP2010, Shimadzu) on polar column BPX 90 (60 m x 0,25 mm x 0,25 μm), SGE Incorporated. The identification of FAME including conjugated linoleic acid was done comparing the retention time and mass spectrum with standard of FAME and CLA (FAME standard C4:0 – C22:5n6, GLC – 674, Nu – Chek – Prep., USA; CLA standard UC–59–M, Nu – Chek – Prep., USA). The free fatty acid fraction (FFA) was extracted and fractionated from cheese models (1.5 g) using solid phase extraction (SPE Strata – NH_2, 513 m^2 / g, Phenomenex) according to De Jong i Baddings (1990). Free fatty acids were than esterified with 1% H_2SO_4 in methanol [Christie, 1993]. The free heneicosanoic fatty acid was used as a internal standard (C21:0, ≥ 99%, N – 21 – A, Nu – Chek – Prep., USA, 2 mg / cm^3). Quantitative calculations of FAME and FFA were performed on the basis of internal standard using correction factors. Results were given as mg / 100 g of fat of model cheese. Measurement of pH was carried using a pH- meter LPH33OT, TOCUSSEL according to Polish Standard [PN-73/A-86232]. Statistical analysis. Analyses of significant statistical differences for FAME and FFA content in samples of cheese from different times of ripening were estimated with analysis of variance ($\alpha = 0.05$) using HSD Tukey test and statistic packet Statgraphics V. 8. Each analysis was performed in triplicate. The presented results were obtained from two series of cheeses made.

Results and discussion

According to the procedure of model cheeses production all variants of models were inoculated with starter bacteria *Lactococcus lactis* subsp. lactis R – 603 on level app. 7.7 log c.f.u / g (Fig. 1). During the whole ripening period the growth of starter bacteria was statistically important. During the eight weeks of ripening the cells number of R – 603 in the largest degree enlarged in models with *Lb. casei* DN – 114001 and it was increase about 1.5 log c.f.u.. / g. The number of cells of starter bacteria grew up to the 4. first weeks of ripening reaching app. 8.6 log cycle following slow dropping (about 0.4 log cycle) in the last period of the maturation. The inoculum of anaerobic lactic rods in cheese slurry of models with Lb. casei was above 7.5 log cycle. In the whole period of ripening the number of probiotic *Lactobacillus casei* was growing up to reach the value above 9.0 log cycle at the end.

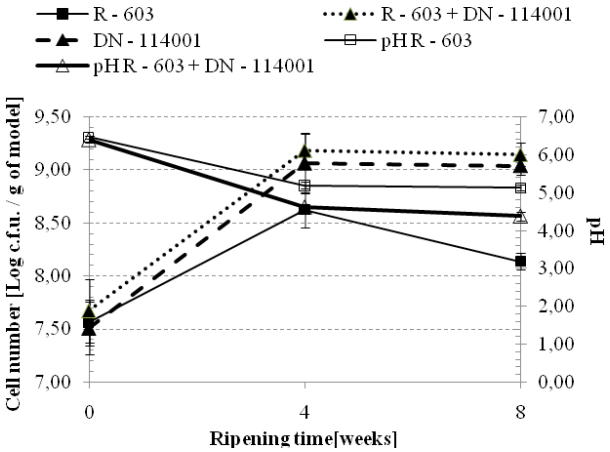


Fig. 1. Changes in cell number of starter bacteria starterowych in control and probiotic models (R – 603 oraz R – 603 + DN – 114001), number of cells of *Lb. casei* (DN – 114001) and pH

The most intensive changes considering active acidity of model cheeses, were observed during ripening of probiotic models. The pH lowered here in 8. weeks to about 4.4 units of active acidity, from pH 6.5 in zero time. This value was not typical for ripening cheeses, what could probably result with higher content of water as well as lactose comparing models composition with natural products. It was also influence of *Lb. casei.* Less intensive changes followed in control models (R – 603). The pH reached here 5.14.

The results of investigations of the changes in esterified fatty acids content were introduced in Table 1. Conducted chromatographic analyses permitted on identifying and quantitative setting over 40. fatty acids which composition was typical for milk fat. The average content of saturated fatty acids (SFA) was about 72.5 g / 100 g of fat. Taking into account the amount of individual SFA the palmitic acid (over 31.8 g / 100 g of fat), myristic acid (over 11.1 g / 100 g of fat) as well as stearic acid (over 10.4 g / 100 g of fat) were present in the largest quantities. In app. 4.3 g / 100 g butyric acid was present, while lauric acid in over 3.3 g / 100 g of fat.

Table 1

Changes in fatty acid kontent in gliceryde fraction during ripenig of control models cheeses (R – 603) and models with *Lb. casei* (R – 603 + DN –114001)

Fatty acid	R – 603 time 0	R – 603 4 weeks	R – 603 8 weeks	R – 603 + DN – 114001 time 0	R – 603 + DN –114001 4 weeks	R – 603 + DN – 114001 8 weeks
	$\bar{X}$ ± SD [mg / 100 g tłuszczu]					
1	2	3	4	5	6	7
C4:0	4288 ± 95 a	4259 ± 146 a	4214 ± 197 a	4266 ± 124 a	4242 ± 81 a	4220 ± 99 a
C6:0	2253 ± 68 a	2278 ± 63 a	2239 ± 119 a	2217 ± 166 a	2202 ± 104 a	2226 ± 94 a
C8:0	1223 ± 42 a	1219 ± 25 a	1238 ± 94 a	1212 ± 204 a	1203 ± 49 a	1210 ± 72 a
C10:0	2891 ± 23 a	2859 ± 43 a	2868 ± 51 a	2861 ± 88 a	2804 ± 148 a	2801 ± 64 a
C11:0	260 ± 19 a	275 ± 41 a	236 ± 20 a	255 ± 25 a	231 ± 17 a	239 ± 10 a
C12:0	3354 ± 79 a	3314 ± 134 a	3364 ± 52 a	3361 ± 66 a	3330 ± 74 a	3345 ± 95 a
C13:0	307 ± 8 a	309 ± 20 a	304 ± 21 a	330 ± 32 a	312 ± 22 a	293 ± 43 a
iso – C14:0	170 ± 23 a	173 ± 24 a	163 ± 18 a	152 ± 59 a	130 ± 14 a	101 ± 26 a
C14:0	11170 ± 142 a	11036 ± 221 a	10986 ± 216 a	11107 ± 235 a	11049 ± 213 a	11026 ±221 a
C14:1 trans – 9	265 ± 16 a	265 ± 20 a	265 ± 20 a	287 ± 19 a	246 ± 17 a	244 ± 15 a
iso –15:0	555 ± 44 a	496 ± 60 a	468 ± 16 a	532 ± 29 a	513 ± 17 a	493 ± 13 a
C14:1 cis – 9	1068 ± 89 a	1057 ± 41 a	1076 ± 26 a	1087 ± 39 a	1007 ± 47 a	1007 ± 42 a
C15:0	1247 ± 69 a	1214 ± 27 a	1190 ± 71 a	1246 ± 64 a	1210 ± 46 a	1171 ± 69 a
iso – 16:0	380 ± 3 a	343 ± 3 a	293 ± 36 a	344 ± 37 a	294 ± 23 a	262 ± 11 a
C16:0	31857 ± 122 a	31966 ± 302 a	32367 ± 210 a	31838 ± 229 a	32115±30 a, b	32594±261 b
iso – C17:0	502 ± 42 a	460 ± 30 a	427 ± 17 b	523 ± 41 a	499 ± 29 a	463 ± 34 a
C16:1 cis – 9	1464 ± 76 a	1463 ± 108 a	1494 ± 47 a	1486 ± 40 a	1488 ± 53 a	1439 ± 14 a
anteiso – C17:0	434 ± 51 a	436 ± 30 a	416 ± 25 a	422 ± 17 a	394 ± 10 a	383 ± 14 a
16:1 cis	319 ± 46 a	287 ± 8 a	231 ± 24 a	300 ± 17 b	271 ± 32 a,b	233 ± 34 a
C17:0	784 ± 118 a	716 ± 68 a	655 ± 33 a	715 ± 24 a	695 ± 31 a	649 ± 30 a
17:1 cis – 10	207 ± 20 a	234 ± 72 a	230 ± 30 a	205 ± 41 b	166 ± 17 a,b	128 ± 17 a
C18:0	10440 ± 37 a	10437 ± 245 a	10442 ± 81 a	10456 ± 133 a	10587 ± 180 a	10570±265 a
C18:1 trans – 9	176 ± 14 a	194 ± 9 a	208 ± 38 a	194 ± 8 a	161 ± 16 a	139 ± 9 a
C18:1 trans – 11	887 ± 21 a	876 ± 20 a	861 ± 67 a	890 ± 9 a	847 ± 11 a	887 ± 16 a
C18:1 cis – 9	18171 ± 290 a	18575 ± 350 a	18813 ± 287 a	18628 ± 245 a	19074 ± 143 a	19082±260 a
C18:1 cis – 11	323 ± 8 a	312 ± 12 a	294 ± 10 a	305 ± 12 a	304 ± 12 a	308 ± 22 a
C18:1 cis – 12	198 ± 36 a	172 ± 26 a	143 ± 18 a	188 ± 12 a	162 ± 10 a	150 ± 30 a
C18:1 cis – 15	296 ± 42 a	301 ± 10 a	296 ± 15 a	245 ± 18 a	234 ± 38 a	226 ± 25 a
C18:2 trans –9, trans–12	196 ± 24 a	203 ± 28 a	216 ± 27 a	182 ± 17 a	171 ± 3 a	149 ± 15 a
C18:2 cis – 9, cis – 12	1465 ± 49 a	1463 ± 30 a	1367 ± 29 a	1473 ± 75 a	1412 ± 86 a	1399 ± 20 a
C20:0	350 ± 61 a	321 ± 68 a	294 ± 12 a	325 ± 30 b	217 ± 52 a,b	217 ± 4 a
C18:3 all cis – 9, 12, 15	665 ± 11 a	642 ± 14 a	637 ± 38 a	687 ± 84 a	650 ± 37 a	621 ± 33 a
C20:1 cis – 11	80 ± 23 a	78 ± 10 a	73 ± 26 a	82 ± 12 a	74 ± 7 a	72 ± 16 a
C18:2 cis – 9, trans – 11	453 ± 11 a	454 ± 10 a	442 ± 21 a	449 ± 11 a	509 ± 13 b	527 ± 18 b
C20:3 all cis – 8, 11, 14	142 ± 21 a	121 ± 27 a	120 ± 30 a	101 ± 35 a	111 ± 10 a	105 ± 15 a
C22:0	138 ± 38 a	139 ± 30 a	114 ± 29 a	109 ± 27 a	104 ± 26 a	102 ± 10 a
C20:4n6 all cis – 5, 8, 11, 14	135 ± 38 a	141 ± 27 a	121 ± 26 a	119 ± 13 a	132 ± 38 a	98 ± 18 a

Table 1 cd.

1	2	3	4	5	6	7
C20:5n3 all cis – 5, 8, 11, 14, 17	116 ± 51 a	152 ± 34 a	114 ± 24 a	100 ± 50 a	122 ± 31 a	113 ± 19 a
C24:0	86 ± 11 a	67 ± 28 a	58 ± 25 a	59 ± 23 a	57 ± 29 a	56 ± 17 a
C22:5n3 all cis – 7, 10, 13, 16, 19	135 ± 41 a	140 ± 11 a	112 ± 17 a	113 ± 16 a	121 ± 26 a	104 ± 17 a
∑ SFA	72689±1097a	72317±1582 a	72336±1344 a	72330±1631 a	72188±1467 a	72418±1464a
∑ MUFA	22125 ± 629 a	22478 ± 637 a	22649 ± 483 a	22524 ± 436 a	22779 ± 360 a	22645 ±460 a
∑ PUFA	2657 ± 210 a	2659 ± 142 a	2471 ± 163 a	2591 ± 294 a	2548 ± 228 a	2440 ± 112 a
∑ trans isomers	1523 ± 74 a	1539 ± 76 a	1550 ± 152 a	1553 ± 53 a	1424 ± 46 b	1419 ± 55 b
CLA	453 ± 11 a	454 ± 10 a	442 ± 21 a	449 ± 11 a	509 ± 13 b	527 ± 18 b

Explanation: $\bar{X}$– mean value (n = 6), SD – standard deviation; a, b, …– mean value with the same letter are not statistically different (α = 0,05).

During 8. weeks of ripening of models the change in total saturated fatty acid content did not show statistically important differences. Considering the individual saturated fatty acids contents of short – and average carbon chain length, what means C4:0 – C10:0 as well as C12:0 and C14:0, their reduction was observed in acyloglicerol content. The character of palmitic and stearic acid changes was different. In cheeses modelswith *Lb. casei* content of palmitic acid enlarged from 31.8 to 32.6 g / 100 g of fat being statistically important. The increase in C16:0 was about ok. 760 mg / 100 g of fat. The range of changes was not significant in control models. In case of stearic acid the increase was not essential in both variants of models, however the dependence between content of octadecanoic acid and some other fatty acids was noticed. In models with *Lb. casei* addition, stearic acid correlates with oleic acid. Coefficient defines this dependence was even r = 0.813.

The monounsaturated fatty acid (MUFA) was the next appointed group of lipids, which stated over 22 g / 100 g of fat. The monounsaturated acid about the highest content was oleic acid (average 82% MUFA). The change in MUFA quantity during ripening of cheese models showed increasing tendency, but it was not essential alteration. Mentioned growth in MUFA quantity the growth of content of MUFA was conditioned by oleic acid amount (C18:1 cis – 9). In probiotic models there was strong negative correlation between oleic acid and the changes in pH value of model cheese curd. In these matrices the lowest pH values were noticed (Fig. 1). Additionally, in models with *Lactobacillus casei* DN – 114001, the growth of number of *Lb. casei* cells was positively correlated with the content of discussed acid. Figure 2 presents the three – dimentional correlation between oleic acid, pH as well as the logarithm of the number of Lb. casei. The correlation indicates that the growth of oleic acid content in these models followed with growth of number of anaerobic *Lactobacillus* together with increasing in acidity of cheese matrices. The correlation coefficients describing mentioned dependences were r = 0.712 and r = – 0.730.

Analysing the quantity of polyunsaturated fatty acids (PUFA) after models production it was affirmed, that PUFA were present app. at 2.6 g / 100 g of fat. The linoleic fatty acid content was about 1.5 g / 100 g of fat, while the linolenic acid content was over 0.6 g / 100 g of fat. The remaining PUFA from the group of eicosanoic acids as well as docosapentaenoic acid were present at the concentrations app. 0.1% each. Eight weeks of maturation of models did not cause essential changes considering mentioned group of lipids. In spite of general ten-

dency in dropping in linoleic and linolenic fatty acid quantity the changes were unimportant statistically.

The *trans* fatty acids isomers amounted app. 1.5% of the fat of models after production. The main trans isomer was *trans* – 11 vaccenic acid (C18:1 *trans* – 11) and elaidic acid (18:1 *trans* – 9). Their content was 890 mg / 100 g of fat as well as app. 200 mg / 100 g of fat suitably. The lower content was observed in case of C14:1 *trans* – 9 isomer as well as C18:2 *trans* – 9, *trans* – 12, what means about 290 and 180 mg / 100 g of fat respectively. After eight weeks of ripening the total content of this fatty acids showed statistically essential lowering in models with Lb casei, (about 130 mg / 100 g of fat). The reduction in individual isomer content (C18:1 *trans* – 9, C18:1 *trans* – 11 and C18:2 *trans* – 9, trans – 12) was statistically unimportant. The statistical analysis showed, that the change in the quantity of trans isomer were correlated with some other fatty acids content. For example, in models with *Lb. casei* DN – 114001 there was a correlation between elaidic acid (C18:1 *trans* – 9) and *trans* – 9, *trans* – 12 octadecadienoic acid (r = 0.873). Elaidic acid negatively correlated with stearic acid (r = – 0.532) and oleic acid (r = – 0.622) as well as with *Lb. casei* cell number (r = – 0.526). Fritsche and Steinhart [1998] postulated that elaidic acid, but also oleic acid, are products of microbial hydrogenation of unsaturated fatty acids to stearic acid, that is why observed dependences between individual lipid components could result from processes about similar character.

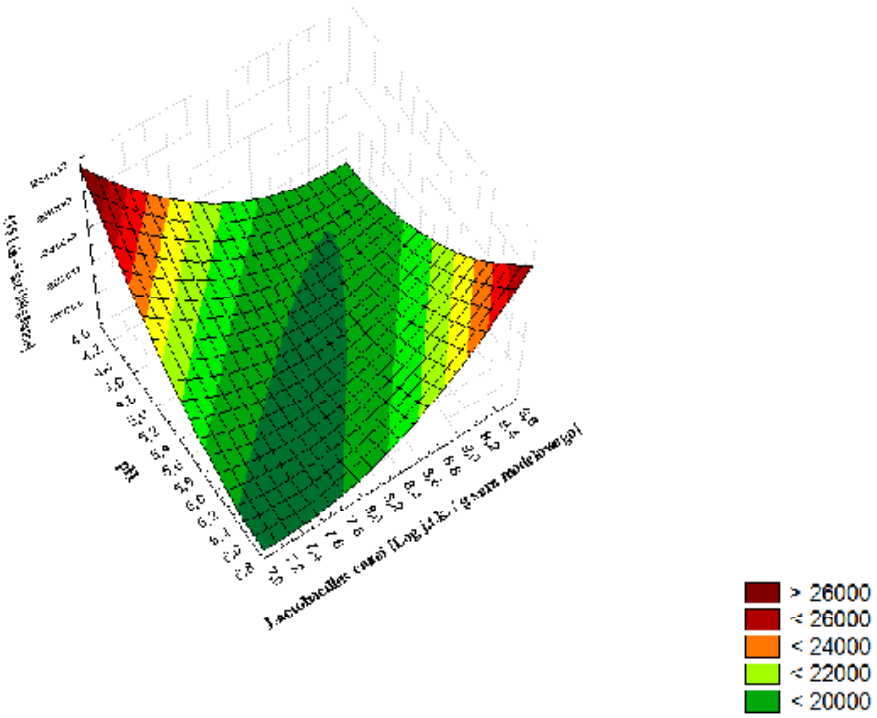


Fig. 2. Three – dimensional matrix of correlation between oleic acid (18:1 cis – 9), pH and number of anaerobic lactic acid bacteria *Lactobacillus casei* in cheese models ripening at 14 ºC during 8 weeks

Conjugated linoleic acid (CLA) was present at average quantity 450 mg / 100 g of fat of model cheeses. In control models maturation did not cause essential changes the in CLA content. On the other hand, during six weeks of ripening of cheese slurry with Lb. casei DN – 114001 statistically important increase in CLA quantity was observed. It was app. 99 mg / 100 g of fat in relation to the amount after models production (449 mg / 100 g of fat). In next two weeks of maturation the CLA content decreased unimportantly (about 20 mg). It was noticed that the increase in CLA mass stayed in positive correlation with oleic acid C18:1 cis – 9 ($r = 0.587$), meanwhile in negative with pH changes ($r = 0.568$). The CLA changes were not correlated with linoleic and linolenic acid.

According to introduced results of present investigations, during the time of ripening of models the increase in some components of glycerol lipid fraction of milk fat was observed. Slačcanac et al. [2005] also noticed similar changes, although they concerned not cheeses but fermented cow's milk. During the fermentation process they observed the increase in percentage content of some fatty acids in the glycerol fraction. After two hours of fermentation the content of palmitic acid grew up from app. $36.0 \pm 0.4\%$ to app. $37.1 \pm 0.3\%$. In case of oleic acid the growth was from app. $28.8 \pm 0.3\%$ to app. $30.8 \pm 0.,2\%$ in models of milk fermented with *Lb. acidophilus* La – 5, *Bifidobacterium* and *Streptococcus thermophilus*. During fermentation the content of linoleic acid and stearic acid decreased, what in case of second acid was not similar with direction of changes observed in discuses cheeses models. Also different tendencies were affirmed in case of short – and medium chain length fatty acids (increase in content). Differences could result with using different strain of lactic acid bacteria. Rao and Reddy [1984] studied the influence of fermentaton of milk on composition of fatty acids. Fermentation of the full – cream milk using *Lb. acidophilus*, *Lb. delbrüeckii* subsp. *bulgaricus* and *Streptococcus thermophilus* brought to moderated, but statistically important, increase in saturated fatty content, mainly in case of palmitic acid and stearic acid, as well as in unsaturated fatty acid like oleic acid. At the same time the content of linoleic and linolenic acids amount unimportantly decrease in glycerol fraction of milk fat. During the fermentation the complete hydrolysis of monoacylogliceryde was observed. Alm [1982] defined changes in milk fat of fermented Swedish products. The analyses confirm described already tendencies of changes result from the activity of lactic acid bacteria including *Lactobacillus* and *Bifidobacterium* strains. Additionally, similarly like at present work, scientists observed decreasing in the amount of short – and medium chain length fatty acids. In investigations of Boylston et al. [1999] the differences in the profile of fatty acids in Cheddar cheeses were confirm by the differences in metabolism of starter cultures. Also Yadaw et al. [2007] observed the increase of content of palmitic, oleic and stearic acids in studied fermented products with the addition of probiotic *Lb. acidophilus* and *Lb. casei*. According to Guerzoni et al. [2001] the change in the fatty acids composition of cellular membrane of *Lactobacillus helveticus* resulted from cellular stress because of the presence of salt, acids, components with strong oxidising proprieties (H_2O_2), as well as thermal stress. Mentioned authors affirmed the strong correlation among oleic acid content and temperature condition of the growth environment, with salt amount, pH or presence of hydrogen peroxide. The higher concentration of oleic acid in cell membranes of studied bacteria were present in lower pH values (higher environment acidity), as well as when the NaCl concentration was higher. Moreover authors put the hypothesis, that in higher temperatures oleic acid connected with phospholipides of cellular membrane could be converted to linoleic acid. It is an mechanism of cellular protection against thermal stress. Lowering the fluidity of cellular membrane limits also the Na^+ ions, protons or hydrogen

peroxide permeability. Goldberg and Eschar, already in year 1977, observed similar defensive system based on the principle of fatty acids composition change in cellular membrane because of low temperatures condition of LAB growth. However biosynthesis of fatty acids of lactic acid bacteria (LAB) and the rules of this mechanisms are still not well know. In last time Fernandez et al. [2008] conducted the investigation applied to adaptation of *Lactobacillus bulgaricus* to the environmental stress connected with high acidity conditions. The experience included analysis on the level of functioning of gene responsible for including suitable defensive mechanisms. Scientists identified the proteins induced during mentioned kind of stress. It was considered, that described adaptive mechanism was used in case of LAB growth in fermented milk products, where bacteria have to deal with the condition of pH reduction because of transforming the pyruvate to lactic acid. Described defensive mechanism of studied bacteria cells showed favouring *de novo* synthesis of cellular phospholipids about suitable composition of fatty acids. The content of oleic acid can therefore increase in result of pH dropping, but also when the exposition to lower temperatures is applied. Such dependences confirm mentioned correlation that were identified in work of Guerzoni et al. [2001], Kankaanpää et al. [2004] as well as in discussed cheese models.

The increase in CLA content in studied models could also result with LAB adaptation to conditions of dropping pH as well as relatively low ripening temperatures of cheese models, that could influence on composition of cellular membranes lipids. Harnandez – Mendoza et al. [2008] explain, that enlarged production of CLA in lower temperatures can result from cellular membrane importance in cell life. Scientists led the experiment over bioconversion of linoleic acid (5, 10, 20 and 30 mg / cm^3) in cultivation medium to CLA by Lactobacillus reuteri at different temperature conditions. The following temperatures of incubation were applied: 4, 10, 16, 22 and 30°C. The higher concentration of CLA was got at 10°C temperature and the addition of free linoleic acid on level 5 – 20 mg / cm^3. How it was mentioned, normal cell functioning requires fluid structure of lipid of cytoplasmic membrane. In lower temperatures fluency of this layer drops similarly like in conditions of high acidity, so bacteria regulate the change of fluency of cellular membrane across incorporating proper fatty acids into cellular membrane phospholipids. The mechanism of cis / *trans* isomerization also regulates protective proprieties of membrane in case of toxicological stress, in presence of lypolitic factors or other unfavorable factors [Bessa et al., 2000].

Positive correlation between CLA and oleic acid was confirmed by Boylston et al. [1999] what is in agreement with results of discussed work. According to authors in biological systems, C18:1 cis – 9 isomer can undergo desaturation to CLA or linoleic acid, at the same time oleic acid is a product of biohydrogenation themselves, similarly like CLA. Lin [2006] achieved similar observations. Enzymes isolated from *Lb. delbrueckii* subsp. bulgaricus cells were able to carry out the conversion of oleic acid to CLA, what was the result of desaturase activity probably. Additionally, utilization of oleic acid in synthesis of CLA depended on the pH of reaction condition. Near to pH 5 oleic acid was a preferred substrate of transformations to CLA, while higher pH values (pH 6,5) influenced on biohydrogenation of linoleic acid. The positive correlation between CLA and oleic acid was observed also in commercial cheeses, other fermented milk products as well as in milk [Lin et al., 1999]. The presence of CLA isomers in cell membrane of LAB influenced on increase in CLA in environmental where microorganisms growth. Sieber et al. [2004] show that lyophilized biomass of five strains of *Lactobacillus* species contained 32 – 45 mg CLA per gram of fatty acid methyl esters.

In control models with the addition of starter culture R – 603 the initial content of free fatty acids (FFA) amounted about 421 mg / 100 g of fatty acids (Fig. 3). The initial contents of FFA in probiotic models did not differ ($\alpha = 0.05$) from quantity of FFA in control models. According to Partidário et al. [1998], who made reference to results of Deeth et al. [1983] as well as De Jong and Badings [1990], the content of free fatty acids carried out in the cow's milk was app. 120–270 mg / l of milk. Partidário et al. [1998] affirmed the growth in FFA content during initial stages of cheeses production. In milk applied to production there was app. 215 mg FFA / l of milk (269 mg / 100 g of fat). In fresh cheese slurry, before whey impression, FFA stated app. 626 mg / 100 g of fat. Hickey et al. [2006] defined range of lypolise in Cheddar cheeses in dependence on starter bacteria used. In one – day curds the level of FFA was 580 mg / kg of cheese (187 mg / 100 g of fat). Freitas and Malcata [1998] in cheeses about 20% of fat the fat noticed app. 460 mg FFA / 100 g of fat. Mangia et al. [2008] certified between 328 and 392 mg FFA / 100 g of fat in cheeses with 17–19% of fat. The ripening process of model cheeses contributed to increase of FFA content. The increase in FFA in control cheese reached app. 89 mg / 100 g of fat, what means about 21.0% increase. This changes were higher from affirmed by Hickey et al,. [2007] in Cheddar cheeses with *Lactococcus lactis* subsp. lactis 303 after 56 days of maturation at 8ºC temperature.

The addition of probiotic cultures Lactobacillus casei to model cheeses showed significant influence on lypolitic alteration. In models with Lb. casei the degree of milk fat hydrolysis was more intensive in comparison with control models. Also Madkor et al. [2000] observed that *Lb. casei* influenced on degree of free fatty acids liberation in cheeses and it depended on strain of used bacteria. Menéndez et al. [2000] also confirmed that *Lactobacillus* strains were more effective in fat hydrolysis in comparison with *Lactococcus bacteria*. Kondylii et al. [2003] noticed that after 90 days of cheeses ripening with addition of Lb. casei subsp. *rhamnosus* the total content of FFA reached 945 mg / 100 g of fat, which shows similarity with changes affirmed in presented model changes. In models with Lb. casei the amount of FFA was higher about 820 mg / 100 g of fat at the end of ripening time. McSweeney and Sousa [2000], as also Woo et al. [1984] show, that the range of fat hydrolysis depends on cheese kind, what generally is connected with the kind of used microflora. For example, in Cheddar cheeses the content of FFA carries out the average ok. 1000 mg / kg of cheese, in Edam cheeses about 400 mg FFA / kg, Camembert above 680 mg FFA / kg, while in mould chesses the level could be above 32000 mg FFA / kg. The level of lypolize should not be higher than 2% of triacyloglicerole in cheeses Gouda, Gruyere or Cheddar, while in mould cheese can reach about 20%. According to literature the values of lypolitic index (the content of FFA) were at range typical for ripening cheeses.

In presented model cheeses tendencies in fat hydrolysis consist of changes of near thirty FFA. The predominant free fatty acids were palmitic and stearic (over 360 mg / 100 g fat) while the individual concentration of myristic, oleic and butyric acids averaged 100 mg / g fat. The content of free linoleic, linoleic and CLA fatty acids were respectively 19.0; 7.7 and 7.6 mg / 100 g fat. Comparing results of free fatty acids profile in cheese models with literature data, it was affirmed, that the content of individual fatty acids were similar to represented for natural products [Freitas and Malkata, 1998; Kandylii et al., 2003; Hickey at al., 2006; Mangia et al., 2008, Perotti et al., 2005]. In mentioned literature, mirystic, palmitic, stearic and oleic acids were the main free fatty acids present in fat of cheeses with lactic acid bacteria addition. In variants of probiotic models also the stearic acid content changed significantly. The range of increase in mass of acids like butyric, heksanoic, dekanoic, lauric, myristic

acids was smaller (10 – 50 mg / 100 g of fat). The preferences in liberation of short – and medium chain length fatty acids was not observed. Lopez et al. [2006] studied the lipolitic activity of lactic acid bacteria also did not perceive such selectivity in enzymes activity.

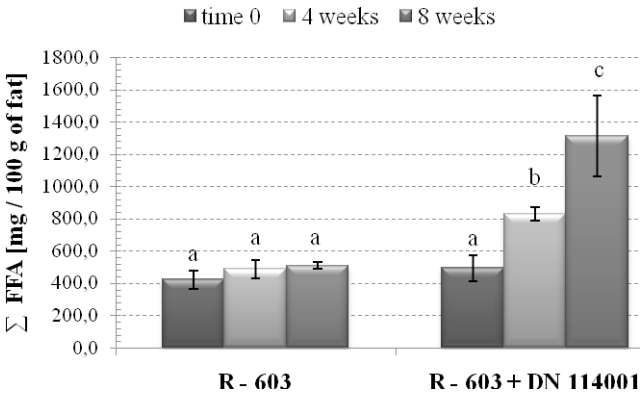


Fig. 3. Changes in total free fatty acids content during ripening of model cheese at 14ºC during 8 weeks. Explanation: R – 603 control models, R – 603 + DN models with *Lb. casei*

Conclusions

The addition of lactic acid bacteria *Lactobacillus casei* DN-114001 influenced on fatty acids in gliceryde fraction during the ripening of model cheeses, including the content of conjugated linoleic acid with important nutritional properties. It is possible that the character of changes results with the bacteria cell adaptation to conditions of decreasing pH as well temperature of ripenig.

In models with *Lb. casei* the more intensive fat hydrolysis was observed. Therefore *Lb. casei* DN-114001 could be used to accelerate the process of maturation and the flavor formation in cheese.

References

Alm L., 1982. Effect of fermentation on milk fat of Swedish fermented milk products. J. Dairy Sci., 65, 521–530.

Bessa R.J.B, Santos–Silva J., Ribeiro J.M.R., Portugal A.V., 2000. Reticulo – rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. Livestock Production Sci., 63, 201–211.

Boylston T.D., Beitz D.C., 2002. Conjugated linoleic acid and fatty acid composition of yogurt produced from milk of cows fed soy oil and conjugated linoleic acid. J. Food Sci., 67, 1973–1978.

Crespo P., Kneubühler H., Bising W., Schindler M., Fröhlich-Wyder M.T., Bachmann H.P., 2004. Method for the characterization and evaluation of cultures for the use in semi – hard cheese. IDF Symposium on Cheese: Ripening, Characterization and Technology, March 21–25, 115.

De Jong C., Badings H.T., 1990. Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up and capillary gas chromatographic analysis. J. High Resol. Chromatography, 13, 94–98.
De Wit M., Osthoff G., Viljoen B.C., Hugo A., 2005. A comparative study of lipolysis and proteolysis in Cheddar cheese and yeast – inoculated Cheddar cheese during ripening. Enzyme Microb. Technol., 37, 606–616.
Fernandez A., Ogawa J., Penaud S., Boudebbouze S., Ehrlich D., M. van de Guchte, Maguin E., 2008. Rerouting of pyruvate metabolism during acid adaptation in Lactobacillus bulgaricus. Proteomics, 8, 3154–3163.
Folch J., Lees M., Stanley G.G.S., 1957. A Simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., 226, 497–509.
Freitas A.C., Malcata F.X., 1998. Lipolysis in Picante cheese: influence of milk type and ripening time on free fatty acid profile. Lait, 78, 251–258.
Fritsche J., Steinhart H., 1998. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z Lebensm Unters Forsch A, 2006, 77–82.
Guerzoni M.E., Lanciotti R., Cocconcelli P.S., 2001. Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and termal stresses in Lactobacillus helveticus. Microbiol., 147, 2255–2264.
Hernandez-Mendoza A., Lopez-Hernandez A., Hill Jr C.G., Garcia H.S., 2008. Bioconversion of linoleic acid to conjugated linoleic acid by Lactobacillus reuteri under different growth conditions. J. Chem. Technol. Biotechnol., www.interscience.com, DOI 10.1002/JCTB.2021,
Hickey D.K., Kilcawley K.N., Beresford T.P., Wilkinson M.G., 2006. Starter bacteria are the prime agents of lipolysis in Cheddar cheese. J. Agric. Food Chem., 54, 8229–8235.
Hickey D.K., Kilcawley K.N., Beresford T.P., Wilkinson M.G., 2007. Lipolysis in Cheddar cheese made from raw, therminized and pasteurized milks. J. Dairy Sci., 90, 47–56.
Kankaanpää P.E., Yang B., Kallio H., Isolauri E., Salminen S., 2004. Effects of polyunsaturated fatty acids in growth medium on lipid composition and on physicochemical surface properties of Lactobacilli. App. Environ. Microbiol., 70, 129–136.
Kondyli E., Massouras T., Katsiari M.C., Voutsinas L.P., 2003. Free fatty acids and volatile compounds in low-fat Kefalograviera-type cheese made with commercial adjunct cultures. Int. Dairy J., 13, 47–54.
Lin T.Y., 2006. Conjugated linoleic acid production by cells and enzyme extract of Lactobacillus delbrueckii ssp. bulgaricus with additions of different fatty acids. Food Chem., 94, 437 – 441.
Lin T.Y., Lin C.W., Wang Y.J., 2002. Linoleic acid isomerase activity in enzyme extracts from Lactobacillus acidophilus and Propionibacterium freudenreichii ssp. shermanii. Food Microbiol. Safety, 67, 1502–1505.
Lin T.Y., Lin Ch–W., Lee Ch–H., 1999. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chem., 67, 1–5.
Lopez C., Maillard M.B., Briard-Bion V., Camier B., Hannon J.A., 2006. Lipolysis during ripening of Emmental cheese considering organization of fat and preferential localization of bacteria. J. Agric. Food Chem., 54, 5855–5867.
Madkor S.A., Tong P.S., Soda M.El., 2000. Ripening of Cheddar cheese with added attenuated adjunct cultures of lactobacilli. Journal Dairy Science, 83, 1684–1691.
Mangia N. P., Murgia M. A., Garau G., Sanna M. G., Deiana P., 2008. Influence of selected lab cultures on the evolution of free amino acids, free fatty acids and Fiore Sardo cheese microflora during the ripening. Food Microbiol., 25, 366–377.
Menéndez S., Centeno J.A., Godinez R., Rodrigez-Otero J.L., 2000. Effects of Lactobacillus strains on the ripening and organoleptic characteristics of Arzúa – Ulloa cheese. Int. J. Food Microbiol., 59, 37–46.

Meyers S.A., Cuppett S.L., Hutkins R.W., 1996. Lipase production by lactic acid bacteria and activity on butter oil. Food Microbiol., 13, 383–389.
Partidário A.M., Barbosa M., Boas L.V., 1998. Free fatty acids, triglycerides and volatile compounds in Serra da Estrela Cheese – changes throughout ripening. Int. Dairy J., 8, 873–881.
Perotti M.C., Bernal S.M., Meinardi C.A., Zalazar C.A., 2005. Free fatty acid profiles of Reggianito Argentino cheese produced with different starters. Int. Dairy J., 15, 1150–1155.
Rao D.R., Reddy J.C., 1984. Effects of lactic fermentation of milk on milk lipids. J. Food Sci., 49, 748–750.
Sieber R., Collomb M., Aeschlimann A., Jelen P., Eyer H., 2004. Impact of microbial cultures on conjugated linoleic acid in dairy products– a review. Int. Dairy J. 14, 1–15.
Slačanac V., Hardi J., Pavlović H., Vlainić M., Lučan M., 2005. Promjena udjela masnih kiselina tijekom fermentacije kozjeg i kravljeg mlijeka ABT-2 kulturom. Mljekarstvo, 55, 113–124.
Sousa M.J., Ardö Y., McSweeney P.L.H., 2001. Advances in the study of proteolysis during cheese ripening. Int. Dairy J., 11, 327–345.
Wahle K.W.J., Heys S.D., Rotondo D., 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Progress in Lipid Res., 43, 553–587.
Woo A.H., Kollodge S., Lindsay R.C., 1984. Quantification of major free fatty acids in several cheese varieties. J. Dairy Sci., 67, 874–878.
Xu S., Boylston T.D., Glatz B.A., 2004. Effect of lipid source on probiotic bacteria and conjugated linoleic acid formation in milk model systems. J. Am. Oil Chem. Soc., 81, 589–595.
Yadav H., Jain S., Sinha P.R., 2007.Production of free fatty acids and conjugated linoleic acid in probiotic dahi containing Lb. acidophilus and Lb. casei during fermentation and storage. Int. Dairy J., 17, 1006–1010.
Żegarska Z., 2005. Składniki tłuszczu mlekowego o potencjalnym działaniu przeciwnowotworowym. Przegląd Mleczarski, 6, 4–6.

14

EVALUATION OF THE EFFECT OF ARGINASE AND UREASE ACTIVITIES IN SELECTED STRAINS OF WINE YEAST ON THE PRODUCTION OF ETHYL CARBAMATE

Introduction

Ethyl carbamate (EC), further referred to as urethane, is a natural component of fermented foodstuffs and alcoholic beverages. Due to its carcinogenic effects in humans, the maximum permitted levels of EC in fruit spirits are limited to 0.4 mg/L EC in Canada [Conacher and Page, 1986], Germany and many other countries.

The highest EC concentrations were found in spirits derived from stone fruit of the genus *Prunus* (L.) (*Rosaceae*) (cherries, various varieties of plums, apricots, etc.) [Battaglia et al., 1990, Zimmerli and Schlatter, 1991]. The hydrolysis of cyanogenic glycosides (such as amygdalin) contained in these fruit species catalyzed by specific enzymes (mainly β-glucosidase) leads to the formation of cyanide which is the most important precursor of EC in spirits. Cyanide is oxidized to cyanate, which reacts with ethanol to form EC [Battaglia et al., 1990, Wucherpfennig et al., 1987, MacKenzie et al., 1990, Aresta et al., 2001]. The wide range of EC concentrations in stone fruit spirits reflects its light-induced and time-dependent formation after distillation and storage [Balcerek and Szopa, 2006, Mildau et al., 1987, Baumann and Zimmerli, 1988].

One of the major precursors of ethyl carbamate in wines is urea which is a product of catabolism of arginine – one of the most abundant amino acids occurring in grape juice. The first step of L-arginine degradation in *S. cerevisiae* is catalyzed by arginase (EC 3.5.3.1) which hydrolyzes L-arginine to L-ornithine and urea. The latter compound reacts with ethanol produced through fermentation thereby leading to synthesis of ethyl carbamate [Sumrada and Cooper, 1984]. Strains of the species *S. cerevisiae*, applied in the fermentation of fruit mashes, synthesize enzymes that can, to some extent, affect the content of urethanes.

It is known that *S. cerevisiae* strains produce urea through reaction catalyzed by arginase, which is encoded by the CAR1 gene [Sumrada and Cooper, 1982]. An arginase – deficient mutant was constructed by CAR1 gene disruption from a laboratory yeast strain, YNN27 and this mutant was incapable of urea producing [Suizu et al., 1990].

The enzyme catalyzing hydrolysis of urea to CO_2 and two molecules of NH_3 is urease (EC 3.5.1.5), *i.e.* urea amidohydrolase, whose activity is affected by Ni(II) ions. Unfortunately, the urease activity of wine-producing strains of *S. cerevisiae* is insufficient to provide complete hydrolysis of urea. Its excess, accumulated in yeast cells, is released to the environment where it reacts with ethanol thereby yielding ethyl carbamate. To decrease the quantity of urea, and thus to decrease the level of urethane in wine, innocula are enriched with an acid

urease isolated either from bacterium *Lactobacillus fermentum* or yeast *Schizosaccharomyces pombe* containing a mutated gene encoding that enzyme [Fujinawa et al., 1990, Pigeau, 2000].

The study aimed at evaluating the yield of biosynthesis of intracellular arginase (EC 3.5.3.1) and urease (EC 3.5.1.5) by selected strains of wine-producing yeast and characterizing the effect of these enzymes on concentration of ethyl carbamate and its precursors in aronia mashes and raw spirits.

Materials and methods

Yeast strains and culture conditions

Experiments were carried out with the following strains of wine-producing yeast *(S. cerevisiae)*: *Syrena, Tokay, Burgund, Bordeaux* and *Steinberg,* originating from the Pure Culture Collection at the Institute of Fermentation Technology and Microbiology, Technical University of Łódź, and dried *Saccharomyces bayanus* yeast purchased from the Prochimica Varese SRL (Italy). The pure cultures of yeast strains were stored on YPG slants at 4°C.

Prior to cultures in a liquid mineral medium (250 mL, 5% v/v inoculum) described below, the yeast strains were activated through stationary, 24 h cultures in 30 mL of the liquid YPG medium. The inocula were prepared through 48 h shaken cultures in 2 variants of mineral medium described below.

The modified mineral medium was prepared according to Romano (1976) and contained (per1 L): 80.0 g of glucose, 2.0 g of KH_2PO_4, 0.25 g of $CaCl_2$, 0.25 g of $MgSO_4 \times 7H_2O$, 1.0 mg of $ZnSO_4 \times 7H_2O$, 0.5 mg of $FeCl_3 \times 6H_2O$, 0.1 mg of KJ, and 0.1 mg of $CuSO_4 \times 5H_2O$. It was supplemented either with urea (1.3 g/L) to stimulate urease synthesis or with L-arginine (1.74 g/L) to stimulate arginase production. pH of these 2 variants of culture medium was adjusted to 3.8 with 0.5 M citric acid. Sterile medium was supplemented with the following doses of vitamins (per 1 L): 25 μg of biotin, 300 μg of thiamine, 300 μg of niacin, 300 μg of Ca-pantothenate, 300 μg of HCl-pyridoxine, and 25 mg of mesoinositol. The vitamins were added in the form of a concentrated solution (filtered through Milex®-GV with pore size of 0.22 μm). The cultures were run for 72 h at 28–30°C under conditions of reduced oxygen availability.

Preparation of cell-free extracts

On completion of cultures the cells were harvested by centrifuging (3000 x g, 30 min, 4°C) and the pellet was rinsed either with sodium phosphate buffer, pH 7.0 (prior to assays of urease activity) or with 50 mM Tris-HCl buffer, pH 7.5 (prior to assays of arginase activity). The pellet was suspended in the same buffer, respectively (at a buffer to biomass ratio of 2:1, v/v) and yeast cells were subjected to disintegration with sterile sand (ø=0.45–0.50 mm) in an ice-water bath until 99% cells were broken (microscopic evaluation). The crude extract was centrifuged (12000 x g, 30 min, 4°C) and sand and cell debris were discarded.

Total protein Protein content in the cell-free extracts was determined according to Lowry et al. [1951] with crystalline bovine serum albumin as a standard.

Determination of urease activity The activity was assayed by a modified method of Weatherburn [Ghasemi et al., 2004] consisting in determination of ammonia released through urease-catalyzed hydrolysis of urea. Ammonia reacts at alkaline pH with sodium nitroferricyanide (in the presence of phenol and sodium hypochlorite) and produces a colorful derivative. On completion of this reaction the absorbance was measured at 630 nm. Standard $(NH_4)_2SO_4$ solutions were used to prepare the calibration curve.

To initiate the reaction, 100 µL portion of the cell-free extract was mixed with 500 µL of 100 mM sodium phosphate buffer, pH 8, and 500 µL of urea solution (50 mM). This mixture was incubated for 30 min at 37°C. The reaction was terminated by transferring 50 µL of reaction mixture to test tubes containing 500 µL of phenol-sodium nitroferricyanide solution (0.05 g sodium nitroferricyanide and 1 g phenol dissolved in 100 mL distilled water). A 500 µL aliquot of alkaline hypochlorite (3.56 g Na_2HPO_4 and 1 mL sodium hypochlorite dissolved in 100 mL distilled water) was added to the test tubes and incubation at room temperature was continued for 30 min. The relevant controls contained the thermally denatured enzyme.

Results are expressed as micromoles of ammonia released in 1 h under presented reaction conditions. The specific activity (expressed in these units per 1 mg protein) was calculated based on protein content in the cell-free extract.

Determination of arginase activity The assay was performed according to Konarska and Tomaszewski [1986] method. L-ornithine produced from L-arginine by arginase reacts with the ninhydrin solution to give a colorful derivative. L-ornithine standard solutions were used to prepare the calibration curve.

To activate the arginase, an aliquot of the cell-free extract (25 µL) was added to 10 mM $MgCl_2$ (25 µL) and incubated at 55°C for 20 minutes. Then the solution was cooled to 37°C and to initiate the reaction sodium carbonate buffer (150 µL, 100 mM, pH 9.5) was added along with 100 mM L-arginine in 100 mM Tris-HCl buffer (pH 9.5, 50 µL). The reaction catalyzed by arginase was stopped after 10 minute incubation at 37°C by addition of glacial acetic acid (750 µL). Then the ninhydrin solution (250 µL) (2.5 g ninhydrin dissolved in 40 mL of 6 M phosphoric acid mixed with 60 mL of glacial acetic acid) was added and the samples were incubated for 1 hour at 100°C. On completion of this incubation the samples were cooled and the absorbance of reaction product was measured at a wavelength of 515 nm. Results are expressed as nanomoles of ornithine released in 1 minute. The specific activity (expressed in these units per 1 mg protein) was calculated based on protein content in the cell-free extract.

Fruit mashes To prepare mashes from aronia berries (Aronia melanocarpa Elliot) for fermentation, portions of thawed berries were homogenized and supplemented with $(NH_4)_2HPO_4$ at a dose of 0.2 g/kg.

Dried wine yeast *Saccharomyces bayanus* were re-hydrated before addition to mashes (the dose of 1.0 g d.m./kg of the pulp). For activation, *S. cerevisiae* strains from agar YPG slants were inoculated under sterile conditions to malt wort (30 mL, with density of 10°Blg and pH 5) and cultured at 30°C for 24 h. To prepare inocula for fermentation of aronia mashes the yeast strains were cultured for 48 h at 28-30°C in 0.5-L flasks, each containing 150 mL of medium supplemented with malt wort and aronia must (1:1, v/v), both with density of 10°Blg and pH 5. To initiate fermentation of aronia mashes, portions of separated and washed yeast were added at a dose of 1.0 g d.m./kg of fruit pulp.

The process was carried out in 4 L flat-bottom flasks (each containing *ca.* 3 kg of the mash) which were closed with stoppers equipped with fermentation pipes filled with glycerol,

at a temperature of 28–30°C, with occasional stirring and measurement of intensity of carbon dioxide emission (as an index of fermentation dynamics). The process was continued until the mass of fermented mashes (measured every few hours) was constant.

On completion of fermentation processes, ethanol was separated from mashes through distillation. The distillates with the strength of *ca.*15% v/v ethanol were concentrated to 40–45% v/v ethanol by using a birectifier.

The content of arginine in supernatants of culture media and fruit mashes before and after fermentation was determined with Perkin-Elmer HPLC system equipped with UV detector (operating in a wavelength range of 190-360 nm). The standard and tested samples were treated with 9-fluorenylmethyl chloroformate (FMOC-Cl) according to Bauza et al. [1995] and analyzed by using NUCLEOSIL C18 (250 x 4.6 mm) column and UV detector. The column was eluted with the solvent gradient from 100% buffer A (50 mM sodium acetate – 3% acetonitrile pH 5.4) to 100% buffer B (32 mM sodium acetate – 70% acetonitrile pH 6.1) for 15 minutes, at a flow rate of 0.5 mL/min. Retention time of arginine derivative was 2.8 min. Its concentration was measured at a wavelength of 250 nm.

The concentration of urea in supernatants of culture media and in fermented mashes was determined with the use of enzymatic test produced by R-Biopharm.

The content of ethyl carbamate in culture media supernatants and in aronia spirits was determined by gas chromatography coupled with mass spectrometry (GC-MS) [Conacher et al., 1987]. Samples (50 mL) of culture broth supernatants and samples of spirits (the volume providing ethanol concentration not higher than 10% vol. after filling up to the volume of 50 mL) were saturated with NaCl (30 g) and subjected to extraction with 2 portions (75 mL) of methylene chloride. The extracts were pooled, filtered through Whatman 1 filter paper and dried on a column packed with 40 g of anhydrous Na_2SO_4. Then, a portion of ethyl acetate (3 mL) was added to the extract and concentrated in an evaporator at 28°C to a volume of *ca*. 2 mL. The residue after evaporation was transferred quantitatively into a test-tube by three-fold rinsing the evaporator's flask with 1 mL of ethyl acetate, and filled up to the volume of 5 mL. The extract of ethyl carbamate in ethyl acetate was injected (2-µL aliquots) onto a capillary column filled with a stationary phase INNOWAX 19091N-113 (the length of 30 m, internal diameter of 0.32 mm and film thickness of 0.25 µm). The analysis was conducted by using 6890N gas chromatograph (Agilent) with a flame-ionization detector (FID) and SSL splitless injector. Conditions of the GC analysis were as follows: temperature program - 50°C (5 min) → 20°C/min → 140°C (10 min) → 20°C/min → 250°C (10 min); injector temperature - 260°C; detector temperature - 250°C; flow rate of carrier gas (helium) – 1 mL/min. Ethyl carbamate was identified based on the presence of m/z 62, 74 ad 89 ions, using a GC 8000 gas chromatograph (Fissons) coupled with an MD 800 mass spectrometer. The capillary column and analytical conditions were as shown above (the energy of ionization of 70 eV). The concentration of ethyl carbamate in examined samples was determined based on a calibration curve. The recovery of ethyl carbamate ranged between 90 and 104%, and detection limit was 0.01 mg/l.

Statistical methods Standard deviation and Pearson's coefficient of correlation (r) were calculated with the use of Origin 6.0 software.

Results and discussion

Biosynthesis of arginase (EC 3.5.3.1) and urease (EC 3.5.1.5) by selected strains of wine-producing yeast strains and influence of these enzymes on arginine and urea concentrations in fermented aronia mashes and ethyl carbamate content in distillates obtained from these mashes were investigated.

Biosynthesis of arginase and urease by selected wine yeast strains

Amongst the investigated yeast strains, the most efficient producer of arginase (under anaerobic conditions) was the strain *Steinberg* (specific activity of 9.355 U/mg protein in the cell-free extract) whereas the lowest specific activity of this enzyme (only 1.416 U/mg protein) was found in cell-free extracts from strain *Bordeaux*. In consequence, the decrease in arginine content in culture broth of the strain *Steinberg* was the greatest (45.4%) as compared to the other tested yeast strains (Table 1). These data imply that more urea can be produced during fermentation of must or fruit pulp with this yeast strain as compared to the other investigated yeast strains.

However, the cell-free extracts from biomass of *Steinberg* strain were characterized by the relatively high specific urease activity (0.569 U/mg protein) and this explains the greatest decrease in the concentration of urea (by ca. 19%) in the culture broth under conditions of the limited availability of oxygen (Table 2).

Irrespective of the activities of arginase and urease in cells of the examined yeast strains, the concentration of ethyl carbamate in culture broth supernatants was at a similar level (ca. 0.01 mg/L) with an exception of strain *Tokay* (0.02 mg/L) grown in the medium supplemented with urea. It can be explained by the lowest specific urease activity in cells of this yeast (0.192 U/mg protein in the cell-free extract) (Table 2).

Table 1

Specific activity of arginase in cell-free extracts from biomass of selected strains of wine-producing yeast and concentrations of arginine and EC in culture broth supernatants

Yeast strain	Arginase activity U*/mg protein	Culture broth supernatant	
		Arginine [g/L]	EC [mg/L]
Bordeaux	1.416 ±0.09	1.55±0.05	<0.01
Burgund	1.612 ±0.12	1.58±0.05	<0.01
S. bayanus	3.000 ±0.18	1.31±0.03	<0.01
Steinberg	9.355 ±0.33	0.95±0.03	0.01
Syrena	2.755 ±0.15	1.37±0.03	<0.01
Tokay	1.899 ±0.12	1.52±0.03	<0.01

*1 U of activity is equivalent to 1 nmol of ornithine produced in 1 minute.

Table 2

Specific activity of urease in cell-free extracts from biomass of selected strains of wine-producing yeast and concentrations of urea and EC in culture broth supernatants

Yeast strain	Urease activity U*/mg protein	Culture broth supernatants	
		Urea (g/L)	EC (mg/L)
Bordeaux	0.420 ± 0.008	1.12±0.02	0.01
Burgund	0.301± 0.005	1.15±0.03	0.01
S. bayanus	0.402± 0.006	1.10±0.03	0.01
Steinberg	0.569 ± 0.012	1.05±0.01	<0.01
Syrena	0.367 ± 0.006	1.12±0.02	0.01
Tokay	0.192 ± 0.004	1.25±0.04	0.02

*1 U of activity is equivalent to 1 μmol of NH_3 produced in 1 h.

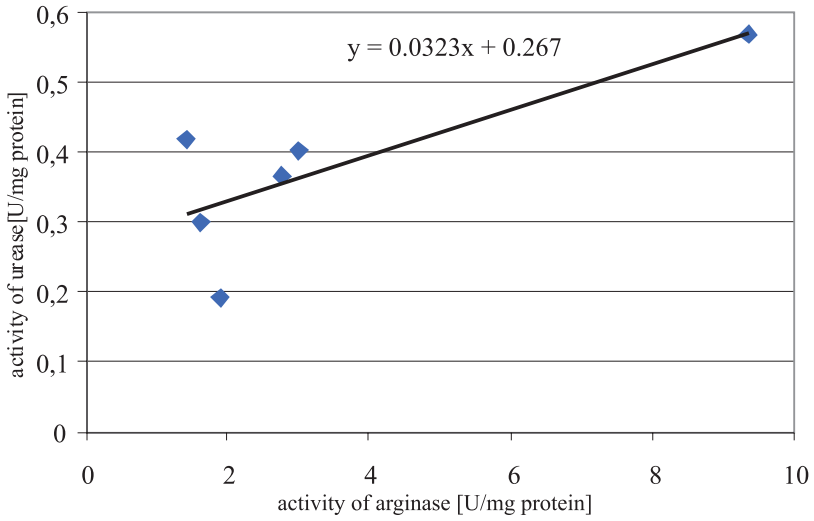


Fig. 1. Activity of infracellular urease of selected wine-producing yeast strains as a function of activity of their intracellular arginase

Chan and Cossins [1972] postulated that urea could regulate the activity of arginase by repression of enzyme synthesis. They found that the specific activity of arginase depended on nitrogen sources in culture media and that the products of arginase catalyzed reaction repress biosynthesis or inhibit the activity of this enzyme while low concentrations of the substrate stimulate biosynthesis of arginase.

Schehl et al. [2007] genetically engineered a diploid yeast strain to reduce the arginase activity and thereby blocked the pathway of urea production. The constructed strains with either a heterozygous CAR1/car1 deletion or a homozygous defect (car1/car1) were compared

to the parental wild-type strain and to an industrial yeast strain applied in cherry mash fermentations and spirit production. Spirits produced by using the strain with the homozygous car1 deletion contained less EC than spirits obtained with the aid of the wild-type strains. However, EC level was not reduced in stone fruit mashes fermented with the mutant strain but these mashes are thought to contain the other sources of this compound.

Characteristics of aronia mashes and distillates

The influence of yeast strain on arginine and urea concentrations in fermented aronia mashes and ethyl carbamate content in distillates obtained from these mashes is presented in Table 3.

The content of arginine in aronia mashes before fermentation was close to 1.04 mg/kg. After fermentation it ranged between 0.66 and 0.87 mg/kg (mashes fermented with the strains *Syrena* and *Burgund*, respectively).Urea concentrations in all fermented mashes ranged between less than 0.01 mg/kg (mash fermented with *Steinberg* strain) and 0.25 mg/kg (mash fermented with *Burgund* strain).

Table 3

Concentrations of arginine and urea in aronia mashes fermented by the selected wine-producing yeast strains and the content of ethyl carbamate in distillates obtained from these mashes

Yeast strain	Fermented mashes		Ethyl carbamate [mg/L spirit 40% v/v]
	Arginine [mg/kg]	Urea [mg/kg]	
Bordeaux	0.81±0.06	0.15±0.01	0.46±0.03
Burgund	0.87±0.06	0.25±0.03	1.35±0.07
S. bayanus	0.79±0.07	0.06±0.01	0.28±0.01
Steinberg	0.73±0.06	< 0.01	<0.01
Syrena	0.66±0.04	0.03±0.007	0.43±0.03
Tokay	0.82±0.05	0.01±0.003	0.30±0.02

GC analysis of raw spirits obtained from fermented aronia mashes revealed differences in the contents of ethyl carbamate depending on yeast strain. The highest concentration of EC (1.35 mg/L spirit 40% v/v) (Table 3) was found in the distillate obtained from aronia mash fermented with *Burgund* yeast. It could be a consequence of the relatively high content of urea (0.25 mg/kg) in fermented mash and low intracellular urease activity of yeast during fermentation (specific activity of 0.301 U/mg protein in the cell-free extract) (Table 2).

The application of *Steinberg* strain, which displayed nearly 2-fold higher specific urease activity under anaerobic conditions (0.569 U/mg protein) (Table 2) and was characterized by the lowest urea concentration in fermented mash (< 0.01 mg/kg) resulted in a decrease in urethane content to <0.01 mg EC/L spirit 40% v/v (Table 3).

The correlation between the urethane content in aronia spirits (y) and the activity of urease in cells of selected strains of wine yeast (x) was described by a linear equation: $y = -1.6182x + 1.0787$ (Fig. 2). The Pearson's coefficient of correlation (r) was found to be 0.444. This low r value proves that the correlation between the evaluated variables was not statistically significant. Thus the reduced synthesis of urethane in fruit mashes could be achieved only through selection of yeast strains characterized by a relatively high urease activity.

It should be emphasized, that the examined strains of yeast were characterized by a low urease activity. Our results corroborate the thesis of Pigeau (2000) who postulated that urease activity of wine yeast *S. cerevisiae* made the complete hydrolysis of urea impossible.

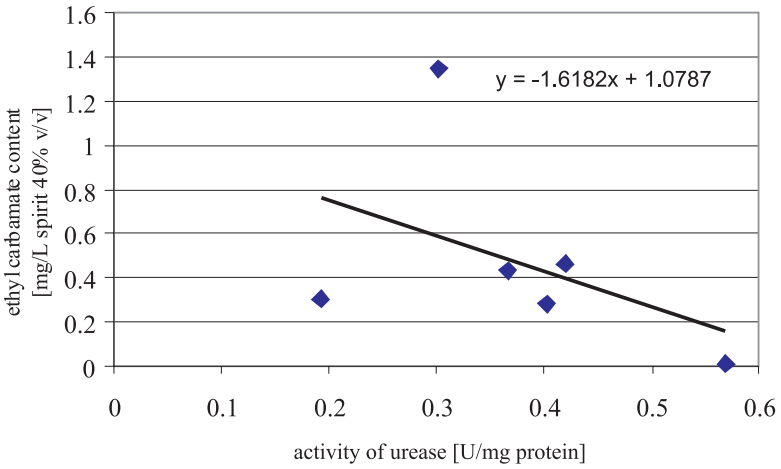


Fig. 2. The relationship between etween ethyl carbamate content in aronia spirits and the activity of infracellular urease of selected wine producing yeast strain

Besides, mashes prepared from stone fruits and others (berries and seed fruits) contain cyanogenic glycosides whose hydrolysis produces hydrocyanic acid, which is the principal precursor of EC in spirits. Under conditions of alcoholic fermentation, this compound can be converted to ethyl carbamate [Battaglian et al., 1990, Wucherpfennig et al., 1987, Mackenzie et al., 1990].

Conclusions

Determination of the yield of arginase and urease biosynthesis by the selected strains of wine-producing yeast cultured under limited oxygen availability conditions showed significant differences between these strains. The lowest activity of arginase (1.416 U/mg protein) was found in cell-free extracts from biomass of strain *Bordeaux* while the less efficient producer of urease (0.192 U/mg protein in the cell-free extract) was the strain *Tokay*. The strain *Steinberg* was characterized by the highest productivity of intracellular arginase (9.355 U/mg protein) and urease (0.569 U/mg protein).

The statistically significant correlation between the activities of these enzymes and urethane content in aronia distillates was not observed. The synthesis of ethyl carbamate is to some extent limited by urease and all the examined strains of wine-producing yeast were characterized by the relatively low activity of this enzyme, too small to totally destroy urea produced during alcoholic fermentation.

The content of ethyl carbamate in distillates received from various fruit mashes depends, first of all, on concentration of cyanogenic glycosides contained in fruit tissue.

References

Aresta M., Boscolo M., Franco D.W., 2001. Copper (II) catalysis in cyanide conversion into ethyl carbamate in spirits and relevant reactions. J. Agric. Food Chem., 49, 2819–2824.

Balcerek M., Szopa J.S., 2006. Ethyl carbamate content in fruit distillates, Żywność, Nauka, Technologia, Jakość, 46, 91–101.

Battaglia R., Conacher H.B.S., Page B.D., 1990. Ethyl carbamate (urethane) in alcoholic beverages and foods: a review. Food Addit. Contam., 7, 47–496.

Baumann U., Zimmerli B., 1988. Accelerated formation of ethyl carbamate in spirits. Mitt. Geb. Lebensmittelunters. Hyg., 79, 175–185.

Bauza T., Blaise A., Daumas F., Cabanis J.C., 1995. Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection. J. Chrom. A., 707, 373–379.

Chan P.Y., Cossins E.A., 1972. Regulation of arginase levels by urea and intermediates of the Krebs--Henseleit cycle in Saccharomyces cerevisiae. Febs. Letters, 19, 335–339.

Conacher H.B.S., Page B.D., 1986. In: Proceedings of Euro food tox II. European society of toxicology, Schwerzenbach Switzerland, 237–242.

Conacher H.B.S., Page B.D., Lau B.P., Lawrence J.F., Bailey R., Calway P., Hanchay J. P., Mori B.. 1987. Capillary gas ghromatographic determination of ethyl carbamate in alcoholic beverages with confirmation by GC/MS. J. Assoc. Off. Anal. Chem., 4, 749–751.

Fujinawa S., Todoroki H., Ohashi N., Toda J., Terasaki M., 1990. Application of an acid urease to wine: determination of trace urea in wine. J. Food Sci., 4, 1018–1022, 1038.

Ghasemi M.F., Bakhtiari M.R., Fallahpour M., Noohi A., Moazami N., Amidi Z., 2004, Screening of urease production by Aspergillus niger strains. Iran. Biomed. J., 1, 47–50. http://ibj.pasteur.oc.ir/ibj21/html/bakhtiari.htm

Konarska L, Tomaszewski L., 1986. A simple quantitative micromethod or arginase assay in blood spots dried on filter paper. Clin. Chim. Acta, 154, 7–17.

Lowry O.H., Rosenbrough N.J., Farr A.L., Randell L.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265–268.

Mackenzie W.M., Clyne A.H., Mcdonald L.S., 1990. Ethyl carbamate formation in grain based spirits. Part II. The identification and determination of cyanide related species involved in ethyl carbamate formation in Scotch whisky. J. Inst. Brew., 96, 223−232.

Mildau G., Preuß A., Frank W., Heering W., 1987. Ethyl carbamate (urethane) in alcoholic beverages: improved analysis and light dependent formation., Dtsch. Lebensm.-Rundsch., 83, 69–74.

Pigeau G., 2000. Characterization of wild-type and mutants ureases of the fission yeast S. pombe. Project Administered by dr Debra Inglis, http://www.bracku.ca/ccovi /res/ inglis7.html-8k

Romano P., 1976. Biosynthesis of sulphur amino acids in Saccharomyces cerevisiae, Arch. Microbiol., 108, 211–215.

Schehl B., Senn T., Lachenmeier D.W, Rodicio R., Heinisch J.J., 2007. Contribution of the fermenting yeast strain to ethyl carbamate generation in stone fruit spirits. Appl. Microbiol. Biotechnol., 74, 843–850.

Suizu, T., Iimura Y., Gomi K., Takahashi K., Hara S., Yoshizawa K., Tamura G., 1990. Construction of urea nonproducing yeast Saccharomyces cerevisiae by disruption of the CAR1 gene. Agric. Biol. Chem., 54, 537–539.

Sumrada R.A., Cooper T.G., 1982. Isolation of the CAR1 gene from Saccharomyces cerevisiae and analysis of its expression. Mol. Cell. Biol., 2, 1514–1523.

Sumrada R.A., Cooper T.G., 1984. Nucleotide sequence of the Saccharomyces cereviasiae arginase gene (CAR1) and its transcription under various physiological conditions. J. Bacteriol., 160, 1078–1087.

Wucherpfennig K., Clauss E., Konja G., 1987. Formation of ethyl carbamate in alcoholic beverages based on the maraschino cherry. Dtsch. Lebensm. Rundsch., 83, 344–349.

Zimmerli B, Schlatter J., 1991. Ethyl carbamate: analytical methodology, occurrence, formation, biological activity and risk assessment. Mutat. Res., 259, 325–350.

15

THE INFLUENCE OF THE IN VITRO DIGESTION PROCESS CONDITIONS ON THE PHENOLIC COMPOUNDS OF WINE

Introduction

In the recent years polyphenols in wine have raised a lot of attention due to their health-promoting effects and the antioxidant role they play in biological and food systems. Results of certain studies have indicated that these effects could be ascribed to the antioxidant properties of polyphenols, an important group of secondary plant compounds. Phenolic compounds that are important in terms of the wine quality (in particular, colour and astringency) could be classified into two groups: flavonoids and non-flavonoids [Davalos et al., 2006].

Manny *in vitro* and *in vivo* researches show, that wine, especially red one, contains natural phenolic antioxidants that have beneficial effects on our health. Polyphenols are not only important for the antioxidant properties but also influence the sensorial properties of wine [Zern and Fernandez, 2005; Muselik et al., 2007].

After birth the human gastrointestinal tract becomes readily colonized with microorganisms from the mother and the environmental. The composition of this microbial community is relatively simple in infants but very complex in adults. In has been estimated that more than 400 different bacterial species contribute to an estimated total number of to 10^{13} bacterial cells resident in the human gastrointestinal tract [Gawęcki and Libudzisz, 2006]. Although there are certain similarities with respect to the general makeup of the bacterial community it has to be emphasized that the composition of the intestinal microbiota is highly variable between subjects and, when analyzed in detail, actually unique for each individual. The density of the bacterial cells in the gastrointestinal contents increases by up to 11 orders of magnitude from levels of 10^{1} to 10^{3} colony forming units (cfu) per g of contents in the stomach to 10^{12} cfu per g of contents in the distal colon [Blaut et al., 2003; Konishi and Kobayashi, 2004].

Unfortunately, there is still very little data concerning the behavior of the polyphenol compounds in the man's gastrointestinal tracts, in particular, their transformations under the influence of the intestinal microflora as well as their toxicity.

The aim of this study was to assess the impact of digestion in the in vitro tract model on the antioxidant activity and changes in the level of phenolic compounds in selected wines.

Materials and methods

The experimental material comprised wines: red grape "Szekszardi Kadarka"-semisweet and white "Solarra" from the 2007 year.

Conditions of the "*in vitro*" digestion process. The "*in vitro*" digestion was conducted in a glass bioreactor equipped in 4 inlets allowing the introduction of the pH electrode, programming of the active acidity, dosage of biochemical agents and appropriate media as well as collecting of analytical samples.

Samples for the "*in vitro*" digestion process were prepared by taking 200 cm^3 of products.

The bioreactor was thermostable and the reactions were carried out at the temperature of 37°C. The conditions of the "digestion" process in the bioreactor were designed in such a way as to comprise the following stages of the model: the "stomach", the "small intestine" and the "large intestine" (Fig. 1). The parameters of the digestion process were selected on the basis of our own investigations Gumienna et al. [2006]; Goderska et al. [2008] but also taking into consideration studies carried out by Aura et al. [1999]; Gil-Izquierdo et al. [2001].

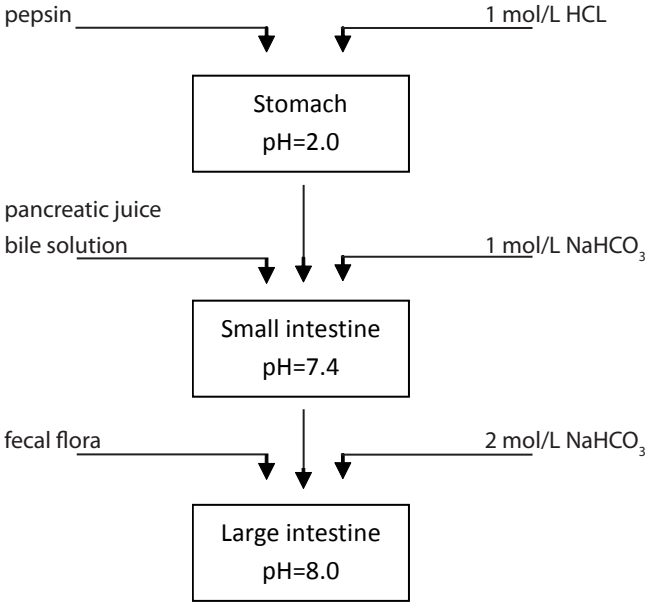


Fig. 1. Gastrointestinal tract model.

The employed intestinal microflora was collected from faeces of 3 volunteers 25 to 30 years of age. The standardisation of the intestinal microflora was conducted in accordance with the methodology prepared by Knarreborg *et al*. [2002].

In order to control the influence of the conditions prevailing in the gastrointestinal tract on the growth of microorganisms, control inoculations were made after 2 hours from the moment of the introduction of microorganisms into the environment and at the moment of termination of the digestion process (after 21 hours). The determined groups of microorganisms (cfu·dm^{-3}) included: *Entrobacteriaceae* (MacConkey selective medium), *Lactobacillus* (MRS medium – agar), *Enteroccocus* (substrate – agar with kanamycin, esculin and sodium azide), *Bifidobacterium* (Garch's medium). Plates were incubated at 37°C for 48 h in anaerobic conditions.

Analytical methods. During the digestion process the total polyphenol content, quantitative and qualitative analysis, and the antioxidation potential was determined.

The **total polyphenol** content was measured using modified Folin-Ciocalteu method and its values were estimated from a standard curve of gallic acid. All results have been corrected for the presence of phenols in the pancreatin/bile salts mixture. Results are expressed as equivalents of gallic acid per mg/ml wine [Singleton and Rossi, 1965].

The **antioxidative activity** (TEAC) was determined against the ABTS reagent (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) according to the method described by Re et al. [1999]. Results of the TEAC assay are expressed as the capability of antioxidants to scavenge ABTS radicals relative to that of Trolox (a water-soluble vitamin E analogue) and given as mg Trolox·cm^{-3} of wine.

HPLC methods. The quantitative and qualitative phenol compounds was determaind by a HPLC method using a Waters (USA) system, including UV-VIS 966 detector (Waters, USA). Chromatographic separation was performed on a X-Terra C_{18} (Waters) column (3.9x 150 mm, 4μm). The mobile phases considered of acetonitrile, formic acid and water mixture 30/10/60% (v/v) with flow rate of 1cm·min was used. Column were placed in a column oven set at 30°C, autoinjector was used with a fixed 10 μl loop. The amount of phenolic compounds was calculated based on a standard curve prepared with flavonoid and phenolic acids standards (Sigma).

The number of live bacterial cells was determined using the Koch's plate method. The number of microorganisms introduced into the digestion process amounted to about 10^6 cfu·cm^{-3}.

Data analysis. Each experiment was repeated three times. Analysis of data was done using Excel 2003 Microsoft Office Program.

Results and discusion

Products subjected to the "digestion" process were characterised by a different initial concentration of phenolic compounds and antioxidative activity. The highest concentration of phenolic compounds as well as the highest antioxidative potential was observed in the red wine (Table 1). The higher content of phenolic compounds as well as higher antioxidant activity of red wine is caused by the fact that red grapes in comparison to white grapes contain higher concentration of poliphenols from the group of flavonoids, flavanoids and tannins [Czyżewska and Pogorzelski, 2004].

Significant differences in the content of phenolic compounds as well as their antioxidative activity were found at all stages of the performed process of digestion. The highest content of phenolic compounds was recorded in the case of all products after the process of digestion carried out in the "stomach" (50.57 mg·cm^{-3} – white wine, 238.80 mg·cm^{-3} – red wine). Following the stage of digestion in the "small intestine" and the "large intestine", significant reductions in the concentrations of these compounds were recorded: 8.84 mg·cm^{-3} – white wine, 52.40 mg·cm^{-3} – red wine (Table 2, 3). However, the highest antioxidative activity was determined at the stage of digestion in the "stomach" – white wine 60.74 mgTx·cm^{-3} and the red wine: 724.23 mgTx·cm^{-3} but significant reductions of these activity were recorded and the "large intestine" 13.45 mgTx·cm^{-3} and the red wine: 159.88 mgTx·cm^{-3} (Table 3).

Table 1

Antioxidant capacity of wines and polyphenols content

Products	Antioxidant capacity [mg Trolox·cm^{-3}]	Total polyphenols content [mg gallic acid·cm^{-3}]
White wine "Solarra"	39.81 ± 14.66	53.68 ± 4.96
Red wine – "Szekszardi Kadarka"	625.80 ± 28.50	281.76 ± 29.97

Table 2

Antioxidant capacity of white wine and polyphenols content detected in wine during digestion in the gastrointestinal tract model

Treatment	Antioxidant capacity [mg Trolox·cm^{-3}]	Total polyphenols content [mg gallic acid·cm^{-3}]
2 h at pH 2.0 "after stomach"	60.74 ± 10.48 d	50.57 ± 3.91 d
at ph 7.4 "in small intestine"	32.14 ± 10.08 c	29.73 ± 2.68 b
10 min after digestion with fecal flora	26.67 ± 5.41 b	29.09 ± 1.98 b
2.5 at pH 7.4 "after small intestine"	22.41 ± 4.89 b	24.93 ± 1.32 c
21 h at pH 8.0 "after large intestine"	13.45 ± 3.66 a	8.84 ± 2.85 a

Values are means ± SD of three independent experiments. Means with different letters:
^{a,b,c,d} are significant (in the columns) at $p<0.05$

Table 3

Antioxidant capacity of red wine and polyphenols content detected in wine during digestion in the gastrointestinal tract model

Treatment	Antioxidant capacity [mg Trolox·cm^{-3}]	Total polyphenols content [mg gallic acid·cm^{-3}]
2 h at pH 2.0 "after stomach"	724.23 ± 81.51 d	238.80 ± 24.32 c
at ph 7.4 "in small intestine"	496.60 ± 12.05 b	281.92 ± 12.48 d
10 min after digestion with fecal flora	470.53 ± 27.42 b	201.48 ± 10.98 c
2.5 at pH 7.4 "after small intestine"	528.81 ± 14.65 c	145.44 ± 11.02 b
21 h at pH 8.0 "after large intestine"	159.88 ± 8.46 a	52.40 ± 6.85 a

Values are means ± SD of three independent experiments. Means with different letters:
^{a,b,c,d} are significant (in the columns) at $p<0.05$

The determined relatively high antioxidative potential determined at the stage of digestion in the "stomach" can be attributed to the activity of digestive enzymes. During this process, phenolic compounds are probably liberated from the glycoside flavones as a result of hydrolysis of the glycoside bond, which occurs between the sugar residue and hydro cyclic ring. The above transformation leads to the development of an active aglycon which is more reactive than the glycoside form and which can be recognized as one of the more favourable changes taking place in the course of the "in vitro" digestion [Sakakibara et al., 2003; Grajek, 2003]. However, during the *in vitro* digestion process a decrease of the concentration of polyphenolic compounds as well as the antioxidant capacity was stated (Tables 2, 3). Furthermore after the successive stages of the process quality changes of those components, especially phenolic acids like: chlorogenic acid, caffeic acid and coumaric acid were determined (Table 4).

Table 4

Qualitative and quantitative changes of selected phenolic compounds in the wines digestion

Wines	Treatment	Polyphenols [μg·cm^{-3}]				
		Resveratrol	Chlorogenic acid	Caffeic acid	Coumaric acid	3-(4-hydroxyphenyl) propionic acid
White	products	1.80±0.02	0.65±0.01	0.112±0.02	24.62±0.79	–
	2 h at pH 2.0 "after stomach"	1.45±0.01	0.26±0.01	0.137±0.01	21.62±0.69	–
	2.5 at pH 7.4 "after small intestine"	1.34±0.03	0.15±0.01	0.15±0.01	20.19±0.34	2.98±0.02
	21 h at pH 8.0 "after large intestine"	1.21±0.02	0.08±0.01	0.013±0.0	19.21±0.21	1.3±0.04
Red	products	15.07±0.12	2.86±0.06	0.96±0.2	23.83±0.22	–
	2 h at pH 2.0 "after stomach"	18.11±0.43	2.34±0.06	0.76±0.2	23.06±0.19	–
	2.5 at pH 7.4 "after small intestine"	17.78±0.21	1.97±0.04	0.63±0.3	21.87±0.22	13.23±0.11
	21 h at pH 8.0 "after large intestine"	8.57±0.09	0.12±0.01	1.23±0.3	20.57±0.11	9.80±0.09

It is known, that chlorogenic acid is metabolized in the human gastrointestinal tract into caffeic acid and chinic acid. Moreover, the chlorogenic acid is quite stable and does not hydrolyze in the stomach stage at low pH. Its partial hydrolysis occurs in the small intestine as result of esterase activity. Next in the large intestine, the chlorogenic acid is metabolized by the fecal microflora. The esterase found in the small intestine as well as the one produced by the bacteria cause the release of caffeic acid from the chlorogenic acid, and the caffeic acid become a substrate in a following metabolic processes. The activity of the fecal flora cause the appearance of new 3-(3,4-dihydroxyphenyl)propionic acid and 3-hydroxyphenylpropionic acid [Konisi and Kobayashi, 2004]. Furthermore some of the bacteria from the genus *Escherichia, Lactobacillus* and *Bifidobacterium* (*Escherichia coli,* two strains of *Lactoba-*

cillus gasseri and *Bifidobacterium lactis*) produce the esterase to hydrolyses the chlorogenic acid and next to use it for their growth [Budryn and Nebesny, 2006]. This can be a reason of the significant decrease of the concentration of this acid in the digested material, as well as the decrease in the antioxidant capacity.

It was found by other authors that phenolic acids keep the antioxidant capacity in the gastrointestinal tracts, although in the successive stages of the digestion process a decrease of the activity was observed. The final metabolites formed as result of degradation of this compounds do not express the antioxidant capacity which is caused because of the lack of the hydroxylic group – like hippuric acid (the metabolite of chlorogenic acid digestion) [Konisi and Kobayashi, 2004].

Changes in the concentration of resveratrol were also found during the digestion process. The highest increase of its amount was observed after 2.5 h in the small intestine and a significant decrease after 18 h in the large intestine. The differences result from the activity of digestion enzymes, which cause the release of resveratrol in the stages from stomach to small intestine. Next the resveratrol is metabolized by the fecal microflora and decrease of its concentration is observed (Table 4).

After finishing the process of digestion an impeding effect of the red grape win on *E. coli* was noted. It was recorded that after "small intestine" value of 2.99 cfu·cm^{-3} of this bacteria increased only up to 3.68 cfu·cm^{-3} in the red grape wine after "large intestine". It is worth mentioning that no influence was recorded in other kind of wine by selected microorganisms (Table 5).

Table 5

Changes in the number of intestinal microflora in the course of digestion of wines

Sample	*Bifidobacterium*	*Lactobacillus*	*Enterococcus*	*Enterobacteriaceae*
	Log 10 cfu·cm^{-3} after digestion ± SD			
Inoculum	6.68 ± 0.19	6.45 ± 0.13	6.95 ± 0.34	6.78 ± 0.15
White wine				
2.5 at pH 7.4 "after small intestine"	6.87 ± 0.09	6.65 ± 0.22	6.08 ± 0.37	6.89 ± 0.23
21 h at pH 8.0 "after large intestine"	7.59 ± 1.19	6.70 ± 0.62	7.60 ± 1.39	5.60 ± 0.11
Red wine				
2.5 at pH 7.4 "after small intestine"	5.84 ± 0.11	6.15 ± 0.09	5.40 ± 0.10	2.99 ± 0.08
21 h at pH 8.0 "after large intestine"	5.04 ± 0.14	5.37 ± 0.08	5.03 ± 0.15	3.68 ± 0.19

It is believed that traits that support the antimicrobial activity of extracts from plant compounds include hydrophobicity and their antioxidising capacity. Some flavonoids can inhibit proliferation and reduce the determined number of bacteria without damaging cells

and making it possible for them to develop colonies. However, until now little is known about what kind of interactions constitutes the basis of the phenomena taking place in the intestinal ecosystem [Vattem et al., 2004].

Conclusions

During the *in vitro* digestion process of the tested wines a changes in polyphenols concentration as well as antioxidant activity were determined.

The qualitative and quantitative analysis of polyphenols demonstrated a decrease of concentration of this compounds during the digestion process, mainly: resveratrol, derivatives of hydroxicinnamic acids as well as theirs metabolites (caffeic acid, 3-(4-hydroxyphenyl) propionic acid)

A differential influence of the digested wines on the tested fecal microflora was observed. The highest inhibiting effect was determined against *Enterobacteriacea*e when digested red wine. After the digestion process number of bacteria amounted to 10^3 cfu·cm^{-3} while the number of other investigated microorganisms ranged from 10^5 to 10^7 cfu·cm^{-3}.

References

Aura A.M., Harkonen H., Fabritius M., Poutanen K., 1999. Development of in vitro enzymatic digestion method for removal of starch and protein and assessment of its performance using rye and wheat breads. J. Cereal Sci., 29, 139–152.

Blaut M., Schoefer L., Braune A., 2003. Transformation of flavonoids by intestinal microorganisms. Int. J. Vitam. Nutr. Res., 73, 79–86.

Budryn G., Nebesny E., 2006. Phenolic AIDS- their properties, occurrence in plant materiale, absorption and metabolizm. Bromat. Chem. Toksykol., XXXIX, 2, 103–110.

Czyżewska A., Pogorzelski E., 2004. Changes to polyphenols in the process of production of must and wines from blackcurrants and cherries. Part II. Anthocyanins and flavanols. Eur. Food Res. Technol., 218, 355–359.

Dávalos A., Fernández-Hernando C., Cerrato F., Martínez-Botas J., Gómez-Coronado D., Gómez--Cordovés C., Lasunción M. A., 2006. Red grape juice polyphenols alter cholesterol homeostasis and increase LDL-receptor activity in human cells in vitro. J. Nutr., 136, 1766–1773.

Gawęcki J., Libudzisz, Z. 2006. Microorganisms in Food and Nutrition. The August Cieszkowski Agricultural University of Poznań Press, 6, 31–40.

Gil-Izquierdo A., Terreres F., Barberan T., 2001. *In vitro* availibility of flavonoids and other phenolics in orange juice. J. Agric Food Chem., 49, 1035–1041.

Goderska K., Gumienna M., Czarnecki Z., 2008. Release of phenolic compounds from bean flour, bean-derived chips and black chokeberry juice and changes in their antioxidant activity during digestion in an *in vitro* gastrointestinal model. Pol. J. Food Nutr. Sci., 58, 497–501.

Grajek W., 2003. Changes of antioxidative potential of plant materials during processing and intestine digestion. Food – Science, Technology, Quality, 37, 26–35.

Gumienna M., Goderska K., Nowak J., Czarnecki Z., 2006. Changes in the antioxidative activities of bean products and intestinal microflora in the model of the gastrointestinal tract "in vitro". Pol. J. Food Nutr. Sci., 56, 29–32.

Knarreborg A., Simon M.A., Engberg R.M., Jensen B.B., Tannock G.W., 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol., 68, 5918–5924.

Konishi Y., Kobayashi S., 2004. Transepithelial transport of chlorogenic acid, caffeic acid, and their colonic metabolites in intestinal Caco-2 cell monolayers. J. Agric. Food Chem., 52, 2518–2526.

Muselík J., García-Alonso M., Martín-López M., Žemlička M. ,Rivas-Gonzalo J., 2007. Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. Int. J. Molec. Sci., 8, 797–809

Re R., Pellegirini N., Protegente A., Pannala A., Yang M., Rice-Evans C., 1999. Antioxidant activity applying an improved ABTS radical cation decolonization assay. Free Rad. Biol. Med., 26, 1231–1232.

Sakakibara H., Honda Y., Nakagawa S., Ashida H., Kanazawa K., 2003. Simultaneous determination of all polyphenols in vegetables, fruits and teas. J. Agric. Food Chem., 51, 571–581.

Singelton V.L., Rossi J.A., 1965. Colorymetry of total phenolics with phosphomolybdic-phodphotungstics acid reagents. Am. J. Etanol. Vitic., 16, 144–158.

Vattem D.A., Lin Y.-T., Labbe R.G., Shetty K., 2004. Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus *Lentinus edodes* and effect on antimicrobial activity against selected food borne pathogens. Innov. Food Sci. Emerg. Technol., 5, 81–91.

Zern T. L., Fernandez M.L., 2005. Cardioprotective effects of dietary polyphenols. J. Nutr. 135 (10): 2291–2294.

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SEA BUCKTHORN AND APPLE: A NEW MIX OF HIGH ANTIOXIDANT ACTIVITY JUICES RICH IN PHENOLIC COMPOUNDS

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.; Elaeagnaceae) is a temperate bush and is native to Europe and Asia [Rousi, 1971]. It was used as a medicinal plant in Tibet as early as 900 A.D. [Lu, 1992]. Since the 1950s, many medicinal preparations of sea buckthorn, from both wild and cultivated sources, have been clinically used to treat radiation damage, burns, oral inflammation, and gastric ulcers in China and the former Soviet Republics [Lebedeva et al., 1989; Fu et al., 1993]. These positive effects include lowering plasma cholesterol level, inhibiting platelet aggregation, regulating immune function, promoting the repair of injuries on skin and mucosa, and anticancer effects [Li et al., 1993; Jiang et al., 1993; Yang et al., 1999].

Sea buckthorn berries is a yellow-orange berry contain organic acids, sugars, protein, amino acids, elements, flavonoids, vitamins [i.e., A, C, and E (tocols)], carotenoids, fatty acids, and phytosterols [Beveridge et al., 1999]. Yang et al. [2002] reported that a characteristic property of sea buckthorn berry pulp/peel oil is the high content of C16:1*n*7 (palmitoleic acid), 20–33% of total fatty acids. However, the commercial value of sea buckthorn oil is commonly based on levels of carotenoids [Oomah et al., 2003] and tocols [Kallio et al., 2002]. Tocol content represented by tocopherols (R-T, _-T, *γ*-T, and *δ*-T) and tocotrienols (R-T3, _-T3, *γ*-T3, and *δ*-T3) is higher in pulp/peel oil than in seed or juice oil [Oomah et al., 2003]. Phytosterol content of berry oil is attributed to fruit-coat lipids (~50%), pulp (~20%), and seeds (~30%) [Mironov et al., 1989]. *β*-Sitosterol is the major sterol present in pulp/peel and seed fractions [Yang et al., 2001].

The fruits of sea buckthorn are rich sources of flavonols (1500–2000 mg/kg), which are reported to consist of glycosides of isorhamnetin, quercetin, and kaempferol [Lachman et al., 1985; Królikowska et al., 1972]. The most important flavonol glycosides in sea buckthorn are isorhamnetin-3-O-sophoroside-7-O-rhamnoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, quercetin-3-O-rutinoside, and quercetin-3-O-glucoside [Kallio et al., 2005; Rosh et al., 2004], detailed structures of phenolic acids and flavan-3-ols are mostly unknown.

These beneficial effects have made sea buckthorn products, especially its oils, desirable for medicinal and cosmetic purposes. Products on the market from sea buckthorn range from oil, juice, and food additives to candies, jellies, cosmetics, and shampoos [Schroeder et al., 1995]. The literature describing the formation of these products is both scattered and limited. The aroma is characterized by several aliphatic esters (*2*), and sea buckthorn juice has been

characterized and compared with aromas such as strawberry, peach, mango, apricot, papaya, and citrus.

Nowadays, there is a trend and worldwide pursuit of designing new functional foods and healthy food products. In this sense, a design of new beverages combining apple juice with other fruits (rich in polyphenols and vitamin C) interesting healthy product.

The aim of this work was to evaluate the antioxidant activity and the content of the bioactive compounds present in the new mixed beverages such as apple and sea buckthorn juice being the new health-promoting food products.

Materials and methods

Plant Materials and juices preparation. Fruits of sea buckthorn (*Hippophae rhamnosides* L.) were obtained from the Garden of Medicinal Plants herbarium at the Medical University in Wrocław, Poland. Fresh fruit samples were frozen and stored at -20°C until analysis. Apple (cv. Szara Reneta) were from a local market

In this study was preparaed three type of juices: 100% of apple juices and sea bucthorn and one mix juices of 80:20 of apple:sea buckthorn. The fruits were crushed and heated at 80°C for 5 min with the use of Thermomix (Vorwerk, Germany). After cooling, the pulp was depectinized at 50°C for 1 h by adding enzyme (0.5 mL of Pectinex Yield Mash (Begerow, Germany)) per kg was added. Pulp was pressed under final pressure of 3000 kPa for 3 min in a laboratory hydraulic press (Type Zoodiak). The juices were heated in Thermomix to 90°C for 5 min, 0.2 L glass jars were hot filled and immediately inverted for 10 min to sterilize the lids, and then cooled to 20°C. All processing procedures were replicated. Directly after processing and 6 months of storage in a different conditions, at 4°C and at 30°C the juices were subjected to analyses.

Dry matter analysis. Dry matter content was determined according to PN-90/A-75101/03. The method entails drying at defined pressure and temperature until the sample attains a constant mass.

Total extract analysis. Extract content was analyzed in accordance with PN-90/A-75101/02. The extract content was determined using a digital Pocket PAL-1 refractometer (ATAGO; Japan). The measuring range of the apparatus was between 0 and 53 Brix degrees.

HPLC Analysis of Polyphenols. The juices (5mL) was from10 min of centrifugation at 15 000 rpm (20.878 g). The analyses of flavan-3-ols, hydroxycinnamates, dihydrochalcones, and flavonol glycosides were carried out on a Merck-Hitachi L-7455 liquid chromatography with a diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM multisolvent delivery system (Merck-Hitachi, Tokyo, Japan) and autosampler L-7200. Separation was performed on a on a Cadenza CDC18 (75mm x 4.6mm, 5 μm) column (Imtakt, Japan). Oven temperature was set to 20°C. The mobile phase was composed of solvent A (2.5% acetic acid, v/v) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 25% B in 36 min, followed by washing and reconditioning of the column. The flow rate was 1.0 mL/min, and the runs were monitored at the following wavelengths: flavan-3-ols and dihydrochalcones at 280 nm, hydroxycinnamates at 320 nm, and flavonol glycosides at 360 nm. Diode array detector (DAD) spectra were measured over the wavelength range 200-600 nm in steps of 2 nm. Retention times and spectra were compared with those of

pure standards within 200-600 nm. The calibration curves were made from (-)-epicatechin, (+)-catechin, chlorogenic acid, phloretin-2-O-glucose, quercetin-3-O-glucoside, and procyanidin B2, C1, B1 as standards.

Color Measurement. Color properties (L*, a*, b*) of juices were determined with Color Quest XE Hunter Lab colorimeter. The samples were filled in a 1 cm cell, and L*, a*, b* values were determined using Illuminant D65 and a 10° observer angle. L* denotes 'lightness', and its value ranges from 0 to 100 (0 for ideal black, and 100 for ideal white). A positive value of a* indicates 'red colour', a negative value of a* indicates 'green colour', a positive value of b* indicates 'yellow colour', and a negative value of b* indicates 'blue colour.

Analysis of total carotenoids. Carotenoids were analyzed according to PN-90/A-75101/12. In this method carotenoids are extracted from the sample with hexane and their content in the extract is determined by colorimetry at 450 nm. In the present study extinction was measured with a Spekol 11 (Carl Zeiss Jena) spectrophotometer at 450 nm, in the presence of hexane.

Vitamin C. Ascorbic acid was estimated by using a LiChroCART(R) 125-3 Purospher(R) RP-18 column (5 µm) (Merck). The elution was carried out using of 0.1% *m*-phosphoric acid, the flow rate was 1 ml/min. The absorbance was monitored at 254 nm. The ascorbic acid was identified by comparison with standard. Calibration curve was prepared by plotting different concentrations of standard versus area measurements in HPLC.

DPPH Radical Scavenging Spectrophotometric Assay. The DPPH radical scavenging activity of investigated juices was determined according to the method proposed by Yen et al., [1995]. The juices (0.5 mL) was added to 0.5 mL of DPPH and 1.5 mL of ethanol. The mixture was shaken and let to stand at room temperature for 10 min. Antioxidant capacity was measured by recording absorbance at 517 nm in a Shimadzu UV-2401 PC spectrophotometer. Ethanol was used as blank. All determinations were performed in triplicate. The results were corrected for dilution and expressed in µM Trolox per 100 ml of juices.

Result and discussions

The fruit juices yield varied from 81.31 to 74.19%, for sea buckthorn having the highest juice yield (Table 1). The fruit juice yield of sea buckthorn berries has previously been reported to vary between 60 and 80% [Sabir et al., 2005a]. Oszmiański et al. [2009, in press] obtained yield of apple juices that ranged from 73.70 to 77.00% and dependent on the type of enzymatic mash treatment. The cultivar has a significant influence on juice yield, possibly due to the origin of the plant material, geographical conditions or the applied agronomic practices.

The highest level of total soluble solids (TSS) was found in the apple juices (15.1 °Brix), and the lowest in the mixed and sea buckthorn juices (12.4 and 9.7 °Brix, respectively). Statistical analysis has revealed significant differences (Table 1). The TSS content of new beverages was in accordance with the range established by the European National regulations for commercial drinks/beverages. pH values of juices varied from 2.78 for sea buckthorn to 3.8 for apple juices, and 2.7 for mixed juices. The pH value for sea buckthorn was in agreement with the previously reported results published in Finland [2.7–2.9 pH; Tiitinen et al., 2005]. Total acidity (TAA) varied widely depending on the type of juice (Table 1). TAA was

the highest (100%) for the sea buckthorn juices (2.48). It was 32% lower for the mixed juices and 56% lower for the apple juices. These values are still very similar to lemon juice with its pH amounting to 2.37 and TAA equal 5.73 mg/100 ml [Gonzalez-Molina et al., 2008], and higher than virtually all other fruits and vegetables [Holland et al., 1991]. This means that sea buckthorn berries are an excellent source of vitamin C.

Table 1

Average yield, pH, titratable acidity (TA), total soluble solids (TSS), vitamin C and carotenoids in the different juices

	Yield (%)	TSS	pH	TA	vitamin C	carotenoids
sea buckthorn	81.4±2.3	9.7±0.5	2.78±0.1	2.48±0.6	2191.6±23.5	6.80±0.3
mix juice	79.4±1.4	12.4±0.7	2.70±0.2	1.68±0.4	388.1±12.4	1.65±0.1
apple juice	74.2±3.7	15.1±1.3	3.08±0.1	1.08±0.1	9.8±1.3	n.d.

Values are mean ± standard deviation (n = 3).
TSS (total soluble solids) is expressed as ^{o} Brix (20 ^{o}C).
TA (titratable acidity) is expressed as g citric acid/100 ml juice.
Vitamin C and carotenoids is expressed as mg/100 ml juice.
n.d. – not detected

The ratio of extracts and total acidity has been reported to constitute the major promoter of fruit juices taste. In this study we have observed the highest values of the ratio for apple juices, whereas the smallest ratio was observed for sea buckthorn juices.

The differences in vitamin C content among these three types of juices were statistically significant ($P < 0.05$; Table 1). The content of vitamin C was only moderate, ranging from 8.9 (apple juice) and 388.1 (mixed juices) to 2191.6 mg/100 ml for sea buckthorn juice. 20% of sea buckthorn added during the production of mixed juice makes the juice a rich source of vitamin C, a better one than pure apple juice. According to earlier reports, sea buckthorn berries are rich in vitamin C and it is the genetic background that is the most important factor determining the vitamin C content; also the degree of ripeness affects it more than other factors such as climatic conditions. Wild types of sea buckthorn have been reported to have lower vitamin C content than cultivated varieties. The vitamin C concentration in sea buckthorn ranges from 28 to 310 mg/100g of berries in the European subspecies *rhamnosides* [Leong et al., 2002], and from 200 to 2500 mg/100g of berries in Chinese subspecies *sinesnsis*. Ascorbic acid accounted for 65–100% of the total antioxidant capacities of the juices derived from citrus fruits but for less than 5% in juices derived from apples, pineapples and vegetables. This supports the observation that ascorbic acid is the major antioxidant in orange juice but is not a major contributor to antioxidant capacity in apple juice [Gil et al., 2006].

The content of total carotenoids in the samples examined varied from 0.08 to 6.80 mg/100 ml (Table 1). Higher values were determined in the sea buckthorn juice, medium for mixed juices (1.65 mg/100 ml). In the apple juice the carotenoid content was not measured. According to the previous study mentioned, total carotenoid content in sea buckthorn fruits ranged from 3.0 to 15.0 mg/100 g [Raffo et al., 2004] and depended on the cultivar. In these fruits the main carotenoids were zeaxanthin, β-carotene and β-criptoxanthin, which were the principal pigments of the bright orange-coloured berries. Sea buckthorn total carotenoid content was similar to that of other plant foods particularly those rich in carotenoids (containing more than 10.0 mg/100 g), such as tomatoes, carrots, kale and spinach. Among carotenoids

detected in the sea buckthorn fruits and juices β-carotene possesses active provitamin A. Moreover, it has been reported that a diet rich in carotenoids increased their accumulation in the macula and that these compounds could protect against age-related macular degeneration, by preventing light-initiated oxidative damage to the retina and retinal pigment epithelium [Johnson et al., 2000]. Seglina et al. [2004] suggests that the carotenoid pigments in the juice are primarily associated with the suspended solids and oil and its content probably varies depending on the method of juice production.

The values of colour parameters (CIE Lab system) are summarized in Table 2. Tiitinen et al. [2005] reported that L*, a* and b* values in sea buckthorn berries are 33.6–57.8, 4.0–18.5 and 22.8–45.7, respectively. These results support our findings for sea buckthorn juice too (Table 2). The 100% sea buckthorn juice had the higher *b** value than the rest of juices. The sea buckthorn juice is yellow in colour; the high amount of carotene is responsible for this yellow colouration. *b** value was 46.60, 35.44 and 12.92 for sea buckthorn, mixed juice and apple juices, respectively. The value of parameter *a** was the highest (27.62) in the sample of sea buckthorn and mixed juice, whereas the lowest (4.52) value was measured in pure apple juice. Lightness (L*) of the investigated juices ranged from 53.02 to 58.03 and is negligibly small. The brightest were the apple juice samples, and the darkest those with the sea buckthorn juice. Nevertheless, changes in the lightness of juices were impossible to evaluate by the human eye.

Table 2

The colour parameter L*, a*, b* for sea buckthorn, apple and mix juices

	L*	a*	b*
Sea buckthorn	58.03	27.62	46.6
mix juice	55.7	16.48	35.44
Apple	53.02	4.13	12.92

Sea buckthorn fruits and juices are an excellent source of phytochemicals such as ascorbic acid, tocopherols, unsaturated fatty acids, organic acids, phytosterol, and carotenoids [Beveridge et al., 1991]. A number of these compounds have been extensively studied, but there is still little information on sea buckthorn's polyphenol contents. The total content of phenolics in sea buckthorn juice was ca. 1301.8 mg/L (Table 3). Sea buckthorn juice was the richest in flavonols (quercetin-3-*O*-rutinoside, -glucoside, and –rhamnoside), and flavan-3-ols ((+)-catechin and (-)-epicatechin) but only trace amounts of hydroxcinnamic acid were quantified. High amount of carotene is responsible for yellow colour of these juices because quantification of anthocyanins in these juices was very low. The content of (+)-Catechin and (-)-epicatechin was comparable (11.7 and 10.9, respectively), which was consistent with the result obtained by Rösch et al. [2003]. However, HPLC analysis suggested that the predominating subunits of sea buckthorn were oligomeric proanthocyanidins (792.1 mg/L).

A total of 12 kinds of polyphenolic compounds found in apple juice were identified: two hydroxycinnamates (chlorogenic acid, p-coumaroylquinic acid), four flavan-3-ols ((+)-catechin, (-)-epicatechin, and procyanidin B2, and C1), two dihydrochalcones (phloretin-2′-O-xyloglucoside and phloretin-2′-O-glucoside), and four flavonols (quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-arabinoside, and quercetin-3-O-xyloside). The amounts of polyphenolic classes such as hydroxycinnamates, catechins, and oligomeric procyanidin were found to be higher in apple juice than in sea buckthorn juice.

Table 3

The total phenolic compounds content in sea buckthorn (SB), mix (apple + sea buckthorn) and apple (A) juices

	SB	A+SB	A
chlorogenic acid	7.1±0.3	204.7±4.3	242.3±5.2
p-coumaric acid	n.d.	15.0±1.5	14.0±1.3
(+)-catechin	11.7±1.2	27.3±2.0	15.4±2.5
(-)-epicatechin	10.9±0.8	123.7±1.3	71.0±2.8
Procyanidins B2	n.d.	60.0±2.9	51.9±1.9
Procyanidins C1	n.d.	51.8±1.1	53.1±2.1
Phloretin-2'-O-xyloglucoside	n.d.	22.5±1.8	23.0±2.4
Phloretin-2-O'-glucoside	n.d.	53.7±3.5	53.9±1.9
Quercetin-3-O-galactoside	2.8±0.1	14.3±2.2	13.9±0.6
Quercetin-3-O-glucoside	6.2±0.3	3.7±0.3	1.9±0.2
Quercetin-3-O-arabinoside	344.0±3.2	2.8±0.8	6.5±0.3
Quercetin-3-O-xyloside	127.0±1.8	66.9±2.7	4.1±0.3
Oligomeric procyanidins	792.1±2.7	1795.3±5.9	1547.6±7.2
TOTAL OF POLYPHENOLS	1301.8	2441.7	2098.5

Values are mean ± standard deviation (n = 3).

There has been a considerable increase of total phenolic content in mixed juice as compared to apple and sea buckthorn juices (14 and 47%, respectively) (Table 3). The high content of phenolic compounds found in mixed juice is a beneficial result of mixing components rich in natural antioxidants. Sea buckthorn fruits are an excellent natural source of vitamin C and mixing them with apple during grinding had a protective effect on phenolic compounds The addition of 20% of sea buckthorn to apple juice during their preparation resulted in the increase of flavan-3-ols content in final products, especially of oligomeric procyanidins. Proanthocyanidins are of great interest for nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health [Santos-Buelga et al., 2000]. It has recently been hypothesized that the free radical scavenging properties of procyanidins may reduce the risk of cardiovascular diseases [Reed, 2002; Steinberg et al., 2003], cancer [Bagchi et al., 2000] and blood clotting.

Antioxidant activity of investigated juices described by DPPH method was presented in Figure 1. There was a significant difference in antioxidant activity caused by type of juices. Similarly as with the difference in total phenolics content, mixed and sea buckthorn juices showed a wider range of antioxidant capacity than the apple juices. The effects of juice preparation type on antioxidant capacity measured by DPPH radical scavenging ranged from 312.3.2 to 46.5 μM of Trolox equivalent. The highest level of antioxidant activity (P <0.05) was observed in sea buckthorn than in mixed juice ≥ apple juices. The high antioxidant activity measured for sea buckthorn juice was probably a consequence of high content of natural antioxidant vitamin, vitamin C, than of polyphenols content. The vitamin C content correlated positively with the free-radical scavenging capacity of food products (*21*).

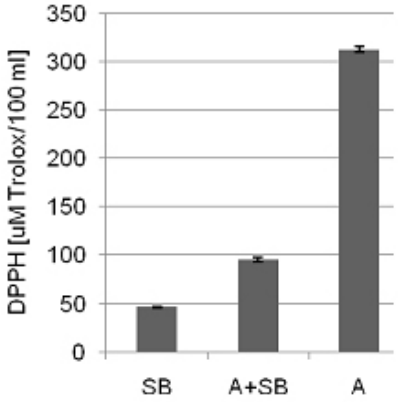

Fig. 1. The antioxidant activity for sea buckthorn (SB), mix (apple + sea buckthorn) and apple (A) juices measured by DPPH method

Conclusions

The 100% of buckthorn juice had the very interesting components, especially vitamin C and carotenoids and it had a very attractive yellow colour. Obtained results showed that the addition of sea buckthorn fruits had beneficial influence on conservation of phenolic compounds in apple juices during preparation and had a good effect on antioxidant activity. Therefore, the new drink based on apple juice plus sea buckthorn fruits showed potential for the development of interesting functional products, rendering an interesting beverage in the growing market of food consumed for the benefit of health.

References

Bagchi D., Bagchi M., Stohs S.J., Das D.K., Ray S.D., Kuszynski C.A., 2000. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology, 148(2–3), 187–197.

Beveridge T., Li T.S.C., Oomah B.D., Smith A., 1999. Sea buckthorn products: Manufacture and composition. J. Agric. Food Chem., 47, 3480–3488.

Fu Q., Yang Q., Yang G., 1993. Analysis of alpha-tocopherol contents in sea-buckthorn oil by reversed phase-high performance liquid chromatography. J. Xi'an Med. Univ. 14, 181–183.

Gil M.I., Aguayo E., Kader A.A., 2006. Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. J. Agric. Food Chem., 54, 4284–4296.

Gonzalez-Molina E., Moreno D.A., Garcia-Viguera C., 2008. Aronia-enriched lemon juice: A new highly antioxidant beverage. J. Agric. Food Chem. 56, 11327–11333.

Holland B., Welch A.A., Unwin I.D., Buss D.H., Paul A.A., D.T.A. Southgate. McCance and Widdowsons, 1991. The Composition of Foods. 5th edition. the Royal Society of Chemistry, Science Park, Milton Road, Cambridge CB4 4WF, UK.

Jiang Y.D., Zhou Y.C., Bi C.F., Li J.M., Yang J.X., Yu Z.D., Hu Z.Y., Zhao S.X., 1993. Clinical investigation of effects of sea buckthorn seed oil on hyperlipeamia. *Hippophae,¨ 6* (3), 23–24.

Johnson E.J., Hammond B.R., Yeum K.J., Qin J., Wang X.D., Castaneda C., Snodderly D.M., Russell R.M., 2000. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. Am J. Clin. Nutr., 71, 1555–1562.

Kallio H., Yang B., Halttunen T. 2005. Flavonol glycosides of berries of three major sea buckthorn subspecies, *Hippophae¨ rhamnoides* ssp. *rhamnoides*, ssp. *sinensis*, and ssp. *mongolica.* Proc. InVited Speeches Second Int. Seabuckthorn Assoc. Conf. 29–35.

Kallio H., Yang B., Peippo P., Tahvonen R., Pan R., 2002. Triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols in berries and seeds of two subspecies (ssp. *sinensis* and *mongolica*) of sea buckthorn (*Hippophae rhamnoides*). J. Agric. Food Chem., 50, 3004–3009.

Krolikowska M., 1972. Umber die Flavonolglykoside von Sanddornbeeren *Hippophae rhamnoides*. Planta Med., *22*, 418–427.

Lachman J., Pivec V., Hubacek J., Rehakova V., 1985. Flavonoid substances in the fruits of sea buckthorn (*Hippophae rhamnoides*). Sci. Agric. Bohem. 3, 169–182.

Lebedeva,L., Rachmov I., Kchaidarov K., 1989. Screening investigation of the antiinflammation activity of sea buckthorn oil. Proc. Int. Symp. Sea Buckthorn, Xi'an, China, 398–399.

Leong L.P., Shui G., 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem., 76, 69–75.

Li Z.R., Tan S.Z., 1993. Clinical observation of the effects of orally administered sea buckthorn seed oil on patients with malignant tumor under chemotherapy. *Hippophae¨* 6 (4), 41–42.

Lu R., 1992. Sea buckthorn: A multipurpose plant species for fragile mountains; ICIMOD Publication Unit: Katmandu, Nepal.

Mironov V.A., Guseva-Donskaya T.N., Dubrovina Yu., Osipov G.A., Shabanova E.A., Nikulin A.A., Amirov N.Sh., Trubitsina I.G., 1989. Chemical composition and biological activity of extracts from sea buckthorn fruit components. Khim. Farm. Zh. (Russian) 23, 1357–1364.

Oomah B.D. 2003. Sea buckthorn lipids. In *Sea Buckthorn (Hippophae rhamnoides L.): Production and Utilization*; Li, T. S. C., Beveridge, T., Eds.; NRC Research Press: Ottawa, Canada, 51–68.

Oszmiański J., Wojdyło A., Kolniak J., 2009. Effect of enzymatic mash treatment on phenolic composition, antioxidant activity, and turbidity of cloudy apple juice. JAFC, 57 (15), 7078–7085.

Reed J., 2002. Cranberry flavonoids, atherosclerosis and cardiovascular health. Crit. Rev. Food Sci. Nutr., 42(Suppl), 301–316.

Rösch D., Bergmann M., Knorr L.W., 2003 Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. J. Agric. Food Chem., 51, 4233–4239.

Rosch D., Krumbein A., Mugge C., Kroh L.W., 2004. Structural investigations of flavonol glycosides from sea buckthorn (*Hippophae ¨ rhamnoides*) pomace by NMR Spectroscopy and HPLCESI-MSn. J. Agric. Food Chem*., 52*, 4039–4046.

Sabir S.M., Maqsood H., Ahmed S.D., Shah A.H., Khan M.Q., 2005. Chemical and nutritional constituents of sea buckthorn (*Hippophae rhamnoides* ssp. turkestanica) berries from pakistan. Ital. J. Food Sci., 17, 455–462.

Santos-Buelga C., Scalbert A., 2000. Proanthocyanidins and tanin-like compounds: nature, occurrence, dietary intake and effects on nutrition and health (review). J. Sci. Food Agric., 80, 1094–1117.

Schroeder W.R.; Yao Y., 1995. Sea Buckthorn: A Promising Multipurpose Crop for Saskatchewan; PFRA Shelterbelt Center, Supplementary Report, 95–102.

Steinberg F., Bearden M., Keen C., 2003. Cocoa and chocolate flavonoids: Implications for cardiovascular health. J. Am. Diet Assoc., 103, 215–223.

Tiitinen K.M., Hakala M.A., Kallio H.P. ,2005. Quality components of sea buckthorn (*Hippophae rhamnoides*) varieties. J. Agric. Food Chem., 53, 1692–1699.

Yang B., Kalimo K.O., Mattila L.M., Kallio S.E., Katajisto J.K., Peltola O.J., Kallio H.P., 1999. Effects of dietary supplementation with sea buckthorn (*Hippophae¨ rhamnoides*) seed and pulp oils on atopic dermatitis. J. Nutr. Biochem., 10, 622–630.

Yang B., Kallio H., 2002. Effects of harvesting time on triacylglycerols and glycerophospholipids of sea buckthorn (*Hippophae rhamnoides* L.) berries of different origins. J. Food Compos. Anal., 15, 143–157.

Yang B., Karlsson R.M., Oksman P.H., Kallio H.P., 2001. Phytosterols in sea buckthorn (*Hippophae rhamnoides* L.) berries: Identification and effects of different origins and harvesting times. J. Agric. Food Chem., 49, 5620–5629.

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THE EVALUATION OF POSSIBILITIES OF APPLE POMACE UTILIZATION FOR ACETIC ACID BIOSYNTHESIS

Introduction

Acetic acid is the main component of vinegar and it is widely used in food and chemical industries. The production of acetic acid is commonly known and applied as a traditional fermentation process in which glucose is first fermented by yeast to ethanol and it is then oxidized to acetic acid by a bacteria of the genus *Acetobacter* or *Gluconobacter*. This process is characterized by a very high oxygen demand, connected with bacteria requirements as well as their high nutritional demand which increase the production costs [Castro-Martinez et al., 2005]. Furthermore, because of the loss of one carbon through CO_2 in the glucose to ethanol fermentation, the theoretical maximum yield of the whole reaction is 2 moles of acetic acid from 1 mole of glucose what correspond to 0.67g of acetic acid per gram of glucose. Another microorganism, a spore forming thermophilic bacteria *Clostridium thermoaceticum*, is able to ferment various sugars like fructose, glucose and xylose and produce 3 moles of acetic acid from 1 mole glucose [Danner and Braun, 1999; Martin et al., 1985; Poston et al., 1966]. Unfortunately, the toxicity of acetate and low production rates do not allow successful applications of these microorganisms for industrial production of acetic acid. The ability to produce enhanced amounts of acetic acid has been also reported for the wine yeast from the genus *Brettanomyces* [Aguilar Uscana et al., 2003; Castro-Martinez et al., 2005; Freer, 2002; Freer et al., 2003]. In the beverage industry, *Brettanomyces* is well known yeast, as it causes a microbial spoilage of wine. Its presence in wine is often associated with off-odours described as phenolic, horse sweat, Band-Aid, mousy, wet wool, medicinal, smoky, spicy, etc. The analysis of *Brettanomyces* metabolism shows also their ability to direct bioconversion of glucose to acetic acid, excluding the alcoholic fermentation, and under minimal nutritional requirements [Guadalupe et al., 2000; Loureiro and Malfeito-Ferreira, 2003; Shinorara et al., 2000; Suárez et al., 2007]. It was found that resting cells of *Brettanomyces* under aerobic conditions fermented glucose at a higher rate than in the absence of oxygen. This phenomenon, called "Custer effect" (negative Pasteur effect), is attributed to the inhibition of the alcoholic fermentation under anaerobic conditions and to the ability of *Brettanomyces* to produce acetic acid directly from glucose with the parallel reduction of NAD^+ [Scheffers and Winken, 1969; Carrascosa et al., 1981]. This knowledge inspired us to check the possibility to use this yeast as an alternative microorganism to *Acetobacter* bacteria commercially used for acetic acid production. The aim of this study was to evaluate the ability of the yeast of the genus *Brettanomyces* to produce the acetic acid. The raw material containing two carbon sources: glucose and fructose, was an apple pomace which is a primary by-product in the apple pro-

cessing industry. The utilization of this by-product in combination with the sufficient yield of acetic acid synthesis could contribute to the application of an innovative technology and production of an added value bioproduct.

Materials and methods

Microorganism

Three *Brettanomyces bruxellensis* strains: CE116, CE120 and CE254 from the Cornell Enology collection (NYSAES, Cornell University, NY, USA) were used in the performed experiments. Yeast strains were maintained in YM medium (Difco) with monthly transfer and cultivated at 30ºC for 48 h.

Material

Apple pomace was obtained from apple juice production process in the Food Biotechnology and Microbiology Pilot Plant of Poznań University of Life Science. The material was stored at -22ºC until needed.

The medium used for acetic acid biosynthesis was a suspension of 250 g of apple pomace in one dm^3 of distilled sterile water.

Methods

Acetic acid production process was performed in 300 cm^3 Erlenmeyer flasks. A sample of 30 g of apple pomace was suspended in 120 cm^3 of the sterile water, inoculated with 48h culture of the *Brettanomyces* strain (5% v/v) and cultured in shake conditions (150 rpm) at the temperature of 30ºC, without pH regulation for 14 days.

Growth of the yeast (expressed as number of cells in one cm^3 of the medium) during the fermentation process was evaluated microscopically and the vitality of yeast cells was determined by using the methylene blue staining. Glucose, fructose, acetic acid as well as lactic acid and ethanol were determined by HPLC equipped with Biorad Aminex HPX-87H column and RI detector, at 30ºC. The mobile phase was 5mM H_2SO_4, flow rate 0.6 cm^3/min and the volume of the injected sample 20 µl.

Results and discussion

Apple pomace is a primary by-product of apple processing industry. It constitutes about 25–35% of the weight of fresh fruit. This waste material contains approximately 10–20% of sugars, mainly glucose and fructose. A low protein concentration in the pomace, cause that only small part of this by-product can be used as an ingredient in livestock feed supplementation [Vendruscolo et al., 2008]. Because of that, the disposal of this waste material presents a serious environmental problem.

In this study investigations on the utilization of apple pomace for production of an added value product – acetic acid were performed. Three strains of *Brettanomyces bruxellensis CE116, CE120* and *CE254* from Cornell Enology Strain Collection (NYSAES, NY, USA)

Table 1

Changes of pH, number and vitality of yeast cells, concentration of glucose, fructose, acetic acid, lactic acid and ethanol during fermentation of *B. bruxellensis CE116* strain

Time (days)	pH	log cell·cm^{-3}	Cell vitality (%)	Glucose (g·dm^{-3})	Fructose (g·dm^{-3})	Total sugar (g·dm^{-3})	Sugar consumption (%)	Acetic acid (g·dm^{-3})	Lactic acid (g·dm^{-3})	Ethanol (% v/v)
0	3.44 ± 0.00	7.62 ± 0.00	97.86 ± 0.00	9.7 ± 0.19	7.03 ± 0.07	16.73 ± 0.13	–	0	1.40± 0.04	0
1	3.33 ± 0.30	7.09 ± 0.30	96.05 ± 2.29	7.31 ± 0.11	5.18 ± 0.08	12.49± 0.29	25.34	0.8 ± 0.01	1.40± 0.02	0.05 ± 0.01
3	3.15 ± 0.10	7.83 ± 0.10	92.70 ± 2.49	1.78 ± 0.03	0.4 ± 0.02	2.18± 0.04	86.96	2.50 ± 0.06	1.30± 0.01	0.90 ± 0.02
7	3.07 ± 0.05	8.23 ± 0.05	95.37 ± 1.37	0.87 ± 0.02	0.4 ± 0.01	1.27± 0.03	92.41	11.20 ± 0.17	1.00± 0.03	0.06 ± 0.01
10	2.71 ± 0.02	8.36 ± 0.02	95.66 ± 0.88	0	0	0	100	12.30 ± 0.22	1.01± 0.07	0
14	3.57 ± 0.16	7.98 ± 0.16	88.18 ± 3.20	0	0	0	100	3.60 ± 0.04	0.90± 0.11	0

Table 2

Changes of pH, number and vitality of yeast cells, concentration of glucose, fructose, acetic acid, lactic acid and ethanol during fermentation of *B. bruxellensis CE120* strain

Time (days)	pH	log cell·cm^{-3}	Cell vitality (%)	Glucose (g·dm^{-3})	Fructose (g·dm^{-3})	Total sugar (g·dm^{-3})	Sugar consumption (%)	Acetic acid (g·dm^{-3})	Lactic acid (g·dm^{-3})	Ethanol (% v/v)
0	3.44 ± 0.00	7.36 ± 0.00	97.41 ± 0.00	9.70 ± 0.19	7.03 ± 0.07	16.73 ± 0.13	–	0	1.40 ± 0.02	0
1	3.32 ± 0.06	7.42 ± 0.05	96.29 ± 0.97	6.11 ± 0.27	4.94± 0.05	11.05 ± 0.11	33.59	0.40 ± 0.01	1.40 ± 0.06	0.20 ± 0.01
3	3.09 ± 0.11	8.15 ± 0.18	95.24 ± 1.20	1.83 ± 0.06	0	1.83 ± 0.06	89.06	2.10 ± 0.07	1.40 ± 0.01	0.60 ± 0.01
7	2.95 ± 0.06	7.91 ± 0.14	96.32 ± 1.26	0	0	0	100	9.50 ± 0.26	1.10 ± 0.03	0.20 ± 0.01
10	2.82 ± 0.19	8.40 ± 0.10	95.42 ± 0.58	0	0	0	100	11.70 ± 0.22	1.03 ± 0.01	0
14	4.51 ± 1.19	7.78 ± 0.18	88.65 ± 1.89	0	0	0	100	4.60 ± 0.11	1.01 ± 0.02	0

Table 3

Changes of pH, number and vitality of yeast cells, concentration of glucose, fructose, acetic acid, lactic acid and ethanol during fermentation of *B. bruxellensis CE254* strain

Time (days)	pH	log cell·cm^{-3}	Cell vitality (%)	Glucose (g·dm^{-3})	Fructose (g·dm^{-3})	Total sugar (g·dm^{-3})	Sugar consumption (%)	Acetic acid (g·dm^{-3})	Lactic acid (g·dm^{-3})	Ethanol (% v/v)
0	3.44 ± 0.00	7.55 ± 0.00	98.81 ± 0.00	9.70 ± 0.21	7.03 ± 0.07	16.73 ± 0.13	–	0	1.40 ± 0.09	0
1	3.30 ± 0.03	7.31 ± 0.16	97.02 ± 1.90	7.14 ± 0.16	6.11± 0.11	13.25 ± 0.19	20.81	1.20 ± 0.04	1.40 ± 0.09	0.20 ± 0.01
3	3.07 ± 0.03	8.19 ± 0.20	94.53 ± 0.34	2.08 ± 0.05	1.18± 0.07	3.26 ± 0.07	80.51	5.80± 0.12	1.20 ± 0.06	0.40 ± 0.01
7	2.85 ± 0.01	8.04 ± 0.07	94.93 ± 0.63	0	0	0	100	10.80± 0.19	1.00 ± 0.05	0
10	3.28 ± 0.04	8.37 ± 0.21	95.04 ± 0.77	0	0	0	100	7.30± 0.25	1.04 ± 0.08	0
14	4.76 ± 0.15	7.81 ± 0.14	80.57 ± 6.70	0	0	0	100	0.03± 0.01	1.00 ± 0.04	0

were tested from the point of view of their ability for biosynthesis of acetic acid from the sugars contained in apple pomace. The cultures were run for two weeks in aerobic conditions at the temperature of 30ºC. In all the analyzed fermentation processes sugars were completely utilized by the yeast. The highest dynamic of glucose and fructose consumption was observed during *B. bruxellensis CE120* cultivation (Tab. 2). In the third day 89% of total amount of sugars was utilized and in the seventh day no more sugars in the medium were found. A total consumption of sugars after three days of the process was also observed when strain *B. bruxellensis CE254* was applied (Tab. 3). Much slower dynamic of sugar utilization was found when *B. bruxellensis CE116* was cultured (Tab. 1). All the three tested strains utilized both sugars: glucose and fructose almost simultaneously.

The yield and dynamic of acetic acid biosynthesis was similar for all tested microorganisms. The synthesized acetic acid was detected already after first day of the process. The highest concentration was determined after 10 days of *B. bruxellensis CE116* and *B. bruxellensis CE120* fermentation, and reached 12.3 ± 0.22 and 11.7 ± 0.22 g·dm^{-3} respectively. Significantly faster but also less effective synthesis was observed when *B. bruxellensis CE254* was used. In this case the highest concentration of acetic acid reached 10.8 ± 0.19 g·dm^{-3} and was determined in seventh days of fermentation process. The highest acetic acid yield reached 0.74 g of acetic acid per 1 g of consumed sugar which corresponded with 49.2 of acetic acid per 1 kg of utilized apple pomace and was determined during fermentation of *B. bruxellensis CE116* (Tab. 4).

The production of acetic acid corresponded with increasing acidity of growth medium and the pH decreasing from 3.44 to 2.71, 2.82 and 2.85 during culturing *B. bruxellensis CE116, B. bruxellensis CE120 and B. bruxellensis CE254* respectively. This could cause an inhibitory effect on growth and metabolic activity of the yeasts. Similar observations were described as a result of investigations on the inhibitory effect of acetic acid synthesized by *B. bruxellensis* isolated from a contaminated alcoholic fermentation in a distillery plant [Aguilar et al., 2003]. Those authors confirmed previous results of others [Pamphula and Loureiro-Dias, 1989; Rasmussen et al., 1995] that the acetic acid toxicity acts at the cytoplasmic level where the enzymes involved in glycolysis are present. They also found that the toxicity of this acid is dependent on the external pH, because the active form is the undissociated form, which penetrates the cellular membrane more easily and, once inside, can dissociate, causing modification of the internal pH of the cell. In the presented experiment an inhibiting effect of the synthesized acetic acid was also observed. After reaching the highest concentration of acetic acid the vitality of yeast cells significantly decreased. Similarly, the presence of acetic acid was found to disturb the growth and fermentative activity of yeast when its concentration exceed 2 g·dm^{-3} [Yahara et al., 2007] in both aerobic and anaerobic conditions.

The yeast belonging to the genus *Brettanomyces* and *Dekkera* were previously investigated by Freer [2002] from the point of view of possibilities for acetic acid production. From the sixty strains tested during batch aerobic cultures in medium initially containing 100 g glucose in dm^3, twenty-eight strains produced at least 5 g acetic acid in dm^3. Of these, sixteen strains produced over 20 g acetic acid in dm^3. The highest productivity (over 29 g acetic acid in dm^3) was observed for strains: *Dekkera intermedia, Brettanomyces intermedius* and *Dekkera bruxellensis*, however, non of these cultures completely utilized the 100 g glucose from dm^{-3} initially present in the medium [Freer, 2002]. They also found that the cultures grown at 30ºC tended to produce the product more rapidly. When cultured at 30ºC, the fermentations were completed in 3–5 days, while the cultures grown at 24ºC required 5–7 days completion,

Table 4

Comparison of the yield of acetic acid production during fermentation of *B. bruxellensis CE 116, 120 and 254* strains

Time (days)	*B. bruxellensis CE116*		*B. bruxellensis CE120*		*B. bruxellensis CE254*	
	g of acetic acid per 1 g of consumed sugar	g of acetic acid per 1 kg of apple pomace	g of acetic acid per 1 g of consumed sugar	g of acetic acid per 1 kg of apple pomace	g of acetic acid per 1 g of consumed sugar	g of acetic acid per 1 kg of apple pomace
1	0.18	3.2	0.07	1.6	0.34	4.8
3	0.17	10.0	0.14	8.4	0.43	23.2
7	0.72	44.8	0.56	38.0	0.64	43.2
10	0.74	49.2	0.69	46.8	0.43	29.2
14	0.21	14.4	0.27	18.4	0.001	0.12

as measured by the presence of a constant residual carbon source and no further increase in acetic acid production.

In the presented investigations, every experiment was characterized by a certain concentration of lactic acid and ethanol in the fermented medium. Lactic acid most probably was coming from the lactic acid bacteria contamination of apple pomace. The amount of lactic acid decreased during the process from the initial concentration of 1.4 to 0.9 $g \cdot dm^{-3}$ (Tab. 1–3). Ethanol was found in the medium between the first and the seventh day in the amount from 0.05 to 0.9% v/v and next was totally utilized. It is postulated that because of the lack of sugars in the medium after seventh and tenth day of the process, the yeast could use ethanol as well as acetic acid and lactic acid as the only available carbon sources.

The influence of availability of low-cost carbohydrates on *Brettanomyces bruxellensis* growth and acetic acid production was also studied by Aguilar et al. [2007] in order to ascertain the potential of this yeast to become an alternative microorganism in industrial acetic acid production. It their study six different raw materials were tested as a carbon source: glucose, sugarcane molasses, refined cane sugar, pineapple, sugarcane and beet juices. In the result it was showed that *B. bruxellensis* developed in a complex culture medium like plant juices and sugarcane molasses better than in a medium with a simple carbohydrate such as glucose. The maximum acetic acid yield (0.24 g of acetic acid per 1 g of consumed sugar) and productivity (0.14 g of acetic acid in one dm^3 in one hour) were attained in tests carried out with sugarcane molasses medium containing 60 g sucrose in one dm^3.

Conclusions

The three tested *Brettanomyces bruxellensis* strains showed the ability to produce acetic acid from sugars coming from apple pomace. The efficiency of acetic acid production did not exceed 12.3 g acetic acid in dm^3 of the applied medium, which corresponds to the yield of 49.2 g acetic acid from kg apple pomace. But, it has to be pointed out, that the sugars were totally consumed in the process and after approximately seven days no more carbon sources were available for the fermentation process. So addition of other sugar sources such as molasse or other by-product as well as control of pH to reduce the inhibiting effect of the acidity of the medium, can significantly increase the yield of the acetic acid producing process and make the yeast of the genus *Brettanomyces* an alternative microorganism in the innovative technology of acetic acid production by its direct bioconversion from glucose excluding the ethanol fermentation.

References

Aguilar Uscanga M.G., Escudero Abarca B.I., Gomez Rodrigues J., Cortes Garcia R., 2007. Carbon sources and their effect on growth, acetic acid and ethanol production by *Brettanomyces bruxellensis* in batch culture. J. Food Process. Eng., 30, 13–23.

Aguilar Uscanga M.G., Delia M.L., Strehaiano P., 2003. *Brettanomyces bruxellensis:* effect of oxygen on growth and acetic acid production. Appl. Microbiol. Biotechnol., 61, 157–162.

Carrascosa J.M., Viguera M.D., Nuez de Castro I., Scheffers W.A., 1981. Matabolism of acetaldehyde and Custer effect in the yeast *Brettanomyces abstinens*. Antonie Van Leeuwenhock, 47, 209–215.

Castro-Martinez C., Escudero-Abarca B.I., Gomez Rodriguez J., Hayward-Jones P.M., Aguilar-Uscanga M.G., 2005. Effect of physical factors on acetic acid production in *Brettanomyces* strains. J. Food Process. Eng., 28, 133–143.

Danner H., Braun R., 1999. Biotechnology for the production of commodity chemicals from biomass. Chem. Soc. Rev., 28, 395–405.

Freer S.N., 2002. Acetic acid production by *Dekkera/Brettanomyces* yeasts. World J. Microbiol. Biotechnol., 18, 271–275.

Freer S.N., Dien B., Matsuda S., 2003. Production of acetic acid by Dekkera/Brettanomyces yeasts under conditions of constant pH. World J. Microbiol. Biotechnol., 19, 101–105.

Guadalupe Aguilar Uscanga, M., Delia, M.-L., Strehaiano, P., 2000. Nutrional requirements of *Brettanomyces bruxellensis*: growth and physiology in batch and chemostat cultures. Can. J. Microbiol., 46, 1046–1050.

Loureiro V., Malfeito-Ferreira M., 2003. Spoilage yeasts in the wine industry. Int. J. Food Microbiol., 86, 23–50.

Martin D.R., Misra A., Drake H.L., 1985. Dissimilation of carbon monoxide to acetic acid by glucose-limited cultures of Clostridium thermoaceticum. Appl. Environ. Microbiol., 49 (6), 1412–1417.

Poston J.M., Kuratomi K., Stadtman E.R., 1966. The conversion of carbon dioxide to acetate. J. Biol. Chem., 241 (18), 4209–4216.

Pamphula M.E., Loureiro-Dias M.C., 1989 Combined effect of acetic acid, pH and ethanol on intracellular pH of fermenting yeast. Appl. Microbiol. Biotechnol., 31, 547–550.

Rasmussen J.E., Schulz E., Snyder R.E. Jones R.S., Smith C.R., 1995. Acetic acid as a causative agent in processing stuck fermentations. Am. J. Enol. Vitic., 46, 278–280.

Scheffers W.A., Wiken T.O., 1969. The Custer effect (negative Pasteur effect) as a diagnostic criterion for the genus *Brettanomyces*. Antonie Van Leeuwenhock, 35, 119–202.

Shinorara T., Kubodera S., Yanagida F., 2000. Distribution of Phenolic Yeasts and Production of Phenolic Off-Flavors in Wine Fermentation. J. Biosci. Bioeng., 90(1), 90–97.

Suarez R., Suarez-Lepe J.A., Morata A., Calderon F., 2007. The production of ethylphenols in wine by yeasts of genera *Brettanomyces* and *Dekkera*: a review. Food Chem., 102, 10–21.

Vendruscolo F., Albuquerque P.M., Streit F., Esposito E., Ninow J.L., 2008. Apple pomace: A versatila substrate for biotechnological applications. Crit. Rev. Biotechnol., 28, 1–12.

Yahara G.A., Javier M.A., Tulio M.J.M., Javier G.R., Guadalupe A.U.M., 2007. Modeling of yeast *Brettanomyces bruxellensis* growth at different acetic acid concentrations under aerobic and anaerobic conditions. Bioprocess Biosyst. Eng., 30, 389–395.

18

WORTS PRODUCTION WITH ADDITION OF MILLED CORN PRODUCTS

Introduction

Worts produced with the addition of unmalted corn grits or fine grits owe their popularity to the considerable number of advantages achieved using this process [Jurek at al., 2004; Zembold-Guła and Błażewicz, 2007]. These are: maintenance of good mash extractivity without any significant changes in the mass and volume of wort; shorter time of wort flow and ensuring good apparent attenuation of wort. The disadvantageous characteristics include the deficiency of protein hydrolysis (especially α-amino acid nitrogen), minerals and other typical malt components [Agu, 2002; Jurek and Petrów, 2003; Jurek, 2004].

Malt contains ingredients that are subject to initial enzymatic processing during germination and drying of malt. Malt enzymes are responsible for the conversion of grain ingredients into soluble forms during mashing, i.e. the hydrolysis of carbohydrates, proteins, non-starch polysaccharides and other malt components. The components of unmalted grits and fine grits, including starch, are not suitable for enzymatic hydrolysis and require gelatinization. Gelatinization, which facilitates enzymatic conversions of starch, is based on warming unmalted grits in the presence of water to a temperature that enables the formation of water-containing gels from starch granules, so-called amorphous starch. It allows the hydrolysis of corn starch by malt enzymes during mashing malt with corn grits or fine grits.

The increased doses of adjuncts require the use of enzymatic preparations, eliminating not only the physical disadvantages of the mashed mass (e.g. high viscosity) but also facilitating the extraction of mash ingredients [Kunze, 1999].

Unmalted adjuncts are usually the only the source of carbohydrates, and malt remains the main source of enzymes, protein hydrolysis products and other specific wort ingredients. In the technology of wort production using a large proportion of unmalted raw materials, the most difficult processes are the adequate preparation of the unmalted material and the selection and practical application of enzymatic preparations that are capable of hydrolyzing starch of this material. The substitution of malt with unmalted materials in brewing may disrupt the balance between the soluble components of the extract which influence the physiology of yeasts and sensory properties of beer. The minimization of disadvantageous effects of these changes is interesting not only for research centers and breweries, but also for the producers of unmalted additives and enzymatic preparations. During the production of worts for malt concentrates, the requirements for worts are considerably smaller than in beer production.

Enzymes used in the brewing industry facilitate the technological operations, support the enzymatic activity of defective malts, enable the application of adjuncts, facilitate fast filtration of wort and beer, help regulate the attenuation of wort, and shorten the time of beer maturation [Solarek and Cissowski, 2003].

In using adjuncts in the production of brewing worts, Novozymes recommend the use of Termamyl SC. Boiling the unmalted material at 100ºC in the presence of Termamyl SC results in starch gelatinization and liquefying which significantly increases the extraction rate. The application of this preparation also results in a lower mash viscosity. Boiling the dense mash is not associated with a risk of thermal inactivation of the α-amylase present in Termamyl, as the maximum activity of this enzyme during mashing occurs between 85ºC and 100ºC, and the enzyme remains active for 30 min during boiling at 100ºC [Solarek and Cissowski, 2003].

In the brewing industry worldwide, the most commonly applied adjuncts are corn, wheat and rice [Debyser at al., 1998]. Corn is the most reliable adjunct during processing, although corn grits and fine grits require gelatinization when used as malt substitutes [Hug and Pfenninger, 1976a, b, c].

Malt concentrate is concentrated or dried wort. Malt wort is a water solution of malt components: carbohydrates (glucose, maltose and dextrins), protein substances (amino acids and peptides), enzymes (amylolytic, proteolytic, cellulolytic and other), minerals (sodium, potassium, calcium, phosphorus, iron), vitamins (B_1, B_2, B_6, PP) and many other substances. Wort may be produced using malts of Pilsner type and other special malts with or without the addition of unmalted grain [Kunze, 1999].

Malt concentrates are usually produced as paste with a 70–80% d.m. concentration. The stability of the concentrates may be improved through, for example, drying in spray driers which is more expensive than using a vacuum evaporator but enables the increase of stability of the concentrate without using any preservatives [Błażewicz and Borek, 1999a, b; Błażewicz, 2004; Fraj and Sąsiadek, 1984].

There is presently a great deal of knowledge on malt concentrate production using only malt and also with the addition of unmalted grain. Modern brewing technology enables the production of worts from various compositions of malt and adjuncts in a controlled and repeatable manner, even under industrial brewing conditions [Kunze, 1999; Błażewicz 2004].

Classical malt concentrate production technology is based on wort production with the same methods utilized in breweries, and fixing it through vacuum evaporation or spray drying.

This paper shows the effect of various factors on the efficiency of mashing Pilsner malts with corn adjuncts. The examined variables were doses of corn grits or fine grits (40% to 80% of the charge); gelatinization of the corn adjuncts or lack of gelatinization, and the addition of enzymatic preparations Termamyl SC, Ceremix 6X MG and Ceremix Plus MG.

Materials and methods

This experiment used Pilsner type malt taken from the malthouse in Głubczyce, corn grits (500–1250 μm), fine corn grits (250–750 μm) and enzymatic preparation manufactured by the Novozymes company (Termamyl SC, Ceremix 6X MG, Ceremix Plus MG). The enzymatic preparations were added in excess amounts in order to facilitate the enzymatic

conversions during mashing of Pilsner malt or with the addition of corn grits or fine grits. It was assumed that the milled corn products are a good extract source that do not contain components that hinder wort filtration. It was also assumed that the application of enzymatic preparation should increase mash efficiency.

Termamyl SC – added to the mashing tun at the beginning of mashing in the dose of 0.5–1.0 kg/t of adjunct. Termamyl SC decreases mash viscosity, is stable in a wide pH and temperature range and is not sensitive to the deficiency of stabilizing calcium ions. It is characterized by a high activity of thermoresistant α-amylase and also ensures a greater mash efficiency.

Ceremix 6X MG – exhibits the activity of β-glucanase, protease, α-amylase and pentosanase. It is used during the mashing of malt with adjuncts. Added to mash tun in the dose of 0.5–0.6 kg/t of adjunct. It enables an increase in the content of adjuncts up to 50% dry matter.

Ceremix Plus MG – similarly to Ceremix 6X MG, it is added to the mash tun during mashing malt with adjuncts. The preparation is added in the dose of 0.7–1.0 kg/t of unmalted grain. It is characterized by a high activity of β-glucanase, protease, pentosanase oraz thermostable α-amylase. Similarly to other preparations from the Ceremix group, it enables an increase in the content of adjuncts up to 50% dry matter.

During congress mashing, charge in each tun contained 50 g dry matter of malt or mixture of malt and various proportions of the adjunct. The gelatinization of corn grits or fine grits before mashing was carried out at 90ºC for 10 min, mixing them with water at the 1:5 ratio. In the obtained worts the following parameters were determined: wort filtration time, volume and extract content using the pycnometric method. The assessment of worts obtained from malt and the mixture of malt and adjuncts was carried out according to the requirements of EBC analytics. Homogenous groups, described with the subsequent letters of the alphabet, were determined using the method of multiple comparisons using the Duncan's test. All the statistical calculations were carried out using the *STATISTICA 8.0* software by StatSoft.

Determination of mash efficiency (% d.m.)

Due to the considerable content of adjuncts in malt charge (even 80%), the term 'malt extractivity' was substituted by 'mash efficiency' in this paper, a term describing the content of dry mass in wort obtained from 100 grams of dry matter. This dry matter is the sum of dry matter of malt and adjuncts prepared in various ways and mashed with or without enzymatic preparations. Mash efficiency was calculated using the values of dry matter of the malt charge, wort extract and the volume of produced wort.

Results and discussion

Malt extracts obtained in this experiment are not brewing worts. These are extracts which may be used in the production of food concentrates and which contain mainly the products of enzymatic hydrolysis of barley and corn starch. The content of these products results from the method of preparation of adjuncts and methods of wort production. Malt used in a grist with the content ranging from 20 to 60% is not only the source of enzymes but also of extractive substances. The content of malt in dry matter influences the amount of typical malt ingredients that can be extracted to wort. These ingredients include the products of protein enzymatic hydrolysis, non-starch polysaccharides, tannins and dyes [Błażewicz, 2004].

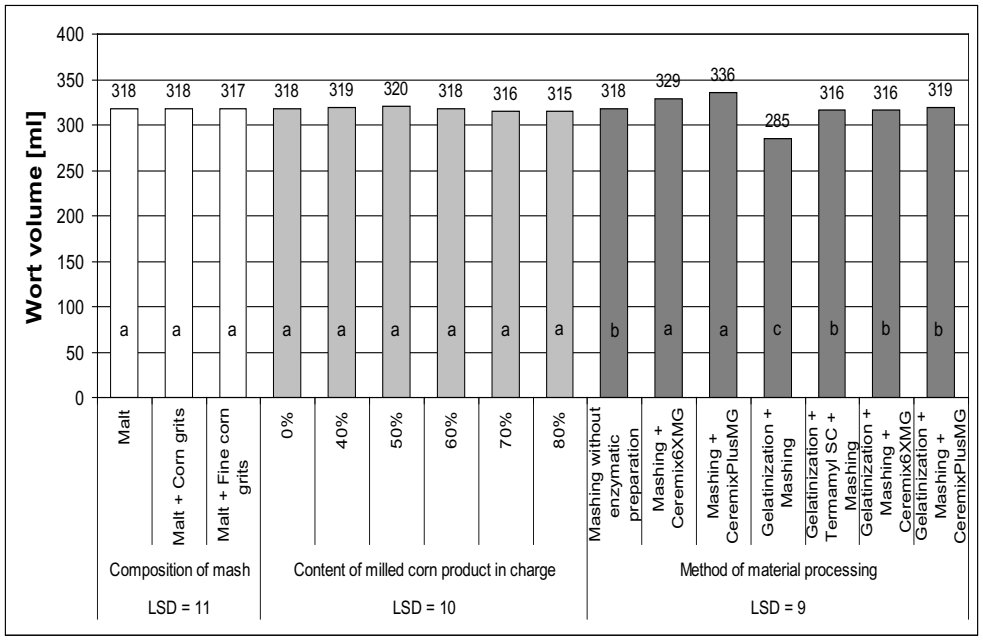
Adjuncts (unmalted grain) used in this article are corn grits and fine grits. These products, professionally milled corn grain, are characterized with different granulation and starch content. A review of literature shows that these products are a good carbohydrate source. They also do not hinder wort filtration thanks to the lack of β-glucans and pentosans, commonly present in other grains. These compounds, due to their amount or lack of enzymes which could hydrolyze them, could hinder the filtration of worts [Pawlikowska-Mandziak, 1997; Błażewicz, 2003; Błażewicz, 2006]. However, the applicability of adjuncts in the production of worts for beer production is limited. Unmalted grain of barley, wheat, triticale and other grains introduce to mash so many components that hinder filtration, that additional enzymatic preparations are needed to hydrolyze non-starch polysaccharides [Błażewicz, 2002; Solarek, 2001].

In wort production, one can also use cereal starch as extruded cereal grain or potato starch [Błażewicz, 2004]. These products are rich in extractive ingredients but they are also expensive. Various methods of obtaining wort for food concentrates lead to final products with different compositions and prices. The most expensive to produce is the pure malt wort and the cheapest are worts produced with adjuncts. Adjuncts dilute typical malt ingredients as they introduce mainly the products of starch enzymatic hydrolysis to wort [Błażewicz, 2004].

The method of adjunct preparation for mashing is connected mainly with the process of gelatinization and carbohydrate enzymatic hydrolysis. When the proportion of adjuncts is low it is possible to obtain extract with a high extract content, thanks to the activity of malt enzymes. Increased doses of malt substitutes hinder carbohydrate conversions due the increased amounts of the substrate and decreased amount of enzymes. It is therefore necessary to apply enzymatic preparations for wort production using a large amount of adjuncts [Solarek, 2001; Solarek, 2002; Solarek and Cissowski, 2003].

In this paper two methods were used for the application of enzymatic preparations. The first enzymatic preparation – Termamyl SC was used in the process of gelatinization of milled corn products. It was assumed that this method of preparation will facilitate the hydrolysis of corn starch by enzymes during mashing. This assumption proved right as the mash efficiency for malt and corn grits and fine grits was high in the presence of Termamyl SC. Another method was based on application preparations with multi-directional enzymatic activities (Ceremix 6X MG or Ceremix Plus MG) during mashing malt with the adjunct. The activity of Ceremix preparations is complex and, thanks to their enzymatic composition, it is compared to the complex activity of natural malt enzymes. It was assumed that the application of Ceremix preparations with the high share of non-gelatinized adjunct will enable appropriate mash efficiency. It was observed that mash efficiency after the application of Termamyl SC and Ceremix 6X MG and Ceremix Plus MG is similar both with and without the gelatinization of the corn grits (Fig. 4). Similar mash efficiencies were observed for a short filtration time, high volume and high extract content in the obtained worts (Figs. 1–3).

In this paper, worts were obtained using laboratory infusion mashing, a method with a relatively short duration. Modification of mashing that would introduce an additional thermal and (potentially) enzymatic processing of spent grain could increase mash efficiency. In the production of worts for malt concentrates it is important to ensure the greatest content of extract possible in wort. It is important to obtain worts with the lowest content of water, which needs later to be removed during extraction.



a, b, c, d – homogenous groups (α=0.05); LSD – last significant differences (p=0.05)

Fig. 1. Volume of laboratory worts in relation to the type and content of material in dry matter and to a processing method

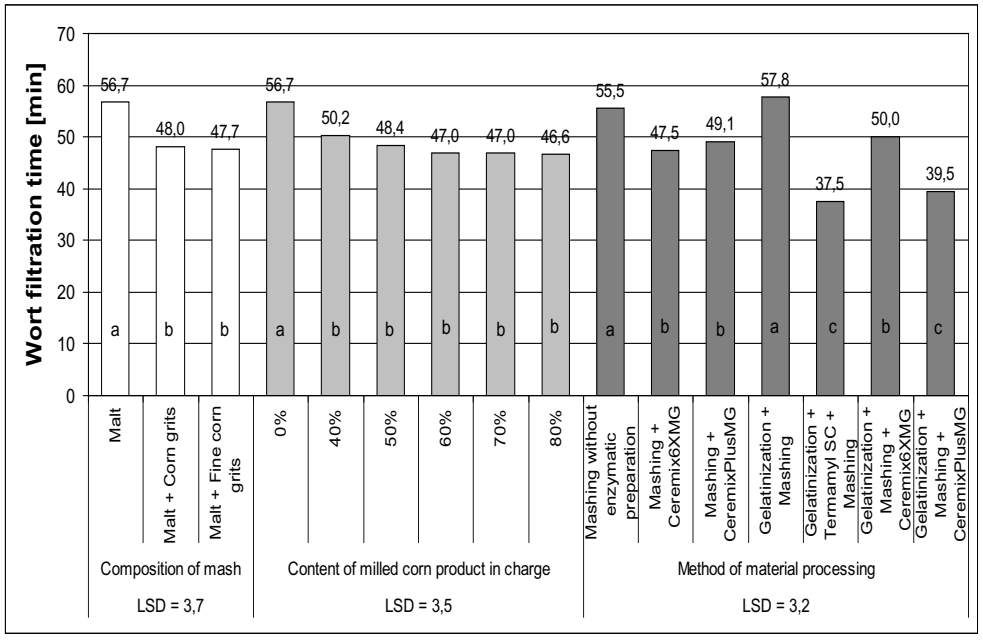


Fig. 2. Time of worts filtration in relation to the type and content of material in charge and to a processing method

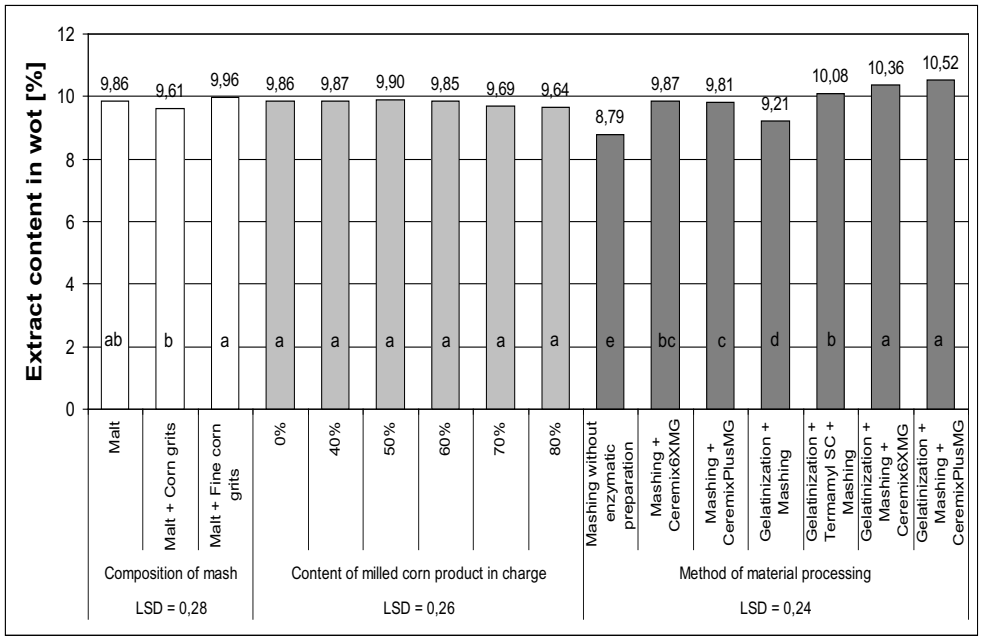


Fig. 3. Content of extract in worts in relation to the type and content of material in charge and to a processing method

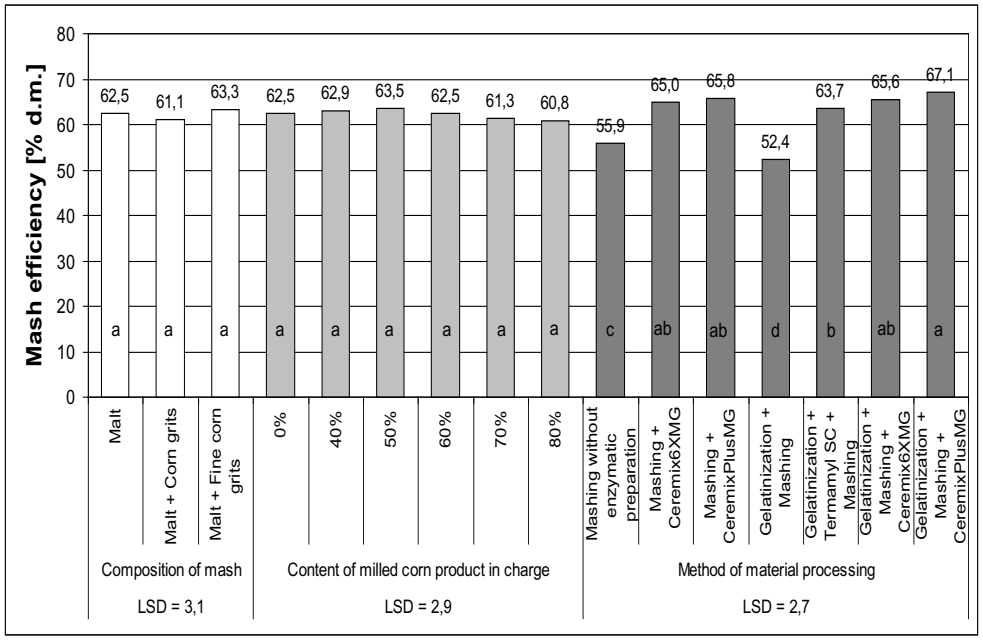


Fig. 4. Mash efficiency in relation to the type and content of material in charge and to a processing method

The obtained results show a high usefulness of corn grits and fine grits in the production of worts with a high extracts content. Interesting is the observed insignificance of gelatinization of these adjuncts during congress mashing in the presence of Ceremix 6X MG and Ceremix Plus MG preparations. Typical parameters connected with obtaining malt worts (filtration time, mass and volume of worts, extract content and mash efficiency) indicate that the milled corn products only slightly hinder the technological process of infusion mashing. When compared with literature data on the application of unmalted grain these results are very promising [Błażewicz, 2004].

It was observed that the application of corn grits or fine grits in the range of 40 to 80% in dry matter did not significantly influence the volume of wort. Differences resulted from gelatinization and the use of enzymatic preparations. A high volume of worts, independent of the dose of adjuncts and type of grits, show indicate no effects that hinder wort filtration, which is also reflected in the shortening of wort filtration time (Fig. 2) and a high extract content in worts (Fig. 3). Statistical analysis showed the gelatinization of corn grits surprisingly decreased the volume of worts.

The application of corn grits or fine grits significantly enhanced the filtration of worts, regardles of their content in the mash. Time of filtration decreased even by 10 min compared with wort produced from malt only. Contrary to expectations the gelatinization of the unmalted grain did not affect filtration time. A decrease in wort filtration time was to a varying extent brought about by the addition of enzymatic preparations during the gelatinization of corn grits or during mashing malt with the adjuncts. The shortest filtration time was observed when the milled corn products were gelatinized with the addition of Termamyl SC or the gelatinized grits were mashed in the presence of Ceremix Plus MG.

The results clearly show that the applied malt substitutes did not decrease the extract content in worts obtained with a large share of the corn adjuncts in dry matter (40–80%), and maintained it at a high level, similar to worts produced from malt only. Worts produced from malt and fine corn grits had a higher extract content than with the addition of corn grits. Starch content is similar in these two corn products, so the difference could arguably have resulted from various levels of granulation (Table 1). Increasing the content of the adjuncts in the range from 40 to 80% did not result in different extract levels in worts. The best extract content was observed when gelatinized grits were mashed with the addition of Ceremix Plus MG or 6X MG preparations.

Mash efficiency is not a typical parameter of malt and adjunct assessment in brewing technology. EBC Analytics uses the term ‘extractivity’, described by given formulas and coefficients concerning Pilsner malt. In this paper, results concern not only worts obtained from pure malt, but also with the addition of unmalted grain. Some results concern worts obtained from 80% of corn grits or fine grits and 20% of malt. Mash efficiency in this paper includes the extract content and the volume of laboratory worts obtained using a congress method. It is an indicator showing the percentage of dry matter of extractive ingredients obtained from malt and adjuncts in the mash. Results show that infusion mashing with a 40–80% content of milled corn products may transport about 60% of dry matter to wort, mainly the products of enzymatic hydrolysis of barley and corn starch (Fig. 4). Most often 40% of the dry matter used in mashing remains as spent grain.

It is very significant that the increase in the content of adjuncts – corn grits or fine grits, even up to 80% dry matter, did not significantly affect the mash efficiency. However, it depended on the method of adjunct preparation for mashing and the method of mashing

itself. Worts produced without enzymatic preparations had a lower mash efficiency than for mashing malt only. The application of the aforementioned enzymatic preparations during the gelatinization of adjuncts or mashing malt with the adjuncts resulted in about a 10% greater mash efficiency.

Table 1

Starch and protein content in Pilsner type malt, corn grits and fine grits

Material	Starch [% d.m.]	Total protein [% d.m.]
Barley malt of Pilsner type	63.6	9.4
Corn grits of 500–1250 μm granulation	85.4	8.3
Fine corn grits of 250–750 μm granulation	84.4	8.3

Table 2

Variants of laboratory wort production

Content of materials [% d.m.]		Preparation of corn grits or fine grits	Enzymatic preparation during mashing
Malt of Pilsner type	Corn grits or fine grits		
100	0	–	Without enzymatic preparation
100	0	–	Ceremix 6X MG
100	0	–	Ceremix Plus MG
60, 50, 40, 20	40, 50, 60, 80	Without gelatinization	Without enzymatic preparation
60, 50, 40, 20	40, 50, 60, 80	Without gelatinization	Ceremix 6X MG
60, 50, 40, 20	40, 50, 60, 80	Without gelatinization	Ceremix Plus MG
60, 50, 40, 20	40, 50, 60, 80	Gelatinization	Without enzymatic preparation
60, 50, 40, 20	40, 50, 60, 80	Gelatinization with the addition of Termamyl SC	Without enzymatic preparation
60, 50, 40, 20	40, 50, 60, 80	Gelatinization	Ceremix 6X MG
60, 50, 40, 20	40, 50, 60, 80	Gelatinization	Ceremix Plus MG

Conclusions

It is possible to obtain worts with high extract contents and short filtration time from infusion mashing malt with corn grits or fine grits (40–80%), with grits being subjected to gelatinization prior to mashing or mashed in the presence of enzymatic preparations Ceremix 6X MG or Ceremix Plus MG.

The greatest mash efficiency can be achieved through the gelatinization of milled corn products in the presence of Termamyl SC or mashing malt with the adjuncts in the presence of preparations Ceremix 6X MG or Ceremix Plus MG.

References

Agu R.C., 2002. A comparison of maize, sorghum and barley as brewing adjuncts. J. Inst. Brewing, 108/1, 19–22.

Analytica – EBC, Verlag Hans Carl Getraenke-Fachverlag, 1998, Nuernberg.

Błażewicz J., Borek M., 1999. Próby otrzymania pszenżytnich ekstraktów słodowych. Zesz. Nauk. AR Wroc., Technol. Żyw., VIII, 53–60.

Błażewicz J., Borek M., 1999. Zastosowanie ekstraktów słodowych typu piekarniczego w przemyśle spożywczym. Cukiernictwo, 2, 15.

Błażewicz J., 2003. Możliwości zastosowania produktów przemiału polskiej kukurydzy w piwowarstwie, in: VIII Szkoła Technologii Fermentacji, Enzymy w piwowarstwie – dziś i jutro. Kliczków, 102–117.

Błażewicz J., 2004. Właściwości brzeczek i koncentratów słodowych otrzymanych z użyciem skrobi ziemniaczanej, ziarna pszenżyta i jęczmienia jako zamienników słodu. Zesz. Nauk. AR Wroc., 491, 1–94.

Błażewicz J., 2002. Wpływ zastosowania w czasie zacierania preparatu enzymatycznego Ultraflo L na właściwości zagęszczonych i wysuszonych ekstraktów słodowych. Acta Sci. Pol., Biotechnologia, 1, 19–28.

Błażewicz J., 2006. Zastosowanie surowców niesłodowanych w produkcji piwa. in: XI Szkoła Technologii Fermentacji, Łódź, 132–146.

Debyser W., Delvaux F., Delcour J.A., 1998. Activity of arabinoxylan hydrolyzing enzymes durgin mashing with barley malt or barley malt and unmalted wheat. J. Agric. Food Chem., 46, 4836–4841.

Fraj A., Sąsiadek R., 1984. Produkcja i zastosowanie ekstraktu słodowego. Przegląd Piekarski i Cukierniczy, 2, 11–12.

Hug H., Pfenninger H., 1976a. Maize as raw material for brewing. Part 1. Fundamentals. Schweizer Brauerei Rundschau, 87, 65–68.

Hug H., Pfenninger H., 1976b. Maize as raw material for brewing. Part 2. Fundamentals. Schweizer Brauerei Rundschau, 87, 105–108.

Hug H., Pfenninger H., 1976c. Maize as raw material for brewing. Part 3. Fundamentals. Schweizer Brauerei Rundschau, 87, 135–138.

Jurek K., Błażewicz J., Petrów A., 2004. Właściwości piw wytwarzanych z dodatkiem produktów przemiału ziarna kukurydzy w warunkach uproszczonej technologii. Żywność, Nauka, Technologia, Jakość, 40, 109–118.

Jurek K., Petrów A., 2003. Wpływ substytucji słodu przetworami kukurydzianymi na zawartość azotu α-aminowego w brzeczkach laboratoryjnych. Żywność, Nauka, Technologia, Jakość, 35, 49–60.

Kunze W., 1999. Technology of Brewing and Malting, VLB, Berlin.

Pawlikowska-Mandziak M.B. 1997. Możliwości zastosowania kukurydzy w przemyśle piwowarskim. Przem. Ferm., 11, 12–14.

Solarek L., Cissowski J., 2003. Mikrobiologiczne preparaty enzymatyczne "Novozymes S.A." w technologii piwowarstwa. in: VIII Szkoła Technologii, Jamrozowi Polana, 118–137.

Solarek L., 2001. Kleikowanie i upłynnianie surowców skrobiowych niesłodowanych z zastosowaniem enzymów mikrobiologicznych Novozymes. Przem. Ferm., 12, 10.

Zembold-Guła A., Błażewicz J., 2007. Wpływ modyfikacji czasu słodowania ziarna jęczmienia na cechy brzeczek otrzymanych z udziałem grysu kukurydzianego. Żywność, Nauka, Technologia, Jakość, 54, 75–81.

19

MICROBIOLOGICAL AND TECHNOLOGICAL QUALITY OF MALTING BARLEY GRAIN PRESERVED USING NEAR-AMBIENT DRYING

Preservation of malting barley grain

Grain after harvest, together with contaminants and infesting pests and microorganisms, constitutes a biological system in which numerous life processes occur, is referred to in literature as an ecosystem of preserved and stored grain [Ryniecki, 2002]. These processes are especially intensive in wet and non-cooled grain, which is an ideal medium for the development of fungi constituting the biggest threat for its quality. Leading the stored grain mass to the state of anabiosis during the process of grain preservation and treatment, as well as the maintenance of this state throughout the entire storage, makes it possible to reduce to the minimum adverse life process occurring in the ecosystem of stored grain mass, while preserving its technological and nutritive value.

Malting barley to be used in malt production, due to the necessity to preserve high enzymatic activity of grain, requires the application of especially mild drying conditions [Gąsiorowski, 1997, Wawrzyniak et al., 2006]. This is of particular importance in case of barley with very high moisture content, being sensitive to higher temperatures which may considerably reduce its germination capacity [Kunze, 1996]. One of the primary methods of post-harvest preservation of malting barley grain in Poland, first of all on producers' farms, is convection drying and cooling in a thick bed using the so-called near-ambient drying [Ryniecki, 1994, Kaleta, 1996, Ryniecki and McLean, 2006].

The near-ambient drying method consists in ventilation of grain stored in a thick bed using air at a temperature close to ambient temperature. The drying potential of atmospheric air depends on its temperature and moisture content and changes stochastically depending on weather conditions. In case when the drying potential of air is too low, it is increased by heating the air pumped into the drying chamber. In modern near-ambient drying systems its drying potential is controlled by a special controller, which task is to prevent wetting of grain mass, providing safe drying conditions to ensure grain quality and to optimize energy consumption [Ryniecki et al., 2007b].

Preservation using near-ambient drying is run under mild conditions. Temperature of drying air in near-ambient drying preservation is close to ambient temperature, which results in a long-lasting process (from several to around a dozen days). This is connected with the risk of mould development and the occurrence of negative changes in grain mass. It needs to be stressed that harvested grain, which moisture content in years with high precipitation may be as high as 20% while the temperature frequently exceeds 20°*C*, is an ideal environment for the development of microorganisms. Under such conditions processes of grain preservation

in a thick bed are commonly perceived as a race against moulds [Ryniecki, 2005]. In view of the above threats it seems advisable to conduct a series of studies including quality monitoring of plant materials preserved using the above mentioned method.

Hazards connected with inadequate preservation of malting barley grain

Barley grain to be used for malt production should meet some of the strictest quality requirements [Fornal and Filipowicz, 2005], thus during storage it needs to be properly protected, especially against the development of storage fungi. Inappropriate grain storage leads to a reduction of its technological quality, which is manifested in a deterioration of parameters crucial in case of malting barley, such as energy and germination capacity as well as enzymatic activity [Nellist, 1973, Gąsiorowski, 1997]. Moreover, the development of fungal microflora may cause a deterioration of both the nutritive value and organoleptic attributes of grain. In extreme cases, when infestation with storage fungi is considerable, there is a risk of the appearance of mycotoxins, which presence condemns grain both as a raw material for malting and as feedstuff.

Microbiological purity of malting barley grain determines the quality of malt produced from this grain and as a consequence also the quality of beer. An elevated level of fungal microflora in stored malting barley grain results in the inhibition of grain germination as a result of damage to kernel germ by developing fungi [Chełkowski, 1985]. Moreover, mould growth may also contribute to the formation of an atypical enzymatic profile of grain, manifested in increased intensity of beer colour [Satora and Tuszyński, 2008]. Secondary metabolites, i.e. polypeptides and peptidoglucanes produced during malting, e.g. by moulds from genera *Fusarium, Rhizopus, Aspergillus* or *Penicillium*, also initiate uncontrolled beer gushing. Fungi present in grain may produce mycotoxins, which when penetrating through malt to beer constitute a health hazard to humans. Baxter et al. [2001], while investigating the effect of beer production process on degradation of ochratoxin A contaminating (at approx. 52 $\mu g \cdot kg^{-1}$) malt obtained from malting barley inoculated with *Penicillium verrucosum*, found that from 13 to 32% ochratoxin A found in analyzed malt samples penetrated to produced beer.

Methods to assess the degree of grain contamination with fungi

Microbiological methods

Primary methods to assess microbiological quality of food and feed, including cereal grain, are plate methods used to determine the number of colony forming units (CFU), [Schwadorf and Müller, 1989, Olsson, 2000, Castro et al., 2002]. They consist in culturing a specific volume of a sample or its dilutions on solid medium and the determination of the number of colonies grown after several days of incubation. An element having a significant effect on obtained results is the type of used medium. The composition of medium used for qualitative and quantitative determination of fungi should be selected so that it provides optimal conditions for growth and development of all inoculated propagation units of fungi, and thus facilitates identification of genera and species of these microorganisms, at the same time inhibiting growth of bacterial colonies and reducing development of fast growing moulds.

The number of colony forming units still remains the most frequently used parameter, recommended by standards concerning the assessment of microbiological quality of cereal grain [PN-ISO 7698:2004, EN ISO 6887-1:2000], being a measure of development of fungi. The medium recommended by ISO for the determination of counts of yeasts and moulds in cereal grain is agar medium containing yeast extract, glucose and chloramphenicol [PN-ISO 7698:2004]. Using the above mention technique it needs to be remembered that in case of fungi plate methods make it possible only to determine the number of live propagation units (in the form of fragments of hyphae and spores) of these microorganisms, particularly live fungal spores [Schwadorf and Müller, 1989, Gutarowska and Żakowska, 2002]. We need to stress here that the content of spores in the suspension used for inoculation is much higher than that of hyphal fragments and thus they do not yield information on the amount of fungal biomass contaminating grain [Marín et al., 2005]. The specific character of the described method results in the number of determined CFU being dependent first of all on the fact whether fungi infesting grain have developed a new generation of spores and not on the current fungal growth [Marín et al., 2005]. An advantage of plate methods is the possibility to identify species of fungi infesting grain. However, these methods are time- and labour-consuming and they do not provide current information on the microbiological condition of grain, since the time of analysis is 3–7 days. For this reason the application of plate methods in practice as a routine method to determine fungi on cereal grain and in their processing products is impossible.

Ergosterol as an indicator of the degree of grain infestation with fungal microflora

Drawbacks of the plate methods described above result in a situation when techniques are being constantly searched for, which would facilitate easy and rapid estimation of the degree of grain contamination with fungi. Due to the precision and relatively short time of analyses performed using chemical methods, they are more and more frequently used as auxiliary techniques supplementing traditional methods used in microbiology, both in taxonomy and when assessing the degree of microbial contamination of agricultural products and foodstuffs. Biomarkers used in chemical methods include specific components of cell walls, i.e. ergosterol [Seitz et al., 1977, Christensen and Sauer, 1982, Zhao et al., 2005, Pronyk et al., 2006], chitin [Christensen and Sauer, 1982, Ekblad et al., 1998] and metabolites, i.e. ATP [Suberkropp et al., 1993, Hawronskyj and Holah, 1997] or volatile compounds [Kamiński and Wąsowicz, 1991, Jeleń and Wąsowicz, 1998, Olsson et al., 2000, Presicce et al., 2006].

Numerous research studies have been devoted to problems concerning methodology of ergosterol determination in food products (including cereal grain) and the possibility to apply this sterol in the assessment of the level of fungal biomass next to other measures of growth of moulds. Ergosterol, found in most fungi [Maupetit et al., 1993, Gutarowska and Żakowska, 2002], is formed very early and is detected at all stages of their development [Janińska, 2002]. It is the primary sterol of cell membranes of hyphae and spores [Seitz et al., 1977, Pasanen et al., 1999, Mille-Lindblom et al., 2004]. Ergosterol is contained in the phospholipid layer of cell membranes [Gutarowska and Piotrowska, 2007], in which it is found mainly in the free form as free sterol and to a lesser extent in the form of esters with fatty acids or glycosides [Stoudt and Foster, 1954, Maupetit et al., 1993, Robine et al., 2005]. Slight amounts of ergosterol may also be found in protozoa, algae [Newell, 1992 after Zhao et al., 2005],

as well as certain bacteria [Schubert et al., 1968 after Schwadorf and Müller, 1989]. In higher plants ergosterol is not detected or it is only in trace amounts [Zhao et al., 2005, Gutarowska and Piotrowska, 2007].

Due to the fact that a predominant group of microorganisms in which ergosterol is found are fungi, starting from the 1970's increasing interest has been observed in ergosterol as a biomarker of moulds [Mille-Lindblom et al., 2004]. Seitz et al. [1977] were the first to use ergosterol in the determination of the level of contamination with moulds in cereal grain. In the opinion of many authors ergosterol may also be used indirectly to estimate hazard connected with the presence of mycotoxins in different products [Abramson et al., 1999, Saxena 2001, Castro et al., 2002, Abramson et al., 2005, Varga, 2006].

The first stage in the determination of ergosterol is its isolation from the biological material, consisting in three successive actions: methanol extraction, basic saponification and extraction with pentane or hexane]Seitz et al., 1977]. The last stage is quantitative determination of ergosterol content. The presence of the characteristic absorption spectrum in the ultraviolet range at wavelength of over 240 nm up to maximum length of 282 nm makes it possible to distinguish it from the other compounds in this group, together with ergocalciferol [Schwadorf and Müller, 1989, Maupetit et al., 1993, Dawson-Andoh, 2002]. Its quantitative determination is performed most often using high performance liquid chromatography (HPLC) with an absorption detector (Fig. 1).

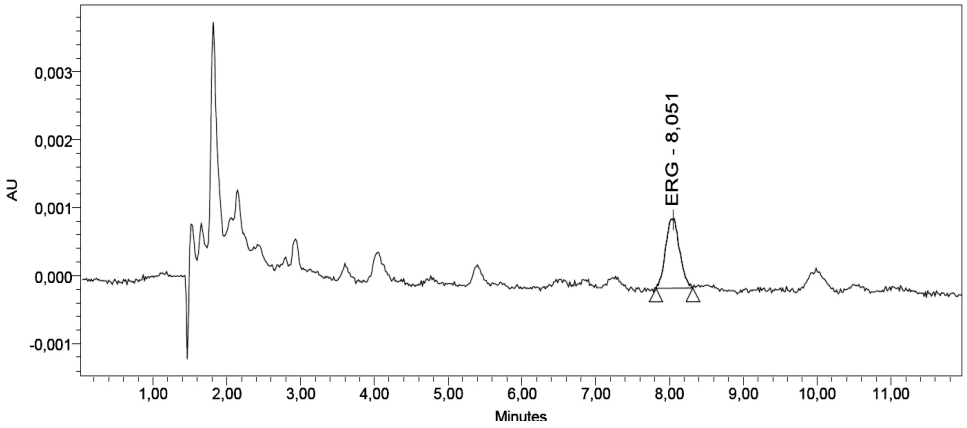


Fig. 1. A chromatogram with marked peak coming from ergosterol. AU – Absorbance Units

The method using ergosterol to assess the degree of infestation of analyzed products with fungi is less time- and labour-consuming than microbiological methods [Chirstensen and Sauer, 1982]. Certain limitations of this technique are related to the species and environmental conditions of moulds accompanying the synthesis of ergosterol. Ergosterol content in fungal biomass is not constant and depends on the fungal species, medium and growth conditions as well as the development phase of mycelium, i.e. growth stage and the degree of mycelium development as well as sporulation [Christensen and Sauer, 1982, Marín et al., 2005, Zhao et al., 2005]. Due to the fact that ergosterol is found in most fungi, this method does not make it possible to determine the species composition of fungal microflora [Schnürer et al., 1999]. Its limited application in routine analyses results also from the fact that the analysis of ergosterol determination requires specialist apparatus and qualified staff

[Schnürer and Jonsson, 1992, Reeslev et al., 2003]. Another problem stems from the fact that so far no standards have been developed to determine admissible ergosterol levels in plant origin products and considerable discrepancies are observed between admissible ergosterol levels reported by different authors. According to Schnürer and Jonsson [1992], ergosterol content of 3 $mg \cdot kg^{-1}$ is the concentration limit of ergosterol in grain to be used for human consumption, while in the opinion of Maupetit et al. [1993] barley grain containing no more than 9 $mg \cdot kg^{-1}$ ergosterol still classifies it as healthy grain of regular quality.

Selected parameters in assessment of technological quality of malting barley grain

Quality of barley used in malt production determines the quality of produced malt and as a consequence also the quality of produced beer, thus next to microbiological purity its technological value is significant when selecting raw material for malt production. One of the most important parameters of technological quality in malting barley is germinative energy of grain. High germinative energy, which for raw material to be used in malt production should be at least 95%, shows that grain is healthy and the malting process will run properly [Kunze, 1996]. The Schönfeld method recommended by Polish Standard PN-R-74110:1998 containing physico-chemical requirements for malting barley is used to assess germinative energy of grain. This method consists in germination of grain on a Schönfeld germinating bed and calculation of the number of ungerminated kernels after 72 h.

Chemical changes taking place during malt and beer production are to a considerable extent the effect of the action of enzymes [Kunze, 1996], thus – apart from germinative energy – another important biochemical indicator describing the suitability of malting barley for malt production is also amylolytic activity of grain. Appropriate post-harvest treatment of grain is a crucial factor affecting preservation of adequate enzymatic activity of grain used to produce brewers malts. Appropriately preserved grain is characterized by low amylolytic activity. The enzymatic mechanism in kernels is activated only during the malting process, which makes it possible to produce malt with desirable amylolytic activity [Weiner et al., 2008]. High amylolytic activity of grain indicates intensive life processes occurring in grain, which may lead to adverse changes in the mass of stored grain or it may result from sprouting of grain. Changes caused by too intensive, uncontrolled action of amylases in malting barley grain may have a negative effect on final parameters of produced malt [Kunze, 1996].

Quality of grain preserved using near-ambient drying

At the Institute of Food Technology of Plant Origin (Food Engineering Group) of the Poznan University of Life Sciences studies are conducted on the optimization of control systems for drying of plant products run at temperatures close to ambient temperature [Ryniecki, 1994, Gawrysiak-Witulska and Ryniecki, 2004, Gawrysiak-Witulska et al., 2006, Ryniecki et al., 2006, Gawrysiak-Witulska et al., 2007b] and development of methods of automatic endpoint identification in near-ambient drying [Ryniecki, 2005, Ryniecki and Gawrysiak-Witulska, 2005, Tomkiewicz et al., 2006, Ryniecki et al., 2007a]. Numerous research papers are also devoted to issues concerning microbiological and technological quality of plant raw materials preserved using the above mentioned method [Gawrysiak-Witulska et al., 2005, Wawrzyniak et al., 2006**,** Gawrysiak-Witulska et al., 2007a, Gawrysiak-Witulska et al., 2008, Ryniecki et al., 2007b].

In studies concerning the determination of quality of malting barley preserved by near-ambient drying two malting barley cultivars were investigated, ie. Annabell and Sebastian [Wawrzyniak et al., 2006, Ryniecki et al., 2007b**,** Gawrysiak-Witulska et al., 2008]. Authors of those studies stressed that harvests occurred in the so-called wet years with adverse ripening and harvest conditions, thus grain was characterized by high initial moisture content, which in case of both cultivars was 20.0 and 20.5% [Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008]. It may be added here that moisture content of 20% is seen as the maximum value at which near-ambient drying may still be used [Ryniecki et al., 2007b].

In the conducted experiments barley grain after harvest was dried by near-ambient drying in a thick bed of approx. 2 m thickness. The experimental station was a flat-bottom silo BIN28 with perforated floor and capacity of 36 m^3. The station was equipped with a centrifugal fan, an air heater and a control and measurement system for near-ambient drying BIT-04 [Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008]. Drying of barley was run until grain in the outlet layer for drying air reached moisture content of 14%. Drying of grain in case of cv. Annabell was completed after 8 days since the onset of the process, while for cv. Sebastian it was after 12 days. Microbiological and technological quality of barley grain was assessed in samples freshly collected from the field and in samples after the completion of drying, collected from the bottom (0.2 m) and top layer in the silo (2.0 m).

Malting barley grain contamination with fungal microflora was determined by the microbiological method and by determination of ergosterol concentration. Results showed that the applied preservation method of near-ambient drying effectively reduced the development of moulds [Wawrzyniak et al., 2006, Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008]. The degree of grain contamination with moulds after the completion of drying was similar to the initial level of fungi (Figs. 2 and 3).

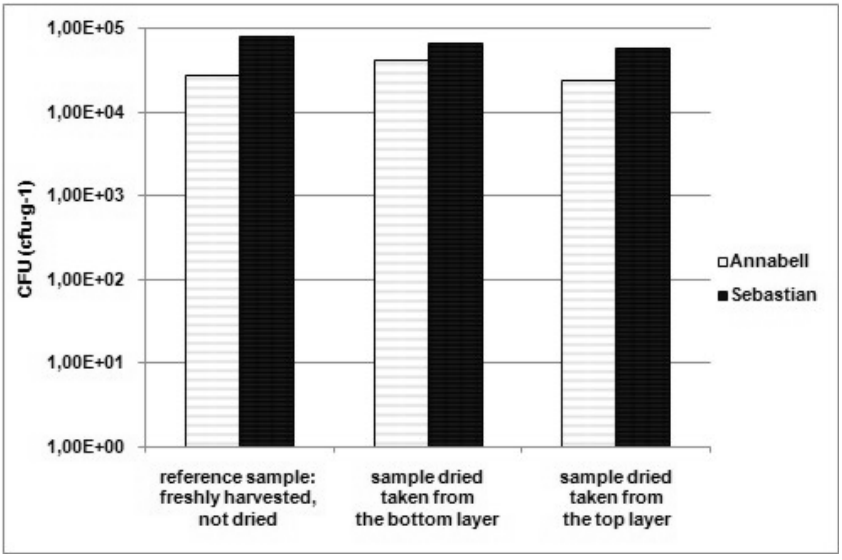


Fig. 2. Changes in the number of colony forming units of moulds (cfu·g-1) in samples of malting barley grain dried using near-ambient drying controlled using a process controller in a BIN silo, collected immediately after harvest (reference) and from the bottom and top layers of the silo after drying was finished

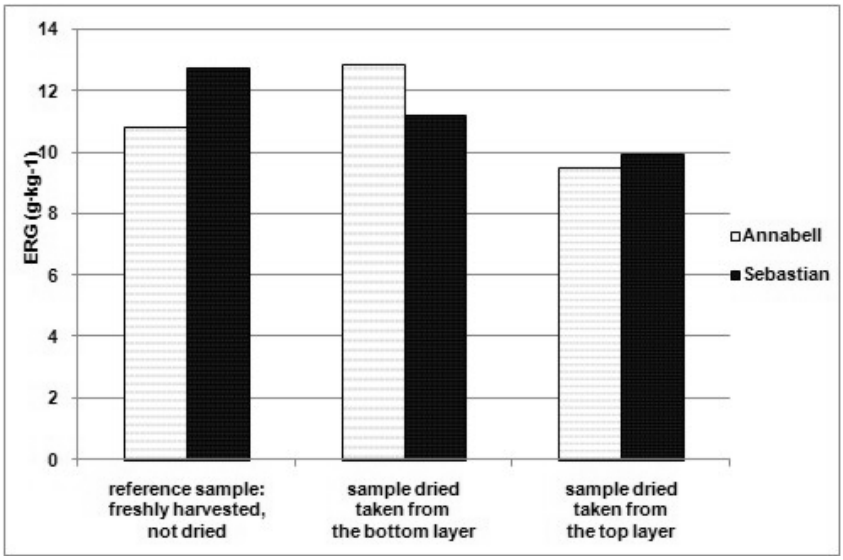


Fig. 3 Changes in ergosterol content (mg·kg-1) in samples of malting barley grain dried using near-ambient drying controlled using a process controller in a BIN silo, collected immediately after harvest (reference) and from the bottom and top layers of the silo after drying was finished

The determined level of grain contamination with moulds expressed in the number of colony forming units of fungi per gram grain (CFU), (according to the procedure described in standards PN-ISO 7698:2004 and EN ISO 6887-1:2000) in all tested barley samples ranged from 2.8×10^4 to 4.3×10^4 for cv. Annabell [Gawrysiak-Witulska et al., 2008] and from 6.8×10^4 to 8.1×10^4 for cv. Sebastian [Ryniecki et al., 2007b]. Assessment of grain contamination with moulds was performed using ergosterol, which determination was conducted following the procedure described by Perkowski et al. [2007, 2008], confirmed results obtained by the microbiological method. In barley grain samples after drying no significant changes were observed in ergosterol content in relation to the concentration of this sterol in barley samples directly after harvest (Fig. 3). In case of both cultivars barley grain after harvest was characterized by the relatively high initial level of contamination with fungi and high ergosterol content, which in the opinion of the authors could have been connected with adverse weather conditions, promoting the development of field microflora during grain ripening and harvest [Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008]. Identification of fungal microflora infesting kernels conducted for the analyzed barley cultivars showed only the presence of typical field fungi from genera *Alternaria, Fusarium, Cladosporium, Mucor, Epiccocum Rhizopus* as well as occasionally fungi from the genus *Drechsleraand Acremonium*. Thus, conducted microbiological analyses showed that malting barley grain, preserved using the above mentioned method, was also protected against contamination with storage fungi, and thus against contamination with mycotoxins which these fungi could have produced.

One of the basic parameters used in the assessment of technological quality of grain is germinative energy. The main threat for grain viability in near-ambient drying may be the development of moulds. These microorganisms first of all infest the least protected part of kernels, which are germs, covered only by the seed coat and a thin layer of fibre [Chełkowski,

1980], in this way leading to a reduction of germinative energy of grain, so important for the malting process. Values of grain germinative energy recorded for the above mentioned malting barley cultivars dried using the near-ambient drying method, determined by the Schönfeld method according to the procedure described in standard PN-R-74110:1998, showed that the applied preservation method in barley makes it possible to retain grain viability [Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008]. Germinative energy in investigated barley cultivars preserved by near-ambient drying (Fig. 4), despite the high initial moisture content of grain did not fall below 95% and fell within the admissible values specified for malting barley in the above mentioned standard.

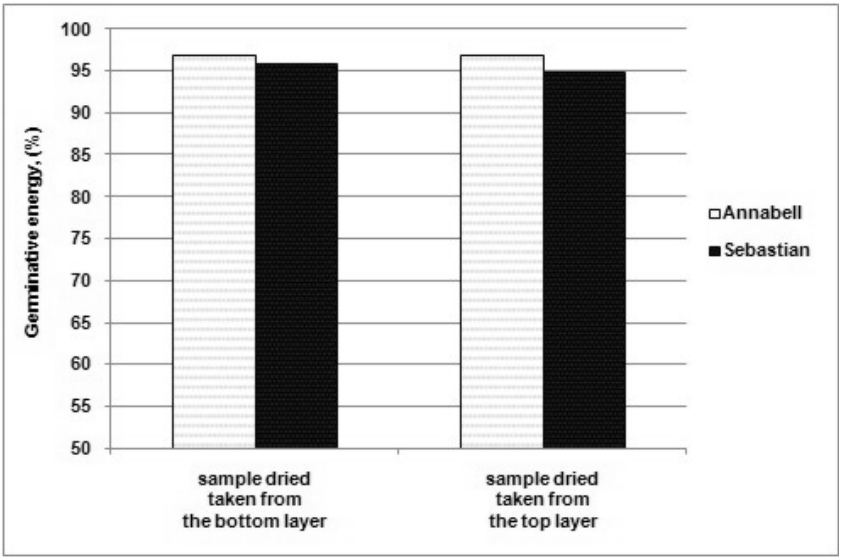


Fig. 4. Germinative energy of malting barley in samples of grain dried by near-ambient drying controlled by a process controller in a BIN silo, collected from the bottom and top layers of the silo

In the course of grain germination the process of enzyme formation is initiated, being one of the most important phenomena occurring in the malt-house and next during the preparation of mash in the brewery during beer production [Kunze, 1996]. Dormant barley grain contains several enzymes. However, they are found in slight amounts and mostly in the form of insoluble bonds. During inadequate grain storage or in case of too long near-ambient drying the activation of amylolytic enzymes in kernels may occur much earlier, thus leading to reduced technological value of grain [Gawrysiak-Witulska and Ryniecki, 2005].

Ryniecki et al. [2007b] and Gawrysiak-Witulska et al. [2008] also conducted studies on enzymatic activity of malting barley dried by near-ambient drying. In case of grain of barley cv. Annabell these studies included assessment of quality of produced malt, while in case of cv. Sebastian the determination of amylolytic enzyme activity in grain. Analysis of malt produced by micromalting of malting barley cv. Annabell showed that obtained malt was characterized by enzymatic activity adequate for beer production. Activity of β-amylase expressed in terms of values of diastatic activity in Windisch-Kolbach units, in the produced malt was 240 WK for grain dried in the top layer and 230 WK for grain dried in the bottom layer of the silo. It is assumed for pale malt that diastatic activity should be more than 240 WK.

In case of the discussed cultivar the authors indicate that the value of 240 WK was not the effect of inappropriately run drying, but rather a species-specific characteristic of barley, which for this reason is used as an admixture to other malts. The value of the Hartong factor VZ 45° illustrating the activity of proteolytic and cytolytic enzymes in malt fell within the admissible range, amounting to 38.9 for grain from the top layer of the silo and 39.2% for grain from the bottom layer. In case of cv. Sebastian relatively low activity of amylolytic enzymes (determined according to Bernfeld method [1955]) in grain dried by near-ambient drying, amounting to 7064 JAA for grain collected from the bottom layer of the metal silo and 9495 JAA for grain after drying collected from the top layer of the silo, also showed that this grain was adequately protected against loss of enzymatic activity.

Summing up studies on quality of malting barley dried by near-ambient drying, conducted at the Institute of Food Technology of Plant Origin, the Poznań University of Life Sciences it may be stated that an adequately applied near-ambient drying may be an effective method of post-harvest preservation of malting barley, making it possible to preserve their microbiological and technological quality appropriate for malt production [Wawrzyniak et al., 2006**,** Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008].

Summing up studies on quality of malting barley dried by near-ambient drying, conducted at the Institute of Food Technology of Plant Origin, the Poznan University of Life Sciences it may be stated that an adequately applied near-ambient drying may be an effective method of post-harvest preservation of malting barley, making it possible to preserve their microbiological and technological quality appropriate for malt production [Wawrzyniak et al., 2006**,** Ryniecki et al., 2007b, Gawrysiak-Witulska et al., 2008].

References

Abramson D., Hulasare R., White N.D.G., Jayas D.S., 1999. Mycotoxin formation in hull-less barley during granary storage at 15% and 19% moisture content. J. Stored Products Res., 35, 297–305.

Abramson D., Hulasare R., York R.K., White N.D.G., Jayas D.S., 2005. Mycotoxins, ergosterol, and odour volatiles in durum wheat during granary storage at 16% and 20% moisture content. J. Stored Products Res., 41, 67–76.

Baxter E.D., Slaiding I.R., Kelly B., 2001. Abstract: Behavior of Ochratoxin A in Brewing. J. Am. Soc. Brewing Chem., 59, 98–100.

Bernfeld P., 1955. Amylases, alpha and beta. In: Met Enzymol. Ed. S.O. Colowick, N.O. Kaplan. Accademic Press New York 1, 149–158.

Castro M.F.P.M., Bragagnolo N., Toledo Valentini S.R., 2002. The relationship between fungi growth and aflatoxin production with ergosterol content of corn grains. Braz. J. Microbiol., 33, 22–26.

Chełkowski J., 1980. Powstawanie mikotoksyn (ochratoksyn) i ich detoksykacja w ziarnie zbożowym [Formation of mycotoxins (ochratoxins) and their detoxication in cereal grain]. Rocz. AR w Poznaniu, Rozpr. Nauk., 100, Wyd. AR w Poznaniu.

Chełkowski J., 1985. Mikotoksyny, wytwarzające je grzyby i mikotoksykozy [Mycotoxins, mycotoxin-producing fungi and mycotoxicoses]. Skrypty SGGW AR w Warszawie, Wyd. SGGW-AR.

Christensen C.M., Sauer D.B. 1982. Microflora, nn: Storage of cereal grains and their products (ed.: Christensen C. M.), Am. Ass. Cereal Chem., Minnesota, 219–238.

Dawson-Andoh B. E., 2002. Ergosterol content as a measure of biomass of potential biological control fungi in liquid cultures. Holz Als Roh- Werkstoff, 60, 115–117.

Ekbald A., Wallander H., Nasholm T., 1998. Chitin and ergosterol combined to measure total and living fungal biomass in ectomycorrhizas. New Phytologist, 138, 143–149.
Fornal Ł., Filipowicz A., 2005. Ocena jakości jęczmienia browarnego [Quality assessment of malting barley]. www.eurequa.pl
Gawrysiak-Witulska M., Ryniecki A., 2004. Analiza procesu niskotemperaturowego suszenia rzepaku w silosie typu "BIN" (An analysis of near-ambient drying of rapeseed in a BIN silo). Postępy Techniki Przetwórstwa Spoż. (Poland) 14/25, 46–48.
Gawrysiak-Witulska M., Ryniecki A., 2005. Wartość technologiczna pszenicy wysuszonej metodą niskotemperaturową [Technological value of wheat dried using near-ambient drying method]. Inż. Rol., 11, 71, 137–143
Gawrysiak-Witulska M., Ryniecki A., Rudzińska M., 2005. Wpływ temperatury i techniki suszenia na wybrane wyróżniki jakościowe nasion rzepaku (The effect of temperature and drying method on selected quality attributes of rapeseed). Inż. Rol. (Poland) 11(71), 129–136.
Gawrysiak-Witulska M, Ryniecki A; Wawrzyniak J., 2006. Związki korelacyjne między wybranymi parametrami powietrza i ziarna jęczmienia suszonego metodą niskotemperaturową w grubej nieruchomej warstwie [Correlations betwen selected parameters of air and barley grain dried by near-ambient method in a thick bed], Inż. Rol., 7 (82), 173–179.
Gawrysiak-Witulska M., Rudzińska M., Ryniecki A., 2007a. Wpływ metody suszenia oraz przechowywania na wybrane wyróżniki jakościowe nasion rzepaku [The effect of drying method and storage on selected quality attributes of rapeseed]. Inż. Rol., 5 (93). 153–159.
Gawrysiak-Witulska M., Ryniecki A., Wawrzyniak J., Banaszak J., 2007b. Badanie wpływu prędkości czynnika suszącego na korelacje między wybranymi parametrami powietrza i ziarna jęczmienia suszonego metodą niskotemperaturową [Studies on the effect of drying agent velocity on correlations between selected parameters of air and barley grain dried by near-ambient drying method]. Inż. Rol., 5 (93), 161–167.
Gawrysiak-Witulska M., Wawrzyniak J., Ryniecki A., Perkowski J., 2008. Relationship of ergosterol content and fungal contamination and assessment of technological quality of malting barley preserved in a metal silo using the near-ambient method. J. Stored Products Res., 44, 360– 365.
Gąsiorowski H. 1997, Jęczmień. Chemia i Technologia [Barley. Chemistry and Technology], Państwowe Wyd. Rolne i Leśne, Poznań.
Gutarowska B., Żakowska Z., 2002. Elaboration and application of mathematical model for estimation of mould contamination of some building materials based on ergosterol content determination. Int. Biodeterioration Biodegradation 49, 299–305.
Gutarowska B., Piotrowska M., 2007. Methods of mycological analysis in buildings. Building and Environment 42, 1843–1850.
Hawronskyj J.M., Holah J. 1997. ATP: A universal hygiene monitor. Trends in Food Sci. Technol., 8, 79–84.
Janińska B., 2002. Metody oceny skażenia obiektów budowlanych grzybami pleśniowymi [Methods to assess contamination of building structures with mould]. Fundations of Civil and Environmental Engineering 3, 52–58.
Jeleń H., Wąsowicz E., 1998. Volatile fungal metabolites and their relation to the spoilage of agricultural commodities, Food Rev. Int., 14, 391–426.
Kaleta A., 1996. Modelowanie procesu konwekcyjnego suszenia ziarna w silosach [Modelling convection drying of grain in silos] Post-doctoral dissertation, Fundacja "Rozwój SGGW", Warszawa.
Kamiński E., Wąsowicz E., 1991. The usage of volatile compounds produced by moulds as indicators of grain deterioration. In: Cereal grain – Mycotoxins, Fungi and Quality in Drying and Storage (ed.: Chełkowski J.), Elsevier Sci. Pub. B.V., Amsterdam, New York, 229–258.
Kunze W., 1996. Technology of Brewing and Malting. VLB Berlin Versuchs - und Lehranstalt fur Brauerei in Berlin, Germany.

Marín S., Ramos A. J., Sanchis V., 2005. Comparison of methods for assessment of growth of food spoilage moulds in solid substrates. Int. J. Food Microbiol., 99, 329–341.
Maupetit P., Gatel F., Cahagnier B., Botorel G., Charlier M., Collet B., Dauvillier P., Laffiteau J., Roux G., 1993. Quantitative estimation of fungal infestation of feedstuffs by determining ergosterol content. 44th An. Meet EAAP. 16–19 August 1993, Comm. Anim. Nutr. Aarhus, Denmark,
Mille-Lindblom C., Wachenfeldt E., Tranvik L.J., 2004. Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. J. Microbiol. Met, 59, 253– 262.
Nellist M. E., Hughes M., 1973. Seed Sci. Technol., 1, 613–643.
Olsson J., Börjesson T., Lundstedt T., Schnürer J., 2000. Volatiles for mycological quality grading of barley grains: determinations using gas chromatography–mass spectrometry and electronic nose. Int. J. Food Microbiol., 59, 167–178.
Pasanen A.L., Yli-Pietila K., Pasanen P., Kalliokoski P., Tarhanen J., 1999. Ergosterol Content in Various Fungal Species and Biocontaminated Building Materials. App. Environm. Microbiol., 65, 138–142.
Perkowski, J., Wiwart, M., Buśko, M., Laskowska, M., Berthiller, F., Kandler, W., Krska, R., 2007. *Fusarium* toxins and total fungal biomass indicators in naturally contaminated wheat samples from north-eastern Poland in 2003. Food Additives and Contaminants, 24, 1292–1298.
Perkowski, J., Buśko, M., Stuper, K., Kostecki, M., Matysiak, A., Szwajkowska-Michałek, L., 2008. Concentration of ergosterol in small-grained naturally contaminated and inoculated cereals. Biologia 63, 542–547.
Presicce D.S., Forleo A., Taurino A.M., Zuppa M., Siciliano P., Laddomada B., Logrieco A., Visconti A., 2006. Response evaluation of an E-nose towards contaminated wheat by *Fusarium poae* fungi. Sensors and Actuators B, 118, 433–438.
Pronyk C., Abramson D., Muir W.E., White N.D.G., 2006. Correlation of total ergosterol levels in stored canola with fungal deterioration. J. Stored Products Res., 42, 162–172.
Reeslev M., Miller M., Nielsen K.F., 2003. Quantifying mold biomass on gypsum board: comparison of ergosterol and beta-N-acetylhexosaminidase as mold biomass parameters. App. Environm. Microbiol., 69, 3996–3998.
Robine E., Lacaze I., Moularat S., Ritoux S., Boissier M., 2005. Characterisation of exposure to airborne fungi: Measurement of ergosterol. J. Microbiol. Met, 63, 185– 192.
Ryniecki A., 1994. Optymalizacja systemów sterowania procesem suszenia ziarna pszenicy w temperaturze bliskiej temperaturze otoczenia [Optimization of control systems for near-ambient drying of wheat grain]. Post-doctoral dissertation. Wyd. AR, Poznań.
Ryniecki A., 2002, Warunki bezpiecznego przechowywania [Safe storage conditions]. In: Poradnik - Dobrze przechowywane zboże [Manual – Good cereal storage] (eds.: Ryniecki A., Szymański P.), MR INFO i Towarzystwo Umiejętności Rolniczych, Poznań, pp. 14–17.
Ryniecki A., Gawrysiak-Witulska M., 2004. Automatyczna identyfikacja zakończenia procesu suszenia nasion rzepaku w grubej nieruchomej warstwie [Automatic identification of endpoint of rapeseed drying in a thick bed]. Inż. Rol., 5 (60), 313–320.
Ryniecki A., 2005. Drying and Cooling Grain in Bulk – Handbook (Part 1). Mr Info, Poznań, Poland and KBN Handelsselskab v/Karlo B, Nielsen, Esbjerg, Denmark.
Ryniecki, A., Pawłowska, A., Moliński, K., 2006. Stochastic analysis of grain drying with unheated air under two different climates. Drying Technol., 24 (9), 1147–1152.
Ryniecki A., McLean K., 2006. Pielęgnacja ziarna mokrego [Treatment of wet grain], in: Poradnik – Dobrze przechowywane zboże [Manual – Good Cereal Storage] (eds.: Ryniecki A., Szymański P.). MR INFO i Towarzystwo Umiejętności Rolniczych, Poznań, 30–50.
Ryniecki A., Gawrysiak-Witulska M., Wawrzyniak J., 2007a. Correlation for the automatic identification of drying endpoint in near-ambient dryers: Application to malting barley. Biosystems Eng., 98, 437–445.

Ryniecki A., Gawrysiak-Witulska M., Wawrzyniak J., 2007b. Pożniwne suszenie i chłodzenie jęczmienia browarnego [Post-harvest drying and cooling of malting barley]. Chapter 15, in: Właściwości fizyczne suszonych surowców i produktów spożywczych [Physical properties of dried materials and foostuffs] (eds.: B. Dobrzański jr and L. Mieszkalski). Komitet Agrofizyki PAN, Wyd. Nauk. FRNA, ISBN-13: 978-83-60489-06-2, 159–170.

Satora P., Tuszyński T., 2008. Zakażenia mikrobiologiczne piwa [Microbiological contamination of beer]. Katedra Technologii Fermentacji i Mikrobiologii Technicznej, Akademii Rolniczej w Krakowie, http://www.ar.krakow.pl/tz/ktfimt/dydaktyka/materialy/mp/04a.doc.

Saxena J., Munimbazi C., Bullerman L.B., 2001. Relationship of mould count, ergosterol and ochratoxin A production. Int. J. Food Microbiol., 71, 29– 34.

Schnürer, J., Jonsson, A., 1992. Ergosterol levels and mould colony forming units in Swedish grains of food and feed grade. Acta Agriculturae Scandinavica, Section B, Soil and Plant Sci., 42, 240–245.

Schnürer J., Olsson J., Börjesson T., 1999. Fungal Volatiles as Indicators of Food and Feeds Spoilage. Fungal Genetics and Biology, 27, 209–217.

Schwadorf K., Müller H.M., 1989. Determination of ergosterol in cereals, mixed feed components, and mixed feeds by liquid chromatography. J Assoc. Anal. Chem., 72 (3), 457–462.

Seitz I.M, Mohr H.E., Rurroughs R., Sauer D.B., 1977. Ergosterol as an indicator of fungal invasion in grains. Cereal Chem., 54, 1207–1217.

Stoudt T.H., Foster J.W., 1954. The Microbiological Synthesis of Ergosterol. I. Assay Procedure. Research Laboratories, Chemical Division, Merck & Co., Inc., Rahway, New Jerseyl, http://aem.asm.org/cgi/reprint/2/6/385.pdf, 385–387.

Suberkropp K., Gessner M.O., Chauvet E., 1993. Comparison of ATP and ergosterol as indicators of fungal biomass associated with decomposing leaves in streams. App. Environm. Microbiol., 59, 3367–3372.

Tomkiewicz D., Ryniecki A., Gawrysiak-Witulska M. Wawrzyniak J., Perkowski J., 2006. Application of Neural Networks to Design an Algorithm of Automatic Identification of the Termination of the Drying of Barley in Bulk. 2006 CIGR Section VI International Symposium on FUTURE OF FOOD ENGINEERING, Warsaw, Poland, 26–28 April 2006, CD.

Varga M., Bartok T., Mesterhazy A., 2006. Determination of ergosterol in *Fusarium*-infected wheat by liquid chromatography–atmospheric pressure photoionization mass spectrometry. J. Chromatography A, 1103, 278–283.

Wawrzyniak J., Gawrysiak-Witulska M., Ryniecki A., Wiewiórowska M., 2006. Jakość mikrobiologiczna jęczmienia browarnego konserwowanego w silosie metalowym [Microbiological quality of malting barley preserved in a metal silo]. Przegląd Zbożowo-Młynarski, 50 (10), 25–27.

Weiner W., Gozdecka G., Korpal W., 2008. Badania możliwości pozyskiwania słodu z ziarna wybranych zbóż. [Study on the possibility of obtaining malt from grain of selected cereals]. Acta Agrophysica, 12, (3), 813–823.

Zhao X.R., Lin Q., Brookes P.C., 2005. Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol. Biochem., 37, 311–317.

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ETHANOL FERMENTATION OF EXTRUDED RAW MATERIAL WITH NONCONVENTIONAL YEAST

Introduction

The need of intensified production of ethanol as an alternative carrier of energy causes the necessity of finding new technological solutions as well as alternative microorganisms for *Saccharomyces cerevisiae* yeast [Alper et al., 2006, Linde et al., 2007, Ohgen et al., 2007]. Nowadays, *Saccharomyces cerevisiae* yeast are nearly the exclusive producer of ethanol. A considerable disadvantage of starch bioconversion to alcohol by yeast is the need of preliminary preparation of raw material involving the processes of starch grueling, liquefaction and then saccharification with the use of amylolytic enzymes [Słomińska and Garbacik 2002, Słomińska et all 2003]. Fermentation kinetics and its final effects depend on initial technological treatments. The most energy – consuming are the first two stages (grueling and liquefaction) because of the need of applying high temperatures for a relatively long time.

Intensive, long-term heating of plant products containing protein and carbohydrates leads, as a rule, to diminishing their value because of resulting products of Maillard reaction. There are formed enzyme – resistant chemical combinations between free amino-acids and aldehyde groups. At high temperature there also takes place caramelization of sugars which do not undergo the process of fermentation and their presence is disadvantageous for yeast cells.

Highly uneconomical results and loses in raw material quality indicate the need of searching new solutions shortening the time of starch liquefaction. Such a solution regarding preparation of starch raw material can be provided by the process of extrusion HTST (high temperature, short time) consisting in alteration of starch globules by simultaneous effect of heat, coagulation forces and stresses [Mercier, 1986]. According to Czarnecki and Nowak [1997], as well as to Grossman et al. [1998] application of extrusion process ensures appropriate preparation of starch raw material to conduct ethanol fermentation eliminating the need of using energy – consuming pressure process of starch deliquescence. Investigation proves that energy outlay is not high and it ranges for 1 kg of starch extrudate from 0.1 to 0.2 kWh [Vervaet et al., 1995]. Moreover, under the influence of high temperature and pressure there takes place elimination of dangerous microflora affecting mash microbiological stability when the environment is controlled by biomass of seed yeast.

The aim of the work was the assessment of extrusion effect on potato starch ability to be hydrolyzed and fermented by *Schwaniomyces occidentalis* ATCC 48086 yeast.

Materials and methods

Biological material was *Schwaniomyces occidentalis* ATCC 48086 yeast strain originating from American type Culture Collection (Rockville, USA). It is UV mutant resistant to deoxy – D – glucose, capable o starch digestion and fermentation. This strain possesses amylolytic enzymes: alpha –amylase, glucoamylase and enzymes hydrolyzing α – 1.6 glycoside bonds [Kawa-Rygielska, 2002]. *Schwanniomyces occidentalis* ATCC strain was stored and activated on YM medium.

The material for investigation was potato starch subjected to the process of extrusion in different temperature conditions. Control sample involved not extruded starch.

Raw material preparation

Extrusion of potato starch of 25% moisture was conducted in AEV 650 single – screw extruder produced by Brabender firm at the temperatures 60°C, 120°C and 180°C with a worm of 1:2 compression ratio and diameter of die 3mm. Obtained extrudates were subjected to milled. Viscosity of 2% extruded starch gruels was determined using Haaske Rheostress 50 viscometer.

Determination of speed of starch hydrolysis

The speed of starch hydrolysis by enzymes of *Swanniomyces occidentalis ATCC 48086* yeast excreted in the course of their growth were assessed on YPS medium containing 1% of potato starch extruded at the temperature of 60°C, 120°C and 180°C. Yeast culture was conducted for 72 h, the samples for analysis were collected every 24 h. After yeast centrifuging, resulting supernatant liquid, stained with Lugol fluid 1:3 water diluted, was subjected to absorbance measurement using Beckman DU 650 spectrophotometer at $\lambda = 620$ mm. Starch content in supernatant was read from analytical curves of relation between absorbance and starch concentration.

On this basis there was calculated the degree of starch utilization [%] and the speed of its hydrolysis expressed by the amount of hydrolyzed starch in a time unit [g/h].

The assessment of fermentation activity

Fermentation tests with the examined yeast participation were done in conical flasks of 250 ml capacity containing 100 ml of appropriate fermentation medium. The latter one involved mash containing 4% of potato starch extruded in already mentioned temperatures, enriched in mineral salts: $MgSO_4x7H_2O$, $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2$. Control sample was the medium containing not extruded potato starch. In the samples there were not used any amylolytic enzymes. Fermentation process was carried out at the temperature of 30°C. Dynamics of fermentation, expressed as the amount of released CO_2 in the course of the process in relation to total amount of released CO_2 [%], was assumed as a criterion of the assessment of yeast fermentation activity. After completed fermentation the samples were distilled twice and ethanol content was assayed in the resulting liquid according to HPLC method. In the brew there were determined remaining indirectly reducing sugars and total sugars using Nizowkina–Jemielianowa reduction method. On the basis of the results obtained there was estimated the degree of starch utilization [%], as well as practical efficiency of fermentation process in relation to a theoretical one.

Physiological state of yeast after fermentation was assessed in a microscopic preparation stained with methylene blue. Yeast physiological state was expressed as percentage contribution of budding cells and getting stained with methylene blue.

Results

Viscosity of 2% potato starch gruels subjected to extrusion was shown in Table 1. The highest viscosity featured potato starch gruel extruded at low temperature ranging 60°C. As extrusion temperature increased there was recorded decreased viscosity of the gruels prepared. The most dramatic decrease in gruel viscosity was observed as extrusion temperature was growing from 60 to 120°C and the difference amounted 3.77 mPa/s, while further increase by successive 60°C slightly effected on the value of the parameter in question and gruel viscosity dropped only by 1.23 mPa/s.

Table 1

The viscosity of 2% potato starch gruels subjected to extrusion

Potato starch gruels	Potato starch extruded at the temperature of 60°C	Potato starch extruded at the temperature of 120°C	Potato starch extruded at the temperature of 180°C
Viscosity of 2% potato starch gruels mPa/s	12.36	8.59	7.36

Extrudates obtained became a raw material for the assessment of the speed of starch hydrolysis by amylolytic enzymes of *Schwanniomyces occidentalis ATCC 48086* yeast excreted to the environment during their growth. The highest diversity of starch hydrolysis speed was observed during the first 24 hours of the examined yeast culture (Table 2).

Table 2

The speed of potato starch hydrolysis by *Schwanniomyces occidentalis ATCC 48086*

Starch	The speed of potato starch hydrolysis $x10^{-4}$ [g/h]		
	Time [h]		
	24	48	72
Potato starch	1.41	1.28	1.26
Potato starch extruded at the temperature of 60°C	0.63	0.52	0.45
Potato starch extruded at the temperature of 120°C	3.25	1.68	1.29
Potato starch extruded at the temperature of 180°C	2.85	1.94	1.33

Within that period the most susceptible to hydrolysis proved potato starch extruded at the temperatures: 120°C and 180°C. Definitely less susceptible to the effect of amylolytic enzymes of the examined strain was not – extruded potato starch, as well as the one extruded at the temperature of 60°C. The speed of not extruded starch hydrolysis was twice lower and the speed of the one extruded at 60°C was five times lower as compared to hydrolysis speed recorded for starch extruded at higher temperatures. The amount of starch decomposed within first 24 hours ranged the values from 15 to 78% (Tab. 3).

Table 3

The amount of starch decomposed by *Schwanniomyces occidentalis ATCC 48086* in the samples initially containing 1% of starch [in%]

Starch	The amount of starch decomposed [%]		
	Time [h]		
	24	48	72
Potato starch	33.8	61.4	90.7
Potato starch extruded at the temperature of 60°C	15.0	24.9	32.4
Potato starch extruded at the temperature of 120°C	78.0	80.6	92.8
Potato starch extruded at the temperature of 180°C	68.4	93.1	95.8

During three-day-lasting culture starch extruded at high temperatures (120°C and 180°C) was over 90% hydrolyzed. Not extruded starch and starch extruded at the lowest temperature was hydrolyzed to a lower degree. The course of not hydrolyzed starch hydrolysis featured constant speed throughout the whole process, while hydrolysis sped in the remaining samples decreased according to the time of yeast culture.

The further part of investigation involves the assessment of *Schwanniomyces occidentalis ATCC 48086* yeast strain capability of fermenting potato starch.There was conducted ethanol fermentation of potato starch , both not extruded and extruded at the temperatures: 60°C, 120°C and 180°C, without enzymes added. Figure 2 shows the dynamics of fermentation (expressed as% of total amount of released CO_2 [g] after 24, 48, 72, 96 and 120h of fermentation).

The process of potato starch extrusion did significantly effected on the improvement of fermentation dynamics involving participation of *Schwanniomyces occidentalis ATCC 48086.*The amount of CO_2 released during first 24 h of fermentation ranged from 9 to 26%, while that of not extruded one was only 3%. It was also recorded that the higher temperature of extrusion, the higher dynamics of ethanol fermentation. The mentioned tendency lasted for three days, as the amount of released CO_2 within 96th hour of fermentation was the highest in the samples containing starch extruded at 120°C amounted 95%.

The highest yield of ethanol fermentation was obtained using as raw material potato starch extruded at the temperature of 120°C. The quantity of ethanol produced was twice higher than the one obtained for raw material extruded at 60°C. The latter one did not differ from control sample involving not extruded material. The increase in extrusion temperature to 180°C did not improve final effects of fermentation. Ethanol yield was by 4.2% lower in comparison to the sample extruded at 120°C (Table 4).

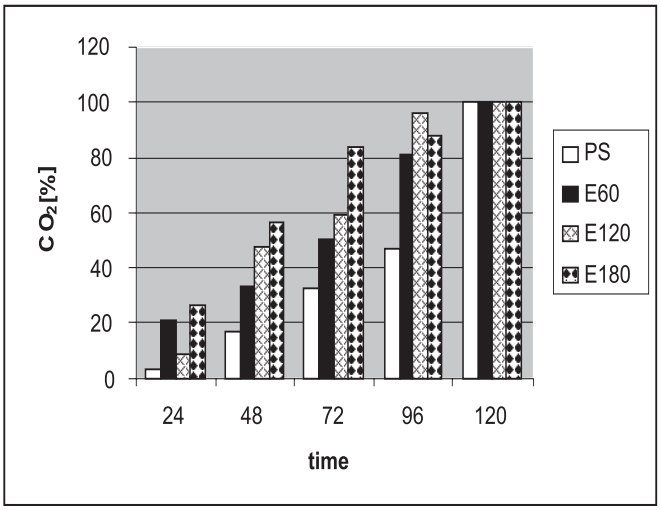

Fig. 1. PS – Potato starch, E 60, E120, E180 – Potato starch extruded at the temperature of 60°C, 120°C, 180°C

Table 4

Final effects of ethanol fermentation extruded potato starch by using *Schwanniomyces occidentalis ATCC 48086* yeast

Final effects of ethanol fermentation	Potato starch	Potato starch extruded at the temperature of 60°C	Potato starch extruded at the temperature of 120°C	Potato starch extruded at the temperature of 180°C
Ethanol yield% theoretical	39.6	42.3	75.0	70.8
pH of the medium	4.1	4.7	3.8	3.6
Reducing sugars [g/dm^3]	2.1	1.8	2.2	2.4
Total sugars [g/dm^3]	12.0	10.8	4.1	6.5
Degree of starch consumption [%]	73.0	75.7	91.0	86.5

Post-fermentation liquids pH value ranged from 3.6 to 4.7. The lowest pH, similar to the initial culture medium before fermentation, was observed in the samples prepared from starch extruded at the temperature of 60°C.

Reducing sugars content in a medium after fermentation amounted about 2%, while total sugars value ranged from 12 to 4% and it was lower for starch extruded at 120°C.

The process of extrusion carried out at 120°C and 180°C did considerably effected on yeast strain *Schwanniomyces occidentalis* ATCC 48086 ability to use potato starch (91 and 86.5% respectively). Lower starch consumption was recorded for fermentation of media prepared from potato starch extruded at 60°C (45.7%) and for potato starch, which did not undergo extrusion (43%).

Comparing physiological state of *Schwanniomyces occidentalis ATCC 48086* after potato starch mash fermentation and its extracts it is possible to state that contribution of budding cells was quite alike and it ranged about a dozen percent (Table 5).

Table 5

The physiological condition of *Schwanniomyces occidentalis ATCC 48086*

	Potato starch	Potato starch extruded at the temperature of 60°C	Potato starch extruded at the temperature of 120°C	Potato starch extruded at the temperature of 180°C
The number of inactive cells [%]	2.5	14.5	17.0	8.8
The number of budding cells [%]	20.5	16.3	17.8	11.0

More considerable diversity was observed in the presence of inactive cells. Relatively low percentage of dead cells occurred in fermentation bases prepared from potato starch extruded at 180°C – 8.8% and from not extruded one. The remaining samples containing extruded starch featured much worse physiological state and the quantity of dead cells amounted 14.5–17.8%.

Discussion

In the recent years numerous investigation have focused on the assessment of yeast biotechnological properties regarding their amylolytic capability in the view of starch mash fermentation. The most important biotechnological property of *Schwanniomyces* species yeast is their rich complex of amylolytic enzymes. For that reason Ingledew [1987] called *Schwanniomyces*, "super yeast''. Strains belonging to the same species excrete extracellular α – amylase, one or two glucoamylase forms and the enzyme hydrolyzing branches in a starch chain (so-called debranching enzyme) to their environment. *Schwanniomyces occidentalis* yeast are also capable of fermentation [Jurgen et al., 1996; Kręgiel, 1998, Laluce et al., 1988]. Application of this yeast type in potato starch fermentation process allows eliminating or reducing the amount of enzymatic preparations and thus effects on economy of ethanol production. Concerning advantages of the strain there was undertaken an attempt to hydrolyze native potato starch by *Schwanniomyces occidentalis ATCC 48086* yeast enzymes .

As it can be concluded from the works by other authors native starch globules (i.e. raw starch) feature diversified, generally very high resistance to enzymes effect, while starch susceptibility to amylase activity is different and it depends on starch origin as well as the kind of enzyme and its dose [Sawicka-Żukowska et al., 1999].

Laluce et al. [1998], quoting the other authors, stated that glucoamylases synthesized by some yeast and another microorganisms like: *Aspergillus niger, A. awamori, A. sp. Rhizopus, Corticumrolfsii and Chalara paradoxa,* as well as alpha–amylases produced by *Bacilus circulans* F–2, *B.subtilis* 65, *B.cereus* NY – 14 *Clostridium butiricum* characterize the abilities of raw starch degradation. The mentioned authors isolated, from potato processing plant sewage, the strain belonging to *Bacillus firmus/lentus* group which in the culture with raw

potato starch medium produced amolytic enzymes complex enabling entire decomposition of raw potato starch, maize and wheat starch globules at the temperature of 37°C, with glucose as the main product. In our own investigation application of amolytic enzymes complex of *Schwanniomyces occidentalis ATCC 48086* allowed to hydrolyze native potato starch in 40%.

Numerous authors also confirm that potato starch is much more resistant to amolytic enzymes activity than other cereals starch, which results from potato starch structure. It is generally believed that raw starch granules are practically resistant to the effect of many chemical factors unless they are subjected to initial processing.

One the ways of directed modification of potato starch is the process of extrusion. The effect of extrusion on starch is interpreted as the phenomenon of "specific grueling" and it is connected with the change of physical-chemical properties, as well as with molecular transformation of amylase and amylopectins [Mercier, 1986, Miladinov and Hanna, 2001]. This process causes considerable increase in starch solubility. The increase in temperature from 90 to 210°C results in altered solubility from 61 to 95%. Conducting extrusion at 110°C it was possible to obtain starch featuring satisfactory solubility at room temperature. Therefore, manipulating parameters of this process, it is possible to produce modified potato starch of diversified functional properties [Jamroz at al., 2005, Mościcki, 2002, Thymi et al., 2005].

Application of potato starch extrusion in our own investigation did significantly improve the effects of ethanol fermentation conducted by *Schwanniomyces occidenalis ATCC 48086* yeast strain. Yet there were recorded considerably diversified results obtained after extruded starch fermentation according to extrusion temperature applied. Investigation carried out by Miladinova and Hanna [2001] proved that temperature and moisture of the material subjected to extrusion are the most essential factors effecting the degree of grueling and partial saccharization. Observations of starch texture proved that if the temperature of was too low starch did not undergo any significant physical – chemical alterations. Our own investigation showed that raw material extrusion at the temperature of 60°C did not effect on the improvement of the examined features.

The results obtained in this work are regarded as an introduction to further investigation on utilizing *Schwanniomyces occidentalis ATCC 48086* yeast strain in unconventional starch raw materials, especially extruded starch.

Conclusions

The process of extrusion at the temperature of 120 and 180°C increased potato starch susceptibility to the effect of α –amylase and glucoamylase produced by *Schwanniomyces occidentalis ATCC 48086* yeast strain and it did improve final effects of potato starch fermentation.

As extrusion temperature grows, dynamics of ethanol fermentation grows along with it to reach the highest value of ethanol efficiency and the highest degree of starch use for potatostarch extrusion at 120°C.

The quantity of ethanol produced during fermentation of starch extruded at 120°C was twice higher in comparison to the amount of ethanol obtained when not extruded starch was involved in fermentation.

The process of potato starch extrusion at 60°C did not influence the improvement of final effects of ethanol fermentation.

References

Alper H., Moxley J., Nevoigt E., Fink G.R., Stephanopoulos G., 2006. Engineering yeast transcription machinery for improved ethanol tolerance and production. Sci. Mag., 5805, 1565–1568.

Czarnecki Z., Nowak J. 1997. Ethanol fermentation of HTST extruded rye grain by bacteria and yeasts. Acta Biotechnol., 17, 63–71.

Grossman M.V.E., El – Dash, Carvelho J.F., Campinas., 1988. Extrusion cooking of cassava starch for ethanol production. Starch, 40, 303–307.

Ingledew W.M., 1987. *Schwanniomyces:* A potential super- yeast?. Crit. Rev. Biotechnol., 5, 159–176.

Jamroz J,, Ciesielski W., Pielichowski K., Tomasiak P., 1998. Extrusion – cooking of potato starch and selected properties of the extrustrudates. Pol. J. Nutr. Sci., 48, 89–97.

Dohmen J.R., Hollenberg C.P., 1996. Schwanniomyces occidentalis, nonconventional yeasts in biotechnology. Handbook, Springer- Verlag Berlin Heidelberg, New York.

Kawa- Rygielska J., 2002. Ulepszanie drożdży przemysłowych metodą fuzji protoplastów. Część 1. Otrzymywanie hybrydów. Acta Scientiarum Polonorum Biotechnologia, 1(1–2) 37–46.

Kręgiel D., 1998. Amylazy drożdży *Schwanniomyces* SP. Post. Microbiol., 37.

Linde M., Galbe M., Zacchi G., 2007. Simultaneous saccharification and fermentation of steam-pretreated barley straw at low enzyme loadings and low yeast concentration. Enzyme Microb. Technol., 40, 1100–1107.

Laluce C., Bertolini M.C., Ernandes J.R, Martini A.V, Martini A., 1988.New amylolytic yeast strains for starch and dextrin fermentation. App. Environm. Microbiol., 54, 2447–2451.

Linko P., Hakulin S., Linko Y-Y., HTST – extrusion cooking in ethanol production from starchy materials, from Enzyme Mikrob. Technol., 6, 457–46

Mercier C., 1986. Comparative modifications of starchy products by extrusion-cooking and drum- drying. Pasta and Extrusion Cooked Foods. Elsevier Appl. Sci.

Miladinov V.D., Hanna M.A., 2001. Temperatures and ethanol effects on the properties of extruded modified starch. Elsevier, 13, 21–28.

Mościcki L., 2002. Zmiany właściwości fizykochemicznych surowców roślinnych poddanych procesowi ekstruzji. Przegląd zbożowo-młynarski, 27–29.

Ohgren K., Bura R., Leśnicki G., Saddler J., Zacchi G., 2007. A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover. Process Biochem., 42, 834–839.

Sawicka – Żukowska R., Zielińska K., Wędrychowska B., 1999. Enzymatyczna degradacja różnych rodzajów skrobi surowej. Przem. Spoż., 5, 33–36.

Słomińska L., Wiśniewska D., Grześkowiak A., 2003. Liquefaction of starch by thermostable alfa – amylase, ACTA Scientiarum polonorum, 2,17–26.

Słomińska L., Garbacik M., 2002. Porównanie właściwości hydrolitycznych dwóch termostabilnych preparatów enzymatycznych. ACTA Scientiarum Polonorum, Technologia Alimentaria, 1, 21–30.

Thymi S., Krokida M.K., Pappa A., Maroulis Z.B., 2005. Structural properties of extruded corn starch, Elsevier, 68, 519–526.

Verveat Ch., Beart L., Remon J.P., 1995. Extrusion–spheronisation. A literature review, Elsevier, Int. J. Pharmaceutics, 116, 131–146.

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THE PROPERTIES OF EXTRUDATES OBTAINED FROM POTATO STARCH DURING ONE - AND TWOFOLD EXTRUSION PROCESS

Introduction

The extrusion is a mechanical-thermal process where in relatively short time the raw material (carbohydrate, proteinaceous) is subjected to the temperature, mechanical forces and changing pressure. The raw material (flour or groats) is converted into plastic mass. The new product has characteristic texture and shape as the result of rapid decreasing pressure and water evaporation.

For the first time the extrusion process was applied in the food industry in the 60s of XX century. Nowadays, it is a common technology because of the extrusion parameters like: the temperature, the pressure, the intensity of mechanical forces which often create the different properties of ready- products [Gambus et al., 1999, Jamroz et al., 1998, Singh and Smith, 1997]. During the extruding starch gelatinizes and partial hydrolyzes. The extrusion causes the deranging of structure's starch granules, changes the spatial organization of starch chains, the degree of crystanillity and the partial molecular degradation [Gambus et al., 1999, Nabeskima and Grossmann, 2001]. In the food industry a lot of new products are obtained because of their interesting properties.

The range of extruded products was widen by applying different chemical substances during the extrusion process. A lot of different reactions like: starch phosphorylation, starch acetylation, starch oxidation and many others, are carried out on extruders by adding the chemical components. As the result there are created new products with interesting properties compared with products obtained without using the chemical substances [Ganjyal and Hanna, 2004, Xu and Hanna, 2005].

New innovative methods to obtain new unknown products are still searched. To this end not only raw materials or chemical substances can be used but also new extrusion parameters like: the temperature of process, the moisture of extruded material, the type of flexibilizer, the size of pressure and mechanical forces [Gambus et al., 1999, Jamroz et al., 1998, Singh and Smith, 1997, Thymi et al., 2005].

Nowadays the functional foods play an important role since the consumer's consciousness about the healthy foods has increased. Many authors have been working at the producing the functional extruded foods. Researches tested the samples with addition of fiber in extrudated foods [Rzedzicki et al., 2004, Rzedzicki and Zarzycki, 2005]. Products with the additive like for example bran, though many nutritious qualities are unacceptable by numerous group of consumers due to its unattractive shape. Previous investigations revealed that the addition of insoluble fiber fraction in the quantity of more than 4% influenced the quality

deterioration of the final product. The extrudates with high fiber addition were characterized by slight hardness and inappropriate consistency. Therefore they were rejected by consumers. Moreover it was stated that insoluble fiber fraction transforms into soluble fraction during the extrusion process which positively influenced the texture and doesn't increase the hardness (its like hydrocolloid). Probably the twofold and every next stage of the extrusion process could increase the water soluble fiber fraction and improve the consistency of obtained snacks.

Starch is the main texture- made component that influences the quality of extrudated products. After investigating the changes of extrudated starch it can be likely to predict the changes that could appear in ready- products with starch and other additives, therein with fiber addition. On this account extremely essential seems to determine the changes of preparations properties which based mainly on starch. These preparations were obtained as a result of already extruded products ready to the second stage of the extrusion process. Probably the unknown products could be obtained by applying the procedure of twofold extrusion process [Kollengode et al., 1996, Willett et al., 1997]. Owning to the second and the following extrusions it is probably to obtain products included to the functional foods, for example products with high fiber content which could be accepted by a large consumer market

The purpose of the work was to determine the physical and mechanical properties of extrudates obtained from potato starch during the one- and twofold extrusion process.

Materials and methods

The experimental material was potato starch from Przedsiębiorstwo Przemysłu Ziemniaczanego S.A. in Niechlów in 2005.

The starch was adjusted to 25% moisture and then subjected to the extrusion process in a single screw laboratory Brabender extruder (of 20DN type). The extrusion process was carried out using the screw with a compression ratio of 2:1 and round nozzle of 3 mm in diameter. The screw turns were 80 turns/ min, and the load – 30 turns/ min. The extrusion process was carried out with three temperature variants presented in Table 1.

Table 1

The temperatures of the first and second stage of the extrusion process

Experimental variant	Extrusion temperature [ºC]		
	I zone	II zone	III zone
1	50	90	140
2	60	100	150
3	70	120	170

The obtained preparations were granulated to 1 mm in *Pulversisette 19* cutterhead Fritsch mill. A part of extrudates was granulated to 400 μm in the laboratory mill of WZ-1 type. The remaining part was adjusted to 21% moisture and then re-subjected to extrusion process (a screw compression ratio of 2:1, a nozzle of 3 mm in diameter and temperature parameters like in the first extrusion). The screw turns were 80 turns/min and the load- 6 turns/ min. The obtained modified products were granulated to 400 μm.

Before granulating, in preparations obtained from one- and twofold extrusion processes, were determined:

- degree of expansion – as the proportion of diameter of extrudate to diameter of used nozzle,
- bulk density – as the proportion of mass of extrudate to its volume,
- bending moment – with the use of Instron 5544 with the bend Fixture attachment, head loading was 2 kN and its speed of travel was 4 mm/min.

Statistical analyses were conducted using a Statistica package version 7.1 (one- and two-way variation analyse).

Results and discussion

The degree of expansion is the value which depends on the parameters of: extruder (for example: the type and kind of nozzle used [Ganjyal and Hanna, 2004], screw [Jamroz et al., 1998, Kollengode et al., 1996]), extrusion process (for example: rotation screw rate [Thymi et al., 2005], material travel speed [Hagenimana et al., 2006], temperature process [Hagenimana et al., 2006, Jamroz et al., 1998, Kőksel et al. 2004]) as well as extrudated mixture ratio (for example: kind of raw material [Gambus et al., 1999, Singh and Smith, 1997], kind of flexibilizer [Miladinov and Hanna, 2001], moisture [Gambus et al., 1999, Hagenimana et al., 2006, Singh and Smith, 1997, Thymi et al., 2005] and used additives [Trimnell et al., 1993]).

As it is shown in Table 2 the degree of expansion of obtained extrudates run from 1.79 to 2.44 and depended on the temperatures of extrusion. The preparations obtained in onefold extrusion were characterized by the increase of the degree of expansion with the higher temperature. The similar results were presented by other authors [Cha et al., 2001, Hagenimana et al., 2006, Jamroz et al., 1998, Kőksel et al. 2004]. The volatile components of extrudates at higher temperatures cause the higher pressure what can lead to the increase the elastic force and, in the consequence, the degree of expansion [Miladinov and Hanna, 2001]. During the extruding, the water included in material increases the vapour pressure as the result of increasing the temperature. According to Clapeyron rule, as the result of violent transition of material to the area of lower pressure it comes to increase the pressure of water vapour from the extrudates and to very fast its decompresses what leads to relief the accumulated water and to increase the tensile force. This force increase causes the formation of the air bells in elastic material. Their size are the bigger, the higher is the temperature and pressure in an extruder [Della Valle et al., 1997, Ganjyal and Hanna, 2004, Miladinov and Hanna, 2001]. The degree of expansion is the resultant of two phenomenons- presented above the formation of the air bulks and the phenomenon presented by Cha and others [Cha et al., 2001]. According to these authors the increase of the temperature in the extruder can cause the decrease the viscosity of material what can lead to a decrease the resistance in extruder's nozzle and the degree of expansion. Probably, in the conducted investigation, during the second stage of extrusion process, this second phenomenon was more significant. Possible, it was because the lowest degree of expansion in extrudates was noted at the preparations obtained in medium temperature of extrusion process. Moreover the properties of extruded material influenced the degree of expansion. In the second stage of the extrusion process the raw material was onefold extruded starch, highly depolymerizated because of lower pastes viscosity. The second

extrusion process, carried out in two lower temperatures, influenced the decrease the degree of expansion. The similar results were obtained by Kollengode and others under insufficient mixing conditions [Kollengode et al., 1996]. It is also worth noticing that the second stage of the extrusion process carried out at the highest temperature caused the significant increase the degree of expansion compared with the material onefold extruded. This relationship was noticed in each extrudated products obtained at the highest temperature of the second stage of the extrusion, regardless of temperatures of the first stage of the extrusion process. Probably, this tendency can be applied in the snacks produced by hot extrusion technology. Presumably, owning to the products obtained in the second stage of the extrusion the degree of their expansion can be improved what can influence the better impression which is connected with higher lightness of product.

Table 2

The influence of temperatures on the size of expansion's degree of preparations obtained during one- and twofold extrusion process

Temperature of the extrusion process	Expansion ratio [mm/mm]				
	First stage of the extrusion	Temperature of second stage of the extrusion			
		Variant I	Variant II	Variant III	LSD
Variant I	1.85	1.80	1.79	2.15	0.11
Variant II	1.97	1.89	1.83	2.44	
Variant III	2.09	1.88	1.86	2.13	
Average	1.97	1.86	1.80	2.24	
LSD	0.43				

The bulk density of obtained preparations run from 0.81 [g/cm^3] to 1.77 [g/cm^3] and depended on the temperatures of both extrusion processes. The bulk density of obtained extrudates decreased with the increase the temperature of extrusion. The increase of the temperature of the second stage of extrusion also caused the decrease the bulk density [Cha et al., 2001, Guha et al., 1997, Hagenimana et al., 2006, Jamroz et al., 1998, Kollengode et al., 1996, Kőksel et al. 2004, Singh et al., 2007]. The results of statistical analyze confirm the essential effect of correlation between temperatures of both extrusions on the bulk density of obtained extrudates. The bulk density of preparations obtained at the lowest extrusion temperature decreased after carried out the second extrusion. However, the bulk density of preparations obtained at the highest temperature increased after subjected them to the second stage of extrusion process.

As it was reported in the literature, the bulk density is closely connected with the degree of expansion [Cha et al., 2001, Gambus et al., 1999, Hagenimana et al., 2006, Kollengode et al., 1996, Singh et al., 2007]. Comparing the size of expansion's degree with the bulk density of extrudates obtained in first and second stage of extrusion it was stated the contrary dependence among these parameters. The bulk density of obtained preparations decreased when the expansion's degree increased. The similar results were noticed by the other authors [Cha et al., 2001, Gambus et al., 1999, Hagenimana et al., 2006, Kollengode et al., 1996, Singh et al., 2007, Xu and Hanna, 2005]. Moreover, the preparations obtained in higher temperatures of extrusion (even 260ºC) were characterized by identical dependence [Thymi et al., 2005].

Table 3

The influence of the temperatures on the bulk density of preparations obtained during one- and two-fold extrusion process

Temperature of the extrusion process	Bulk density [g/cm^3]				
	First stage of extrusion	Temperature of second stage of extrusion			
		Variant I	Variant II	Variant III	LSD
Variant I	1.77	1.33	1.24	0.90	0.21
Variant II	1.43	1.58	1.38	0.61	
Variant III	0.81	1.53	1.51	0.95	
Average	1.34	1.51	1.38	0.82	
LSD	0.27				

Many authors confirm the influence of different factors on mechanical properties of obtained extrudates among other things: the temperatures of extrusion process [Jamroz et al., 1998, Miladinov and Hanna, 2001, Thymi et al., 2005, Willett et al., 1997], the moisture of extruded material [Gambus et al., 1999, Trimnell et al., 1993, Willett et al., 1997], the type of starch [Gambus et al., 1999, Trimnell et al., 1993, Xu and Hanna, 2005], the used blowing agent [Xu and Hanna, 2005] as well as the parameters of the second stage of extrusion process [Willett et al., 1997] and even the time of storage [Trimnell et al., 1993]. Dates in Table 4 show that the bending moment of obtained preparations depends on the temperatures and the quantity of cycles of extrusion. The bending moment decreases along with the increase the temperature of first and second stage of the extrusion process. The second stage of the extrusion process significantly influenced the decrease the values of this parameter. The results of statistical analyze confirm the essential effect of the correlation among the temperatures on the mechanical properties of obtained extrudates. The bending moment of preparations obtained in the second stage of the extrusion decreased along with the increase of the temperature in the first stage of the extrusion. This dependence was noticed in all three variants of the temperature of the second stage of the extrusion process. Many authors confirm the dependence between the value of bending moment and the value of the degree of expansion [Gambus et al., 1999, Jamroz et al., 1998, Miladinov and Hanna, 2001, Xu and Hanna, 2005]. The bending moment is the lower, the higher is the degree of expansion. The large expansion causes higher crispness of product. It is characterized by low hardness and soft structure. The preparations are characterized by higher degree of expansion and lower bulk density what directly influenced the decrease the thickness of air bulks walls and the decrease of the resistance of extrudates on mechanical forces [Miladinov and Hanna, 2001]. The extrudates obtained during twofold extrusion significantly differed with the structure from extrudates obtained in onefold extrusion. During this process the products were characterized by more softer and weaker structure compared with the extrudates obtained during the onefold extrusion process (Fig. 1). It is worth noticing that the presented mechanical properties depended (as in the first extrusion process) on the degree of expansion, but these changes had different range of values. The change of these ranges caused that the preparations obtained during twofold extrusion were characterized by lower values of mechanical properties than extrudates obtained in onefold process. The decrease the values of mechanical properties of

the extrudates obtained in twofold extrusion compared with the preparations obtained during onefold process can be explained as the differences in properties of raw material. The onefold extruded starch preparation, being the raw material in the second stage of the extrusion process, was characterized by significant destruction of the structure which became re-weaken during the second stage of the extrusion. The quantity of long- chains starch decreased after extrusion process. It is also possible that the amylopectin fraction in starch can decrease and the amylose fraction can increase what can cause the higher crispness of obtained products [Bhatnagar and Hanna, 1996].

Table 4

The influence of the temperatures on the bending moment of preparations obtained during one- and twofold extrusion process

Temperature of the extrusion process	Bendig moment [Nm]				
	First stage of the extrusion	Temperature of the second stage of the extrusion			
		Variant I	Variant II	Variant III	LSD
Variant I	1.58	1.13	1.05	0.48	0.12
Variant II	1.58	1.06	0.88	0.38	
Variant III	1.37	1.1	0.48	0.36	
Average	1.51	1.10	0.80	0.41	
LSD	0.08				

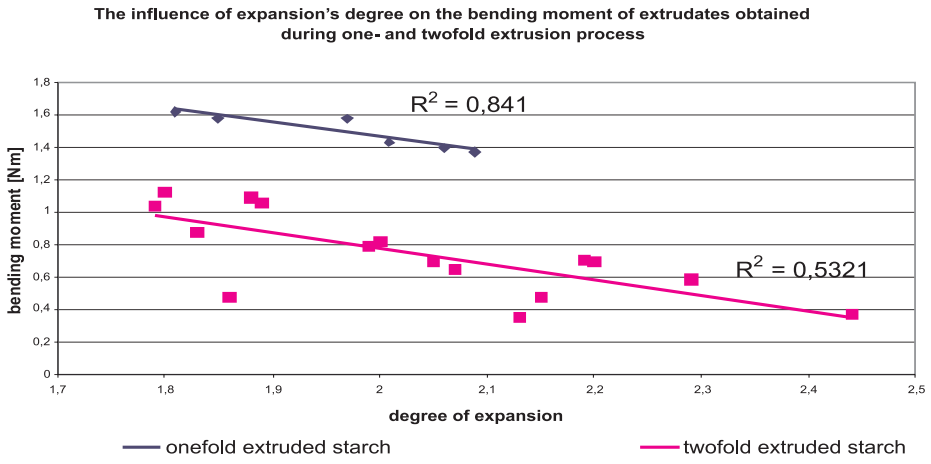

Fig. 1. The influence of expansion's degree on the bending moment of extrudates obtained during one-and twofold extrusion process

Conclusions

As it has been reported in data, the properties of starch extrudates depended on the quantity of extrusion processes and on the conditions under which they were conducted. The properties of onefold extrudated starch depended on the temperature of the extrusion process. The properties of preparations obtained during the second stage of the extrusion process depended on the parameters of both extrusion processes. The higher was the temperature of the extrusion process, the higher was the degree of expansion and the lower was the bulk density. The second stage of the extrusion process conducted at lower temperatures decreased the degree of expansion, but at higher temperatures-increased this parameter. The bulk density of obtained preparations was inversely proportional to the degree of expansion. The mechanical properties of preparations obtained in both stages of the extrusion processes were the weaker the higher was the temperature of the processes, what was detected by the decrease of bending moment of extrudates. Changes of the mechanical properties of the extrudates after second stage of the extrusion process can be applied in production of some extrudated foods.

The presented work is a fragment of doctor's thesis.

References

Alves R.M.L., Grossmann M.V.E., Silva R.S.S.F., 1999. Gelling properties of extruded yam (*Dioscorea alata*) starch. Food Chem., 67, 123–127.

Bhatnagar S., Hanna A.M., 1996. Starch – based plastic foams from various starch sources. Cereal Chem., 76, 601–604.

Della Valle G., Vergnes B., Colonna P., Patria A., 1997. Relations between rheological properties of molten starches and their expansion behavior in extrusion. J. Food Eng., 31, 277–296.

Cha J.Y., Chung D.S., Seib P.A., Flores R.A., Hanna M.A., 2001. Physical properties of starch-based foams as affected by extrusion temperature and moisture content. Industrial Crops and Products 14, 23–30.

Gambus H., Golachowski A., Bala-Piasek A., Ziobro R., Nowotna A., Surowka K., 1999. Functional Properties of starch extrudates. Part 1. Dependence o extrudates properties on starch water content. Electronic J. Pol. Agric. Univ., Food Sci. Technol., 2.

Ganjyal G.M., Hanna M.A. 2004. Effects of extruder die nozzle dimensions on expansion and micrographic characterization during extrusion of acetylated starch. Starch/Stärke, 56, 108–117.

Guha M., Zakiuddin Ali S., Bhattacharya S., 1997. Twin-screw extrusion of rice flour without a die: effect of barrel temperature and screw speed on extrusion and extrudate characteristics. J. Food Eng., 32, 21–267.

Hagenimana A., Ding X., Fang T., 2006. Evaluation of rice flour modified by extrusion cooking. J. Cereal Sci., 43, 38–46.

Jamroz J., Ciesielski W., Pielichowski K., Tomasik P., 1998. Extrusion – cooking of potato starch and selected properties of the extrudates. Pol. J. Food Nutr. Sci., 7/48, 88–97.

Kollengode A.N., Sokhey A.S., Hanna M.A. 1996., Physical and molecular properties of re – extruded starches as affected by extruder screw configuration. J. Food Sci., 61, 596–603.

Kőksel H., Ryu G.H., Basman A., Demiralp H., Ng P.K.W., 2004. Efects of extrusion variables on the properties of waxy hulless barley extrudates; Nahrung Food, 1, 19–24.

Miladinov V.D., Hanna M.A., 2001. Temperatures and ethanol effects on the properties o extruded modified starch. Ind. Crops Products, 13, 21–28.

Nabeskima E.H., Grossmann M.V.E., 2001. Functional properties of pregelatinized and cross-linked cassava starch obtain by extrusion with sodium trimetaphosphate. Carbohydrate Polymers, 45, 347–353.

Rzedzicki Z., Kozłowska H., Troszczyńska A., 2004. Application of pea hulls for extrudate production. Pol. J. Food Nutr. Sci., 54, 13, 363–268.

Rzedzicki Z., Zarzycki P., 2005. Wpływ procesu ekstruzji mieszanek kukurydziano-owsianych na zmiany składu frakcyjnego błonnika pokarmowego. Żywność, Nauka, Technologia, Jakość, 45, 4, 62–73.

Singh B., Sekhon K.S., Singh N., 2007. Effects of moisture, temperature and level of pea grits on extrusion behaviour and product characteristics of rice. Food Chem., 100, 198–202.

Singh N., Smith A.C., 1997. A comparison of wheat starch, whole, wheat meal and oat flour in the extrusion cooking process. J. Food Eng., 34, 15–32.

Thymi S., Krokida M. K., Pappa A., Maroulis Z.B., 2005. Structural properties of extruded corn starch. J. Food Eng., 68, 519–526.

Trimnell D., Swanson C.L., Shorgen R.L., Fanta G.F., 1993. Extrusion processing of granular starch – g-Poly (methyl acrylate): Effect of extrusion conditions on morphology and properties. J. Applied Polymers Sci., 48, 1665–1675.

Willett J.L., Millard M.M., Jasberg B.K., 1997. Extrusion of waxy maize starch: melt rheology and molecular eight degradation of amylopectin. Polymer, 38, 5983–5989.

Xu Y., Hanna M.A., 2005. Physical, mechanical, and morphological characteristics of extruded starch acetate foams. J. Polymers Environm., 13, 221–223.

22

COMPARISON OF EFFECTIVENESS OF HIGH HYDROSTATIC PRESSURES (HHP) AND PASTEURIZATION PROCESS ON BIOSTATIC QUALITY OF ALOE TISSUE TOWARDS CANDIDA ALBICANS

Introduction

Yeasts of *Candida* genera are human's skin and digestion system saprophytes. The easy way for *Candida* invasion is mainly damaged mucous membrane of digestive tube (esophagus). In periods of lower body immunity, during bacterial or viral infections infectious yeasts develop and cause mycosis disease. Mycosis is usually caused by *Candida albicans, Candida glabrata, Candida crusei.* Yeasts of *Candida* genera quickly become resistant to used antibiotics. After treatment yeasts that survived in the weakened immune system circulate in the blood for 2–3 years and in that time may cause another infection. The basis for treatment should be the change of diet, starting by elimination of sugar and white flour products from it. Recommended are products of natural origin based on garlic, noni fruit and aloe, because their main task is to destroy the body of mycosis and remove toxins from the body and to strengthen the immune system by enabling vitamin-mineral compounds [Janus, 2006; Tomaszewska and Jędrzejczak, 2006; Zielińska, 2003].

With digestion system mycosis consumption of low quality milk products can introduce additional source of yeasts. From cottage cheeses opportunistic yeasts of *Candida guillermondii* have been extracted. These yeasts support mycosis development in digestive system [Steinka and Kurlenda, 2001]. Nowadays, importance of products of plant origin is noticeable and rising, especially for health protection reasons. Natural products can be sold as food products, food supplements or as medicines. There are many interesting and biologically active plant ingredients e.g. *Aloe vera* gel added to foods. Significant problem is to maintain their high biostatic activity.

The aim of this research was to evaluate the influence of high hydrostatic pressures (HHP) and the pasteurization process on biostatic activity of aloe pulp in relation to *Candida albicans.*

Material and methods

The subject of research was three-year-old *Aloe aboresces* leaves subjected to preservation process. The sample was rinsed with distilled barren water, dried and then divided into

two parts. The first part was the leaves with skin subjected to homogenization and the second part were the leaves prepared according to the Patent No. 17769. Part of prepared pulp was saved as supervisory sample. Remaining part when high hydrostatic pressure method was applied was being divided and stored in 50 ml barren containers of LDPE type. In HHP a set of pressure level and compression time was applied: 400 MPa / 10 min., 400 MPa / 20 min., 500 MPa / 10 min., 500 MPa / 20 min. in room temperature. The pascalization process was conducted in The Centre of High Pressures PAN in Warsaw. During the pasteurization process the remaining part of the pulp was divided and stored in banner polycarbonic containers of 100ml capacity, and then was being subjected to temperature of 85°C for 20 min. After the pasteurization and pressurization process the samples were stored in cooling conditions (4°C ± 2°C). For evaluation of biostatic activity of aloe pulp, *Candida albicans* received from collection of Clinical Hospital in Gdańsk, were used. Innoculum of *Candida albicans* was growth on agar YGC base with chloramphenicol produced by Merck for 120 hours at 25°C to the concentration of about 10^6 cfu / ml. Defined number of *Candida albicans* cells suspended in the broth was added to 10 or 20% of fresh and preserved aloe pulp solutions. Those concentrations were prepared for observation of the reduction the number of microorganisms. The biostatic activity was analysed by traditional plati method, by sample innoculation onto the YGC base with chloramphenicol for yeasts, in order to notice the difference between the number of yeast in innocula and after incubation with aloe.

Results and discussion

Fresh aloe with skin (AS) added to the culture of *Candida albicans* caused reduction of investigated yeasts from the amount of 7.04 $\log_{10}$ cfu/g on average to 6.58 $\log_{10}$ cfu/g (Figure 1). Addition of 10% of fresh aloe pulp without skin (BS) into *C. albicans* culture caused about 0.3 logarithm circle lower reduction of investigated microorganisms than in 20% of added pulp (Figure 1). Pasteurized homogenate with skin stored for one day and added to the culture in amount of 10% caused the lowest reduction of *Candida albicans* population. In the next days of storage the reduction was higher of about 0.3 logarithm circle (Figure 1). Pasteurized aloe pulp, stored for 30and 60 days in cooling conditions showed weak biostatic activity towards *C. albicans* population. In case of 20% aloe pulp with skin added to the culture of *Candida albicans* after 180 days from the value 6.05 $\log_{10}$ cfu/g to 6.36 $\log_{10}$ cfu/g (Figure 1). Homogenate with skin and without skin added in amount of 10% to the *Candida albicans* culture caused insignificant stimulation of investigated microorganisms (Figure 1). For statistic evaluation of biostatic activity of fresh and pasteurized aloe pulp 3 factors were considered: form of aloe (AS and BS), supplement concentration (10 and 20%) and period of storage (0, 1, 30, 60, 180 days). The statistic data analysis revealed that the level of biostatic activity towards *Candida albicans* depended only on the period of storage ($p<0.05$) (Table 1). It was observed, that for *Candida albicans* population, relatively to the storage time, the biostatic activity of aloe pulp decreased. The interaction factors were of no significant importance (Table 1).

It was stated that, estimated separately regression equations for BS form appeared to be very important ($p<0.05$). Research results affirmed that the concentration of the supplement was of no significant importance for investigated equation but the time of storage ($p<0.01$) was important factor. The exception was the relation between biostatic activity towards

Candida albicans and the time of storage marked for the form with skin where the correlation factor was: R2 = 0.369 and p = 0.20 (Table 2).

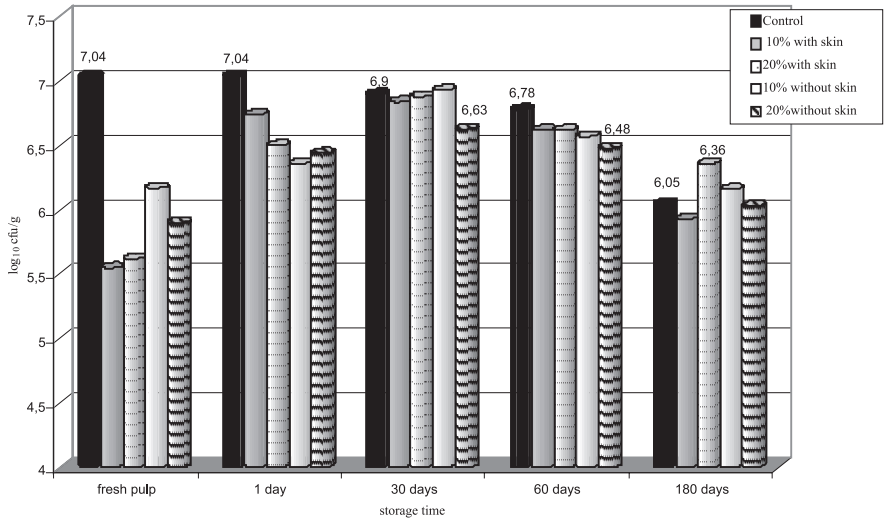

Fig. 1. Biostatic activity of pasteurized and stored in cooling conditions aloe pulp in relation to *Candida albicans*

Table 1

Influence of form, supplement and time of storage on biostatic activity of pasteurized aloe pulp. Results of analysis variation (Test F)

Type of changeability	Analyzed features (changes in micro-organisms number)	
	Candida albicans	
	F	p
Form of aloe (AS, BS)	0.0	0.98
Supplement concentration (10%, 20%)	0.2	0.67
Time of storage (0,1,30,60,180)	32.7	<0.01
Form of aloe * Supplement concentration	1.9	0.24
Form of aloe * Time of storage	2.1	0.25
Supplement concentration * Time of storage	0.5	0.77

p – level of importance

Table 2

Results of analysis of multi-regression. Influence of supplement and time of storage on biostatic activity of aloe pulp towards *Candida albicans*

Independent variables	Aloe pulp with skin (AS)		Aloe pulp without skin (BS)	
	b	p	b	p
Free term	88.828		94.180	
Supplement concentration	0.105	0.83	-0.210	0.47
Time of storage	0.074	0.08	0.062	0.02
Importance of regression equation (R^2)	R^2= 0.369	p=0.20	R2= 0.577	p=0.04

b – agent in regression equation, R^2 – correlation agent, p – level of importance

The researches revealed that fresh and preserved by high pressure aloe pulp, then stored for 1, 30 and 60 days did not show biostatic activity towards *Candida albicans* (Figure 2, 3) with the exception of aloe tissue with skin and without skin preserved by high pressure of 400 MPa for 10 and 20 min and then stored for 30 days because it caused reduction of *Candida albicans* population from the value 6.87 $\log_{10}$ cfu/g to 4.78 and 6.03 $\log_{10}$ cfu/g and 5.2 and 6.06 $\log_{10}$ cfu/g (Figure 2, 3). In the final stage of investigation it was affirmed that biostatic activity of both aloe forms increased. The addition of preserved homogenate with skin caused reduction in number of *Candida albicans* relatively of about 1.1 logarithm cycle for the pulp preserved with the pressure of 500MPa and for about 2 logarithm cycles when the 400 MPa was used (Figure 2, 3). The addition of aloe pulp without skin caused reduction of *Candida albicans* population approximately of about 1.4 $\log_{10}$ cfu/g (Figure 2, 3).

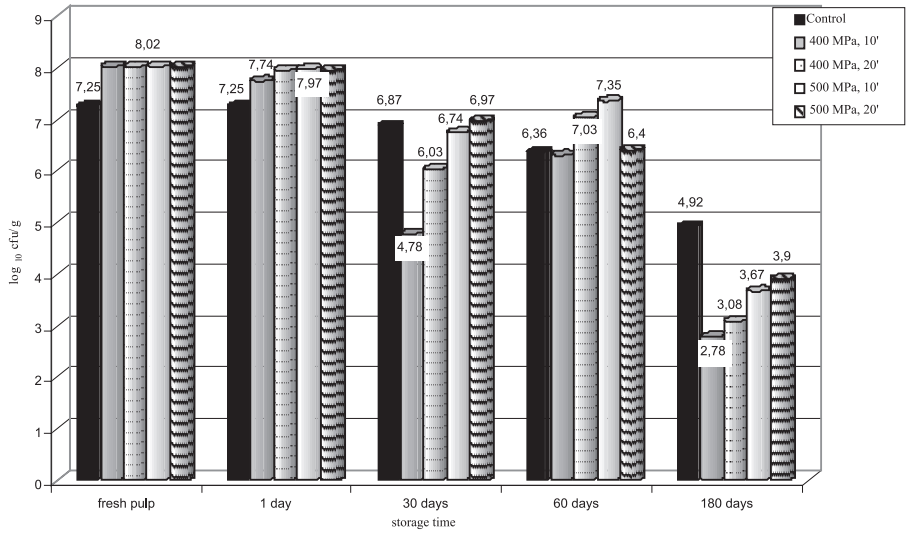

Fig. 2. Biostatic activity of aloe pulp with skin preserved by high pressure method and stored in cooling conditions in relation to *Candida albicans*

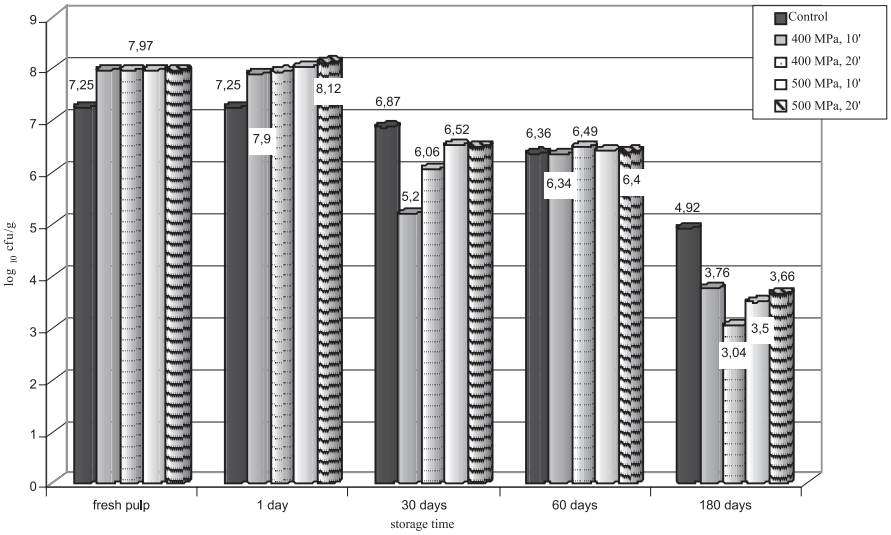

Fig. 3. Biostatic activity of aloe pulp without skin preserved by high pressure method and stored in cooling conditions in relation to *Candida albicans*

For evaluation of biostatic activity of fresh and preserved with high pressures aloe pulp four factors were the subject of consideration: the aloe form (AS and BS), the pressure level (400 and 500MPa), time of pressure treatment (10 and 20 min.), period of storage (0, 1, 30, 60, 180 days). The investigated case of biostatic activity towards *Candida albicans* showed that the level of this activity did not depend on the aloe form ($F = 0.3$; $p = 0.63$) and the time of pressure treatment ($F = 2.2$; $p = 0.21$). The remaining factors were of significant importance ($p < 0.05$) (Table 3). Among the investigated double-agent interactions important was the relation between the value of pressure and the time of storage ($p = 0.04$). For biostatic activity towards *Candida albicans* the analysis of regression equation for the tissue with skin (AS) and without skin (BS) allowed to the statement that biostatic activity depended only on the time of storage at the statistical significance level $p < 0.01$ (Table 4). The result of regression equation for AS and BS form of significant meaning were: $R2 = 0.705$; $p < 0.01$ as well as $R2 = 0.758$; $p < 0.01$ (Table 4).

There are many reports concerning the influence of plants' cells on microorganisms, however there is inadequate data covering the subject of influence of aloe pulp on bacteria and yeasts. Karwowska et al. investigating the biogenic properties of garlic in different forms proved that the strongest properties in relation to *Staphylococcus aureus, Escherichia Coli* and *Candida albicans* had fresh garlic, whereas garlic subjected to thermal processing and garlic extract showed only the activity hindering the growth of *Candida albicans*, what these investigation results present [Karwowska et al., 2007]. Anti-mycosis and anti-germal activity of garlic is connected with presence of allicin (product of disintegration of biologically inactive alliin having that characteristic smell and belonging to the highly important group of biologically active substance present in garlic) and saponins (plant steroids) [Barile et al., 2007; Karwowska et al., 2006; Kączkowski, 1993, Lutomski, 2001]. Saponins showing activity against pathogenic yeasts were isolated from the root of thorny bush *(Smilax medica)*,

plant belonging to the same family *Liliaceae* as aloe [Abad et al., 2007; Stefanowicz – Hajduk and Ochocka, 2006]. The results of aloe biostatic activity pointed to the influence of ingredients of this plant on *Candida albicans* cells. Heck et al. investigated preserved aloe extract produced industrially for public consumption and not preserved aloe extract, similar to the extract growing in households. They proved that 40% concentrated preserved aloe extract was effective when it comes to hindering the growth of selected microorganisms that is *Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae* [Heck et al., 1981]. Whereas, other authors' researches show that 60–90% concentrated aloe extract activity was germicidal for *Staphylococcus the aureus, Escherichia coli, Candida albicans* [Heck et al., 1981; Robson et al., 1982]. Received results concerning biostatic activity prove data presented by Smolarz et al., and pointing out important anti-mycosis influence of aloe-emodin towards *C.albicans* cells [Smolarz and Wegiera, 2004]. Strong biostatic effect of aloe received from *Aloe barbadensis* Mill. on *Candida albicans* is also confirmed by Wynn [Wynn, 2005]. The research results by Enoth-Arthur concerning the influence of fresh aloe juice on *Candida albicans* isolated from skin infections confirm the biostatic influence of aloe tissue on investigated microorganisms [Enoth – Arthur, 2003]. Agarry et al. in their researches concerning the comparison of anti-germal activity of gel and aloe leaves' extract (*Aloe barbadensis* Miller), revealed that only the aloe leaves' extract hindered the activity of *Candida albicans* [Agarry et al., 2005]. They stated that despite the fact that both forms have certain number of the same ingredients they remain different. However both, the leaves and the gel of *Aloe vera* are useful and can make great complementary ingredients of their medical properties [Agarry et al., 2005]. Considering the received results on biostatic activity of fresh and preserved aloe pulp and also the bibliographic data, it can be stated that these are crucial changes in population number of *Candida albicans* influencing good microbiological tissue quality when adding aloe to fermented milk products.

Table 3

Influence of form, pressure level, time of pressuring, storage time on biostatic activity of aloe pulp. Results of analysis variation (Test F)

Type of changeability	Analyzed features (changes in micro-organisms number)	
	Candida albicans	
	F	p
Form of aloe (AS, BS)	0.3	0.63
Pressure level (400 i 500 MPa)	25.4	**0.01**
Time of pressuring (10 i 20 min)	2.2	0.21
Time of storage (0, 1, 30, 60, 180)	159.4	**<0.01**
Form of aloe * Pressure level	4.1	0.11
Form of aloe * Time of pressuring	1.2	0.34
pressure level * Time of pressuring	3.0	0.16
Form of aloe * Time of storage	1.3	0.39
Pressure level * Time of storage	7.1	**0.04**
Time of pressuring * Time of storage	1.9	0.28

p – level of importance

Table 4

Results of analysis of multi-regression. Influence of high pressure level, time of pressuring and storage time on biostatic activity of aloe pulp towards *Candida albicans*

Independent variables	Aloe pulp with skin (AS)		Aloe pulp without skin (BS)	
	b	p	b	p
Free term	64.545		89.609	
Pressure level	0.087	0.09	0.037	0.35
Time of pressuring	0.314	0.52	0.048	0.90
Time of storage	-0.214	<0.01	-0.0201	<0.01
Importance of regression equation (R^2)	R^2= 0.705	p<0.01	R2= 0.758	p<0.01

b – agent in regression equation, R^2 – correlation agent, p – level of importance

Conclusions

At long-term storage in cooling conditions the form of aloe preparation did not affect its biostatic activity towards *Candida albicans*, if the tissue was subjected to high pressure treatment of 400 MPa for 10 min.

Pasteurization at 85°C for 20 min allows to preserve high biostatic activity of aloe tissue.

Homogenate with skin subjected to high pressure treatment showed insignificantly higher biostatic activity towards *Candida albicans* than homogenate without skin throughout the whole storage period. Whereas in case of pasteurized homogenate, form of aloe with skin was more effective only in case of fresh pulp.

It is proved, that received biostatic activity of pasteurized pulp depended only on the time of storage, whereas biostatic activity of pulp subjected to high pressure was additionally influenced by the level of used pressure.

References

Abad M.J., Ansuategui M., Bermejo P., 2007. Active antifungal substances from natural sources. ARKIVOC, 116–145.

Agarry O.O., Olaleye M.T., Bello-Michael C.O., 2005. Comparative antimicrobial activities of *Aloe vera* gel and leaf. Afr. J. Biotechnol., 4, 12, 1413-1414.

Barile E., Bonanomi G., Antignani V., Zolfaghari B., Sajjadi S.E., Scala F., Lanzotti V., 2007. Saponins from *Allium minutiflorum* with antifungal activity. Phytochem., 68, 596–603.

Enoth-Arthur S.M. 2003. Studies on the antiseptic and anti-microbial effects of the fresh juice of *Aloe vera*. J. Tropical Med. Plants, 4, 169-171.

Heck E., Head M., Nowak D., 1981. Aloe vera (gel) cream as a topical treatment for outpatient burns. Burns, 7, 291-294.

Janus A., 2006. Drożdżyca plagą XXI wieku.

Karwowska W., Rusaczonek A., Świderski F., Dąbrowska M., Waszkiewicz-Robak B., 2006. Właściwości antyoksydacyjne, przeciwbakteryjne i przeciwgrzybiczne czosnku i jego przetworów. Bromat. Chem. Toksykol., Supl., 59-63.

Karwowska W., Świderski F., Waszkiewicz-Robak B., 2007. Characteristics of biocide properties of garlic and its products. Pol. J. Food Nut. Sci., 57, 3A, 59–62.

Kączkowski J., 1993. Biochemia roślin. Wydawnictwo Naukowe PWN, Warszawa.

Lutomski J., 2001. Fascynacja czosnkiem – wczoraj i dziś. Postępy Fitoterapii, 5, 1.

Robson M.C., Heggers J., Hagstrom W.J., 1982. Myth, magic, witchcraft or fact? *Aloe vera* revisited. J. Burn Care Rehabilitation, 3, 157–162.

Smolarz H. D., Wegiera M., 2004. Antrachiniony – składniki roślinne o właściwościach nie tylko przeczyszczających. Postępy Fitoterapii, 14, 4/2004.

Stefanowicz-Hajduk J., Ochocka J.R., 2006. Saponiny steroidowe – występowanie, właściwości i zastosowanie w lecznictwie. Postępy Fitoterapii, 1, 36–40.

Steinka I., Kurlenda J., 2001. Ocena zagrożeń mikrobiologicznych związanych z systemem i hermetyką pakowania twarogów. Med. Wet, 57, 10, 757–761.

Tomaszewska A., Jędrzejczak W.W., 2006. Propozycja schematu postępowania w leczeniu inwazyjnych zakażeń grzybiczych u pacjentów hematoonkologicznych. Zakażenia, 6, 4, 67–74.

Wynn R.L. 2005., *Aloe vera* gel: Update for dentistry. General Dentistry, styczeń-luty, 6–9.

Zielińska E. 2003. Kontrowersje dotyczące optymalnej profilaktyki i leczenia zakażeń grzybami w stanach obniżonej odporności. Przegl. Epidemiol., 57, 299–307.

23

CONCENTRATION OF BY-PRODUCTS IN RYE AGRICULTURAL DISTILLATES OBTAINED BY USING THE PRESSURE COOKING AND THE PRESSURELESS (PLS) METHODS OF STARCH LIBERATION

Introduction

Significant changes, related also to raw materials, have been observed in Polish distilleries during the last several decades. In Poland, ethanol has been produced mainly from starch. However, resources of this polysaccharide have changed. About 20–30 years ago, spirit was produced mainly (more than 80% overall production) from potato starch but in 1997 only 12.4% spirit was derived from this commodity [Samborski, 2005]. Recently, spirit production in agricultural distilleries has been based on cereals – mainly on rye, but also on maize [Jarosz, 1998]. Traditional rye breeding in Poland is justified by poor quality of soils because light and very light soils having low or even very low pH account for more than 30% arable land. In consequence, yields of crops are relatively small. Rye is an exception to this rule because its average yield reaches 2.74 t/ha. The volume of ethanol produced in distilleries approaches 390 l per 1 tone of this cereal (for a mean content of starch in rye grains of 62%) [Grabiński, 2006; Łączyński, 1993].

In the global scale, Poland, Germany and Russia are the principal producers of rye, however, the quality of Polish rye is not good. This results from the very low share of qualified seeds (recently, it has been close to only 7%) among rye seeds sown by farmers. Despite motivating financial support from the government Polish farmers do not purchase qualified rye seeds but continue sowing seeds contaminated with seeds of other cereals and susceptible to infections and changes in quality parameters. Application of high quality seeds is advantageous both for farmers and crop processing enterprises like agricultural distilleries because of:

- higher yield of crops,
- good quality of cereals (higher quality of feed for animals, economized processing caused by lesser amounts of by-products and chemical agents used to improve the quality of products),
- high quality of products derived from crops, satisfied demand for food in the country, greater demand for these crops and higher sale prices [Marciniak, 2005].

All these benefits resulting from the usage of qualified seeds apply also to agricultural distilleries. High quality of distillates is of utmost importance for these enterprises and chemical composition of these products depends upon the quality of raw materials. The relevant Polish standard determines the highest permitted concentrations of certain fermentation by-products such as methanol, aldehydes, esters and carboxylic acids in agricultural distillates

[PN-A-79523:2002]. However, this standard does not specify concentrations of higher alcohols (also named fusel oils), which are concerned in the Polish standard related to (bioethanol) PN-A-79521 [PN-A-79521:1999].

Chemistries of by-products formation are very complex and affected by numerous factors like the quality of raw materials, mashing method, density and pH of mashes, yeast strain, volumes of inoculum and sweet mash, and the temperature at which fermentation is carried out [Arrizon and Gschaedler, 2007, Czupryński and Kłosowski, 1994, Kłosowski et al., 2006, Kotarska et al., 2006, Zielińska and Miecznikowska, 1994].

Main factors deciding of aldehyde concentration in the mash on completion of fermentation process embrace concentration of sugars in mashes and fermentation parameters such as pH and temperature. These factors influence enzymatic processes or modify their final steps yielding either ethanol or higher alcohols. Besides, contamination of fermenting mash with sporulating bacteria and heterofermentative lactic acid bacteria results in formation of 3-hydroxypropionic aldehyde (acrolein precursor) [Kłosowski et al., 2003a,b, Kłosowski and Czupryński, 1993, Łączyński, 1995, Wasiak, 2000].

Sources of carboxylic acids in fermented mashes (mainly acetic and lactic acids) are metabolic processes of yeasts and microbial contaminants. Formation of acids takes place only when concentration of oxygen is sufficient because the latter is essential for bacterial strains which are capable of oxidizing ethanol to acetaldehyde and then to acetic acid [Goj, 1992].

Condensation reactions of organic acids and alcohols produce esters. Formation of these compounds is affected by quality of raw materials, yeasts strains, pH of mashes and their microbiological purity [Kłosowski et al., 2003b, Kłosowski and Czupryński, 2006].

Occurrence of methanol in cereal spirits results first of all from thermal pretreatment (at 80÷90^{o}C) of raw materials giving rise to partial hydrolysis of pectic substances [Kłosowski et al., 2003b].

Higher alcohols, which are also termed fusel oils, constitute the other significant group of alcoholic fermentation by-products. Their total concentration and concentration of individual compounds in fermented mashes is determined by many factors like the quality of raw materials, yeast strain, chemical composition of mashes and conditions of fermentation and distillation [Kłosowski et al., 2003b, Kłosowski et al., 2001, Mauricio et al., 1997, Oaxaca and Jones, 2004]. This group of compounds is particularly interesting due to complex pathways of their synthesis which were investigated by Ehrlich, Neubauer and Fromherz, Guymon, Sentheshanmuganathan, Genevois and Lafon and other researchers. Their studies revealed that fusel oils were products of deamination and decarboxylation of proteinogenic amino acids. Ehrlich proved that isobutanol was formed from valine, 3-methyl-1-butanol - from leucine and 2-methyl-1-butanol - from isoleucine. Neubauer and Fromherz described the 3-step enzyme-catalyzed conversion of leucine to isoamyl alcohol beginning from oxidative deamination of this amino acid to α-ketoisocaproate, which is then decarboxylated to isovaleryl aldehyde. The latter compound is reduced to isoamyl alcohol. Sentheshanmuganathan showed that tyrosine was a precursor of tyrosol and thereby corroborated results of studies performed by Ehrlich, Neubauer and Fromherz [Garde-Cerdán and Ancin-Azpilicueta, 2008, Hazelwood et al., 2008, Ingraham and Guymon, 1960, Oaxaca and Jones, 2004, Rao and Jones, 2004, Reazin et al., 1973]. Investigations of Nordstrŏm and Guymon revealed that certain α-keto acids were intermediates of synthesis processes of both amino acids and higher alcohols [Guymon, 1960]. Another pathway of higher alcohols synthesis was postulated by Genevois and Lafon, who found that some of these compounds were sugar derivatives. Some

yeast and bacterial species convert pyruvate derived from sugar catabolism to α-acetolactate and decarboxylate the latter to produce acetone. This ketone is either reduced to isopropanol or condensed with acetaldehyde to β-methylocrotonyl aldehyde and then converted to 3-methyl-1-butanol.

All these investigations provided evidence that fusel oils present in agricultural distillates were products of conversions of amino acids and sugars as well as aldehydes derived from the first compounds.

This study aimed at determination of the effect of such factors as the method used to liberate and saccharify starch, the strain of fermenting yeasts and pH of mashes subjected to fermentation on qualitative and quantitative composition of cereal distillates derived from 3 rye varieties.

The scope of the study embraced:

- preparing of sweet mashes by the pressure cooking (steaming) method,
- preparing of sweet mashes by the pressureless starch liberation method (PLS),
- controlled fermentations of mashes,
- distillation of ethanol,
- analysis of raw spirits,
- gas chromatography analysis of raw spirits.

Materials and methods

The following materials were used throughout the study:

1. Grains of 3 winter rye varieties: Dańkowskie Golden, Dańkowskie Diamond and Amilo, purchased from "Danko" Plant Breeding Ltd.,
2. Distillery yeast *Saccharomyces cerevisiae* – 2 strains: As4 and D_2, selected in the Institute of Biotechnology of Agriculture and Food Processing in Warsaw and produced by Kashubian Yeast Factory in Maszew Lęborski.
3. Enzyme preparations from Novozymes (Denmark): Termamyl S.C. (α-amylase), SAN Extra (glucoamylase).

Variants of rye pretreatment and fermentation conditions (*rye variety*; *rye pretreatment method*; *pH of sweet mash*; *yeast strain*) were as follows:

I – Dańkowskie Diamond; steaming; pH = 4.3; D_2
II – Dańkowskie Diamond; steaming; pH = 4.3; As4
III – Dańkowskie Diamond; PLS; pH = 4.8; D_2
IV – Dańkowskie Diamond; PLS; pH = 4.8; As4
V – Dańkowskie Diamond; PLS; pH = 4.3 D_2
VI – Dańkowskie Diamond; PLS; pH = 4.3; As4
VII – Amilo; steaming; pH = 4.8; D_2
VIII – Amilo; steaming; pH = 4.8; As4
IX – Amilo; PLS; pH = 4.8; D_2
X – Amilo; PLS; pH = 4.8; As4
XI – Amilo; PLS; pH = 4.3; D_2
XII – Amilo; PLS; pH = 4.3; As4
XIII – Dańkowskie Golden; steaming; pH = 4.8; D_2
XIV – Dańkowskie Golden; steaming; pH = 4.8; As4

XV – Dańkowskie Golden; PLS; pH = 4.8; D_2
XVI – Dańkowskie Golden; PLS; pH = 4.8; As4
XVII – Dańkowskie Golden; PLS; pH = 4.3; D_2
XVIII – Dańkowskie Golden; PLS; pH = 4.3; As4

Preparing of sweet mashes:

Pressureless liberation of starch

Sweet mashes were produced by using a cylindrical vessel, which was placed in a water bath and equipped with a double blade impeller and a thermometer.

Prior to mashing, rye grains were ground in a disk mill to particles with dimensions below 1.5 mm. Then 3.5 L of water was added per each 1 kg of ground rye grains and the temperature of this suspension was gradually increased to 90°C with continuous agitation. Simultaneously, a starch-liquefying enzymatic preparation (Termamyl S.C.) was added (according to manufacturer's recommendation: 0.12–0.13 L per 1 tone of starch). Starch liquefaction was carried out for 10 minutes. Then the temperature of the hydrolysate was decreased to 66 ÷ 67°C and it was supplemented with a saccharifying enzyme preparation (SAN Extra) in a dose recommended by its manufacturer (0.6 L per 1 tone of starch). Saccharification was carried out at the latter temperature for approximately 30 minutes. The degree of saccharification was determined by using a solution of iodine in potassium iodide.

Steaming

The process of pressure assisted thermal mashing was conducted by using a cylindrical-tapered steamer (of Henze) equipped with a steamer and a mash tub, in which processes of starch liquefaction and saccharification took place. Prior to the steaming the content of the steamer was periodically circulated. The steaming was carried out for 40 minutes at 151.1°C (0.4 MPa). On completion of steaming the mash was pumped to the mash tub (equipped with a stirrer and a coil) and mashing was carried out like in the PLS method.

Yeast cream preparation

Fermentation processes were conducted by using dried distillery yeast (strains D_2 and As4) in a dose of 0.3 g s.s./L sweet mash. To eliminate microbial contaminations, portions of dried yeasts suspended in small water aliquots were acidified with approximately 30% sulfuric acid to pH ≈ 2.5 and kept for 15 ÷ 20 minutes.

Fermentation

Alcoholic fermentation was carried out in 6 L flat bottom flasks containing 4 L of a mash. Their pH was adjusted to 4.3 or 4.8 and then the mashes were supplemented with diammonium phosphate (0.2 g/L) and inoculated with portions of yeast cream (strains D_2 and As4) in a dose of 0.3 g s.s./L mash. The flasks were closed with stoppers equipped with fermentation pipes filled with glycerol. Fermentation was conducted at 28–30°C for 3 days.

Analysis of raw spirits

Alcohol obtained through fermentation was distilled from fermented mashes and concentrated to 40% (v/v) by using birectifiers [PN-93/A-79528/03]. Raw spirits produced by this method were analyzed according to the relevant standard [PN-A-79523:2002] for: carboxylic acids [PN-A-79528-7:2001], esters [PN-A-79528-8:2000], aldehydes [PN-A-79528-

4:2000], fusel oils [PN-A-79528-5:2000] and methanol [PN-A-79528-6:2000]. The concentrations of these compounds were reported as g of the compound per 1000 mL of anhydrous (100% v/v) ethanol produced through fermentation.

Gas chromatography analysis of raw spirits

Raw spirits were quantitatively analyzed for higher alcohols such as: n-propanol, 2-methyl-1-propanol (isobutanol), 2-methyl-1-butanol (optically active isoamyl alcohol) and 3-methyl-1-butanol (achiral isoamyl alcohol). 4-heptanon was the internal standard. Results of this analysis are shown in Table 1 presenting concentrations of higher alcohols reported as g per 1000 mL of anhydrous ethanol in the spirits.

Raw spirit samples were analyzed by using Agilent 6890N gas chromatograph (USA), equipped with flame-ionisation detector (FID), Split/Splitless injector and capillary column HP-FFAP (Agilent) (with dimensions of 30 m x 0.25 mm x 0.25 μm). The stationary phase was polyethylene glycol modified with nitro-terephtalic acid. GC conditions were as follows: injector: splitless (250^{o}C), detector: FID (260^{o}C), temperature program: 40^{o}C; 5^{o}C/min. do 100^{o}C; 6^{o}C/min. do 240^{o}C (5 min.), flow rate of carrier gas (helium) through the column: 1.2 mL/min.

Results and discussion

Raw spirits obtained through distillation of fermented mashes were concentrated to 40% v/v by birectification and subjected to chemical analysis according to the standard [PN-A-79523:2002]. Results of these assays are presented in Figures 1÷5.

These distillates differed in concentrations of by-products that were affected by starch liberation method, pH of mash and yeast strain.

Concentration of aldehydes (shown as concentration of acetaldehyde) in the raw spirits varied between 0.043 and 0.068 g/L alcohol 100% v/v (Fig. 1). The lowest aldehyde concentrations were observed in spirits produced from mashes obtained by PLS method. Other authors [Kłosowski and Czupryński, 1993] postulate that the presence of fermentation by-products (also aldehydes) in raw spirits is a result of inappropriate conditions of pressure cooking, in particular too high temperature of steaming or too long time of this process. The latter conditions stimulate formation of alcohol dehydrogenase inhibitors that slow down reduction of acetaldehyde to ethanol.

Spirits derived from Amilo variety contained by 15 and 12.2% more aldehydes as compared to that produced from varieties Dańkowskie Diamond and Dańkowskie Golden, respectively (Fig. 1). It was found that the strain of fermenting yeast only slightly affected concentration of aldehydes in raw spirits because distillates from mashes fermented by strain D_2 contained only 1.3% more aldehydes than spirits produced by using strain As4 (Fig. 1). According to literature data, aldehyde content in spirits is to some extent dependent on yeast strain and to prevent aldehyde formation, fermentation processes should be conducted only by attested distillery yeast strains [Kłosowski and Czupryński, 1993, Łączyński, 1995].

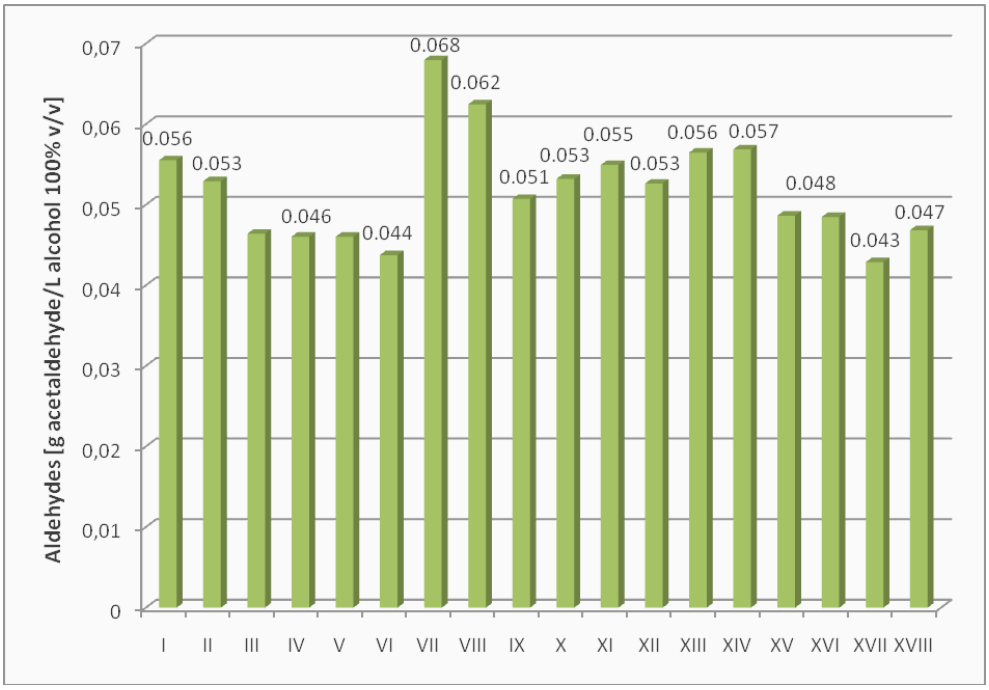

Fig. 1. Concentration of aldehydes in the distillates

Concentration of methanol in the investigated raw spirits was very low and varied between $1.04 \cdot 10^{-3}$ and $8.501 \cdot 10^{-3}$ g/100 mL alcohol 100% v/v (Fig. 2). Spirits based on mashes produced by PLS method contained twice less methanol ($1.04 \cdot 10^{-3} \div 5.791 \cdot 10^{-3}$ g/100 mL alcohol 100% v/v) than their counterparts obtained by means of pressure cooking method. Like formation of aldehydes also generation of methanol is stimulated by too high temperature of steaming or too long duration of this process [Czupryński and Kłosowski, 1994]. It is known that methanol does not rank among alcoholic fermentation by-products but is released at 80÷90°C through partial hydrolysis of pectin which is contained in plant raw materials [Kłosowski et al., 2003b]. When fermentation processes were conducted by strain D_2 raw spirits contained by 13.65% more methanol as compared to spirits produced with the aid of strain As4. It was also found that methanol content in distillates was by more than 50% higher when mashes obtained by means of PLS method had pH of 4.8 (versus distillates from mashes with pH of 4.3).

Our analyses revealed no correlation between the concentration of carboxylic acids in raw spirits, mashing method and pH of mashes. The first quantity was affected only by the yeast strain – spirits produced by using strain As4 contained by 10% less carboxylic acids (Fig. 3).

Concentration of esters in the investigated raw spirits was almost the same irrespective of mashing method, pH of mashes and yeast strain (spirits produced from mashes fermented by strain As4 contained by 6% less esters – Fig. 4).

Concentration of fusel alcohols (shown as an equimolar mixture of isoamyl and isobutyl alcohols) in the spirits varied between 4.784 and 8.134 g/L alcohol 100% v/v (Fig. 5).

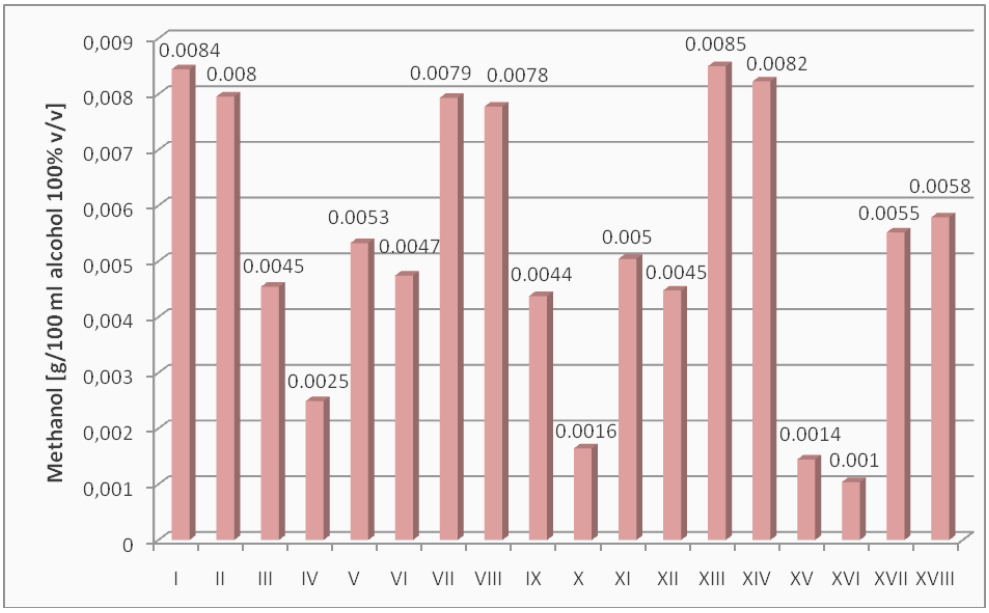

Fig. 2. Concentration of methyl alcohol in the distillates

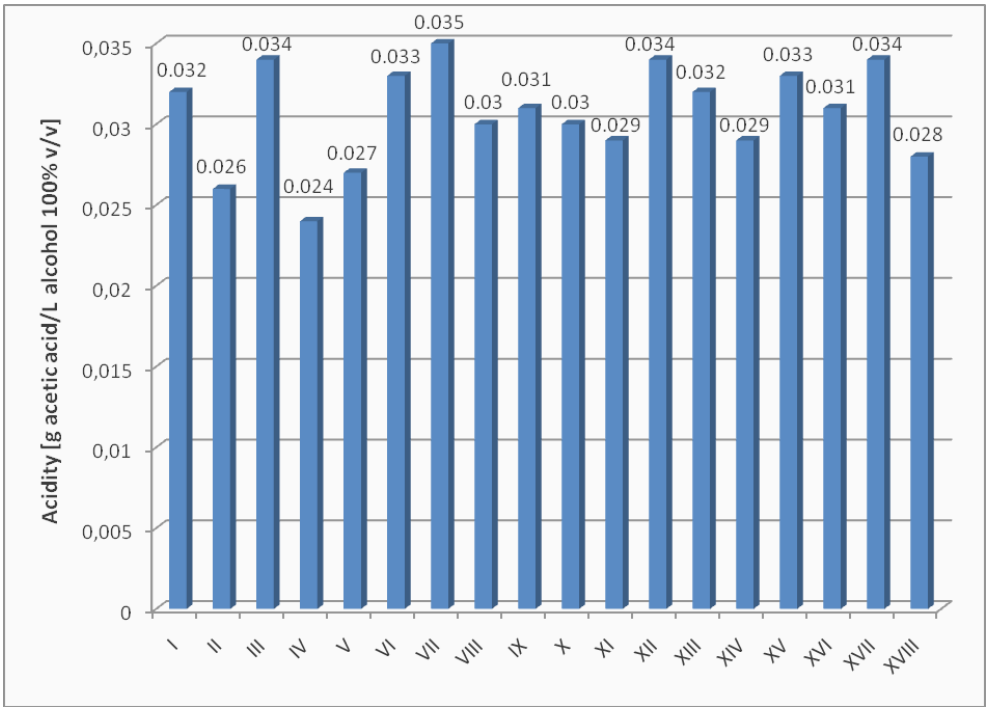

Fig. 3. Concentrations of carboxylic acids in the distillates

Fig. 4. Concentrations of esters in the distillates

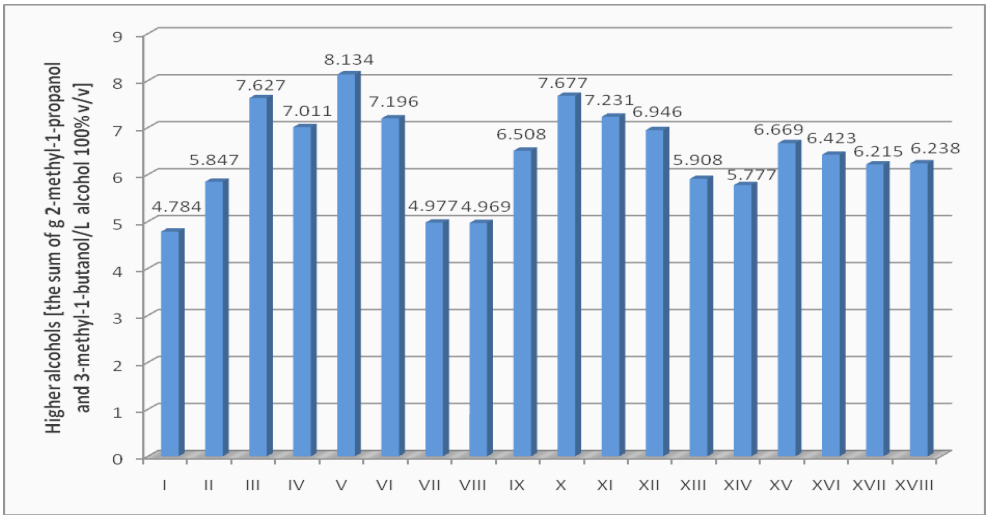

Fig. 5. Concentrations of higher alcohols in the distillates

Chemical analyses revealed no correlation between yeast strain and concentration of fusel alcohols. Also pH of mashes prepared by PLS method had no impact on the latter quantity. Concentration of higher alcohols in distillates produced from the investigated rye varieties was similar with an exception of raw spirits from Dańkowskie Diamond variety which contained by 8.3 and 5.6% more fusel alcohols as compared to that from Dańkowskie Golden and Amilo varieties, respectively. Only raw material preparation method (before mashing) affected the content of higher alcohols in raw spirits which was on average by 23.1% lower for pressure cooking method than for PLS method. This difference is thought to result from

the high temperature inside the steaming which caused denaturation of proteins contained in ground rye grains. In consequence, the content of amino acids which are precursors of fusel oils was increased.

GC analysis of raw spirits showed that the most abundant of them were: n-propanol, 2-methyl-1-propanol (isobutanol), 2-methyl-1-butanol (chiral isoamyl alcohol) and 3-methyl-1-butanol (achiral isoamyl alcohol). Concentrations of these compounds in the analyzed samples are presented in Table 1.

Table 1

Concentrations of higher alcohols in the distillates

Variant	n-propanol (propan-1-ol) [g/L alcohol 100% v/v]	Isobutanol (2-methyl-1-propanol) [g/ L alcohol 100% v/v]	Σ Isoamyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) [g/ L alcohol 100% v/v]
	0.760	0.329	2.177
	0.999	0.428	2.702
	0.148	0.620	4.049
	0.387	0.801	5.227
	0.100	0.497	3.768
	0.324	0.683	5.168
	0.930	0.418	3.119
	0.806	0.425	2.911
	0.375	0.645	3.587
	0.194	0.544	3.307
	0.069	0.378	2.165
	0.272	0.526	3.496
	0.697	0.470	3.734
	0.848	0.343	2.539
	0.203	0.412	3.353
	0.548	0.564	4.925
	0.472	0.581	4.629
	0.415	0.445	3.331

Our assays showed that the concentration of fusel alcohols determined by colorimetric method was approximately 1.35÷1.5 – fold higher than results of GC. This finding is consistent with results of Milewski and Muszkat [1971] who estimated the content of fusel alcohols in various raw spirits by means of these 2 methods. They proved that results from the colorimetric assays were higher than results of GC because also some other components of raw spirits give positive reaction in the first method.

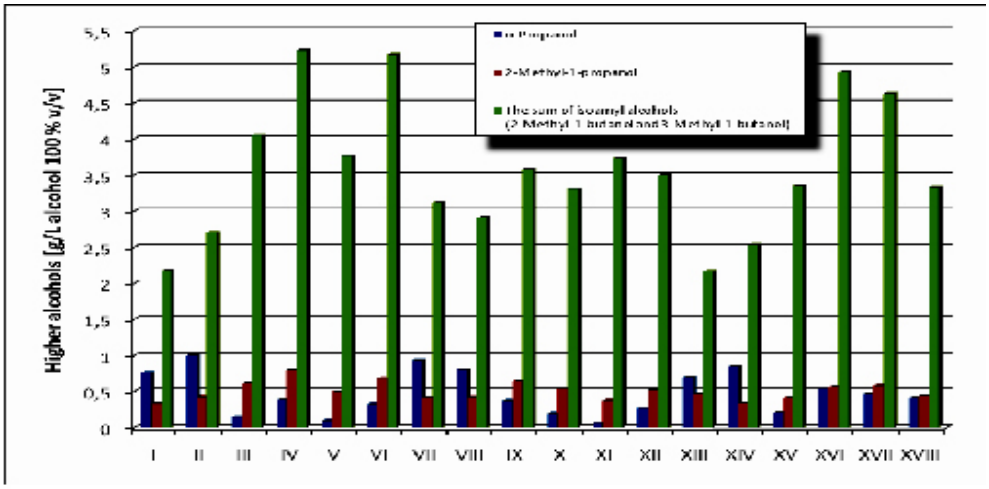

Fig. 6. Concentrations of higher alcohols in rye distillates

Our experiments showed that the method of starch liberation and saccharification considerably affected concentration of higher alcohols in the analyzed raw spirits. GC analysis proved that it was lower (by 24.2%) in the distillates from mashes obtained by pressure cooking method (as compared to that produced with the aid of PLS method). The most abundant of them were isoamyl alcohols (the sum of 3-methyl-1-butanol and 2-methyl-1-butanol) which accounted for 65.44 ÷ 86.32% total fusel oils.

The mean n-propanol : isobutanol ratio in spirits derived by means of pressure cooking method was close to 2.1:1 while in the counterpart spirits it dropped to 1:2.4 (Fig. 6). Thus this ratio is characteristic of the method of mashing. Like the chemical assays also GC analyses of the raw spirits revealed higher content of fusel alcohols (by 10.1 and 8.6%) in spirits from Dańkowskie Diamond variety as compared to distillates from Dańkowskie Golden and Amilo varieties, respectively. pH of sweet mashes produced by PLS method had no impact on fusel oil content in raw spirits. Distillates from mashes fermented by yeast of strain D_2 contained approximately 10% less fusel alcohols (as compared to strain As4). This finding is consistent with results reported by researchers from the Institute of Biotechnology of Agriculture and Food Processing in Bydgoszcz [Czupryński and Kłosowski, 1994].

Regarding these results it is obvious that GC should be applied for qualitative and quantitative analysis of fermentation by-products like fusel alcohols in raw and rectified spirits [Witkowska, 1991].

Conclusions

The method of starch liberation and saccharification has a strong impact on concentration of certain by-products of alcoholic fermentation in raw spirits: The lowest concentrations of methanol and aldehydes were found in spirits derived from mashes prepared by means of PLS method, Concentrations of carboxylic acids and esters in the distillates were

at the same level irrespective of the mashing method, Concentrations of higher alcohols in raw spirits obtained from mashes produced by pressure cooking method was by 24.2% lower as compared to that in spirits derived from mashes produced by PLS method, The mean n-propanol : isobutanol ratios in raw spirits produced from mashes derived by pressure cooking and PLS methods were 2.1:1 and 1:2.4, respectively.

Methanol concentration in distillates derived from mashes produced by PLS method and with pH adjusted to 4.8 was higher (by 50.3%) as compared to raw spirits obtained from mashes with pH adjusted to 4.3.

Analyses of raw spirits derived from mashes produced by PLS method revealed that pH of mashes had no impact on concentration of higher alcohols.

There was no correlation between a rye variety and concentrations of all the assayed by-products contained in raw spirits: Raw spirits obtained from Dańkowskie Diamond variety were characterized by the highest concentration of fusel alcohols, the highest concentrations of aldehydes were found in raw spirits obtained from Amilo variety.

Concentration of some by-products in raw spirits depended on yeast strain: When fermentation was conducted by strain D_2 concentration of higher alcohols was approximately 10% lower (as compared to strain As4), Agricultural distillates from spirits fermented by strain D_2 contained by 7.5% more carboxylic acids, esters, methanol and aldehydes than spirits obtained with the aid of strain As4.

Among the assayed higher alcohols dominate isoamyl ones (the sum of 2-methyl-1-butanol and 3-methyl-1-butanol) which account for 65.44 ÷ 86.32% total fusel oils.

References

Arrizon J., Gschaedler A., 2007. Effects of the addition of different nitrogen sources in the tequila fermentation process at high sugar concentration. J. Apl. Microbiol., 102, 1123–1131.

Czupryński B., Kłosowski G., 1994. The effect of yeast strain and enzymes on the quality of rye raw spirit and vinasse. Przem. Ferm. Owocowo-Warzywny, 10, 16–20.

Garde-Cerdán T., Ancin-Azpilicueta C., 2008. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. LWT-Food Sci. Technol., 41, 501–510.

Grabiński J., 2006. Rye- Polish cereal. Wiadomości Rolnicze Polska, 9(25), 20–21.

Goj T., 1992. Dominating carbonyl compounds in spirits and alcoholic beverages. Przem. Ferm. Owocowo-Warzywny, 2, 5–7.

Hazelwood L.A., Daran J.M., Maris A.J.A., Pronk J.T., Dickinson R., 2008. The Ehrlich pathway for fusel alcohol production: a century of research on Saccharomyces cerevisiae metabolism. App. Environm. Microbiol., 74(8), 2259–2266.

Ingraham J.L., Guymon J.F., 1960. The formation of higher aliphatic alcohols by mutant strains of Saccharomyces cerevisiae. Arch. Biochem. Biophys., 88, 157–166.

Jarosz L., 1998. Production and usage of spirits in Poland. Przem. Ferm. Owocowo-Warzywny, 8, 29–31.

Kłosowski G., Czupryński B., Kotarska K., Wolska M., 2003a. Characterization of chemical contaminants worsening the quality of raw spirits (1). Przem. Ferm. Owocowo-Warzywny, 6, 20–21.

Kłosowski G., Czupryński B., Kotarska K., Wolska M., 2003b. Characterization of chemical contaminants worsening the quality of raw spirit (2). Przem. Ferm. Owocowo-Warzywny, 9, 37–38.

Kłosowski G., Czupryński B., Wolska M., 2006. Characteristics of alcoholic fermentation with the application of Saccharomyces cerevisiae yeasts: As-4 strain and I-7-43 fusant with amylolytic properties. J. Food Eng., 76, 500–505.
Kłosowski G., Czupryński B., 1993. Factors responsible for formation of carbonyl compounds, in particular acetaldehyde, in raw spirits – a review. Przem. Ferm. Owocowo-Warzywny, 5, 8–10.
Kłosowski G., Czupryński B., Sieliwanowicz B., Kotarska K., Wolska M., 2001. Monitoring of sugar substrates utilization by D-2 and As-4 yeasts and kinetics of by-products formation during alcoholic fermentation of rye and corn mashes. Pol. J. Food Nutr. Sci., 10/51, 2, 19–24.
Kotarska K., Czupryński B., Kłosowski G., 2006. Effect of various activators on the course of alcoholic fermentation. J. Food Eng., 77, 965–971.
Łączyński B., 1995. Reasons of too high concentration of aldehydes in raw spirits and methods of prevention in agricultural distilleries. Przem. Ferm. Owocowo-Warzywny, 2, 13–15.
Łączyński B., 1993. Production of alternative fuels in agricultural distilleries – tradition and potential. Przem. Ferm. Owocowo-Warzywny, 9, 13–15.
Marciniak K., 2005. Conditions of crop breeding in Poland. Hodowla Roślin i Nasiennictwo, Wydawnictwo Polskiej Izby Nasiennej, 4.
Mauricio J.C., Moreno J., Zea L., Ortega J.M., Medina M., 1997. The effects of grape must fermentation conditions on volatile alcohols and esters formed by Saccharomyces cerevisiae. J. Sci. Food Agric., 75, 155–160.
Milewski J., 1971. ISO, GOST i PN. Przem. Ferm. Rolny, 4, 7.
Oaxaca V.A., Jones L.P., 2004. Formation of ethanol and higher alcohols by immobilized Zymomonas mobilis in continuous culture. Acta Biotechnologica, 11, 6, 523–532.
Polish Standard. PN-93/A-79528/03 Spirit (ethanol). Methods of analysis – assays of concentration, with revisions in PN-A-79528-3/A1:1995.
Polish Standard. PN-A-79521:1999 Absolute ethanol.
Polish Standard. PN-A-79523:2002 Agricultural distillates.
Polish Standard. PN-A-79528-4:2000 Spirit (ethanol). Methods of analysis – Determination of aldehyde concentration.
Polish Standard. PN-A-79528-5:2000 Spirit (ethanol). Methods of analysis – Determination of fusel alcohols concentration.
Polish Standard. PN-A-79528-6:2000 Spirit (ethanol). Methods of analysis – Determination of methanol concentration.
Polish Standard. PN-A-79528-7:2001 Spirit (ethanol). Methods of analysis – Determination of acidity.
Polish Standard. PN-A-79528-8:2000 Spirit (ethanol). Methods of analysis – Determination of esters concentration.
Rao S.C., Jones L.P., 2004. Formation of higher alcohols and phenol by strains of Zymomonas sp. Acta Biotechnologica, 7, 3, 209–219.
Reazin G., Scales H., Andreasen A., 1973. Production of higher alcohols from threonine and isoleucine in alcoholic fermentations of different types of grain mash. J. Agric. Food Chem., 21, 1, 50–54.
Samborski S. 2005. Industrial potato processing. Polska Wieś w Europie, Fundacja Fundusz Współpracy, Biuro Programów Wiejskich, Warszawa, styczeń 2005.
Wasiak M., 2000. Acrolein – formation, occurrence and properties. Przemysł Fermentacyjny i Owocowo-Warzywny, 5, 20–24.
Witkowska E., 1991. Application of gas chromatography for analysis of rectificate and molasses raw spirit. Przem. Ferm. Owocowo-Warzywny, 1, 10–11.
Zielińska K., Miecznikowska A., 1994. Effect of selected steps of technological process on concentration of fermentation by-products in raw spirits. Part I. Effect of yeast strains on concentration of fermentation by-products in raw spirits –model investigations. Przem. Ferm. Owocowo-Warzywny, 7, 7–10.

24

COLOUR CHANGES OF FREEZE-DRIED STRAWBERRIES OSMOTICALLY DEHYDRATED BEFORE DRYING

Introduction

Colour, texture and aroma are the main quality attributes of food influencing consumer acceptability of food products. During processing these attributes may be lost or altered depending on the water content in foods, particularly in dried foods. Many approaches have been taken to improve the quality of fruit products. One common approach is to apply less invasive process and the other is to use specific additives [Rey and May, 1999].

Strawberry is one of the world's largest fruit crops [Doymaz, 2008]. Strawberries assigned to consumption in fresh state have better taste and aroma when they are collected in consumption ripeness state (whole surface is coloured uniformly). They are sensitive on mechanical damage and wrong tolerate transport on farther distance [Płocharski, 2002].

Application of suitable processing technology is necessary because of excessive production of fresh strawberries [Alvarez et al., 1995]. In food technology strawberries have many applications. Preserved fruit occurs as a frozen and dried food, which are obtained with the use of different method: osmotic dehydration, as a fruit charge in dairy industry, in fruit-vegetable industry to juice production, jams and in distilling of alcohol [El-Beltagy and Gamea, 2006]. Every of those methods of preserving and processing cause quality changes in comparison to raw material [Moreno et al., 2000].

Freezing is one of most important preservation method, because cause chemical reaction rate slow down, but simultaneously physical properties become deterioration, especially after defrosting. There is liquid faze concentration and ice crystals increase, which fruit structure damage [Gruda and Postolski, 1999]. This method additionally cause production cost increase and loss of important quality components [Chiralt et al., 2001; Agnelli and Mascheroni, 2002]. Frozen strawberries have received special attention in recent years. Freezing is a preservation process that may cause severe changes to tissues, resulting in excessive softening. The freezing rate is a variable recognized as responsible for tissue damage [Fuchigami et al., 1997] and can result in unacceptable or sub-optimal product characteristics after thawing.

The drying of fruits can be an excellent alternative to make their shelf-life longer and commercialization easier. It allows conversion of perishable materials into stabilized products by lowering water activity to appropriate levels, thus preventing microbial spoilage and quality deterioration due to undesirable biochemical reactions. Facility for transportation, storage and handling of dried fruits are also important factors in globalized world. Moreover, drying reduces wastes and post-harvest losses and might allow that whole production is absorbed by the food industry and distribution sectors. Finally, the development of dried

fruits, which maintain the relevant sensory properties as unaltered as possible and present the convenience of ready-to-eat products, can contribute for commercialization of higher value added products [Marques et al., 2009].

Nevertheless, loss of quality during drying is a major problem that limits the market demand for dry food products. Relatively gentle drying process such as freeze-drying is commonly used since the biological value of the material, structure, flavour, aroma, and colour are retained in the product. Continuous innovation and process optimization have increasingly led to more and more new applications of this drying process on an industrial scale [Rey and May, 1999]. One, probably the most important, way to reduce adverse influence of drying on food quality or to create usual properties of the final product is to design carefully the process and carry it on consciously [Lewicki, 2006].

Freeze-drying has been studied and used as a single process or in combination with other techniques to minimize the adverse quality changes associated with dried products [Shishehgarha etal., 2002].

The use of additives can influence physicochemical properties, aroma, texture and colour, and can improve the overall quality of the product [Kopjar et al., 2008].

Osmotic dehydration is an important technology that enables both the removal of water from the product and the modification of its functional properties by the impregnation of desired solutes. The process is often applied as a pretreatment process for fruits and vegetables, which reduces the physical, chemical and biological changes during drying at high temperature [Long-yuan Li, 2006]. The osmotic treatment has been used mainly as pretreatment to some conventional processes such as freezing, freeze drying, vacuum drying and air drying in order to improve final quality, reduce energy costs or even to develop new products [Sereno and Hubinger, 2001, 1997] and can also minimize drying color losses [Nsonzi and Ramaswamy, 1998].

One of the best method to prevent cell walls damage during freezing is additional substances application [Alvarez et al., 1995]. Fruit freezing with sucrose allows to behavior colour and test of strawberries. Senga Sengana strawberries slices osmotically dehydrated before freezing in 20% glucose solution under reduced pressure caused decrease effluent during defrost a swell as final texture [Bengtsson and Fernquist, 1971]. Usual one part of dry sugar on four or five parts fruit is added before freezing [Wrolstad et al., 1990].

Strawberries can be pre-treated before freezing by osmotic dehydration that is mixing them with sugars or syrups, because these additives sweeten the product, contribute to retain volatile compounds, reduce the water amount to freeze and decrease the browning by acting as a barrier to oxygen [Delgado and Rubiolo, 2005].

It was shown that sugar addition has small but protective effect on anthocyanins monomers in frozen strawberries. Therefore colour intensification during freeze-drying of osmotically dehydrated fruits can be interpreter e.g. by osmotic pre-treatment. Osmotically dehydrated strawberries showed better colour behaviour than samples which were frozen before freeze-drying without osmoltic pre-treatment. Type of osmotic solution was importand [Torreggiani et al., 1995]. Sucrose is considered one of the best osmotic substances,

especially when the OD is employed before drying. The presence of this sugar on the surface of the dehydrated sample is an obstacle for the contact with oxygen [Lenart, 1996] thus reducing the oxidative reactions.

The aim of this work was to investigate the effect of osmotic dehydration, type of osmotic solution and freezing on colour of freeze-dried strawberries. A study was undertaken to

define pretreatment conditions before freeze-drying of strawberries which could affect on colour of dried fruit.

Materials and methods

The frozen Senga Sengana strawberries were osmotically dehydrated in solutions with water activity equal to 0.9 [sucrose 61.5 g/100 g solution, glucose 49.2 g/100 g solution, starch syrup (glucose equivalent DE 30–35) 67.2 g/100 g solution] in a water bath (EL-PAN-357) at the temperature of 30ºC for 3 hours under atmospheric pressure. The ratio of material to solution was 1:4 w/w. Additionally, the system was shaken with the frequency of 100 Hz and the amplitude of 10 Hz. After the specified period of time the strawberries were separated from the osmotic solution with a sieve, and rinsed twice in water.

Osmotically dehydrated strawberries were frozen in a National Lab GmbH (ProfiMaster Personal Freezers PMU series) freezer at the temperature of -70ºC for 2 hours.

Both osmotically dehydrated and unprocessed frozen strawberries were then dried for 24 hours in an ALPHA1-4 LDC-1m freeze-dryer (Christ, Germany) using contact heating under the pressure of 63 Pa, safety pressure 103 Pa, the dryer shelves temperature being 30ºC. During the process of drying, the fruit temperature was being monitored by a thermo-couple which indicated that the temperature inside dehydrated strawberries rose from -30 to 25ºC. Subsequently, the fruit were put into jars and stored in a dark place at the temperature of 25±3ºC until the time of planned examination.

Colour determination was done for surface of freeze-dried strawberries [Ciurzyńska and Lenart, 2009]. The colour of freeze dried strawberries surface was determinate with the use of detector Chroma – Meter CR-300 Minolta (Austria) company in CIE L*, a*, b* system. The measurements were made in 25 repetitions for every dried fruit. For obtained results average value were calculated.

The colour indicators were calculated with the use of formulas:

SI – saturation index

$$SI = \sqrt{(a^*)^2 + (b^*)^2} \quad [1]$$

H – hue angle

$$H = \text{Tan-1}\ (b^*/a^*) \quad [2]$$

L^* – lightness coefficient

where:

a* – red colour coefficient

b* – yellow colour coefficient

In the ensuing statistical analysis Statgrafics Plus, version 3.0. (Microsoft), Excel 2000 (Microsoft), Table Curve 2D v. 3 (Jadel) software was used. For the obtained averaged results standard deviations (SD) were calculated. Fisher's test for verification of the hypothesis of equality of means for analysed coefficients in measured samples and Pearson's correlation coefficient were used. The analyses were done with the significance level of 0.05.

Results and discussion

To estimate the influence of osmotic dehydration together with the type of osmotic solution and freezing on color changes of freeze-dried strawberries the lightness coefficient (L^*) (Fig. 1) as well as saturation index (SI) (Fig. 2) and Hue angle coefficient (H) (Fig. 3) were determinate.

Osmotic dehydration in fresh state in glucose (1) and sucrose solution (2) caused significant decrease in lightness coefficient for surface of freeze-dried strawberries in comparison to fruit not subjected to osmotic dehydration (3) (Fig. 1). There wasn't significant differences in depend on the type of osmotic solution.

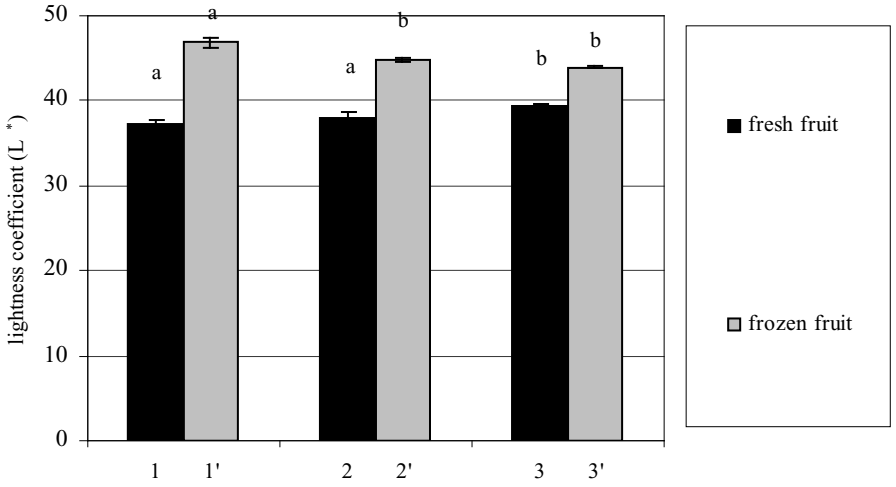

Fig. 1. Influence of osmotic dehydration, type of osmotic solution and freezing on lightness coefficient (L*) for freeze-dried strawberries (surface). Type of osmotic solution: 1 – glucose solution, 2 – sucrose solution, 3 – fruit not subjected to osmotic dehydration. The same letters a, b, c) determine not statistically significant differences (significant level 0,05)

Moreno et al. [2000] and Taiwo et al. [2003] who investigated strawberries osmotically dehydrated in glucose solution and sucrose solution obtained the lightness coefficient increase for the fruit surface in comparison to samples not subjected to osmotic dehydration. The increase of lightness coefficient (L*) for the surface of fruit osmotically dehydrated before freezing can suggest higher colour rinsing out degree during osmotic dehydration [Moreno et al., 2000]. Kopjar et al. [2008] showed that samples freeze-dried after addition of trehalose, had higher L* values than samples without trehalose addition.

Osmotic dehydration of frozen strawberries (1'–2') caused increase in surface lightness coefficient in comparison to fruit not subjected to osmotic dehydration (3'). For sucrose solution (2) differences were not statistically significant.

It was shown that frozen strawberries osmotically dehydrated have higher lightness coefficient (L^*) than in fresh fruit after osmotic pre-treatment (Fig. 1).

Osmotic dehydration of fresh strawberries in glucose (1) and sucrose solution (2) caused significant decrease in saturation index (SI) in comparison to fruit not subjected to osmotic dehydration (3) (Fig. 2). There wasn't significant differences in depend on the type of osmotic solution.

For strawberries osmotically dehydrated in frozen state (1'-2') there was also significant decrease in saturation index in comparison to fruit without osmotic pre-treatment (3') (Fig. 2). Strawberries osmotically dehydrated have significant different saturation index (SI) in depend on the type of osmotic solution. The higher value was observed for fruit osmotically dehydrated in sucrose solution (2').

Storage fruit in frozen state influence on increase saturation index in comparison to strawberries osmotically dehydrated in fresh state. Exception is samples osmotically dehydrated in glucose solution (1') for which there was small decrease in investigated coefficient after long storage time (Fig. 2).

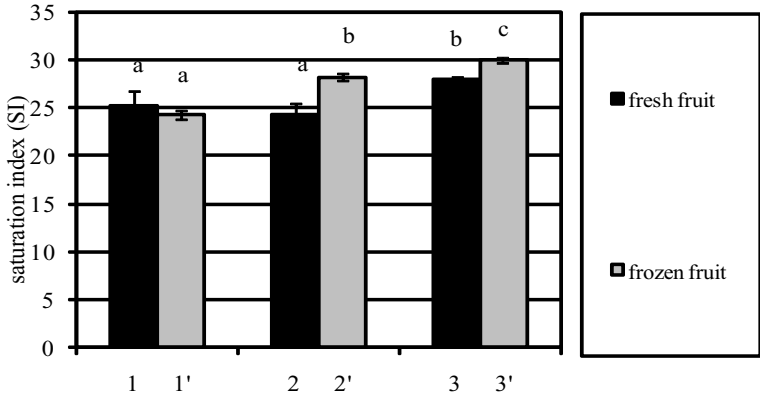

Fig. 2. Influence of osmotic dehydration, type of osmotic solution and freezing on saturation index (SI) for freeze-dried strawberries (surface). Type of osmotic solution: 1 – glucose solution, 2 – sucrose solution, 3 – fruit not subjected to osmotic dehydration. The same letters (a, b, c) determine not statistically significant differences (significant level 0,05)

Strawberries osmotically dehydrated in fresh state (1–2) have not the same hue angle (H) coefficient in comparison to samples without osmotic pre-treatment (3) (Fig. 3). Osmotic dehydration caused statistically significant decrease this coefficient. There wasn't significant differences in depend on the type of osmotic solution.

For fruit osmotically dehydrated in frozen state (1'–2') there was statistically significant decrease of Hue angle (H) coefficient in comparison to strawberries without osmotic pre-treatment (3') (Fig. 3). Strawberries osmotically dehydrated in sucrose solution (2') have higher value of (H) coefficient in comparison to samples osmotically dehydrated in glucose solution (1'). For fruit osmotically dehydrated in fresh state there was decrease hue angle (H) coefficient in comparison to samples not subjected to osmotic pre-treatment (Fig. 3).

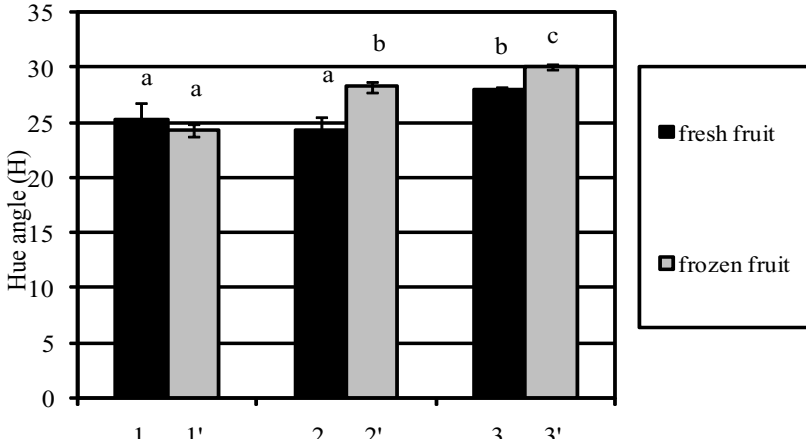

Fig. 3. Influence of osmotic dehydration, type of osmotic solution and freezing on Hue angle (H) for freeze-dried strawberries (surface). Type of osmotic solution: 1 – glucose solution, 2 – sucrose solution, 3 – fruit not subjected to osmotic dehydration. The same letters (a, b, c) determine not statistically significant differences (significant level 0,05).

According to Forni et al. [1997] osmotic dehydration assure colour stability during the drying and freezing process eg. peaches. Wrolstad et al. [1990] consider that sucrose addition has stabilize effect on anthocyanin monomers content and investigations shown that osmotic dehydration in sucrose solution has low but statistically significant protective effect on colour of frozen strawberries.

Conclusions

Fresh strawberries osmotically dehydrated in glucose and sucrose solution obtained lower lightness coefficient (L*) values in comparison to fruit not subjected to osmotic dehydration. For frozen fruit there was increase of this coefficient in comparison to samples without osmotic pre-treatment. Strawberries osmotically dehydrated in frozen state and freeze-dried obtained higher lightness coefficient in comparison to fruit osmotically dehydrated in fresh state.

Osmotic dehydration of fresh and frozen strawberries caused significant decrease of saturation index (SI) in comparison to fruit not subjected to osmotic dehydration. The fruit storage in frozen state influenced on increase of saturation coefficient in comparison to fruit osmotically dehydrated in fresh state. For strawberries osmotically dehydrated in glucose solution there was small decrease of investigated index in long storage time.

Strawberries osmotically dehydrated in fresh state have lower value of Hue angle coefficient (H) in comparison to fruit not subjected to osmotic dehydration. For fruit osmotically dehydrated in frozen state there was decrease of those coefficient in comparison to samples without osmotic pre-treatment. For strawberries osmotically dehydrated in fresh state there was decrease of Hue angle coefficient in comparison to fruit not subjected to osmotic dehydration after freezing. Exception is sample osmotically dehydrated in glucose solution.

References

Agnelli M.E., Mascheroni R.H., 2002. Quality evaluation of foodstuffs frozen in a cryomechanical freezer. J Food Eng., 52, 257–263

Alvarez C.A., Aguerre R., Gómez R., Vidales S., Alzamora S.M., Gerschenson L.N., 1995. Air dehydration of strawberries: Effects of blanching and osmotic pretreatments on the kinetics of moisture transport. J. Food Eng., 25, 167–178

Chiralt A., Martinez-Navarrete N., Martinez-Monzó J., Talens P., Moraga G., Ayala A., Fito P., 2001. Changes in mechanical properties throughout osmotic processes cryoprotectant effect. J. Food Eng., 49, 129–135

Ciurzyńska A., Lenart A., 2009. The influence of osmotic dehydration on colour changes for freeze-dried strawberries. Engineering and chemical apparatus, 48, 20–21.

Doymaz I., 2008. Convective drying kinetics of strawberry. Chem. Eng. Process., 47, 914–919.

El-Beltagy A., Gamea G.R, Amer Essa A.H. 2006. Solar drying characteristics of strawberry. J. Food Eng., 78, 456–464.

Forni E., Sormani A., Scalise S., Torreggiani D., 1997. The influence of sugar composition on the colour stability of osmodehydrofrozen intermediate moisture apricots. Food Res. Int., 30, 87–94.

Fuchigami M., Kato N., Teramoto A. 1997. High-pressurefreezing effects on textural quality of carrots. J. Food Sci., 62, 804–808.

Gruda Z., Postolski J., 1999. Food freezing, WNT, Warsaw, Edition III, 291–293.

Kopjar M., Pilizota V., Hribar J., Simcic M., Zlatic E., Nedic Tiban N. 2008. Influence of trehalose addition and storage conditions on the quality of strawberry cream filling. J. Food Eng., 87, 341–350.

Lenart A., 1996. Osmo-convective drying of fruits and vegetables: technology and application. Drying Technol., 14, 391–413.

Lewicki P.P., 2006. Design of hot air drying for better foods. Trends in Food Sci. Technol., 17, 153–163.

Long-yuan Li., 2006. Numerical simulation of mass transfer during the osmotic dehydration of biological tissues. Computational Materials Sci., 35, 75–83.

Marques Luanda G., Prado Manoel M., Freire Jose T., 2009. Rehydration characteristics of freeze-dried tropical fruits. LWT - Food Sci. Techno. 42, 1232–1237.

Moreno J., Chiralt A., Escriche I., Serra J.A., 2000. Effect of blanching/osmotic dehydration combined methods on quality and stability of minimally processed strawberries. Food Res. Int., 33, 609–616

Nsonzi F., Ramaswamy S., 1998. Quality evaluation of osmoconvective dried blueberries. Drying Technol., 16, 705–723.

Płocharski W., 2002. Starawberries on supply fresh fruit market processing. Food Industry, 6, 12–14.

Rey L., May J.C., 1999. Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products. Marcel Dekker, New York.

Sereno A.M., Hubinger M.D., 2001. Prediction of water activity of osmotic solutions. J. Food Eng., 49, 103–114.

Shishehgarha F., Makhlouf J., Ratti C., 2002. Freeze-drying characteristics of strawberries. Drying Technol., 20, 131–145

Taiwo K.A., Eshtiaghi M.N., Ade-Omawaye B.I. O., Knorr D., 2003. Osmotic dehydration of strawberry halves: influence of osmotic agents and pretreatment methods on mass transfer and product characteristics. Int. J. Food Sci. Technol., 38, 693–707.

Wrolstad R.E., Skrede G., Lea P., Enersen G., 1990. Influence of sugar on anthocyanin pigment stability in frozen strawberries. J. Food Sci., 55, 1064–1065, 1072.

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STRUCTURE CHANGES OF OSMOTICALLY DEHYDRATED APPLES

Introduction

Structure of foodstuff materials plays an important role in processes based on mass transfer. Porous materials, such as fruit and vegetables are characterized by specific physical characteristics (porosity, density, cell membrane permeability, adhesiveness, cell wall resistance), which significantly influence transport of substances taking place within the biological matrix [Salvatorii et al., 1998; Kowalska and Lenart, 2005; Torreggiani and Bertolo, 2003].

Osmotic dehydration is a process of water removal from foodstuff materials using hypertonic solutions (solutions of sugars and salts) without water phase transition. This allows for minimization or elimination of an adverse influence of temperature upon quality of products – especially those rich in thermolabile components. Both the water diffusion and the simultaneous flow of naturally occurring substances (vitamins, mineral components, dyes, aromatic substances etc.) from vacuole to osmotic solution take place during the osmotic dehydration. The counter-current flow of substance stream during the process of osmotic dehydration does not only cause changes to chemical composition of dehydrated material but it also causes changes to its structure [Mavroudis et al., 2004; Kowalska and Lenart, 2005].

Removal of water from analyzed material causes cell plasmolysis along with breaking and disintegration of cell walls and membranes, changes permeability and density of material. All these factors also influence the macroscopic characteristics of a product such as capacity and appearance. Range of those changes depends to a large extent upon conditions of the osmotic process but also upon raw material characteristic. Structure changes point to significant influence of mass transfer within a product during food processing and storage. Because of this it becomes important to conduct an analysis of tissue behaviour at a microstructural level [Khan and Vincent, 1990; Lewicki and Porzecka-Pawlak, 2005; Kowalska and Lenart, 2005].

Plant cell walls are made of cellulose microfibrils immersed in the pectin, hemicelluloses and lignin matrix responsible for hardness of plant tissue. During the ripening of fruit structure f cell walls becomes partially dissolved as a result of enzymatic degradation, which causes their softening. Apart from cell wall structure, cell endurance and macroscopic hardness of a whole fruit is determined by the cell turgor pressure. Fruit loose turgor due to water loss which is caused by transpiration or inhibition of respiration. In consequence the tissue becomes withered causing a dry look and a lack of sheen and colour [Oey et al., 2007].

Plant tissue is composed of capillary porous material composed of both gaseous and iquid phase and the solid matrix. The matrix is composed of cells, capillaries and intracel-

lular spaces forming a continuum (apoplast) able to transport water and small molecules. Protoplasts of neighbouring cells are connected with each other with a plasmodesm forming a structure called symplast which allows for the movement of both organic and inorganic substances. Additionally, connected intercellular spaces form tunnels with a sucking potential [Kowalska and Lenart, 2005; Lewicki and Porzecka-Pawlak, 2005].

The macroscopic mechanical characteristics of the tissue are determined by its microscopic and histologic characteristics such as cell size, the number of intercellular spaces, mechanical characteristics of cell wall and middle lamella as well as the turgor pressure. Thus the micromechanics approach is preferred in order to understand the relative meaning of these characteristics upon the general mechanical behaviour of a fruit [Oey et al., 2007].

Apart from turgor, there are other main factors influencing mechanical characteristics of plant tissue. The adhesion force, cell wall resistance to forces of compression and stretching, density of cell location determining the free space filled with gas or liquid and different factors also typical for other materials such as the sample size and shape, temperature and the pace of applied tension. The main model portraying the mechanical reaction of plant tissue initially shows a linear dependence of stress and tension or an elastic reaction until the critical level of deformation is achieved [Chiralt et al., 2001; Chiralt and Talens, 2005].

Tissue of fresh apples is made of hexagonally shaped cells and shape coefficient of about 0,80. Cell diameter largely depends on their type and place of occurrence in raw material and fits within range from 50 to 500 μm [Pierzynowska-Korniak et al., 2002; Kowalska and Lenart, 2003, 2005]. For the Idared variety of apples the average diameter of parenchyma determined by Kowalska and Lenart [2005] equalled to around 110μm. Parenchyma of apple tissue contains large intercellular spaces formed as a result of combining 8, 10 or 12 cells with smooth cell wall surface [Soliva-Fortuny et al., 2003]. These spaces are mainly filled with air and cover large parts of tissue – around 20–30% of apple parenchyma. Their length amounts to over 400μm and they are 100–200 μm in diameter [Khan and Vincent, 1990] or as stated by Pierzynowska-Korniak et al. [2002] 210–350 μm. Cells and intercellular spaces are loosely arranged. The tissue is characterized by nonhomogeneity and anisotropy. The turgor pressure in cells of fresh tissue enforces tight adherence of membrane plasmas to cell walls [Khan and Vincent, 1990; Nieto et al., 2004; Kowalska and Lenart, 2005].

Those cells which are located directly under the skin surface are small and flattened in tangency to surfaces with the maximum size of around 50 μm. The size of cells increases towards the centre of a fruit up to the maximum value of around 200–300 μm in diameter (depending on an apple variety) within 5–10 mm distance from the surface [Khan and Vincent, 1990].

Density of packed cells is the highest in close proximity to a fruit core and the lowest close to a fruit surface. The differentiated structure of apple tissue does not allow to perceive apple parenchyma as a homogenous material [Khan & Vincent, 1990; Mavroudis et al., 2004].

The aim of this research was to analyze changes in tissue structure of osmotically dehydrated apples in sucrose solution taking into consideration structure indicators (shape coefficient, perimeter, Feret diameter) of cells and intercellular spaces.

Materials and methods

Cubes with sides 10 mm long were cut from apples Idared variety and immersed in 0.20% citric acid solution to present samples darkening.

Osmotic dehydration took place in the water bath type 357 from ELPAN with a thermostat and a mobile platform ensuring constant, light shaking at 100Hz frequency. The ratio of material mass to solution mass amounted to 1:4. Apples samples were dehydrated in 20% sucrose solution at temperatures of 30, 50 and 60ºC for 10, 25, 45, 60, 120 and 180 minutes in atmospheric pressure. Dehydrated samples were separated from osmotic solution, rinsed with water and dried on a filter paper. Tests were conducted in two repetitions.

Changes in structure were determined using a scanning electron microscope of Quanta 200 type made by FEI. Samples were treated after liofilisation: frozen apples were dried using the sublimation drying technique at temperature 15°C for around 24 hours and subsequently the temperature was increased to 25°C. Subsequently during osmotic dehydration a chosen section of apples was observed using 50x magnification. The MultiScan Based program and Microsoft Excel 2007 were used to analyze microscope photographs.

Shape coefficient [Lewicki and Porzecka-Pawlak, 2005]:

$$s_f = \frac{4\pi \cdot A}{P^2} \qquad [1]$$

where: A – cell surface; P – cell perimeter

The shape coefficient acquires values between 0 (for a straight line) to 1 (for a circle).

The Multifactor ANOVA analysis was used for statistical evaluation of obtained results. The statistical inference was conducted at the statistical significance of $\alpha = 0{,}05$.

Results and discussion

The tissue of analyzed raw apples was composed of cube shape cells which formed intercellular spaces as a result of merging. Spherical character of cells determined with the use of the shape coefficient in raw apples equalled to around 0.70 (Tab. 1; Fig. 1), and for apples of the same variety Kowalska and Lenart [2005] obtained about 0.80 and it was a little lower in comparison to 0.82 noted by Lewicki and Porzecka-Pawlak [2005]. Also the range of shape coefficient was narrower and it amounted to 0.54–0.83 while Lewicki and Porzecka-Pawlak [2005] noted values within range of 0.12–0.94 for cells from raw apple tissue. Lewicki and Porzecka-Pawlak [2005] demonstrated that the Feret diameter was 46–346 μm with 72% share of cells with diameter between 75 and 150 μm. They observed a cell perimeter within range of 190–2110 μm, however, 22% of cells had their perimeter below 330 μm and 26.5% above 530 μm.

Ranges of Feret diameter observed in the presented research were moved to higher values (100–250 μm), while the perimeter of osmotically dehydrated apples cells adopted a more wider range between 262–1219 μm. The value of shape coefficient for intercellular spaces acquired by Lewicki & Porzecka-Pawlak [2005] fit between 0.16–0.84 which also constitutes a broader range in comparison to values obtained in this research paper (0.20–0.62) (Tab. 1). Moreover, a smaller share of intercellular space with perimeter between 500–1000 μm was observed against a number of spaces characterized by their perimeter larger than 1000 μm.

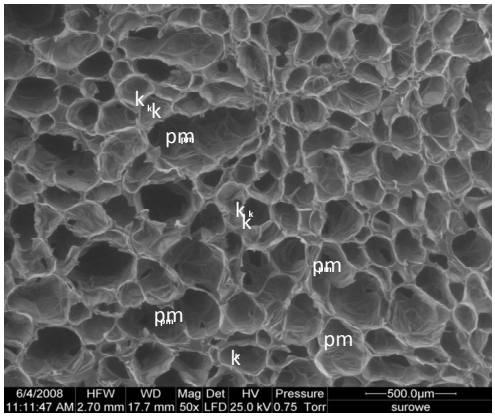

Fig. 1. Raw apples tissue: k – means cell; pm – means intercellular space. Magnification: 50x

Structure of apples tissue depends in a large extent upon the place of sample collection. Many conducted tests have proven that size and arrangement of cells along with number and size of intercellular spaces (responsible for tissue porosity) all depend on the location of a fruit occurrence. Layers located near a fruit core are characterized by the highest density and their porosity increases with distance from the core. Many other tests have confirmed these findings [Khan and Vincent, 1990; Mavroudis et al., 2004; Nieto et al., 2004]. The nonhomogeneity of apples tissue structure may be a cause of differences between obtained results. The osmotic dehydration of apples in the sucrose solution caused significant changes to cell structure of apples. Deformation and deterioration of cell elements took place mainly because of water removal while penetration of the solution into the material had a lower impact [Kowalska and Lenart, 2005].

Table 1

Percentage share of shape coefficient of cells and intercellular spaces for raw and osmotically dehydrated apple tissue

Shape coefficient \ Time [min]		Temp.	30°C			50°C			60°C	
		0	10	60	180	10	60	180	60	180
cells	0.45–0.5	0.00	7.14	3.70	5.60	5.93	7.05	2.51	10.64	1.48
	0.5–0.7	38.60	71.43	71.49	64.80	77.97	77.57	54.78	71.81	54.82
	0.7–0.8	61.40	21.43	24.81	29.60	16.10	15.38	42.71	17.55	43.70
Intercellular spaces	0.20–0.30	8.57	24.00	19.05	25.00	22.22	14.81	28.21	31.71	26.09
	0.30–0.50	62.86	76.00	50.00	57.14	61.11	51.86	46.15	53.66	34.78
	0.50–0.60	28.57	0.00	30.95	17.86	16.67	33.33	25.64	14.63	39.13

Table 2

Percentage share of perimeter coefficient of cells and intercellular spaces for raw and osmotically dehydrated apple tissue

Time [min] / Perimeter [μm]		Temp.	30°C			50°C			60°C	
		0	10	60	180	10	60	180	60	180
cells	0–400	12.09	9.18	12.59	9.60	11.02	10.26	6.53	9.57	2.22
	400–800	67.91	65.31	75.19	52.00	74.57	76.28	58.80	79.26	51.85
	800–1000	20.00	25.51	12.22	38.40	14.41	13.46	34.67	11.17	45.93
Intercellular spaces	0–600	2.86	0.00	0.00	0.00	0.00	0.00	2.56	9.76	0.00
	600–1000	31.43	28.00	23.81	3.57	22.22	18.52	28.21	29.26	4.35
	>1000	65.71	72.00	76.19	96.43	77.78	81.48	71.79	60.98	95.65

Table 3

Percentage share of maximum (Fmax) and minimum (Fmin) Feret diameter of cells and intercellular spaces for raw and osmotically dehydrated apple tissue

Time [min] / Feret diameter [μm]		Temp.	30°C			50°C			60°C	
		0	10	60	180	10	60	180	60	180
Fmax	0–100	17.21	4.08	9.26	8.00	8.47	7.69	8.04	10.11	2.22
	100–250	75.81	67.35	84.44	64.00	80.51	84.62	80.40	85.10	58.52
	250–300	6.98	28.57	6.30	28.00	11.02	7.69	11.56	4.79	39.26
	>300	2.33	14.29	2.96	12.00	1.69	2.56	2.51	2.66	20.00
Fmin	0–100	6.51	9.18	10.37	4.00	11.02	13.46	3.52	5.32	3.70
	100–250	75.35	78.58	82.59	67.20	83.05	79.46	56.78	86.70	74.82
	250–300	18.14	12.24	7.04	28.80	5.93	7.05	39.70	7.98	21.48
	>300	6.98	5.10	1.11	17.60	0.85	1.28	19.40	3.19	5.19

The first stage of osmotic dehydration process involved a significant deformation of cells – mainly those present close to sample surface (Fig. 2d). Those cells were characterized by heavily folded cell walls or they occurred in a compact form along with intercellular spaces. It was a result of a strong influence of osmotic pressure and the initially large water current flowing out of the material. Additionally, surface layers of apples tissue were expose to an osmotic stress from the beginning of the process while layers situated dipper could remain unchanged for a longer time. Microscope photographs of osmotically dehydrated tissue under low temperature and demonstrate that cells located further from the surface did not differ in appearance from cells of fresh tissue (Fig. 1; Fig. 2a, b).

Girlando et al. [2003] also noted significant differences in appearance of cells located at different distances from samples surface during osmotic dehydration of mango tissue. Moreover, they stated that the level of cell content and cell structure breakdown was increasing in

a linear way along with the osmotic solution concentration. Short time dehydration under high temperature (Fig. 2d) or implementation of longer dehydration time (Fig. 2c) caused significant changes in structure of the whole sample which often made it impossible to differentiate between elements of cell structure. There have also been observed cases of cell wall tearing. High temperature strongly influenced changes in cell wall structure. It could cause thermal denaturation of natural membranes and their subsequent tearing and it could influence an increase in flexibility and plasticity of cell membranes and walls.

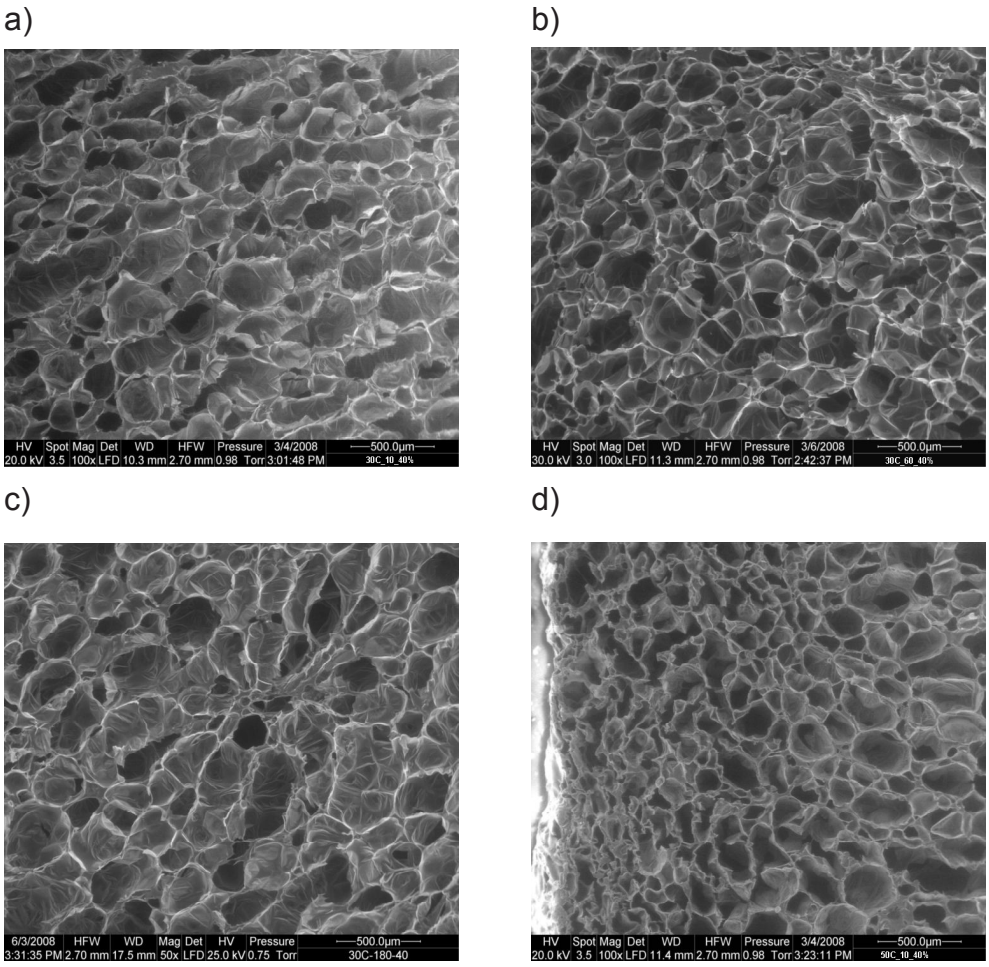

Fig. 2. Osmotically dehydrated apple tissue in 40% sucrose solution. Osmotic dehydration time at temperature 30°C: a) 10 min.; b) 60 min.; c) 180 min. in 40% sucrose solution at temperature. Osmotic dehydration time at temperature 50°C: d) 10 min. Magnification: 50x

In accordance with earlier research [Lewicki and Porzecka-Pawlak, 2005; Kowalska and Lenart, 2005] the cell shape coefficient decreased as a result of the osmotic dehydration of plant tissue (Tab. 1). The coefficient of intercellular spaces decreased less as it demonstrated low values. During the initial stage of dehydration process the shape coefficient of apples

cells decreased considerably. During the first hour of the process the share of cells with shape coefficient higher than 0.7 decreased by 34–55% in comparison to the raw tissue (Tab. 1). At the end of the process (180 min) the shape coefficient regained the initial values to some extent. Then cells took a circular shape once again while their walls became smoother. The share of cells with shape coefficient higher than 0.7 in osmotically dehydrated tissue for 180 min under different process conditions equalled to approximately 30–45% (61% in raw apple tissue).

The level of initial shape recovery by cells of plant tissue and an increase in their shape coefficient depended to a large extent on osmotic dehydration parameters (Tab. 1). Recovery of the initial cell roundness as well as reduction in folding and breaking of cell walls can be explained by the setting of pseudo-equilibrium system after some time of dehydration which generates return streams and new penetration of water into the condensed material. The similar interpretation of sample behaviour was demonstrated by Nieto et al. [2004] using the example of apple tissue. They suggested that the return of primary structure in biological material subjected to osmotic stress might depend upon the rheological characteristics of tissue or the homeostatic mechanism, which serves as a natural mechanism protecting plant tissue from a negative impact of the surrounding environment. In both cases, however, the tissue must maintain its functionality of cell membranes. This becomes impossible when high osmotic dehydration process parameters are implemented – mainly high temperature (over 50°C) which causes thermal degradation of cell membranes (Fig. 2d).

A tendency of initial cell perimeter to shrink during the osmotic dehydration of apples was observed in 40% sucrose solution (Tab. 2). This concerned mainly an increase in the share of cells with perimeter ranging between 480–640 μm for which this change amounted to 6–21% in relation to raw tissue. The higher process temperature caused the more significant change. On the other hand, the sample dehydrated for 180 minutes demonstrated 10.5–16.5% increase in the number of cells with perimeter exceeding 720 μm.

Intercellular spaces demonstrated a smaller changeability than cells themselves (Tab. 2). Similarly to tests conducted by Lewicki and Porzecka-Pawlak [2005] there was no indication of a dominant value of shape coefficient for intercellular spaces in the dehydrated tissue but most fit within the range of 0.3–0.5. A shift in decomposition of shape coefficient towards lower values in comparison to non-dehydrated tissue was observed. Osmotic dehydration also caused an increase in intercellular spaces perimeter. The percentage value of perimeter change for intercellular spaces of tissue dehydrated in 61.5% solution at 30°C for 180 minutes amounted to nearly 22%. The share of spaces characterized by perimeter larger than 1000 μm in raw material was much higher than the one observed by Lewicki and Porzecka-Pawlak [2005]. Moreover, Lewicki and Porzecka-Pawlak [2005] dehydrated apples tissue in 61.5% sucrose solution at 30°C and stated that forces of cramping and stretching affecting cell walls during the osmotic process lead to deformations, wrinkling and breakdown of cell surfaces. This further influenced both shape and perimeter of intercellular spaces. In consequence the tissue of osmotically dehydrated apples was less regular but more homogenous.

Changes to the Feret diameter (minimal Fmin and maximal Fmax value) were directly connected to the shape coefficient. Test results indicate that the osmotic dehydration in a low concentration sucrose solution (40%) in most instances lead to the initial decrease in both diameters and their increase in the last stage (Tab. 3). Regardless of the process parameters, after 180 minutes of apple tissue dehydration in the sucrose solution, the number of cells with F_{max} diameter higher than 200 μm increased in comparison to fresh apple tissue. The highest

increase took place within range of 250–275 μm. Kowalska and Lenart [2005] demonstrated, on the other hand, that the osmotic dehydration of apples and carrot tissue in sucrose, glucose and starch syrup solutions (with concentration resembling water activity 0.9 in 30, 50 and 80°C after 24 h) caused shrinking of cells in the analyzed material. Percentage decrease in cell diameter stayed between 5–18% for apples and depended upon solution type.

They also concluded that the size and shape of cells depended upon the amount of removed water, so the implementation of higher process parameters caused more significant changes in structure (Fig. 2c, d). Nieto et al. [2004] obtained similar results also by dehydrating apple tissue in sucrose and glucose solutions. The high extent of damage made to cell tissue structure of dehydrated material was connected with the initial high rate of mass transfer which was also observed in terms of macroscopic sample changes by Kowalska and Lenart (2005). At the beginning of the process the loss of water was causing a significant reduction in cell shape and thus they lost their initial roundness.

This could lead to gas loss from intercellular spaces by solution penetrating pores located close to the sample's surface (Fig. 2d). In conditions of increased temperature of osmotic dehydration there took place a significant saturation of analyzed material with the osmotic substance and the formation of tight zones of [Giraldo et al., 2003], mainly in samples surface layers. Similar changes were observed by Giraldo et al. [2003] in osmotically dehydrated apples. As the osmotic solution was entering and penetrating the analyzed material pores it replaced a gaseous phase present in them and therefore intercellular spaces underwent total crushing while some cells remained well visible [Chiralt et al., 2001].

Conclusions

Changes to the structure of apples tissues take place during the osmotic dehydration. They mainly depend on osmotic treatment time and less on other dehydration parameters. In comparison to fresh apple tissue osmotic dehydration caused a decrease in the cell shape coefficient by around 9.5% (from 0.70 to 0.64) on average. However, the shape coefficient of intercellular spaces showed less changes within the range of 0.43–0.41.

The temperature increase lead to lower cell shape coefficients at the beginning of the process but also higher rate of their initial shape recovery at the end of the process.

No significant decrease in cell perimeter during mild dehydration conditions was observed. However, implementation of the higher process temperature lead to a decrease in cell perimeter, especially after a longer dehydration time.

Osmotic dehydration of apples in sucrose solution lead to an increase in Feret maximal cell diameter by 5–47% in relation to the raw tissue. Only in some cases a simultaneous increase in share of cells with smaller and bigger maximal diameter was observed. However, for most of the osmotic dehydration conditions a decrease in Feret minimal cell diameter was observed (on average by 13%) in comparison to non-osmotically dehydrated tissue.

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References

Chiralt A., Martinez-Navarrete N., Martinez-Monzo J., Talens P., Moraga G., Ayala A., Fito P., 2001. Changes in mechanical properties throughout osmotic processes Cryoprotectant effect. J. Food Eng., 49, 129–135.

Chiralt A., Talens P., 2005. Physical and chemical changes induced by osmotic dehydration in plant tissue. J. Food Eng., 65, 167–177.

Girlando G., Talens P., Fito P., Chiralt A., 2003. Influence of sucrose solution concentration on kinetics and yield during osmotic dehydration of mango. J. Food Eng., 58, 33–43.

Jokić A., Gyura J., Lević L., Zavargo Z., 2007. Osmotic dehydration of sugar beet in combined aqueous solutions of sucrose and sodium chloride. J. Food Eng., 78, 47–51.

Khan A.A., Vincent J.F.V., 1990. Anisotropy of apple parenchyma. J. Sci. Food Agric., 52, 455–466.

Kowalska H., Lenart A., 2003. Znaczenie wymiany masy w tworzeniu żywności nowej generacji. Postępy Techniki Przetwórstwa Spożywczego, 2, 12–17.

Kowalska H., Lenart A., 2005. Zmiany struktury tkanki roślinnej wywołane odwadnianiem osmotycznym. Inż. Rol., 69, 187–195.

Lewicki P.P., Porzecka-Pawlak R., 2005. Effect of osmotic dewatering on Apple tissue structure. J. Food Eng., 66, 43–50.

Mavroudis N.E., Dejmek P., Sjoholm I., 2004. Studies on some raw material characteristics in different Swedish apple varieties. J. Food Eng., 62, 121–129.

Nieto A.B., Salvadori D.M., Castro M.A., Alzamora S.M., 2004. Structural changes in apple tissue during glucose and sucrose osmotic dehydration: shrinkage, porosity, density and microscopic features. Journal of Food Engineering, 61, 269–278.

Oey M.L., Vanstreels E., De Baerdemaeker J., Tijskens E., 2007. Effect of turgor on micromechanical and structural properties of apple tissue: A quantitative analysis. Postharvest Biol. Technol., 44, 240–247.

Pierzynowska-Korniak G., Zadernowski R., Fornal J., Nesterowicz J., 2002. The microstructure of selected apple varieties. Electronic J. Pol. Agric. Universities, 5(2).

Salvadori D., Andres A., Albors A., Chiralt A., Fito P., 1998. Structural and compositional profiles in osmotically dehydrated apple. J. Food Sci., 63, 4, 606–610.

Soliva-Fortuny R.C., Lluch M.A., Quiles A., Grigelmo-Miguel N., Martin-Belloso O., 2003. Evaluation of textural properties and microstructure during storage of minimally processed apples. J. Food Sci., 68,1, 312–317.

Taiwo K.A., Angersbach A., Knorr D., 2002. Influence of high intensity electric field pulses and osmotic dehydration on the rehydration characteristics of apple slices at different temperatures. J. Food Eng., 52, 182–192.

Talens P., Escriche I., Martinez-Navarrete N., Chiralt A., 2003. Influence of osmotic dehydration and freezing on the volatile profile of kiwi fruit. Food Res. Int., 36, 635–642.

Torreggiani D., Bertolo G., 2001. Osmotic pre-treatments in fruit processing: chemical, physical and structural effects. J. Food Eng., 49, 247–253.

26

STATIC OPTIMIZATION OF OSMOTIC DEHYDRATION AND STORAGE PROCESS OF PLUMS

Introduction

Recently there was a great interest in dehydration process, because of possibility in obtaining a product, which is well preserved and has good nutritious and sensory values. Osmotic dehydration is a technique of water removal from cellular material in order to water activity reduction. Obtained product is not stable for the sake of preservation level. Although osmotic dehydrated fruits, because of its chemical constitution and sensory value may be frozen. Fresh and previously frozen fruits may be used in osmotic dehydration process. Useful and available for whole year form of semi – finished product are frozen fruits, also suitable in osmotic dehydration technology. Received in such process products may be used as ,,bakalie'' for confectionery cakes and in production of yoghurts, milk makes, ice creams [Matusek and Meresz, 2002, Gruda and Postolski, 1999].

Nowadays the new modification of the process are searched to obtain high quality final product [Kamińska and Lewicki, 2005, Khin et al., 2005, Jarczyk et al., 1994, Kowalska and Lenart, 2003].

To receive a product of a high quality and low costs of production, optimal setups of dehydration process are looked for.

Experimental material and methods

Plums Węgierka variety, previously frozen, osmotic dehydrated, frozen and storaged for 3 months were the object of the investigations. Fresh fruits came from integrated production orchard, according to the good pomiculture practice (Certificate of Orcharding Institute in Skierniewice). Experimental material was gathered in 2007.

Halves of plums were osmotic dehydrated. In the process sucrose solutions were used (concentration 30 and 65%). The proportion of fruits mass to solution mass was equal to 1:4. The osmotic dehydration process went on for 6 and 24 hours. The temperature was 25°C. Dependent on sucrose solutions concentrations and time of the process obtained 4 combinations of samples to investigations.

Fruits after osmotic dehydration, filtering off were packed into the plastic bags and frozen. After every 3 weeks samples of each combination were defrosted during 2 hours in the air. After filtering off mass of dry matter (using Radwag WPE 30s), content of extract (refractometry method using refractometer PZO RL 3 according to the PN-90/A-75101.02/AZ1:2002),

general acidity (indicator titration according to the PN-90/A-75101.04/AZ1:2002) and quantity of defrosting leak were examinated. The determinations were carried out 3 times.

Results of the investigations were analyzed using Experiment Planner PL ver. 1.0 and Matlab 7.0. was used for the optimization procedure.

Optimization

Formulation of the optimization problem

Criteria

Optimization criteria were divided into 2 groups connected with the 1) product quality and 2) cost of the production.

Content of the extract k1 is the first quality criterion. Extract is definable as a sum of whole soluble and non – volatile to 100°C compounds in product. Optimum value of this criterion should be as high as possible [Błoński et al., 1986].

Product acidity k2 is the second quality criterion. Acidity is a content of the acids in product, countable on dominant acid. The optimum value of the criterion should be as low as possible [Pukszta, 2003].

The third criterion is dry matter content k3. It is connected with water content in product and its stability also. Dry matter content should be as high as possible in order to obtain the most stable product [Błoński et al., 1986].

The last from quality criteria is the amount of defrosting leak. This is a direct information about the level of cellular structure destruction in product after defrosting and by this reason should be as low as possible [Palich and Pukszta, 2001]. The quantity of the defrosting leak were determinated in percentages as a proportion a difference of product masses before and after dehydration, to product mass before the process.

In the cost criteria one should specify costs connected with osmotic dehydration time, which should be as low as possible. This is directly combined with time of product creation. Next criterion concerns costs of osmotic solutions, which should be as low a possible. The last one is the profit from product storage. It is clear that it should be as high as possible.

In the work were not used any symbolically denotation of the criteria from second group, because they are directly connected with adequate singular decision variables.

Decision variables

The set of osmotic dehydration and storage process setups was chosen as a decision variables set The chosen decision variables set contains:

- Osmotic dehydration time x1
- Concentration of osmotic solution x2
- Storage time x3

Constraints

Constraints imposed on decision variables are:

$$x_1 \in \langle 6\,;\,24 \rangle \,[h] \tag{1}$$

$$x_2 \in \langle 0{,}3\,;\,0{,}65 \rangle \tag{2}$$

$$x_3 \in \langle 0\,;\,12 \rangle \,[weeks] \tag{3}$$

Mathematical model

Acknowledged mathematical model for osmotic dehydration process are Fick's equations [Matusek and Meresz, 2002, Moreira and Sereno, 2003, Crank, 1977]. Most publications which are connected with osmotic dehydration process, contain information about osmotic dehydrations of products of regular shapes (ball, cylinder, cube), which simplifies the problem of solving Fick's equations [Matusek and Meresz, 2002, Moreira and Sereno, 2003]. In this work the product which was osmoticaly dehydrated was the halves of plums. In known literature there are no information about numerical or analytical solutions of Fick's transport equations obtained for objects of arbitrary shapes, thus in this work mathematical model was obtained using experimental data. Each experimental quantity was measured three times and the mathematical model was obtained using nonlinear regression equations [Gruda and Postolski, 1999]. For the sake of this work all experimental quantities were brought to dimensionless quantities using special scaling.

Mathematical formulation of the assessment criteria:

- extract content,

$$\begin{aligned} k_1(x_1,x_2,x_3) &= 9{,}9263 - 0{,}076603\cdot x_1 + 6{,}6838\cdot x_2 + \\ &- 0{,}24968\cdot x_3 + 0{,}50794\cdot x_1\cdot x_2 - 0{,}0018942\cdot x_1\cdot x_3 + \\ &- 0{,}60508\cdot x_2\cdot x_3 + 0{,}026786\cdot x_3^2 + 0{,}013228\cdot x_1\cdot x_2\cdot x_3 \end{aligned} \quad (4)$$

- acidity,

$$\begin{aligned} k_2(x_1,x_2,x_3) &= 0{,}86996 + 0{,}0069733\cdot x_1 + 0{,}51815\cdot x_2 + \\ &+ 0{,}02731\cdot x_3 - 0{,}010709\cdot x_1\cdot x_2 - 0{,}0015355\cdot x_1\cdot x_3 + \\ &- 0{,}07643\cdot x_2\cdot x_3 + 0{,}00034765\cdot x_3^2 + 0{,}0031251\cdot x_1\cdot x_2\cdot x_3 \end{aligned} \quad 5)$$

- dry matter content,

$$\begin{aligned} k_3(x_1,x_2,x_3) &= 8{,}6781 + 0{,}08127\cdot x_1 + 8{,}7048\cdot x_2 + \\ &+ 0{,}5754\cdot x_3 + 0{,}49206\cdot x_1\cdot x_2 - 0{,}038201\cdot x_1\cdot x_3 + \\ &- 1{,}254\cdot x_2\cdot x_3 + 0{,}024603\cdot x_3^2 + 0{,}035979\cdot x_1\cdot x_2\cdot x_3 \end{aligned} \quad (6)$$

- amount of defrosting leak,

$$\begin{aligned} k_4(x_1,x_2,x_3) &= -0{,}99514 + 0{,}62819\cdot x_1 + 14{,}758\cdot x_2 + \\ &1{,}4129\cdot x_3 - 1{,}4073\cdot x_1\cdot x_2 - 0{,}06582\cdot x_1\cdot x_3 + \\ &- 1{,}5111\cdot x_2\cdot x_3 - 0{,}065317\cdot x_3^2 + 0{,}12804\cdot x_1\cdot x_2\cdot x_3 \end{aligned} \quad (7)$$

Examples of graphs illustrating experimental relationships between amount of defrosting leak, time of osmotic dehydration, concentration of osmotic solution and storage time are shown in Figures 1 and 2.

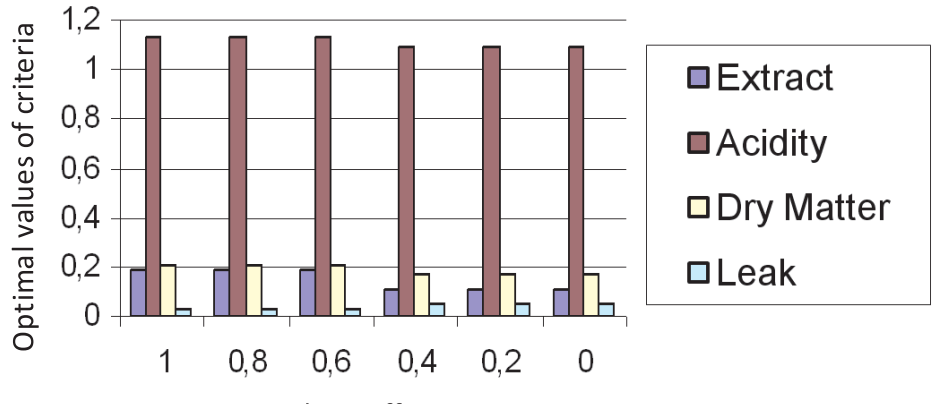

Fig. 1. Experimental model of changes of amount of a defrosting liquid in previously frozen, osmoticaly dehydrated plums for: a) 6 h, b) 24 h and frozen afterwards tp- storage time [weeks], st. – concentration, wy – defrosting leak [%]

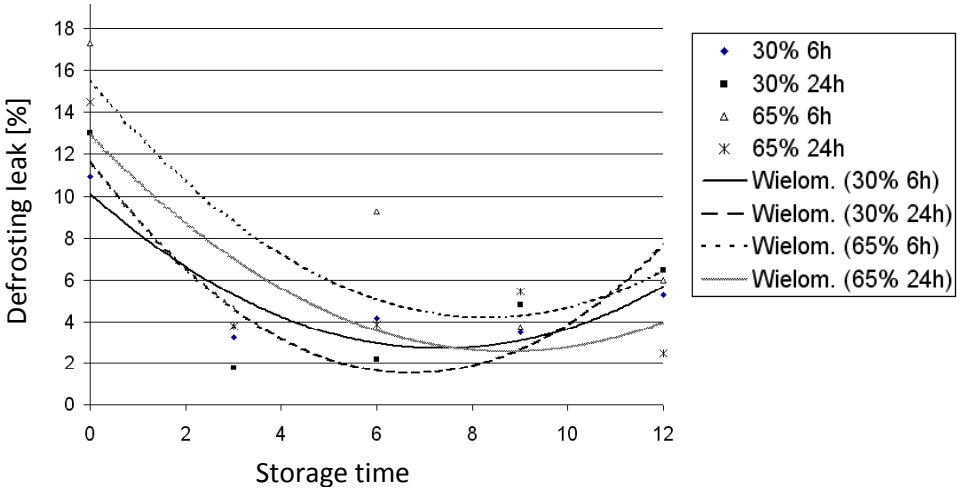

Fig. 2. Percentage value of amount of a defrosting liquid in plums as the function of storage time (polynomial)

The set of designations, values of variables, criteria and weights coefficients are shown in Table 1.

Table 1

List and meanings of used symbols

Name	Symbol	Value	Unit
General weight coefficient	wog	[0;1]	–
Extract weight coefficient	w1	0,1	–
Acidity weight coefficient	w2	0,3	–
Dry matter weight coefficient	w3	0,2	–
Defrosting leak weight coefficient	w4	0,4	–
Osmotic time weight coefficient	wk1	0,33	–
Concentration weight coefficient	wk2	0,33	–
Storage time weight coefficient	wk3	0,33	–
Expected extract content	k1_o	0,211	–
Expected acidity	k2_o	0,971	–
Expected dry matter content	k3_o	0,248	–
Expected defrosting leak	k4_o	0,028	–
Min. Osmotic time	x1min	6	h
Min. Concentration	x2min	0,30	–
Max. Storage time	x3max	12	weeks
Extract content	k1	–	–
Acidity from regression	k2	–	–
Dry matter content from regression	k3	–	–
Defrosting leak from regression	k4	–	–
Searched osmotic time	x1	–	h
Searched concentration	x2	–	–
Searched storage time	x3	–	weeks

Optimization procedure

Scalar optimization criterion has a form [Tarnowski, 2001]:

$$f_{cel} = w_{og} \cdot f_{jakość} + \left(1 - w_{og}\right) f_{koszty} \tag{8}$$

It consists of two elements. The first element represents quality criteria.

$$f_{jakość} = \left(\sqrt[2]{w_1 \cdot f_{cel\_1}^2 + w_2 \cdot f_{cel\_2}^2 + w_3 \cdot f_{cel\_3}^2 + w_4 \cdot f_{cel\_4}^2}\right) \tag{9}$$

The second element represents cost criteria.

$$f_{koszty} = \left(\sqrt[2]{w_{k1} \cdot f_{cel\_5}^2 + w_{k2} \cdot f_{cel\_6}^2 + w_{k3} \cdot f_{cel\_7}^2}\right) \tag{10}$$

where:

$$f_{cel\_(j+4)} = \left(\frac{x_{j\min} - x_j}{x_j}\right) \quad \text{for } i = 1, 2, 3, 4 \tag{11}$$

$$f_{cel\_7} = \left(\frac{x_{j\max} - x_j}{x_j} \right) \quad \text{for } j = 1, 2 \tag{12}$$

$$f_{cel\_7} = \left(\frac{x_{j\max} - x_j}{x_j} \right) \quad \text{for } j = 3 \tag{13}$$

$$w_{og} \in \langle 0\,;1 \rangle \tag{14}$$

$$w_1 + w_2 + w_3 + w_4 = 1 \tag{15}$$

$$w_{k1} + w_{k2} + w_{k3} = 1 \tag{16}$$

Values of weights coefficients for the product quality and cost of its production were established on the basic literature knowledge of the osmotic dehydration processes [Jarczyk et al., 1994, Błoński et al., 1986, Pukszta, 2003, Palich and Pukszta, 2001]. Expected values ek_o, kw_o, sm_o and wyc_o are the features of the most desirable product [Jarczyk et al., 1994, Błoński et al., 1986, Pukszta, 2003, Palich and Pukszta, 2001]. The idea of the aim function is based on the minimization of formula expressing the difference between expected values of the criteria and its values received from the experimental regression equations. Function fjakość is the distance between point (k1_o, k2_o, k3_o, k4_o), and point (k1, k2, k3, k4) which lies on the hypersurface of allowed values of criteria. Function f koszty is the distance between point (x1min, x2min, x3max), and point (x1, x2, x3) belonging to decision variables space. The set of optimal values of decision variables were obtained for the minimal value of the aim function. The minimal value of the aim function was calculated using Matlab Program.

Optimization results

The aim of this work was to determine the set of setups of the process which allow to obtain product of the highest quality simultaneously minimizing the cost of the process. The dependence between quality of the product and its production cost is show in figure 3.

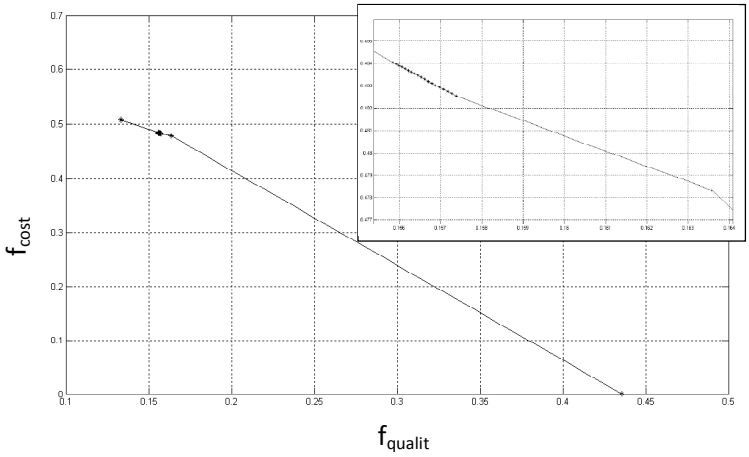

Fig. 3. Quality of a product in function of a production cost. Increment of a quality reply to the decreasing values in abscissal axis

The chart illustrating optimal values of the decision criteria in function of general weight coefficient is shown in Figure 4.

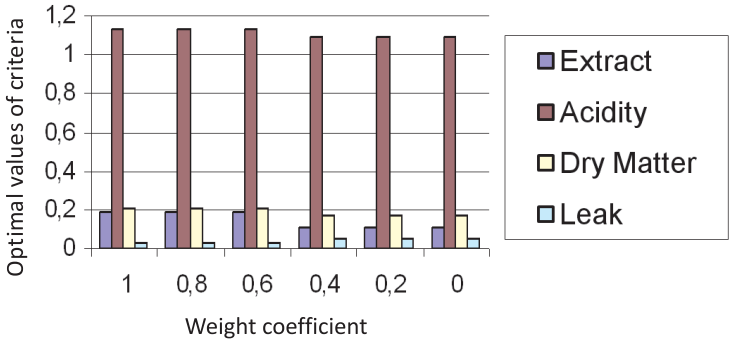

Fig. 4. Optimal values of a single decision criterion as the function of a total weight coefficient

Optimal values of the decision criteria and corresponding to them setups of the decision variables contains Table 2.

Table 2

Optimal values of a single decision criterion and corresponding sets of the decision variables

	Optimization criteria				Decision variables		
Weight	Extract content	Acidity	Dry matter content	Amount of leak	Dehydration time [h]	Solution [-]	Storage time [weeks]
1	0,1836	1,1325	0,2037	0,028	24	0,65	11,5655
0,8	0,1836	1,1325	0,2037	0,0279	24	0,65	11,5685
0,6	0,1836	1,1324	0,2037	0,0279	24	0,65	11,5738
0,4	0,1139	1,0956	0,1658	0,0467	6	0,35	12
0,2	0,1139	1,0956	0,1658	0,0467	6	0,35	12
0	0,1139	1,0956	0,1658	0,0467	6	0,35	12

Conclusions

Formulated optimization algorithm and computer application in Matlab program allow to determine optimal set of decision variables in investigated osmotic dehydration process.

For the defined scalar optimization criterion an explicit dependence between quality and cost of the product was shown.

It was demonstrated that coupling linear additive scalar function with multidimensional, Euclidean metrics, enable to control the distance between points from different spaces simultaneously.

References

Kamińska A., Lewicki P., 2005. Metoda dehydrofreezing (D – F) – znaczenie i przyszłość. Przem. Spoż., 9, 12–15.

Khin M., Zhou W., Perera C., 2005. Development in the combined treatment of coating and osmotic dehydration of food – a review. Int. J. Food Eng., 1,15–27.

Jarczyk A., Witter M., Matuska D., 1994. Charakterystyka składu chemicznego i tekstury wybranych owoców odwodnionych osmotycznie i utrwalonych różnymi metodami. Przem. Fermentacyjny i Owocowo-Warzywny, 9, 22–24.

Błoński Z., Jędrzejczak A., Wąsowicz L., 1986. Ubytki naturalne zamrożonych owoców i warzyw powstające podczas ich przechowywani. Chłodnictwo, XXI5, 19–22.

Pukszta T. 2003. Zmiany kwasowości mrożonych owoców podczas przechowywania. Chłodnictwo, XXXVIII, 2, 34–35.

Palich P., Pukszta T., 2001. Zmiany wielkości wycieku rozmrażalniczego owoców mrożonych. Chłodnictwo, XXXVI, 1, 42–44.

Matusek A., Meresz P., 2002. Modelling of sugar transfer during osmotic dehydration of carrots, Periodica Polytechnica Ser. Chem. Eng., 1–2, 83–92.

Moreira R., Sereno A., 2003. Evaluation of mass transfer coefficients and volumetric shrinkage during osmotic dehydration of apple using sucrose solutions in static and non – static conditions. J. Food Eng., 57, 25–31.

Crank J., 1977. The mathematics of diffusion. Oxford.

Kowalska H., Lenart A., 2003. Zmiany współczynnika dyfuzji wody i substancji rozpuszczonych w owocach i warzywach odwadnianych osmotycznie, X Sympozjum suszarnictwa.

Gruda Z., Postolski J., 1999. Zamrażanie żywności. WNT, Warszawa.

Tarnowski W. 2001. Symulacja i optymalizacja w Matlab'ie, WSM Gdynia.

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PUMPKIN PUREE ENRICHED WITH JAPANESE QUINCE, CORNELIAN CHERRY, STRAWBERRY AND APPLE

Introduction

Puree production is targeted at preserving the nutritive value and vitamins of the starting material used, as well as at extracting the highest possible quantity of ingredients that are beneficial for human health. The nutritive value of the puree is largely influenced by the technological processes applied, and even more importantly, by the thermal processes, which have found wide acceptance in food processing industry. In the course of thermal treatment, the puree samples undergo physical and chemical changes that not only improve the acceptability of the products with respect to taste, smell and colour, but also upgrade their digestibility and storage quality.

Thermal treatment can reduce the biological value of the food. This reduction is, *inter alia*, attributable to the changes that have occurred in the carotenoids during the process. The double bonds that are present there raise the propensity of the carotenoids for oxidation, which not only reduces the nutritive value but also changes the taste and appearance of the carotenoid-rich products. This causes the carotenoids to convert from the more active *trans* form to the biologically less active *cis* form [Gawęcki, 2000].

Free oxygen radicals that form as a result of many reactions initiate oxidative changes in fat, protein and nucleic acid fractions, which may be the underlying cause of pathological changes in the human organism. To resist the influence of the reactive oxygen forms, the organism utilizes its own antioxidant system and supports itself using exogenous antioxidants [Horubała, 1999].

Antioxidant properties are recurrent among many compounds classified as secondary vegetable metabolites, mainly phenolics (phenolic acids, flavonoids) and terpenoids (carotenoids). That is why the consumption of food where such compounds occur in large amounts has an important part in the prophylaxis against many diseases. Epidemiological investigations indicate that the incidence of some cardio-vascular and cancer diseases is lower with regular consumption of fruit and vegetables, regardless of whether consumed fresh or as food-products [Horubała, 1999].

Pumpkins are valued primarily for their high content of carotenoids, and they are believed to protect human organisms against some cancer diseases [Oszmiański and Górska, 2002]. The high amount of carotenoids contributes largely to the antioxidant activity of the pumpkin. Another major benefit linked with this vegetable is the low calorific value (scarce 15 kcal per 100 g). Owing to its easily available low-calorific components, pumpkin has become a valuable part of slimming diets, a constituent that controls metabolism, as well as

exerts a detoxifying and dehydrating effect [Oszmiański and Górska, 2002]. Not surprisingly, with so many health improving properties, hardly any vegetable can compare favourably with pumpkin in the range of applications. Pureed pumpkin, a thermally processed intermediate, may be used for the production of sweets, jams, jellies, savouries, cakes, pasta, or beverages (to name just a few examples). Pumpkin itself is a high-yield vegetable, easy to grow, and, consequently, cheap. However, the changes that occur in colour, flavour and viscosity during thermal processing are some of the factors that undesirably affect the acceptability of the pureed product [Dutta et al., 2006].

Pureed pumpkin with no additives is bland and has a specific flavour that is not commonly accepted. It seems therefore advisable to enrich pumpkin with other pureed raw materials in order to achieve the organoleptic properties desired and, additionally, to upgrade the bioactivity of the product. The aim of this study was to assess the antioxidant activity of pureed pumpkin of the variety Karowita, enriched by the addition of Japanese quince, cornelian cherry, strawberry or apple in different proportions.

Materials and methods

Sampling

In this study use was made of pureed pumpkin of the variety Karowita. The samples were enriched with pureed Japanese quince, cornelian cherry, strawberry or apple of the variety Boskoop.

Pumpkin, Japanese quince and cornelian cherry puree samples were prepared at the stage of fruit maturity, immediately upon harvest time, and then frozen. To prepare strawberry puree, use was made of whole, frozen berries, which were pureed shortly before addition to the pumpkin puree sample. Apple puree samples were prepared from stored fruit, also shortly before addition to the pumpkin puree.

Ten variants of compositions were prepared: 10, 20 and 30% of Japanese quince and cornelian cherry each, and 20 and 30% of strawberry and apple each. The puree samples prepared earlier were stirred, subjected to heat treatment in a Thermomix TM (Vorverk – Germany) kitchen robot at 90°C for 2 min and hot-packed into jars. On the next day the puree was analyzed for dry matter, extract, viscosity, colour, vitamin C, carotenoids, total polyphenols, and antioxidant activity (DPPH, ABTS and FRAP).

Dry matter analysis

Dry matter content was determined according to PN-90/A-75101/03. The method entails drying at defined pressure and temperature until the sample attains a constant mass.

Total extract analysis

Extract content was analyzed in accordance with PN-90/A-75101/02. The method consists in measuring the refractive index of the sample examined. In the present study the extract content was determined using a digital Pocket PAL-1 refractometer made by ATAGO (Japan). The measuring range of the apparatus was between 0 and 53 Brix degrees, with automatic temperature compensation ranging from 10 to 75°C.

Rheological measurements

Rheological measurements were performed with a rotational BROOKFIELD LV, DV-II-Pro viscometer. Approximately 20 ml of the pureed sample were placed in the concentric cylindrical cup. The measuring parameters applied were as follows: spindle no. 64; time, 30s; stirrer speed, 20 rpm (except for the sample consisting of pumpkin and 30% Japanese quince, with 5 rpm).

Instrumental analysis of colour

The colour of the pureed pumpkin samples (reflectance values: L*, a* and b*) was measured using a Color Quest XE (HunterLab) spectrophotometer. Each puree were placed in a glass cuvette, and the colour was recorded using CIE L*a*b* $10^{o}/D_{65}$ colour spaces. L* denotes 'lightness', and its value ranges from 0 to 100 (0 for ideal black, and 100 for ideal white). A positive value of a* indicates 'red colour', a negative value of a* indicates 'green colour', a positive value of b* indicates 'yellow colour', and a negative value of b* indicates 'blue colour'.

Analysis of total carotenoids

Carotenoids were analyzed according to PN-90/A-75101/12. In this method carotenoids are extracted from the sample with hexane and their content in the extract is determined by colorimetry at 450 nm. In the present study extinction was measured with a Spekol 11 (Carl Zeiss Jena) spectrophotometer at 450 nm, in the presence of hexane.

Making use of the standard curve plotted for the potassium dichromate solution, carotenoid content (mg/100 g FW) was calculated in terms of the following equations:

$$x_1 = \frac{E_1 * 2{,}08}{0{,}500}$$

$$\frac{m}{V_0} = x$$

$$x_2 = \frac{x_1 * 100}{x}$$

where:
E_1 = sample absorption
m = weighed amount [g]
V_0 = volume of hexane layer [ml]
X_2 = carotenoid content [mg/100g]

Vitamin C

Vitamin C was analyzed in accordance with PN-90/A-75101/11. The method consists in the oxidation of l-ascorbic acid to dehydroascorbic acid in an acid medium with a blue dye of 2.6-dichloroindophenol, followed by the reduction of the dye to the *leuko* (colourless) form, which takes on a red colour at the pH of 4.2.

Extraction of Polyphenol Compounds for Antioxidant Activity Analysis

An approximately 5 g portion of each puree was weighed into a test tube for antioxidant property analysis. A total of 25 mL of 80% aqueous methanol was added, and the suspension was stirred slightly. The sample was sonicated for 5 min, and left at 4°C. After that the mixture was filtered using Schott funnels. The extracts obtained *via* this route were made subject to analysis. For the extraction of the samples with a cornelian cherry, strawberry or an apple additive use was made of 80% methanol treated with 1% HCl.

Analysis of Polyphenols

Total polyphenols were determined by the Folin-Ciocalteu [Slinkart and Singleton, 1977] method, using gallic acid (GA) as a standard for the calibration curve. The results were read at 765 nm after 1 h in a Shimadzu UV-2401 PC spectrophotometer. All determinations were performed in triplicate. The results of the assay were calculated and expressed as milligrams of GA equivalent (GAE) per 100 grams of fresh weight (FW).

Antioxidant Activity

DPPH Radical Scavenging Spectrophotometric Assay. The DPPH radical scavenging activity of pumpkin puree was determined according to the method proposed by Yen & Chen [1995]. The supernatant (0.5 mL) was added to 0.5 mL of DPPH and 1.5 mL of ethanol. The mixture was shaken and let to stand at room temperature for 10 min. Antioxidant capacity was measured by recording absorbance at 517 nm in a Shimadzu UV-2401 PC spectrophotometer. Ethanol was used as blank. All determinations were performed in triplicate.

$ABTS^{\bullet+}$ Radical Scavenging Spectrophotometric Assay. The $ABTS^{\bullet+}$ radical scavenging activity was measured according to the method developed by Re et al. (1999). The $ABTS^{\bullet+}$ solution was diluted with redistilled water until the absorbance of 0.700±0.02 at 743 nm was achieved. Upon addition of 60 μL of the supernatant to 3 mL of diluted $ABTS^{\bullet+}$ solution, absorbance was read exactly 6 min after initial mixing in the spectrophotometer (Shimadzu UV-2401 PC). All determinations were performed in triplicate.

Ferric Reducing Antioxidant Power (FRAP) Assay. The reducing potential of the sample was determined using the FRAP Assay proposed by Benzie et al. [1996] as a measure of antioxidant power. An antioxidant reduces the ferric ion (Fe^{+3}) to the ferrous ion (Fe^{+2}); the latter forms a blue complex (Fe^{+2}/TPTZ), which increases the absorbance at 593 nm. The supernatant (0.30 mL) and FRAP reagent (3 mL) were added to each well and mixed thoroughly. Absorbance was taken at 593 nm after 10 min. A standard curve was prepared using different concentrations of Trolox. All determinations were performed in triplicate.

The antioxidant activities (DPPH, ABTS, FRAP) of pumpkin puree with additives were expressed as Trolox equivalent antioxidant capacity (TEAC), using the calibration curve plotted against different amounts of Trolox. TEAC values were calculated and expressed as Trolox equivalents (TE) per gram of fresh weight.

Statistical analysis

The data obtained were analyzed statistically. All data were recorded as mean values ± SD and analyzed by Excel 2007. Analysis of variance was performed using ANOVA procedures, and statistical analysis was conducted with Statistica 7.0 (StatSoft Poland). Significant differences ($P \leq 0.05$) between the mean values were determined by Duncan's Multiple Range Test.

Results and discussion

Dry matter

The values of dry matter content, extract, and viscosity found in the samples examined are summarized in Table 1. As shown by these data, dry matter content varies between 10.3 and 14.21%. The highest and the lowest proportion of dry matter was determined in the sample with 30% addition of cornelian cherry and 30% addition of strawberry, respectively. This finding is to be attributed to the fact that the content of dry matter was higher in cornelian cherry than in strawberry puree.

Total extract

The highest level in total extract was found in the pumpkin sample with 30% enrichment by cornelian cherry (11.1), and the lowest in the samples with 20% addition and 30% addition of strawberry (8.3 and 8.4, respectively). Statistical analysis has revealed significant differences (Table 1).

Rheological measurement

As can be seen from the values in Table 1, viscosity varies over a very wide range: from 101227 mPas at 5 rpm to 7258 mPas at 20 rpm. The highest value was determined in the pumpkin sample enriched by 30% addition of Japanese quince, and the lowest value in the one enriched with 30% addition of strawberry.

The study makes it clear that when the proportion of the additive increases, so does the viscosity of the sample. With 10% addition of cornelian cherry, viscosity amounted to 13657.3 mPas, and increased to 15018.3 mPas at 30% enrichment. This finding is to be attributed to the high viscosity of the Japanese quince puree and the low viscosity of the strawberry puree, as well as to the presence of pectins in the starting materials used.

Table 1

Values of dry matter content, extract, and viscosity of pumpkin puree with additives

Sample	Dry matter %	Extract %	Viscosity mPas
Q10	12.23±0.077^{d,e}	9.2±0.071^{c,d}	21887.8±0.092^{c}
Q20	12.17±0.226^{d,e}	8.9±0.072^{d}	23905.0±0.219^{b}
Q30	12.78±0.077^{c}	9.2±0.141^{c,d}	101227.0 ±0.523^{a}
C10	12.31±0.141^{d}	9.8±0.073^{b}	13657.3±0.049^{d,e}
C20	13.34±0.014^{b}	10.7±0.141^{a}	14532.6±0.049^{d}
C30	14.21±0.091^{a}	11.1±0.035^{a}	15018.3±0.247^{d}
S20	10.84±0.113^{f}	8.3±0.071^{e}	8161.3±0.035^{f}
S30	10.30±0.084^{g}	8.4±0.071^{e}	7258.0±0.049^{f}
A20	11.96±0.014^{e}	9.5±0.141^{b,c}	13407.4±0.481^{d,e}
A30	12.16±0.127^{d,e}	9.0±0.072^{c,d}	12342.5±0.445^{e}

[a] Values (within columns) which have been assigned the same letters are not significantly different (Duncan's Multiple Range Test, $P<0.05$).

Q = pumpkin and Japanese quince C = pumpkin and cornelian cherry S = pumpkin and strawberry A = pumpkin and apple.

Colour

The values of colour parameters (CIE Lab system) are summarized in Table 2. The lightness (L*) of the enriched pumpkin puree samples ranges from 39.58 to 60.08. The brightest are the Japanese quince-enriched pumpkin samples (39.58), and the darkest those enriched by 30% addition of cornelian cherry (60.08). The differences in lightness between the samples with 20 and 30% apple additives (51.86 and 51.43, respectively), as well as between the samples with 20 and 30% strawberry additives (45.74 and 45.49, respectively) are negligibly small.

Table 2

Colour parameters of pumpkin puree with additives

Sample	L*	a*	b*
Q10	58.36±0.03 [c]	19.81±0.03 [c]	50.25±0.09 [c]
Q20	59.49±0.03 [b]	18.52±0.02 [d]	51.02±0.22 [b]
Q30	60.08±0.30 [a]	16.98±0.23 [f]	51.79±0.52 [a]
C10	45.38±0.08 [f,g]	18.70±0.02 [d]	28.75±0.05 [g]
C20	41.36±0.05 [h]	22.27±0.08 [b]	20.80±0.04 [h]
C30	39.58±0.19 [i]	23.67±0.19 [a]	17.35±0.25 [i]
S20	45.74±0.02 [f]	16.87±0.02 [f]	30.46±0.03 [f]
S30	45.29±0.19 [g]	17.81±0.04 [e]	28.63±0.05 [g]
A20	51.86±0.13 [d]	15.30±0.05 [g]	40.80±0.48 [d]
A30	51.43±0.27 [e]	13.95±0.29 [h]	38.55±0.44 [e]

[a] Values within columns with similar letters are not significantly differ (Duncan's multiple range test, P<0,05).
Q = pumpkin and Japanese quince C = pumpkin and cornelian cherry S = pumpkin and strawberry A = pumpkin and apple.

Pureed Japanese quince, which displayed the highest lightness values, increased the lightness of the pumpkin puree, whereas pureed cornelian cherry, the darkest of the additives examined, reduced the lightness of the puree to which it had been added. Wojdyło et al. [2008] have reported that in their study on the colour of jams prepared from two strawberry cultivars and different additives the brightest and the darkest jams were obtained with Japanese quince-enriched and chokeberry-enriched samples, respectively.

As for the pumpkin puree with red additives (cornelian cherry, strawberry) examined in the present study, higher parameter a* values were determined in the samples enriched with cornelian cherry. The highest (23.64) and the lowest (16.87) value were measured in the sample with 30% addition of cornelian cherry and in the one with 20% addition of strawberry, respectively. The high value of a* in the samples enriched with cornelian cherry is probably attributable to the high content of anthocyanins in the fresh material. Kucharska et al. [2008] have observed that anthocyanin content differed among the varieties of cornelian cherry, and that it ranged from 46.19 to 122.07 mg/100 g.

In our study, the highest parameter b* values, which varied from 50.25 to 51.79, were obtained with Japanese quince-enriched samples; the lowest values, 17.85 and 20.8, being found in the samples with 20% addition and 30% addition of cornelian cherry, respectively. In the strawberry jam prepared from the cultivar Senga Sengana, the value of b* was 11.66, and increased to 13.37 in the sample with 10% addition of Japanese quince. The value of b*

in the strawberry jam sample obtained from the cultivar Elkant was lower (8.86), but it also increased upon enrichment with a 10% Japanese quince additive (16.59) [Wojdyło, 2008]. It is essential to note that the values reported in the literature were in every instance lower than the values determined in our present study.

Vitamin C

The concentrations of vitamin C in the pumpkin puree varied largely among the samples examined, falling between 1.36 and 23.42 mg/100 g FW (Table 3). The poorest vitamin C content, which ranged from 1.36 to 1.6 mg/100 g FW, was measured in the apple-enriched pumpkin samples. These values are significantly lower as compared to those determined in the other pureed samples, which is to be attributed to the low vitamin C content in the apple variety used. As for the other pumpkin puree samples, the lowest content of vitamin C was determined in the sample enriched with the 30% cornelian cherry additive (10.72 mg/100 g FW). Japanese quince-enriched samples were characterized by the highest vitamin C content, which amounted to 17.88, 20.71 and 23.42 mg/100 g FW for 10, 20% and 30% enrichment, respectively.

A previous study (data not published) has revealed that the content of vitamin C in pumpkin puree of the variety Karowita amounts to 14.12 mg/100 g FW. There was observed decrease of vitamin C concentration in apple as well as cornelian cherry enriched samples of pumpkin puree. With the addition of quince, the level of vitamin C increased significantly.

Carotenoids

Total carotenoid content in pumpkin largely depends on variety, the temperatures that occur during the vegetation period, the cultivation conditions, and more importantly, on the technological processes applied, specifically thermal processing.

The content of total carotenoids in the samples examined varied from 4.89 to 7.27 mg/100 g FW (Table 3). Of this range, higher values were determined in the pumpkin puree samples enriched with lower proportions of the additives, namely: 7.27 mg/100 g FW in the sample with 10% enrichment by cornelian cherry and 6.98 mg/100 g FW in the sample enriched by 10% addition of Japanese quince. The lowest carotenoid content, 4.89 mg/100 g FW, was measured in the sample where quince-enrichment amounted to 30%. According to the previous (data not published) study mentioned, total carotenoid content in the puree prepared from pumpkin of the variety Karowita was 7.4 mg/100 g FW. From the available literature data it can be inferred that in all of the starting materials used as additives to the pumpkin puree examined in our present study total carotenoid content was lower than 7.4 mg/100 g FW.

Polyphenols

The content of polyphenols ranged from 35.31 to 135.15 mg GAE/100 g FW, and differed greatly from one final product to another, depending on the proportion of, as well as the polyphenol content in, the fruit puree added. When the proportion of the polyphenol-rich fruit additive in the sample increased, so did the polyphenol content in the final product. The highest values, 94.03 and 135.15 mg GAE/100 g FW, were measured at 20% addition and 30% addition of Japanese quince, respectively. At 10% addition, the content of polyphenols in the final product was markedly lower, as it amounted to 54.89 mg GAE/100 g FW only.

The lowest content of polyphenols measured in the course of the study, 35.31 mg GAE/100g FW, was determined in the pumpkin puree with 10% cornelian cherry enrichment.

In the study reported by Turkmen et al. [2005] the question of how particular methods of thermal processing influence the polyphenol content and antioxidant activity has not been answered unequivocally. Upon boiling, the content of polyphenols in pepper increased (from 1344.8 mg GAE/100g DM to 1538.4 mg GAE/100g DM), whereas that in leek decreased (from 300.8 mg GAE/100g DM to 193.9 mg GAE/100g DM).

Blanching for 1 min in boiling water was found to reduce (by 12 to 26%) total polyphenols in these vegetables. Sahlin et al. [2004] have observed that cooking by boiling, baking and frying resulted in a significant reduction ($p<0.01$) in the total polyphenol and lycopene contents in tomatoes.

Table3

Content of carotenoids, vitamin C, pholyphenol, and antioxidant activity determined by the DPPH, ABTS, and FRAP

suro-wiec	karoteny	Vitamin C	polifenole	DPPH	ABTS	FRAP
	mg/100g FW	mg/100g FW	mg GA-E/100g FW	µmolTrolox/g puree	µmolTrolo-x/g puree	µmolTrolo-x/g puree
Q10	6.98±0.11 [b]	17.88±0.17 [c]	54.89±1.96 [e]	2.48±0.13 [c]	1.04±0.03 [d]	0.59±0.12 [c,d]
Q20	6.28±0.03 [c]	20.71±0.01 [b]	94.03±1.92 [b]	2.59±0.19 [c]	1.98±0.11 [b]	1.09±0.20 [b]
Q30	4.89±0.01 [g]	23.42±0.35 [a]	135.15±2.89 [a]	2.98±0.06 [b]	2.66±0.06 [a]	1.78±0.38 [a]
C10	7.27±0.04 [a]	14.30±0.01 [d]	35.31±0.28 [h]	2.85±0.10 [d,e]	0.61±0.03 [g]	0.61±0.57 [c,d]
C20	5.91±0.01 [d]	12.70±0.16 [e]	44.11±2.33 [f]	3.16±0.27 [b]	0.98±0.07 [d,e]	0.72±0.16 [c]
C30	5.32±0.04 [f]	10.72±0.18 [f]	60.58±2.21 [d]	3.79±0.12 [a]	1.42±0.09 [c]	0.80±0.02 [c]
S20	5.25±0.09 [f]	12.82±0.34 [e]	66.61±2.17 [c]	2.09±0.16 [d,e]	0.77±0.03 [f]	0.51±0.19 [c,d]
S30	5.86±0.07 [d]	14.55±0.35 [d]	60.75±0.11 [d]	2.54±0.14 [c]	1.02±0.14 [d,e]	0.77±0.14 [c]
A20	5.88±0.03 [d]	1.60±0.18 [g]	38.90±1.30 [g]	1.74±0.15 [e]	0.87±0.15 [e,f]	0.34±0.15 [d]
A30	5.45±0.06 [e]	1.36±0.17 [g]	40.77±2.76 [f,g]	1.81±0.13 [d,e]	0.97±0.13 [d,e]	0.33±0.03 [d]

[a] Values within columns with similar letters are not significantly differ (Duncan's multiple range test, P<0,05).
Q = pumpkin and Japanese quince C = pumpkin and cornelian cherry S = pumpkin and strawberry A = pumpkin and apple.

Antioxidants

All samples were examined for their antioxidant activity. Table 3 summerizes the antioxidant activity of mixed puree determined by the DPPH, ABTS and FRAP methods.

Antioxidant activity expressed as DPPH ranged from 1.75 to 3.79 µmolTrolox/g puree. The highest values were obtained for the puree samples with a 20% additive and 30% additive of cornelian cherry (3.16 µmolTrolox/g puree and 3.79 µmolTrolox/g puree, respectively). With a 10% cornelian cherry additive, DPPH activity amounted to 2.85 µmolTrolox/g puree. The antioxidant activity values of the Japanese quince-enriched pumpkin puree varied between 2.48 and 2.98 µmolTrolox/g puree, showing less distinct differences as compared to those observed in the case of the cornelian cherry additive.

When expressed as ABTS, antioxidant activity ranged from 0.62 to 2.66 µmolTrolox/g puree. The highest values, 1.05, 1.98 and 2.66 µmolTrolox/g puree, were measured in Japanese quince-enriched pumpkin puree samples with a 10%, 20% and 30% additive, respec-

tively. These values correlate both with the content of polyphenols and the Ferric Reducing Antioxidant Power (FRAP): r=0.965 and r=0.923, respectively (Table 4). No correlation has been observed with the antioxidant activity expressed as DPPH.

In their research involving pureed samples prepared from three strawberry varieties, Oszmiański et al. [2009] have measured higher values of ABTS antioxidant activity as compared to the ones obtained in the study reported on in this paper. Seemingly, this is attributable to the low antioxidant activity of the pumpkin.

Antioxidant activity expressed as FRAP was found to vary from 0.34 to 1.78 μmolTrolox/g puree. These were the lowest antioxidant activity values obtained in the present study. As was the case with the ABTS activity, the highest values were measured in Japanese quince-enriched pumpkin puree samples (1.78 μmolTrolox/g puree), and the lowest in the pumpkin samples with apple enrichment (0.34 μmolTrolox/g puree). The FRAP value for strawberry puree reported by Oszmiański et al. [2009] (μmolTrolox/g puree) was approximately ten times as high as the one obtained in our present study (0.34 μmolTrolox/g puree) for the strawberry-enriched pumpkin samples.

In every instance did the antioxidant-rich additives upgrade the antioxidant activity of the samples examined, which increased with the proportion of the additive introduced.

Table 4

Correlation coefficients for vitamin C, polyphenols, and antioxidant activity determined by DPPH, ABTS, and FRAP

	Vitamin C	Polyphenols	DPPH	ABTS
Polyphenols	0.523			
DPPH	0.113	0.478		
ABTS	0.634	**0.966**	0.497	
FRAP	0.519	**0.938**	0.535	0.923

Conclusions

The study into the enrichment of pumpkin puree with fruit additives in various proportions has revealed that even though each of the fruits examined can be used for this purpose, the addition of Japanese quince is the most promising. 30% enrichment by Japanese quince increased the content of polyphenols and consequently the antioxidant activity of the product. These Japanese quince-enriched samples were also characterized by the highest content of vitamin C, as well as by a high value of lightness L* and parameter b*. The highest value of parameter a* was determined in the pumpkin puree with 30% enrichment by the cornelian cherry additive, and the sample also displayed the highest extract value. The colour of the product, however, was not very attractive. The enrichment of the pumpkin puree with apple produced very poor results as compared to Japanese quince or cornelian cherry; the content of vitamin C was very low, and antioxidant activity values were the lowest among those measured in the course of the study.

References

Benzie I.F.F., Strain J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. Anal. Biochem., 239, 70–76.

Dutta D., Dutta A., Raychaudhuri U., Chakraborty R., 2006. Rheological characteristics and thermal degradation kinetics of beta-carotene in pump kin puree. J. Food Eng., 76, 538–546.

Gawęcki J. 2000. Żywienie człowieka. Podstawy nauki o żywieniu. Wydawnictwo Naukowe PWN, Warszawa.

Horubała A., 1999. Pojemność przeciwtleniająca i jej zmiany w procesach przetwarzania owoców. Przemysł Fermentacyjny i Owocowo-Warzywny, 3, 30–32.

Kucharska A.Z., Sokół-Łętowska A., 2008. Skład chemiczny i wykorzystanie owoców dziko rosnących. W: Łuczaj Ł. (red) Dzikie rośliny jadalne – zapomniany potencjał przyrody. Mat. Konf. Przemyśl – Bolestraszyce 13 września 2007, 95–108.

Oszmiański J., Górska M., 2002. Improvement of carotenoid content and colour in carrot juice concentrate. Fruit Proc., 2, 70–73.

Oszmiański J., Wojdyło A., 2009. Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. Eur. Food Res. Technol., 228, 623–631.

PN-90/A-75101/03

PN-90-75101/02

PN-90/A-75101/11

PN-90-75101/12

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., 1999. Antioxidant activity applying an improved abts radical cation decolorization assay. Free Rad. Biol. Med., 26, 1231–1237.

Sahlin E., Savage G.P., Lister C.E., 2004. Investigation of the antioxidant properties of tomatoes after processing. J. Food Comp. Anal., 17, 635–647.

Slinkart K., Singleton V.L., 1977. Total phenol analysis: automation and comparison with manual method. Am. J. Enol. Viticulture, 28, 49–55.

Yen G.C., Chen H.Y., 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 43, 27–32.

Turkmen N., Sari F., Velioglu Y.S., 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem., 93, 713–718.

Wojdyło A., Oszmiański J., Bober I., 2008. The effect of addition of chokeberry, flowering Japanese quince fruits and rhubarb juice to strawberry jams on their polyphenol content, antioxidant activity and colour. Eur. Food Res. Technol., 227, 1043–1051.

28

COMPOSITION OF AROMA COMPOUNDS IN BLACK-CURRANT AND RASPBERRY JUICES WITH HYDROLYSED OAT FLAKES SOLUBLE FRACTION ADDITIVE

Introduction

Oats are currently used because of their positive health image related to the high concentration of β-glucans. Identification of volatile compounds in oats include studies on oat groats, oatmeal porridge [Morello, 1998], rolled oats [Zhou et al., 2000], oat flakes [Schun and Scieberle, 2005]. Oats contain high percentages of unsatured fatty acids that could be the most important factor influencing the formation of aliphatic aldehydes, ketones, alcohols, furans and acids [Klensporf, 2008]. One of the key odorants in oats was reported (E,E.Z)-2,4,6-nonatrienal [Schun and Scieberle, 2005]. Generally in oats compounds belonging to different classes were reported. The following carbonyl compounds were identified: pentanal, hexanal, heptanal, octanal, nonanal, decanal, E-2-nonenal, E-2-decenal and ketones such as heptan-2-one, nonan-2-one, alcohols – pentan-1-ol, hexan-1-ol, nonan-1-ol, octan-1-ol and also 2-pentyl furan. The main volatile compound in oat flakes is hexanal, followed by nonanal, hexan-1-ol [Klensporf, 2008].

Raspberries contain significant amounts of polyphenol antioxidants such as anthocyanin pigments linked to potential health protection against several human diseases. Raspberries are a rich source of vitamin C, manganese. Contents of B vitamins 1-3, folic acid, magnesium, copper and iron are considerable in raspberries. Raspberries rank near the top of all fruits for antioxidant strength, particularly due to their dense contents of ellagic acid (from ellagotannins), quercetin, gallic acid, anthocyanins, cyanidins, pelargonidins, catechins, kaempferol and salicylic acid. Yellow raspberries and others with pale-colored fruits are lower in anthocyanins [Raspberries, 2009].

Raspberries are soft, juicy with a distinct aroma and are a good source of natural antioxidants. In addition to vitamins and minerals, raspberries are also rich in anthocyanin, phenolic acids, and other flavonoids [Wang and Lin, 2000, Heinonen et al., 1998, Wang et al., 1996].The most potent flavour compounds in fresh raspberries were β-damascenone, diacetyl, sotolon, 1-hexen-1-one, 1-nonen-3-one, 1-octen-3-one, cis-3-hexenal [Roberts, 1996], whereas Robertson (1995) reported raspberries with ethyl acetate, trans-β-ocimene, α-ionone,3-pentanone, 2-methyl-1-butanol, α – pinene, camphene, α-phellandrene and β-caryophyllene as main aroma compounds of ripe berries. Volatile compounds varied among cultivars, with benzaldehyde (11.1–31.8%), α-pinene (4.0–11.5%), ethyl heptanoate (6.9–15.2%), β-myrcene (13.2–19.6%) and γ-terpinene (12.2–20.0%) as the predominant volatiles [Shamaila, 1993]. Klesk et al. [2004] reported 75 aromas of different raspberry

cultivars, 22 compounds had equivalent odour impart to tested cultivars: 2,5-dimethyl-4-hydroxy-3-(2H)-furanone, hexanal, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one, (E)-beta-3,7-dimethyl-1,3,6-octatrieneT, 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptaneT, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, ethanoic acid, (Z)-3-hexenalT, 3-methylmercaptopropionaldehyde, (Z)-3-hexenol, 2,6-dimethyl-2,7-octadien-6-ol, butanoic acid, ethyl 2-methylpropanoate, (E)-2-hexenal, hexyl formateT, 2,3-butanedione, heptanalT, thiacyclopentadieneT, cyclohexane carbaldehydeT, (E)-3,7-dimethyl-2,6-octadien-1-olT, and 4-(p-hydroxyphenyl)-2-butanone. Large variations for α-ionone, β-ionone, geraniol, linalool, and (Z)-3-hexenol were observed in different raspberry cultivars [Malowicki, 2008].

Many Ribes species, especially the darker-fruited blackcurrant types, contain high concentrations of polyphenolic compounds, notably anthocyanins and flavonols, and these components are of growing importance due to their link to human health, together with high levels of ascorbic acid (vitamin C) [Brennan, 2008]. Black-currant fruit has an extraordinarily high vitamin C content, good level of potassium, phosphorus, iron and vitamin B5 [Black--currant, 2009]. Black-currant leaves are mainly used for their diuretic property. A tea made from dried black-currant leaves is used against arthritis, urinary problems, diarrhea, bleeding gums and coughs. The scientist Kessler of the University of Bonn found that black-currant juice could support the treatment and metaphylaxis of uric acid stone disease because of its alkalizing effect. Black-currant juice increased the urinary pH level and the excretion of citric acid. The anthocyanidins in the black-currant berries are responsible for their antioxidant and antibacterial action [Black-currant, 2009]. Major anthocyanins in blackcurrant pomace are delphinidin-3-O-glucoside, delphinidin-3-O-rutinoside, cyanidin-3-O-glucoside, and cyanidin-3-O-rutinoside which are retained in the juice concentrate among other yet unidentified polyphenols [Black-currant, 2009].

The aroma profile of black-currant juice comprise of specific profiles of terpenoids, aliphatic esters, carbonyl compounds and alcohols, some of the characteristic compounds are ethyl butanoate, iso amyl acetate, 3-methyl-1-butanol, cis-3-hexene-1-ol, ethyl hexanoate, benzaldehyde, linalool, octanal, 1,8-cineole, urfural, eugenol, diacetyl [Soli, 2008].

The aim of the current research was to determine aroma compounds in black-currant and raspberry juices with hydrolysed oat flakes soluble fraction additive.

Materials and methods

Experiments were performed at the Research Laboratory of Food Products Packing Laboratory of the Department of Food Technology at the Latvia University of Agriculture (LLU).

For preparation of hydrolysed oat flakes oatmeal from ISC "Rīgas Dzirnavnieks" was used. Oat flakes with the content of moisture equaling to 9.8%, water and enzyme preparate Ceremix Plus MG (α-amylase, "NOVOZYMES", Lithuania) were used for the preparation of oat flake hydrolysate. Enzyme characteristics: optimum temperature +65°C, time of fermentation 20–25 min, the recommended dosage of enzyme – 0.5–0.9 kg to 1 ton of grain. Enzyme is obtained from production organisms – Bacillus amyloliquefaciens, Bacillus licheniformis, Humicola insolens.

Concentration and hydrolyse conditions of the enzyme Ceremix Plus MG were chosen the ones to make possible a homogeneus maturation of oat flake mass and a complete starch

gelatinization as a result of which a maximum oat starch modification into reducing sugars would be obtained. The conditions of oat product treatment by enzyme, including the enzyme concentration, temperature and time of the reaction were selected according to the recommendations of enzyme producer.

The technological scheme of preparation of hydrolysed oat flakes is shown on Figure 1.

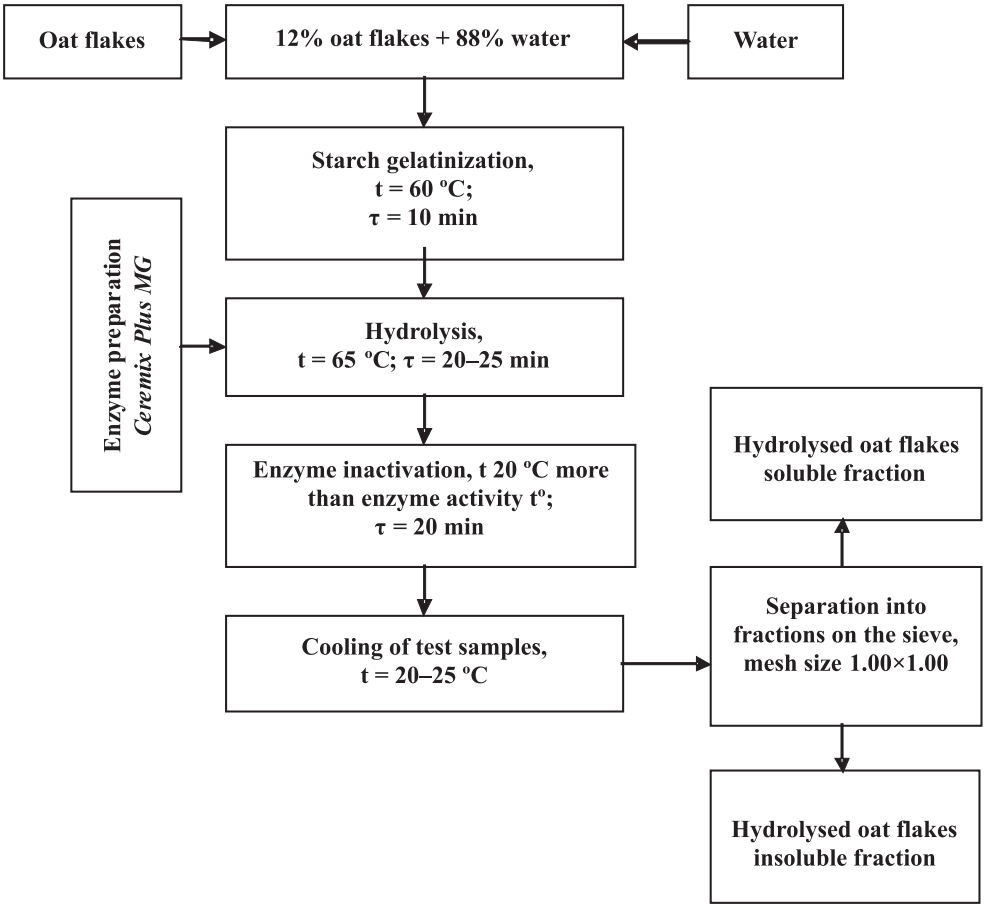

Fig. 1. Technological scheme of hydrolysed oats preparation

The enzyme was inactivated at the end of hydrolysation process by increased temperature. For separation of the hydrolysed oat flakes into fractions, a sieve was used (meshes 1.00×1.00 mm). After separation into fractions, the soluble fractions of oat extracts were mixed with black-currant and raspberries juices in ratios 30:70, 40:60. For drink preparation black-currant and raspberries juices purchased from the Latvia State Institute of Fruit Growing was used.

A sample of 5 g was weighed in 20 ml vials and capped. Volatile aroma compounds were extracted using headspace autosampler Turbomatrix (PerkinElmer). Headspace extraction parameters: temperature – 70 °C, duration – 30 min, injection time 0.1 min, pressurization time 2 min. For the analysis of volatile aroma compounds, a PerkinElmer Clarus 500 GC/MS

equipped with a capillary column Elite-Wax ETR (60 m x 0.25 mm i.d.; DF 0.25 μm) was used. GC working conditions were: injector – 250°C; oven: start – 40°C, hold – 10 min, programmed from 40 to 150°C at 5°C min^{-1}, hold – 10 min and from 150 to 200°C at 5°C min^{-1}, hold 10 min; carrier gas (He) – 1 ml min^{-1}; splitless. Mass spectrometer in Electron impact Ionization (EI+) mode with inlet line temperature at 250°C and source temperature 250°C was used. Acquisition parameters in full scan mode: scanned m/z 40–400. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98 and retention times of the standards. Analyses were performed in triplicate.

Results and discussion

Results of organoleptical evaluation of juices with hydrolysed oat flakes soluble fraction additive showed, that a better proportion of juice and hydrolysed oat flakes soluble fraction additive is 70:30 and 60:40. Aroma compounds were detected in hydrolysed oat flakes soluble fraction, raspberry and black-currant juices and drinks with juices and hydrolysed oat flakes soluble fraction. The biggest amount of volatile aroma compounds was found in black-currant juice (Fig. 2). In raspberry juice amount of total aroma compounds were more than 8 times lower than in black-currant juice. In juices with oat soluble fraction were found lower amount of volatiles than in juices itself, because oat soluble fraction contains small amount of volatiles, and aroma of drinks is mainly given by the added juice. Athough when the drink was made higher proportion of volatiles was preserved in a drink containing raspberry juice.

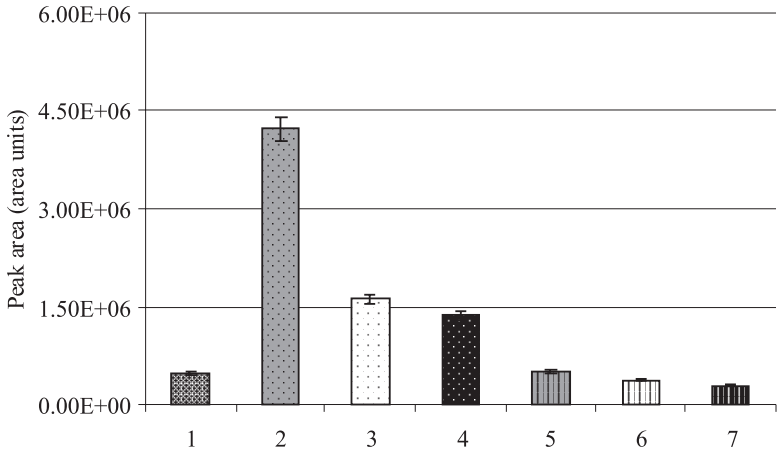

Fig. 2. Total aroma compounds in hydrolysed oat flakes soluble fraction, raspberry and black-currant juices and oat drinks with juices:
1 – Hydrolysed oat flakes soluble fraction; 2 – Black-currant juice; 3 – Black-currant juice + soluble oat fraction (70:30); 4 – Black currant juice + soluble oat fraction (60:40); 5 – Raspberry juice; 6 – Raspberry juice + soluble oat fraction (70:30); 7 – Raspberries juice + soluble oat fraction (60:40)

The studied drinks differ both in quantitative and qualitative content of aroma compounds. In hydrolysed oat flaxes soluble fraction two volatile compounds were identified: 2-aminonadecane and 5-methyl-2-hexanone, respectively 85.4 and 14.65%, some compounds like hexanal, nonanal were identified in trace level.

Totally 7 volatile compounds belonging to different classes were identified in black-currant juice. As the main aromatic compound group was detected monoterpenes (5 compunds) represented by α-pinene, isoterpinolene, trans-β-ocimene, α-thujene. The major aroma forming compounds were α-pinene followed by isoterpinolene and 2-aminotridecane (Fig. 3).

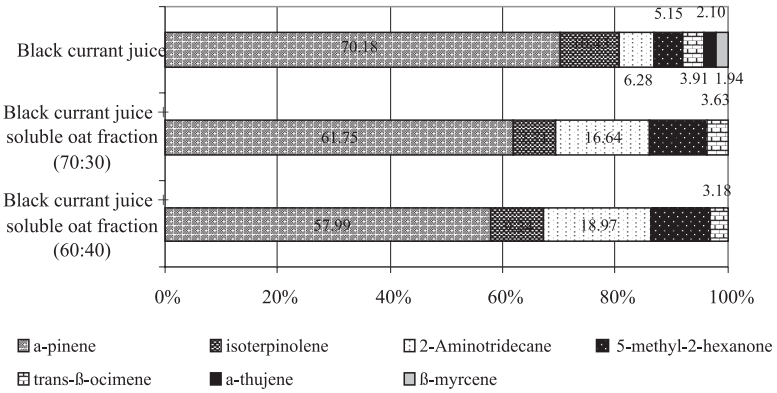

Fig. 3. Aroma compounds in black-currant juice and oat drinks with black currant juice

The composition of headspace of black-currant juice and juice with soluble oat fraction differs. Smaller percentage was detected for α-pinene and isoterpinolene, because these compounds were found only in juice, whereas increase of 2-aminonadecane and 5-methyl-2-hexanone were detected because these compounds were found in both: hydrolysed oat flakes soluble fraction and juice.

In raspberry juice 4 compounds were identified: aminonadecane, ethylacetate, 5-methyl-2-hexanone and 2-(Ethenyloxy)-propane (Fig. 4).

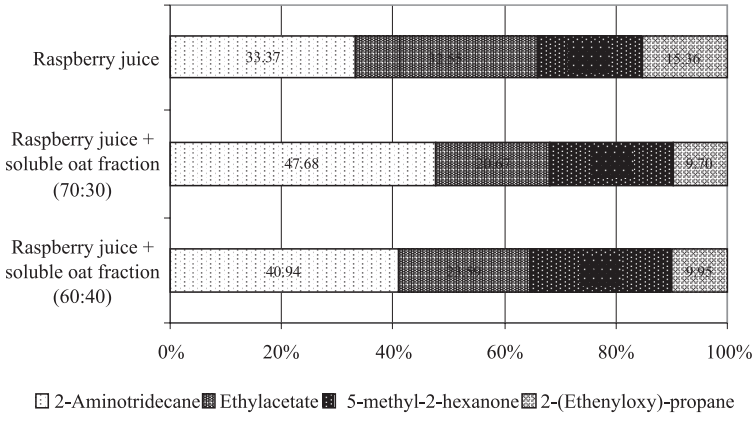

Fig. 4. Aroma compounds in raspberry juice and oat drinks with raspberry juice

The similar tendencies like in drinks with black currant juice were observed - the higher percentages were detected for 2-aminonadecane and 5-methyl-2-hexanone comparing to juice itself.

Conclusions

The studied drinks differ both in quantitative and qualitative content of aroma compounds. Totally 9 volatile compounds were identified in all analysed samples and 2-aminonadecane and 5-methyl-2-hexanone are the major aroma forming compounds. The highest amount of volatile aroma compounds was found in black-currant juice and in drinks with black currant juice. Hydrolised oat soluble fraction contains small amount of volatiles, and aroma of drinks is mainly given by the added juice.

References

Black-currant, 2009. Address of resource is: http://en.wikipedia.org/wiki/Blackcurrant, resource was viewed at 20.05.2009.

Black-currant, 2009. Address of resource is: http://www.phytochemicals.info/plants/blackcurrant.php, resource was viewed at 20.05.2009.

Brennan R.M., 2008. Currants and gooseberries, in: Temperate fruit crop breeding. Ed. by J.F. Hancock, Netherlands, Springer, 176–196.

Heinonen I.M., Meyer A.S., Frankel E.N., 1998. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J. Agric. Food Chem., 46, 4107–4112.

Klensporf D., Jelen H.H., 2008. Effect of heat treatment on the flavour of oat flakes. J. Cereal Sci., 48, 656–661.

Klesk K., Qian M., Martin R.R., 2004. Aroma extract dilution analysis of cv. Meeker (Rubus idaeus L.) red raspberries from Oregon and Washington. J. Agric. Food Chem., 52, 5155–5161.

Malowicki S.M.M., Martin R., Qian M.C., 2008. Volatile composition in raspberry cultivars grown in the pacific northwest determined by stir bar sorptive extraction-gas chromatography-mass spectrometry. J. Agric. Food Chem., 56, 4128-4133.

Morello M., 1998. Influence of preparation on the aroma compounds of oatmeal porridge, in: Contis E.T., Ho C.T., Mussinan C.J., Parliament T.H., Shahidi F., Spanier A.M. Food Flavors: Formation, Analysis and Packaging influences, Elsevier, Amsterdam, 415–422.

Raspberries, 2009. Address of resource is: http://en.wikipedia.org/wiki/Raspberry, resource was viewed at 20.05.2009.

Roberts D.D., Acree T.E., 1996. Aroma analysis of raspberry samples by retronasal simulation combined with headspace gas chromatography – olfactometry. Food Qual. Pref., 7, 322.

Robertson G.W., Griffiths D.W., Woodford J.A.T., Birch A.N.E., 1995. Changes in the chemical; composititon of volatiles released by the flowers and fruits of the red raspberry (Rubus idaeus) cultivar Glen Prosen. Phytochem., 38, 1175–1179.

Shamaila M., Skura B., Daubeny H., Anderson A., 1993. Sensory, chemical and gas chromatographic evaluation of five raspberry cultivars. Food Res. Int., 26, 443–449.

Schuh C., Schieberle P., 2005. Characterization of (E,E,Z)-2,4,6-nonatrienal as a character impact compound of oat flakes. J. Agric. Food Chem., 53, 8699–8705.

Soni V., Abildskov J., Jonsson G., Gano R., 2008 Modelling and analysis of vacuum membrane distillation for the recovery of volatile aroma compounds from black currant juice. J. Membrane Sci., 320, 442–455.

Wang H., Cao G., Prior R.L., 1996 Total antioxidant capacity of fruits. J. Agric. Food Chem., 44, 701–705.

Wang S.Y., Lin H.S. 2000 Antioxidant activity in fruit and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem., 48, 140–146.

29

INFLUENCE OF RESISTANT STARCH ON WHEAT DOUGH AND BREAD QUALITY

Introduction

In the recent years there has been a significant shift in general nutrition patterns, resulting from the increasingly popularized achievements of technological development, informatization and robotization, as well as the changes in people's eating habits and lifestyle [Diowksz, 2006; Dziugan et al., 2006]. Civilizational transformations have led to larger consumption of highly processed foods. This in turn caused impoverishing the diet in dietary fiber, which is essential for proper functioning of the human body. A change of economic conditions has been followed by a shift in views on nutrition. These days a tendency to attempt to reduce the energy content of meals, especially in developed countries, is being observed [Bartnikowska, 1997; Leszczyński, 2004; Mielcarz, 2004a].

In the recent years physiologists, nutritionists and technologists have been increasingly interested in starch resistant to the hydrolytic influence of the human body's digestive enzymes. Resistant starch (RS) is thus the sum of starch and its breakdown products which are not absorbed in the small intestine of a healthy human being [Champ, 1994]. Different forms of resistant starch can be distinguished. Resistant starch type 1 (RS 1) is the kind of starch found in plant cells with intact cell walls, for example in whole wheat grains. Resistant starch type 2 (RS 2) is the starch found in raw (ungelatinized) granules of some plant species, like potato or banana, and type 3 (RS 3) is retrograded starch. Resistant starch type 4 (RS 4) is chemically or physically modified starch [Englyst and Cummings, 1987, Haralampu, 2000]. Resistant starch has been attributed numerous health benefits. Therefore, it is regarded as a component of dietary fiber [Ohr, 2004; Englyst and Hudson, 1997]. The beneficial influence of resistant starch on human digesting process is revealed in better glucose tolerance (glycaemic index reduction), lowering lipids level in blood and the increase of chyme mass. Indigested resistant starch is fermented in the large intestine, as a result of which short chain fatty acids are created (acetate, propionate and butyrate). This in turn results in significantly reducing pH in the bowel, and consequently a selection of microorganisms takes place in the large intestine (thus providing its antineoplastic protection) [Leszczyński, 2004; Soral-Śmietana and Wronkowska, 1999]. Breads and cereal products are a large part of daily human nutrition, thus they may play an important role in the diet of an ill person, aiding their treatment. The importance is no lesser in the case of a healthy human being, where their role might be preventive [Mielcarz, 2004b; 2005].

The aim of this research was the evaluation of the properties of dough and quality of wheat bread in which retrograded acetylated starch (RS 4) is used.

Materials and methods

Materials. The research material was wheat flour type 550, 750 and 2000 incorporated with retrograded acetylated starch (resistant starch RS 4). The starch had been obtained according to the methodology guidelines developed by the Department of Food Storage and Technology at the Wrocław University Of Environmental And Life Sciences. The starch preparations had been obtained according to the P-382126 patent application [Zięba, 2007]. Potato starch had been retrograded and then acetylated. The acetylating process had been carried out analogically to the way it is done in Polish starch production plants [Mężyński, 1972]. The obtained resistant starch preparations had been crumbled and sifted with a sieve with meshes of 265 μm. The content of resistant starch in the achieved flour samples was 0, 10, 20, 30 and 40%. The control sample was wheat flour without the addition of resistant starch.

Methods. The samples used in the research have been marked with the following designations:

- total protein according to Kjeldahl method, using a Nx5,7 conversion coefficient,
- quantity and quality of gluten,
- falling number according to Hagberg-Perten method [1996],
- sedimentation value using Zeleny's test [1998],
- rheological properties of the dough, using Brabender farinograph [1999],
- amylographic properties of wheat flour [2001].

The bread was baked according to the method developed by the Cereal Technology Institute at the Wrocław University Of Environmental And Life Sciences and based on the following recipe: wheat flour – 250 grams, yeasts – 7.5 grams, salt – 3.8 grams, water in amounts allowing to obtain 300 FU consistency. The single-phase method was used when preparing the dough. Blending time was 3 minutes. After that the dough was divided into portions of about 300 grams. Each of them was placed in a baking tin and fermented in a temperature of 30–35°C for 1 hour. After this one-hour fermentation period the dough was again kneaded and placed back in the fermentation chamber. After about 30 minutes the dough was kneaded once more and left to final fermentation. The bread was baked in the laboratory stove for 30 minutes in a temperature of about 240°C. Evaluation of the bread was based on the assessment of its overbake, volume of bread baked of 100 grams of flour measured using a loaf volumeter *Sa-Wy* and the porosity of the crumb according to Dallmann scale.

Statistical analysis. The achieved results have been subject to statistical analysis. O*neway analysis* of *variance* for two variables (the type of wheat flour and the content of resistant starch) with the relevance level of $\alpha \leq 0.05$ has been performed. The average results have been assessed according to the Duncan test. All mean results are shown in tables.

Results and discussion

According to Eerlingen et al. [1994], adding components containing an artificially increased content of resistant starch in the process of bread baking does not lower the quality of the resulting products, and what is more, it results in reducing the caloric value of the makings [Leszczyński, 2004].

Enriching wheat flour with resistant starch, as it has been done in this experiment, has caused change in quality properties of the mixtures (Table 1). The highest values of quality properties of the flour (total protein – 10.1%, gluten yield – 24.1%, deliquescence of gluten – 5.5 mm) have been achieved with flour type 750 (Table 1). The consequence of increasing the content of retrograded acetylated starch in the flour has been a gradual decrease in the total protein 12.0% for the control sample, to 7.2% for the sample with a 40% content of resistant starch. A similar dependence has been observed by other authors [Aparicio-Saguilán et al., 2007]. Also Vergara-Valencia et al. [2007] have observed the reduction of total protein in bread when fiber concentrate from mango fruit has been added. Ajila et al. [2008] have noted a similar tendency in their research, investigating the possibilities of using high-fiber preparations in confectionery products. In bakery practice it is believed, that bread flour should consist of more than 25% of wet gluten. Wet gluten yield has decreased with the increase of resistant starch content in the samples. The highest wet gluten yield has been observed in the control sample (30.8%) and the lowest in the sample including a 40% content of RS4 (7.3%). The baking value of the flour depends on the quantity and quality of gluten, which can be determined by means of measuring its deliquescence and sedimentation value. Strong gluten dissolves only to a small extent when thermostated in a temperature of about 30°C. Flour containing a high level of good quality gluten is characterized by high sedimentation value. The highest deliquescence has been observed in the control sample (6.9 mm) and the lowest in the sample with a maximum content of resistant starch (1.7 mm). In the current research the sedimentation value has ranged from 30.0 to 42.2 ml. What is also important when evaluating the quality of flour is the activity of the amylolytic enzymes. The activity of α-amylase can be indirectly determined basing on the falling number. With a high activity level of this enzyme gelatinized dough is quickly fluidized and the falling number is low. The highest falling number value has been achieved in the control sample (310 s) and the one including a 10% content of RS4 (307 s). Further increase in the quantity of modified starch in the samples has resulted in lowering the value of this quality, causing it to range from 253 to 274 s. In the research carried out by Mielcarz [2004a] adding from 5 to 15% of different kinds of fiber to the flour, has caused minor changes in the falling number.

Table 1

Mean values of wheat flour quality properties depending on the flour type and content level of resistant starch

Factor \ Trait		Total protein [%]	Wet gluten yield [%]	Deliquescence of wet gluten [mm]	Sedimentation value [ml]	Falling number [s]
Flour type	550	9.1 c	18.2 b	3.2 b	38 a	286 a
	750	10.1 a	24.1 a	5.5 a	38 a	287 a
	2000	9.5 b	15.7 c	2.8 b	33 a	267 a
Content level of resistant starch [%]	0	12.0 a	30.8 a	6.9 a	42 a	310 a
	10	10.8 b	25.3 b	4.5 b	39.5 a	307 a
	20	9.6 c	19.1 c	3.5 bc	36.5 a	274 b
	30	8.3 d	13.9 d	2.7 bc	32.5 a	258 b
	40	7.2 e	7.3 e	1.7 c	30 a	253 b

Explanatory notes:

a. b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$;

In Table 2 the values of amylographic properties of wheat flour enriched with resistant starch are presented. The highest initial temperature of gelatinization and the lowest maximum viscosity of gelatinized dough have been achieved for the flour type 2000 (56.2°C and 446 AU respectively). With the increasing quantity of resistant starch in the samples, the initial temperature of gelatinization has lowered from 59.1°C (control sample) to 51.7°C (the sample with 40% of RS4). Lowering the initial temperature of gelatinization, typical of acetylated starch, has been confirmed by other authors' research [Zheng et al., 1998; Zięba et al., 2007]. This kind of dependence has been to be expected due to weakening the structure of starch grains caused by the presence of acetate substituent groups in starch chains [Golachowski, 1998; González and Pérez, 2002, Liu et al., 1999]. For the samples including a 40% content of resistant starch, the lowest final temperature of gelatinization has been achieved (65°C), as well as the shortest gelatinization time (23.3 min) and the largest maximum viscosity of gelatinized dough (840 AU).

Table 2

Means values of amylographic properties of wheat flour depending on the flour type and content level of resistant starch

Factor \ Trait		Initial temperature of gelanization [°C]	Final temperature of gelanization [°C]	Gelanization time [min]	Maximum viscosity of gelatinized doughs [AU]
Flour type	550	54.0 b	82.1 a	34.7 a	817 a
	750	55.0 ab	80.9 a	33.9 a	760 a
	2000	56.2 a	81.1 a	34.0 a	446 b
Content level of resistant starch [%]	0	59.1 a	85.2 a	36.8 a	750 a
	10	56.6 b	85.8 a	37.2 a	635 b
	20	54.4 c	85.7 a	37.1 a	580 b
	30	53.5 c	85.0 a	36.7 a	567 b
	40	51.7 d	65.0 b	23.3 b	840 a

Explanatory notes:
a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$;

Low substitution acetylated starch used in the food industry is characterized by an increased deliquescence in water and water absorption properties [Golachowski, 1998; Zięba et al., 2007]. The highest values of farinographic properties of the dough were discovered in the case of flour type 2000. The increasing content of retrograded starch in flour has had a beneficial influence on water absorption properties of the flour (Table 3), causing its significant increase from 58.9% (control sample) to 88.0% in the samples with the highest content of resistant starch. With the increase of the quantity of the applied resistant starch, the dough development time has lengthened (from 4.7 to 40.8 min) (Table 3) and the quality number has increased (from 91 to 489 mm) (Table 3). Longer dough development time is caused by "diluting" gluten by adding resistant starch. The stability of the dough has spread from 5.6 to 14.7 minutes. The softening of the dough has ranged from 33 to 77 FU, where the highest value of this quality has been observed in the control sample (77 FU), and the lowest in the sample including a 30% content of resistant starch in the mixtures (33 FU) (Table 3).

Table 3

Means values of farinographic properties of wheat flour depending on the flour type and content level of resistant starch

Factor \ Trait		Water absorption of flour [%]	Development time of dough [min]	Dough stability [min]	Softening of dough [FU]	Quality number [mm]
Flour type	550	74.3a	17.6b	7.9b	44 b	267 b
	750	78.5a	16.4b	5.7b	85 a	203 c
	2000	75.3a	27.3a	15.2a	37 b	388 a
Content level of resistant starch [%]	0	58.9c	4.7c	5.6a	77 a	91 c
	10	69.0b	7.3c	6.8a	65 ab	134 c
	20	79.8a	22.0b	11.4a	58 bc	308 b
	30	84.6a	27.4b	14.7a	33 d	408 a
	40	88.0a	40.8a	9.5a	43 cd	489 a

Explanatory notes:

a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$

The lowest volume has been one of breads baked of type 2000 flour (387 cm^3) and the worst porosity (5 points in Dallmann's scale) has been observed in the breads made of type 750 flour (Table 4). With the increased content of resistant starch in the samples, the volume of the bread has decreased from 517 cm^3 in the control sample to 395 cm^3 (40% of RS4) (table 4), which has been caused by "diluting" gluten by the addition of resistant starch to the flour. A similar dependence has also been observed by Korus *et al*. [2009]. According to the research carried out by Wepner *et al*. [1999], the addition of 10% of potato starch esterified with citric acid has not resulted in lowering the volume of bread, compared with breads not incorporated with resistant starch. The decrease in bread volume is a sign of lowering its quality, which may result in decreasing the attractiveness of the bread to consumers. With the increase of the quantity of resistant starch, a significant increase of bread overbake has been observed – from 46.4% (control sample) to 78.7% in the samples with the maximum content of resistant starch (Table 4), which results from a larger water absorption of the mixtures containing RS4. The assessment of porosity of the crumb according to the 8-point Dallmann scale has ranged from 6 to 7 points (Table 4).

Table 4

Means values of quality properties of wheat bread depending on the flour type and content level of resistant starch

Factor \ Trait		Overbake [%]	Bread volume [cm^3/100g flour]	Porosity of the crumb acc. to Dallmann scale
Flour type	550	63.0a	490 a	7.5 a
	750	63.1a	504 a	5.5 b
	2000	65.6a	387 b	7.0 a
Content level of resistant starch [%]	0	46.4d	517 a	6.0 a
	10	56.1c	495 ab	6.5 a
	20	65.0b	467 b	7.0 a
	30	73.4a	428 c	6.5 a
	40	78.7a	395 c	6.5 a

Explanatory notes:

a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$.

Conclusions

Enriching wheat flour with resistant starch, as it has been done in this experiment, has resulted in the following changes regarding flour, dough and bread quality: the increase of water absorption of the flour, quality number, overbake of the bread and lengthening dough development time; the decrease of flour quality index, lowering the initial temperature of gelatinization, softening of the dough and bread volume.

For the samples with a 40% content of resistant starch the lowest final temperature of gelatinization has been achieved, as well as the shortest gelatinization time and the highest maximum viscosity of gelatinized dough.

As a result of the research it has been observed, that applying a 10% or in some cases a 20% content of retrograded acetylated starch can be used as an addition enriching wheat bread in dietary fiber without significantly influencing its quality.

References

Ajila C.M., Leelavathi K., Prasada Rao U.J.S., 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. J. Cereal Sci., 48, 319–326.

Aparicio-Saguilán A., Sáyago-Ayerdi S.G., Vargas-Torres A., Tovar J., Ascencio-Otero T.E., Bello-Pérez L.A., 2007. Slowly digestible cookies prepared from resistant starch-rich lintnerized banana starch. J. Food Campos. Anal., 20 (3/4), 175–181.

Bartnikowska E., 1997. Włókno pokarmowe w żywieniu człowieka. Część I. Przem. Spoż., 5, 43–48.

Champ M., 1994. Definition, analysis, physical and chemical characterization and intake of RS. EURESTA Working Group I. Proceedings of the concluding plenary meeting of EURESTA. Eds. Asp N.-G., Amelsvoort J.M.M., Hautvast J.G.A.J., Wageningen, The Netherlands.

Diowksz A., 2006. Pieczywo hipoalergiczne – poszukiwanie nowych rozwiązań dla szybko rosnącego rynku produktów dietetycznych. Przegl. Piek., 08, 2–4.

Dziugan P., Dziedziczak K., Ambroziak W., 2006. Błonnik w pieczywie. Cuk. Piek., 05, 60–62.

Eerlingen R.C., Van Haesedonck I.P., De Paepe G., Delcour J.A. 1994. Enzyme-resistant starch. III. The quality of straight-dough bread containing varying levels of enzyme-resistant starch. Cereal Chem., 71, 165–170.

Englyst H.N., Cummings. J.H., 1987. Digestion of polysaccharides of potato in the small intestine of man. Am. J. Clin. Nutr., 45, 423–431.

Englyst H.N., Hudson G.J., 1997. Starch and heath. Starch structure and functionality (eds. P.J. Frazier, P. Richmond, A.M. Donald). The Royal Society of Chemistry, Cambridge, 9–21.

Golachowski A., 1998. Stosowanie skrobi i jej przetworów w przemyśle spożywczym. Zesz. Nauk. AR Wroc., Technol. Żyw., 12/328, 117–124.

González Z., Pérez E., 2002. Effect of acetylation on some properties of rice starch. Staerke, 54, 148–154.

Haralampu S.G., 2000. Resistant starch-a review of the physical properties and biological impact of RS3. Carbohydr. Polymers, 41, 285–292.

Korus J., Witczak M., Ziobro R., Juszczak L., 2009. The impact of resistant starch on characteristics of gluten-free dough and bread. 23, 988–995.

Leszczyński W., 2004. Skrobia oporna i jej znaczenie. Przegl. Piek., **7**, 2–5.

Liu H., Ramsden L., Corke H., 1999. Physical properties of cross-linked and acetylated normal and waxy rice starch. Staerke, 51, 249–252.

Mężyński L., 1972. Acetylacja skrobi. Przem. Chem., 51/5, 289–290.
Mielcarz M., 2004a. Żywieniowe i technologiczne aspekty zastosowania błonników pokarmowych do produkcji wyrobów piekarskich i ciastkarskich. Przegl. Piek., 8, 7–9.
Mielcarz M., 2004b. Wartość odżywcza pieczywa i jego przeznaczenie dla konsumentów wymagających określonych diet (cz. I). Przegl. Piek., 10, 12–13.
Mielcarz M., 2005. Wartość odżywcza pieczywa i jego przeznaczenie dla konsumentów wymagających określonych diet (cz. III). Przegl. Piek., 6, 2–3.
Ohr L.M., 2004. Fortifying with fiber. Nutraceuticals&Functional Foods, 58 (2), 71–75.
PN-75/A-04018. Produkty rolno-spożywcze. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko.
PN-77/A-74041. Oznaczanie ilości i jakości glutenu.
PN-ISO 3093, 1996. Zboża. Oznaczanie liczby opadania.
PN-ISO 5529, 1998. Oznaczanie wskaźnika sedymentacyjnego. Test Zeleny'ego.
PN-ISO 5530-1, 1999. Fizyczne właściwości ciasta. Oznaczanie wodochłonności i właściwości reologicznych za pomocą farinografu.
PN-ISO 7973, 2001. Ziarno zbóż i przetwory zbożowe. Oznaczanie lepkości mąki. Metoda z zastosowaniem amylografu.
Soral-Śmietana M., Wronkowska M., 1999. Czy skrobia odporna na hydrolizę enzymatyczną może zastąpić błonnik pokarmowy? Przem. Spoż., 7, 22–24.
Wepner B., Berghofer E., Miesenberger E., Tiefenbacher K., Ng P.N.K., 1999. Citrate starch-application as resistant starch in different food systems. Starch/Stärke, 10, 354–361.
Vergara-Valencia N., Granados-Pérez E., Agama-Acevedo E., Towar J., Ruales J., Bello-Pérez L., 2007. Fibre concentrate from mango fruit: Characterization, associated antioxidant capacity and application as a bakery product ingredient. LWT, 40,722–729.
Zheng G.H., Sosulski F.W., Tyler R.T., 1998. Wet-milling composition and functional properties of starch and protein isolated from buckwheat groats. Food Res. Int., 30/7, 493–502.
Zięba T., 2007. Sposób otrzymywania skrobi o zmniejszonej podatności na działanie enzymów amylolitycznych. BUP, 20 (881), 16.
Zięba T., Kapelko M., Gryszkin A., 2007. Selected properties of potato starch subjected to multiple physical and chemical modifications. Pol. J. Food Nutr. Sci., 57 (4C), 639–645.

30

TECHNOLOGICAL ELABORATION OF BAKERY OF WHOLE-MEAL RYE BREAD WITH REDUCED SALT CONTENT

Introduction

Bread is one of the principal food products in the menu of an average human. It contains valuable energy, construction and regulatory components. Their quantity depends on the type of bread. Investigations indicate that together with bread into the human organism is introduced 25% of the daily amount of consumed sodium chloride.

The World Health Organisation (WHO) informs that almost 200 diseases are the result of improper nutrition. One of the factors leading to an increase in the number of civilisation diseases during the last years is the excessive salt consumption. This element is introduced into the organism principally as kitchen salt, which is consumed by humans to an excessive degree [Gułajski, 1996; Hamułka et al., 1998; Dybkowska et al., 2005].

In many countries one may observe that the consumption of kitchen salt is several times higher that the requirements of the organism. This has a very negative effect on the health because an excessive quantity of sodium chloride retains water in the organism, what in turn burdens the circulatory and secretion systems and may lead to obesity. It is also responsible for arterial hypertension, what in turn may lead to an infarct or cerebral haemorrhage. It is also suspected that excessive quantities of sodium chloride may favour the appearance of tumours [Sikora, 1992, Kozłowska-Wojciechowska, 1999; Wojtasiński, 1999].

According to FAO/WHO, a healthy adult should consume about 7-8 g of sodium chloride per day. In such cases no negative effect of sodium chloride on the blood pressure is observed [Sikora, 1992; Wojtasiński, 1999]. Smaller amounts of salt, up to 3 g per day, should be consumed by children, teenagers, pregnant women, elder people and those with a heightened risk of diseases of the circulatory system. In order to reach such quantities one must resign from adding salt to ready made dishes and, wherever possible, substitute salt with herb mixtures [Sikora, 1992; Gułajski, 1996a; Makarewicz-Wujec and Kozłowska-Wojciechowska, 1999; Hess et al., 1999].

The increasing interest in salt and its negative effect on the human organism induced technologists to search for substitutes that could be used in bread. Salt is a component playing a very important role in the process of baking bread. It affects the gluten, the physical properties of the dough and the fermentation processes. Moreover, bread without salt is not accepted by a majority of consumers [Piesiewicz and Bartnikowska, 2004; Cacak-Pietrzak et al., 1995a; Mielcarz, 2006].

It is necessary to elaborate a method of baking bread in such a way as to make it possible to substitute at least part of the salt. One of the methods of decreasing the amount of

sodium chloride added is its partial substitution by herbs. They give bread a specific taste and aroma and thus draw away the consumers' attention from the decreased quantity of salt. Herbs added to the dough affect not only the sensoric properties of the ready product but also the farinographic and amylographic properties of the dough, together with the fermentation process. With this aim such herbs as thyme, caraway, savory, fennel seed, paprika [Haber et al., 1993; Chojnacka, 1996] may be added to bread.

The present work aimed at decreasing the amount of salt in whole-meal rye bread by substituting part of it with herbs (caraway, thyme, savory and fennel seed).

Materials and methods

The experimental material consisted of rye flours type 2000 and 720 together with wheat bread flour type 750. In order to decrease the amount of salt added to the bread the following herbs were used: fennel seed, thyme, caraway, savory and their mixtures. They were added to the flour at a rate of 0.5 and 1.0%.

The baking value of rye and wheat flours was evaluated on the basis of the following determinations: moisture [PN-ISO 712: 2002], acidity, total protein content according to the method by Kjeldahl, (N x 5.7 for wheat flour, N x 6.25 for rye flour) and using the Foss Tecator apparatus type 1002 [PN-75/A-04018], the Falling Number [PN-ISO 3093: 2000]. Moreover, the amylolytic activity was determined using Brabender's amylograph and the fermentographic evaluation was based on the use of fermentograph SJA. The dough was prepared according to the following recipe: 280 g rye flour type 720, 28 g acid, 4 g salt, 5 g yeasts, 280 cm^3 water, 2.8 g herbs (thyme, savory, fennel seeds or caraway.

The laboratory test baking of bread was conducted according to the three-stage method, basing on the recipe used at the Kowalscy S.c. bakery, according to the following schema:

I. Semi-sour
- 30 kg acid
- 30 kg rye flour type 720
- 50 kg whole meal type 2000
- 60 l water

Time of fermentation 6 h.

II. Acid
- 35 l water
- 50 kg whole meal type 2000

Time of fermentation 3 h.

III. Dough
- 2 kg yeast
- 3.5 kg salt
- 4 kg wheat flour type 750

From such dough 500 bread loaves were formed, 0.57 kg each. The baking took place at a temperature of 240°C for 1 hour.

The acid obtained was after 3 hours supplemented by components added in accordance with the recipe, modifying only the quantity of salt and herbs. Three laboratory baking tests were performed:

- **Series 1**. Baking bread with different quantities of salt: 0.5, 1.0, 1.5, 2.0%.
- **Series 2**. Baking bread containing 1% salt and an addition of herbs.
- **Series 3**. Baking bread containing 1% salt and 0.5% addition of a mixture of herbs.

In order to determine how the supplements affect the quality of the ready product the baked bread was subjected to a laboratory evaluation. All the analyses were conducted 24 hours after baking, in two parallel replicas: volume of bread using granular substances (rapeseed), crumb porosity, crumb hardness using the TA.XTZ texture analyser, crushing force using a cylindrical pivot, 25 mm in diameter, which entered the crumb 9 mm deep (the maximum crushing force was recorded in newtons [N]) and content of chlorides in the bread according to the method by Mohr [Kerłowska-Kułas, 1993]. Moreover, the quality of bread was also evaluated on a score basis [PN-A-74108: 1996]

The statistical analysis of the results obtained was performed using the Statgraphics Plus 4.1 software. The significance of differences between mean values was determined on the basis of an Anova single factor analysis of variance at a level of significance α=0.05, while the least significant difference was determined using Tuckey ‘s test .

Results and discussion

The performed analysis of the physicochemical traits of rye flour type 720 and 2000 as well as wheat flour type 750 demonstrated that they are characterised by good baking properties. The values obtained for selected indicators of flour quality are presented in Table 1.

Moisture, protein content and acidity of the flours used corresponded to standards [PN-A-74032:2003, PN-A-74022:2003]. However, the technological value of rye flours is described more precisely through the properties of starch and especially its ability to gelatinization and susceptibility to proteolytic enzymes.

The activity of amylolytic enzymes contained in the flour is of significant importance for the quality of bread obtained, as it determines the process of dough fermentation and thus obtaining the crumb structure of uniform porosity. The high falling number indicates a low activity of α-amylases, while a low falling number is connected with a high activity of those enzymes. The best value for flours used to produce rye bread is considered to lie between 100 and 200 s. Bread produced from flour with a high falling number does not rise properly, is characterised by a harder crumb, is less acidified and less aromatic. In the case of flours with a low falling number good quality bread may be obtained on condition of a good acidification of the rye dough. Otherwise the bread may prove slack-baked or with a detached crust. When using fouls with a low falling number the yield of dough is as a rule also low [Ambroziak, 1998; Słowik, 2005].

The falling numbers obtained in the present study indicate that rye flour type 2000 had a very high (62 s), while rye flour type 720 (136 s) and wheat flour type 750 (264 s) a medium amylolytic activity [Ambroziak, 2007].

There exists a considerable correlation between the falling number and gruel viscosity, determined with the use of Brabender’s amylograph. The value of amylographic viscosity describes the amylolytic activity of flour, while the final temperature of gelatinization informs about the properties of starch, its susceptibility or resistance to hydrolysis. Viscosity ranging between 400 and 600 j.B. and the final gelatinization temperature of 63–68^{o}C are considered

optimal amylographic parameters of rye flour used for the production of bread [Słowik, 2005; Ambroziak, 2007].

The determination of this parameter showed (tab. 1) that rye flour type 720 and wheat flour type 750 are characterised by optimum parameters for the production of rye bred (maximum viscosity 420 j.B., final gelatinization temperature 68.5°C).

Table 1

Quality characteristics of determined flour

Parametrs determined		Rye flour		Wheat flour type 750
		type 2000	type 720	
Moisture	[%]	14.3	14.2	14.5
Protein content	[% d. m.]	7.3	6.7	9.8
Acidity	[°kw]	3.9	2.3	2.1
Falling number	[s]	62	136	264
Peak viscosity	[j.B.]	120	420	420
Initial temperature of gelatinization	[°C]	52	52	47.5
Final temperature of gelatinization	[°C]	58	68.5	58

The ability of dough to produce dioxide carbonate depends on many factors and among them the hydrate properties of proteins, activity of proteolytic enzymes and the susceptibility of starch to their activity. This trait affects the volume and porosity of bread. As a rule a decrease in the quantity of secreted CO_2 is considered to be an unfavourable property [Czapiewska and Hałasowski, 1980; Ambroziak, 1998].

The addition of herbs affects the ability to produce CO_2 during the fermentation process. This is caused by various substances contained in herbs, some of which have a considerable effect on the activity of baking yeasts. According to data available in literature [Haber et al., 1993] among the herbs examined thyme and caraway lead to a decrease of the quantity of CO_2 produced, what corresponds with the results obtained in the present study (Fig. 1). An addition of thyme or caraway resulted in a decreased CO_2 production when compared with the control sample by 23 and 16%, respectively. In turn, the samples containing savory or fennel seed were characterised by an ability to produce more of this gas by 6 and 10%, respectively (fig. 1).

Volume is one of the indicators of bread quality. In general, with the increasing volume the quality of bread improves. A large bread volume indicates a correct technological process and high quality of the raw materials used. The porosity of such bread is always uniform, the crumb loose and spongy, what indicates a good gelatinization of starch and uniform penetration of heat into the loaf during baking [Ambroziak, 1998, 2007].

With the increase of the salt content the volume of 100 g of bread increased. In the case of the sample containing 1% salt the bread volume increased by 2% compared to sample I, containing 0.5% salt. In the case of bread containing 1.5 and 2% salt the increase in volume amounted to 6 and 7%, respectively, in relation to sample I. Those changes proved to be statistically significant (Tab. 2). An addition of fennel seed or savory resulted in a small

increase of the volume of 100 g of bread – from 0.9 to 6.7%. The remaining herbs lead to a decrease by between 1.7% (0.5% of theme) and 7.5% (1% of caraway) (Tab. 2), the difference between the control sample and samples containing 0.5 and 1% caraway being significant statistically. An addition of 0.5% of a mixture of herbs in most cases resulted in a decrease of the volume of 100 g of bread – by between 3.0 and 3.8%. Only the sample containing 0.5% savory and fennel seed showed a bread volume higher by 1%. The difference between this sample and the control did not prove significant statistically, but the remaining differences were significant.

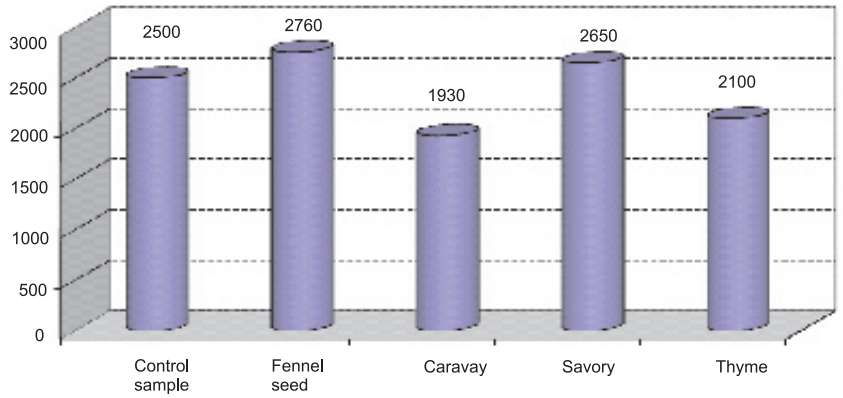

Fig. 1. Herbs influence on the quantity of emitted during fermentation carbon dioxiode

The bread volume is closely related with crumb porosity, which also indicates the bread quality. The porosity is determined by the ratio between the volume of pores and the overall bread volume. On the basis of this value one may examine the progress of the fermentation process and the baking quality of the flour used. According to the data available in literature the porosity of rye bread should amount to 55–70% [Ambroziak, 1998, 2007].

The lowest porosity was observed for samples containing 0.5% salt. With the increasing salt content the porosity increased by 1.9, 2.8 and 5.6%. The addition of herbs and their mixtures resulted in a slight increase in porosity of the bread crumb – from 3.8 to 15.1%. The highest porosity was observed for bread containing 0.5% of a mixture of savory and fennel seed – 17% higher as compared to the control sample and this difference proved statistically significant (Tab. 2).

Texture is a property of food determined by its physical characteristics, estimated by the sense of sight (except for colour), touch and the gestation receptors in the oral cavity. This trait is composed of many factors, such as shape, moisture, chemical composition, structure and mechanical properties. While conducting the evaluation varied impressions are received: softness, tenderness, coarseness. However, the most complete evaluation of the texture of food may be performed only within the oral cavity – while cutting and crushing the product we may observe such traits as fibrousnesses, viscosity, gumminess. Many apparatuses have been constructed to measure the texture of food. They operate on a similar principle and consists of a measurement of the cutting force necessary to cut the product, crushing it at a specified rate and forcing the pivot to a given depth [Ambroziak, 2007]. In the present

study bread with an addition of 2% salt proved to be the hardest. The lower the addition of salt the less hard was the product obtained. The addition of herbs (theme, caraway, savory and their mixtures) resulted in a lower bread hardness by between 4.7% (sample containing 0.5% caraway) and 20.9% (sample containing 0.5% savory). Bred containing fennel seeds demonstrated a hardness higher than that observed for the control sample by 10.5 and 8.1%, depending on the content of the herb (Tab.2.).

Table 2

The effect of addition of salt, herbs and herbs mixture on some traits of the rye bread

Sample \ Determined			Volume of 100 g bread	Structure of crumb	Texture
			[cm^3]	[%]	[N]
Addition of salt		0.5%	204.3^{c}	53.5^{b}	8.2
		1.0%	212.1^{bc}	54.5^{b}	8.5
		1.5%	216.2^{b}	55.0^{ab}	9.3
		2.0%	218.6^{a}	56.5^{a}	9.9
LSD			9.0	1.8	r.n.
Addition of herb	Control sample 1.0% addition of salt		222.6^{ab}	53^{a}	8.6^{a}
	Thyme	0.5%	218.9^{ab}	56^{b}	7.7^{bc}
		1.0%	215.0^{bc}	59^{c}	7.6^{c}
	Savory	0.5%	222.8^{ab}	61^{d}	6.8^{d}
		1.0%	224.1^{a}	52^{a}	7.0^{d}
	Fennel seed	0.5%	223.5^{ab}	55^{b}	9.5^{e}
		1.0%	223.9^{a}	59^{c}	9.3^{f}
	Caraway	0.5%	207.3^{cd}	57^{b}	8.2^{ab}
		1.0%	206.0^{d}	56^{b}	7.3^{cd}
LSD			10.7	9.4	0.7
Addition of herb mixture	Control sample 1.0% addition of salt		222.6^{ab}	53	8.6^{a}
	Caraway Thyme Savory Fennel seed,	0.5%	214.2^{b}	59^{a}	8.9^{ab}
	Thyme Savory Fennel seed	0.5%	214.3^{bc}	52^{a}	8.5^{b}
	Tymianek Savory,	0.5%	215.8^{bc}	56^{a}	7.8^{c}
	Savory Fennel seed	0.5%	224.8^{a}	62^{b}	7.5^{c}
LSD			4.7	6.7	0.5

a, b, c, d, e, f – means in the same column with different superscripts are significantly different at $\alpha = 0.05$.

The role of food is not only to supply the energy and nutrients necessary for a correct functioning of the organism, but also to provide a sensory satisfaction through a pleasant stimulation of the senses of taste, smell and sight. Nowadays, with an increasing supply and variety of food offered on the market the client expects bread of a high quality and a considerable variability of tastes and types [Iwański et al., 2006].

The bread quality is estimated according to the method described in the Polish Standards PN-A-74108: 1996. The sensoric evaluation included such traits as: external appearance of bread, crust properties (colour, thickness), crumb properties (elasticity, porosity) as well as taste and aroma. Depending on the score granted the bread may be classified to one of four quality levels. The maximum score for sensoric and physico-chemical properties amounts to 40.

Basing on the sensoric evaluation conducted one may state that bread containing 2% salt obtained a 100% acceptation of the research team. Such an addition of salt is typically used at the Kowalscy S.c. bakery. Bread containing 0.5% salt obtained the lowest score. One may observe, that the lower the addition of salt the lower the score. Salt had the smallest effect on the aroma of bread. A smaller addition of salt lead to a poorer growth of bread, a darker crust which parted from the crumb after cutting, what resulted in a lower score for external appearance. The quantity of salt added affected also the evaluation of the crumb structure – less salt resulted in the crumb showing a smaller porosity and elasticity. Bread containing 0.5% salt crumbed during cutting.

However, the greatest differences were observed in the taste – bread with a low salt content was not accepted.

As the goal of the study was to decrease the content of salt in whole-meal rye bread further studies were conducted on the sample containing 1% salt. Although such bread was not fully accepted during the sensoric evaluation, is did not show properties that could be considered disqualifying.

The three-stage method of producing dough with a lowered salt content and with an addition of herbs or their mixtures made it possible to obtain bread of a correct external appearance and shape corresponding with the form used. In all cases the crust was browned and shiny, closely adhering to the crumb and without cracks. The samples containing herbs were estimated higher that the control sample. In the case of the control sample containing a lowered salt content the taste received the lowest score. The addition of herbs drew away the attention from the low-salt content. Bread containing herbs rose well, had a pleasant herby aroma, a correct crumb structure and elasticity. The taste of all samples was evaluated as very good (Tab. 3).

Table 3

Results of the sensoric estimation of rye bread (scores)

Sample \ Sensory trait			Appearance	Bread's skin (colour, thickness, other features)	Structure of crumbs (elasticity, porosity, other features)	Taste and aroma	Sum of scores
Bread with addition of salt		0.5%	4.0	8.0	7.0	0	19.0
		1.0%	4.0	8.0	8.0	5.0	25.0
		1.5%	5.0	11.0	9.0	5.0	30.0
		2.0%	5.0	11.0	10.0	6.0	32.0
Bread with addition of herb	Control sample 1.0% addition of salt		4.0	8.0	8.0	5.0	25.0
	Thyme	0.5%	4.0	11.0	9.0	6.0	30.0
		1.0%	4.0	11.0	8.0	6.0	29.0
	Savory	0.5%	5.0	11.0	9.0	6.0	31.0
		1.0%	5.0	11.0	10.0	5.0	31.0
	Fennel seed	0.5%	5.0	11.0	9.0	5.0	30.0
		1.0%	5.0	11.0	10.0	5.0	31.0
	Caraway	0.5%	4.0	11.0	9.0	5.0	29.0
		1.0%	4.0	8.0	9.0	5.0	26.0
Bread with addition of herb mixture	Control sample 1.0% addition of salt		4.0	8.0	8.0	5.0	25.0
	Caraway, Thyme, Savory, Fennel seed,	0.5%	4.0	8.0	9.0	6.0	27.0
	Thyme, Savory, Fennel seed,	0.5%	5.0	11.0	10.0	5.0	31.0
	Thyme, Savory,	0.5%	5.0	9.0	9.0	5.0	28.0
	Savory, Fennel seed,	0.5%	5.0	11.0	10.0	6.0	32.0

Salt plays an important role in the technology of bread production. It is a factor modifying the process of fermentation and dough forming. Moreover, it is an addition that has a considerable effect on the taste of the ready product. Sodium chloride has an inhibiting effect on the activity of yeast enzymes and the addition of different quantities of this component (but not more than 3%) may be used to regulate the fermentation rate. This characteristic is made use of principally during summer months, as by modifying the quantity of salt one may protect the dough from what is known as overswell. Salt has also a favourable effect on the structure of bread. Bread produced without salt may show several faults: faulty taste, decreased elasticity, crumbing [Cacak-Pietrzak et al., 1995; Ambroziak, 2007]. The addition of herbs or their mixtures had no special effect on the content of chlorides in the bread. Only

the sample containing 0.5% of thyme had a content of salt higher by 1.6% than the control sample. The level of salt in the remaining samples was similar or lower (from 0.8 to 7.9%) and those differences were not significant statistically (Fig. 2).

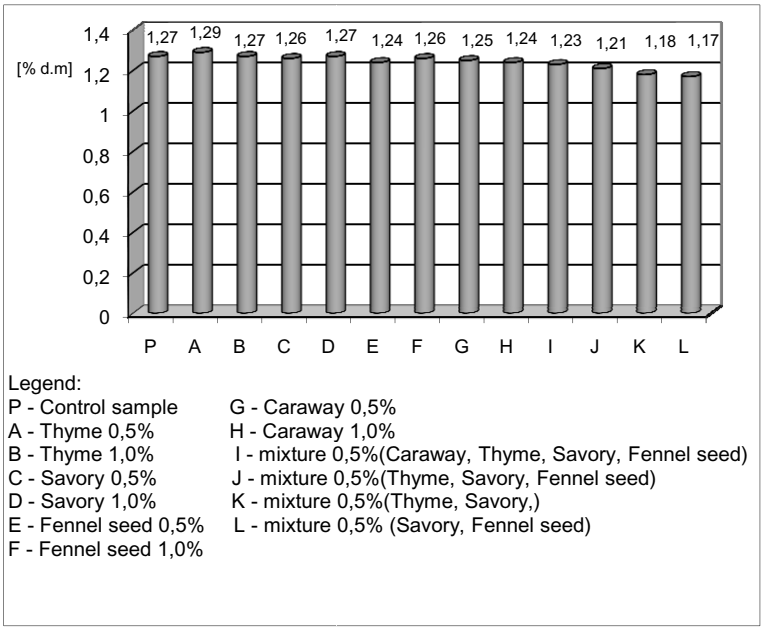

Fig. 2. Chloride conyent in bread with the addition of herbs and herb mixtures

Conclusions

Basing on the results obtained and their analysis one May reach the following conclusions: Rye flour type 720 and 2000, as well as wheat flour type 750, used in the present studies, was characterized by a good quality and could be used for the production of whole-meal rye bread. The addition of herbs affected the fermentation processes in the dough. Fennel seed and savory caused an increased while thyme and caraway a decreased production of CO_2, when compared with the control sample. This was probably caused by substances present in those herbs: thymol in thyme and carvon in caraway, both of which affect the activity of yeasts. The herbs and herb mixtures used had different effects on the physico-chemical properties of bread, the most favourable being that of fennel seed and savory, as well as their mixtures. The bread samples containing those herbs were characterized by a greater porosity and volume and lower hardness as compared to the control sample. The herbs and herb mixtures used affected the sensory evaluation of bread. Samples with a lowered content of salt and simultaneously and addition of herbs were accepted by consumers. Adding herbs is a very good method of lowering the content of salt in bread. An adequate selection of herbs

and their quantities renders it possible to lower the level of salt in the dough without it having a negative effect of the sensoric and physico-chemical properties of bread.

References

Ambroziak Z., 1998. Piekarstwo i Cukiernictwo. WNT, Warszawa.

Ambroziak Z., 2007. Produkcja piekarsko-ciastkarska. WSiP, Warszawa.

Cacak-Pietrzak G., Haber T., Lewczuk J., 1995. Wpływ różnych soli na cechy fizyczne ciasta. Przegl. Piek. i Cuk., 43, 38.

Cacak-Pietrzak G., Haber T., Lewczuk J. 1995a. Wpływ różnych soli na właściwości fermentacyjne ciasta. Przegl. Piek. Cuk., 43, 37.

Chojnacka R., 1996. Ograniczajmy spożycie soli i cukru. Poradnik Gospodarski, 10, 35.

Czapiewska A., Hałasowski T., 1980. Pomiar ilości CO_2 w czasie fermentacji ciasta jako kryterium oceny wartości wypiekowej mąki. Przegl. Piek. Cuk., 28, 217.

Dybkowska E., Świderski F., Waszkiewicz-Robak B., 2005. Spożycie składników mineralnych w polskiej diecie. Żywienie Człowieka i Metabolizm, 32 supl., 200–204.

Gułajski M., 1996. Prawidłowe żywienie podstawą zdrowia. Poradnik Gospodarski, 10, 33.

Gułajski M., 1996a. Wybrane zasady prawidłowego żywienia (II). Poradnik Gospodarski, 12, 33–34.

Haber T., Haberowa H., Lewczuk J., Siwoszek K., 1993. Zioła jako dodatki do chleba o obniżonej zawartości soli. Przem. Spoż., 47, 17–20.

Hamułka J., Kosiorek K., Gronkowska-Senger A., 1998. Daily intake of salt and cholesterol in primary schoolchildren. Pol. J. Food Nutr. Sci., 7, 579–589.

Hess S.Y., Zimmerman M.B., Staubli-Asobayire F., Tebi A., Hurrell R.F., 1999. An evaluation of salt intake and iodine nutrition in reualand urban area of the Cote d´Ivoire. Eur. J. Clin. Nutr., 53, 680–686.

Iwański R., Wianeczki M., Łomaszewicz S., 2006. Analiza sensoryczna wyrobów piekarskich. Przegl. Piek Cuk., 54, 6–8.

Kerłowska-Kułas M., 1993. Badanie jakości produktów spożywczych. PWE. Warszawa.

Kozlowska-Wojciechowska M., 1999. Choroby wadliwego żywienia. Przegl. Gastr., 51A, (1), 3

Makarewicz-Wujec M., Kozłowska-Wojciechowska M., 1999. Nadciśnienie tętnicze lubi grubasów. Przegl. Gastr. 51A, (3), 16

Mielcarz M., 2006. Chleb bez soli? Piek. i Cuk. 61, (7,8), 67–70.

Piesiewicz H., Bartnikowska E., 2004. Sól spożywcza we współczesnych technologiach piekarskich oraz aspekty żywieniowe. Przegl. Piek. i Cuk., 52, (3), 12–15

PN-75/A-04018. Produkty rolno – żywnościowe. Oznaczanie azotu metodą Kiejdahla

PN-A-74022, 2003. Przetwory zbożowe. Mąka pszenna.

PN-A-74032, 2003. Przetwory zbożowe. Mąka żytnia.

PN-ISO 3093, 2000. Zboża. Oznaczanie liczby opadania.

PN-ISO 712, 2002.Oznaczanie wilgotności.

PN-A-74108, 1996. Pieczywo. Metody badań i ocena punktowa.

Sikora E., 1992. Ocena zawartości soli kuchennej w racjach pokarmowych dzieci, młodzieży i osób w wieku starszym. Roczn. PZH XLIII, 3–4, 235–240 (English abstrakt) .

Słowik E., 2005. Właściwości technologiczne i metody oceny żyta. Przel. Piek. I Cuk. 53, (3), 6–9.

Wojtasiński Z., 1999. Dietetyczna łamigłówka. Przegl. Gastr. 51A, (4), 4–5.

31

APPLE POMACE AS A SOURCE OF DIETARY FIBER IN WHEAT BREAD

Introduction

In the last fives decades there is a trend to find new sources of dietary fiber, such as agronomic by-products that have traditionally been undervalued. Dietary fibers from different sources have been used to replace wheat flour in the preparation of bakery products [Ayadi et al., 2009]. Because of their high consumption, baked food products are potential carriers of dietary fiber. Cereal products and bread are perhaps the most important item in our daily diet

According to AACC [2001] definition, dietary fibre consists of edible parts of plants or non-starch carbohydrates, which are resistant to human digestive enzymes and resistant to absorption in small intestine; which undergo total or partial fermentation in large intestine. The dietary fibre is composed of total dietary fibre (TDF), which includes both soluble (SDF) and insoluble dietary fibre (IDF) [Wang et al., 2002]. These species are not biologically active as, for example, vitamins or mineral components, they noticeably affect the metabolic and physiological processes that occur in human organisms. The most common form of DF is insoluble fibre (cellulose, lignin and some hemicellulose), which reduces constipation and is being studied for its potential to reduce the risk of colon/rectal cancer. Although soluble fibre is less common in foods than insoluble fibre, it is believed to have important effects in the digestive and absorptive processes. Dietary fibre contributes to beneficial physiological impact, decreases cholesterol and blood glucose level. The lack of dietary fiber in diets has been associated with constipation, diverticulosis, cardiovascular diseases and cancer [Guillon and Champ, 2000; Ohr, 2004]. Both the nutritional value and technological properties of dietary fibers are important in the potential development of a wide range of fiber-enriched food (e.g. bakery products, snacks, sauces, drinks, cereals, biscuits, dairy products, meat products) [Ayadi et al., 2009].

Fruit dietary fibre concentrates have, in general, better nutritional quality than those found in cereals, because of their significant contents of associated bioactive compounds (flavonoids, carotenoids, etc.) and more balanced composition (higher overall fibre content, greater SDF/IDF ratio, water- and fat-holding capacities, lower metabolic energy value, and phytic acid content) [Chau and Huang, 2003]. Apples give fiber with a well balanced ratio of soluble and insoluble fibre and processed in juice production in large quantities [Gorinstein et al., 2001]. Epidemiological studies have suggested that apple consumption has been inversely associated with lung cancer, cardiovascular disease, and chronic obstructive pulmonary disease [Rupasinghe et al., 2008].

Apple pomace is a by-product resulting from juice pressing. Although for many years regarded as a waste product, at present apple pomace should be considered as a source of bioactive compounds such as flavonoids, polyphenols and carotenoids and also have been considered as a source of better quality dietary fibre [Kołodziejczyk et al., 2007]. Dried pomace, a fruit industry by-product, is considered as a potential food ingredient having high dietary fibre content. Pomace may also become a cheap raw material for food and fodder production [Fronc and Nawirska, 1994].

The aim of the present work was to evaluate the impact of apple pomace content on the quality properties of dough and wheat bread.

Materials and methods

The material used for this research was wheat flour type 750, supplemented with 'Idared' apple pomace, including 93.7% dry mass, 4.1% protein and NDF – 7.76 g/100g of dry mass, ADF – 4.39 g/100g of dry mass and 3.37 g of hemicellulose per 100g of dry mass. The pomace was obtained by pressing apples of technological ripeness in the Fruit Processing Plant of Prusice, Poland. The content of pomace in the obtained samples was 0, 5, 10 and 15%. The control sample was wheat four without the addition of apple pomace.

The samples used in the research have been marked with the following designations:

- total protein according to Kjeldahl method, using a Nx5.7 conversion coefficient,
- quantity and quality of gluten,
- falling number according to Hagberg-Perten method [1996],
- sedimentation value using Zeleny's test [1998],
- rheological properties of the dough, using Brabender farinograph [1999],
- amylographic properties of wheat flour [2001],
- dietary fiber fractions (NDF – neutral dietary fiber, ADF – acid dietary fiber, hemicellulose – calculated by subtracting ADF from NDF) marked according to van Soest detergent method [1963] modified my McQueen and Nicholson [1979].

The bread was baked according to the method developed by the Cereal Technology Institute at the Wrocław University Of Environmental And Life Sciences and based on the following recipe: wheat flour – 250 grams, yeasts – 7.5 grams, salt – 3.8 grams, water in amounts allowing to obtain 300 FU consistency. The single-phase method was used when preparing the dough. Blending time was 3 minutes. After that the dough was divided into portions of about 300 grams. Each of them was placed in a baking tin and fermented in a temperature of 30–35°C for 1 hour. After this one-hour fermentation period the dough was again kneaded and placed back in the fermentation chamber. After about 30 minutes the dough was kneaded once more and left to final fermentation. The bread was baked in the laboratory stove for 30 minutes in a temperature of about 240°C. Evaluation of the bread was based on the assessment of its overbake, volume of bread baked of 100 grams of flour measured using a loaf volumeter **Sa-Wy** and the porosity of the crumb according to Dallmann scale.

Statistical analysis. In order to compare the average values, ***one factor analysis*** of ***variance*** has been performed, with the relevance level of $\alpha \leq 0.05$. The average values have been estimated by means of Duncan test. Statistical analysis of the results has been carried out with the use of the Statistica 8.0 pack. All mean results are shown in tables.

Results and discussion

Enriching wheat flour with apple pomace, as it has been done in this experiment, has resulted in changing quality properties of the mixtures (Table 1). With the increase of apple pomace content in the samples, total protein has been reduced from 12.2% (control sample) to 11.0% (samples with a 10 or 15% content of apple pomace). A similar tendency has been observed by Rupasinghe et al. [2008] in their research on the possibilities of using apple skin powder in muffin production. It has also been noted by Vergara-Valencia et al. [2007], who have observed a reduction of total protein in bread when adding fiber concentrate from mango fruit. In the research by Anioła et al. [2008] the addition of apple pomace to cakes has also caused lowering the value of this discriminant. In bakery practice it is believed, that bread flour should consist of more than 25% of wet gluten. Gluten yield has decreased with the increasing content of apple pomace in the samples, which may have been caused by the lack of this component in the incorporated supplement and the interaction of fiber preparations and gluten, which has been pointed out in the research carried out by Chen et al. [1988]. The highest gluten yield has been observed in the control sample (33.0%) and the lowest the sample with a 15% content of pomace (15.5%). The baking value of the flour depends on the quantity and quality of gluten, which can be determined by means of measuring its deliquescence and sedimentation value. Strong gluten dissolves only to a small extent when thermostated in a temperature of about 30°C. Flour containing a high level of good quality gluten is characterized by high sedimentation value. The highest deliquescence has been noted in the control sample (7.0 mm) and the lowest in the one with a 15% content of pomace (1.5 mm). Sedimentation value has gradually decreased from 34.5 ml to 28.0 ml with the increase of apple pomace content in the samples. What is also important when evaluating the quality of flour is the activity of the amylolytic enzymes. The activity of α-amylase can be indirectly determined basing on the falling number. With a high activity level of this enzyme gelatinized dough is quickly fluidized and the falling number is low. The highest falling number value has been found in the sample with a 5% content of the apple preparation (336 s) and the lowest in the sample with a maximum content of the pomace (302 s). In the research carried out by Mielcarz [2004], supplementing flour with 5 to 15% of different kinds of fiber has caused minor changes in the falling number.

Table 1

Mean values of wheat flour quality properties depending on the content level of apple pomace

Content level of apple pomace [%]	Total protein [%]	Wet gluten yield [%]	Deliquescence of wet gluten [mm]	Sedimentation value [ml]	Falling number [s]
0	12.2 a	33.0 a	7.0 a	34.5 a	324 ab
5	11.6 ab	27.6 b	3.5 ab	33.0 b	336 a
10	11.0 b	25.5 c	2.5 b	31.0 c	318 b
15	11.0 b	15.5 d	1.5 b	28.0 d	302 c

Explanatory notes:
a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$.

The increase in the content of apple pomace has had a significant influence on the values of amylographic properties of wheat flour (Table 2). The highest initial gelatinization temperature has been obtained in the case of the sample with a 10% content of the examined agent (59.6°C), and the lowest for the control sample (58.6°C). As far as the final temperature of gelatinization, time of gelatinization and the maximum viscosity of gelatinized dough are concerned, the sample characterizing with the uppermost values has been the one with the highest content of apple pomace incorporated (89.0°C; 39.3 min and 1302 AU respectively). The lowest maximum viscosity has been achieved for the sample with a 5% content of pomace (605 AU).

Table 2

Means values of amylographic properties of wheat flour depending on the content level of apple pomace

Content level of apple pomace [%]	Initial temperature of gelanization [°C]	Final temperature of gelanization [°C]	Gelanization time [min]	Maximum viscosity of gelatinized doughs [AU]
0	58.6 c	86.0 b	37.3 b	740 c
5	59.2 ab	85.5 b	37.0 b	605 d
10	59.6 a	86.8 b	37.9 b	860 b
15	59.0 bc	89.0 a	39.3 a	1302 a

Explanatory notes:
a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$;

In Table 3 the values of farinographic properties of the wheat dough enriched with apple pomace are shown. The rising content of pomace in the mixtures has resulted in a gradual increase of water absorption of flour from 59.4% for the control sample, to 66.0% for the sample with a maximum content of pomace. Such dependence has also been validated by other authors' research [Ajila et al., 2008; Anil, 2007; Sudha et al., 2007a]. The increase of water absorption of flour supplemented with apple pomace results mainly from the interaction between water and hydroxyl polysaccharides contained in the pomace by hydrogen bonds [Rosell et al., 2001]. The shortest dough development time has been observed in the sample with a 5% content of pomace (3.0 minutes), and the longest in the one with a 15% content (5.6 minutes). For the sample with a maximum content of apple pomace the shortest solidity of dough has been obtained (2.8 minutes). Sudha et al. [2007a] point out that adding apple pomace to dough results in reducing dough solidity and lengthening dough development time, both of which are indicators of flour strength. The higher their values, the stronger dough is obtained. The experiments of Ajila *et al*. [2008] and Sucha *et al*. [2007b], in which they examined the influence of adding fiber concentrate from mango fruit and different kinds of bran to wheat flour on the rheological properties of dough, have led to similar conclusions. In Wang *et al*. research [2002] fiber added to dough has not had any influence on the development time and solidity of the dough. Longer development time and shorter dough solidity are caused by the "dilution" of gluten protein in wheat flour with the fiber content increase and their possible interaction [Ajila et al., 2008; Chen et al., 1988]. In this research dough

weakening caused by adding fruit pomace has been observed. The control sample has appeared to be the most resistant to kneading. In the case of this sample softening value has been 100 FU. The sample with a 10% content of pomace has turned out to be of lowest resistance (softening 130 FU).

Table 3

Means values of farinographic properties of wheat flour depending on the content level of apple pomace

Content level of apple pomace [%]	Water absorption of flour [%]	Development time of dough [min]	Dough stability [min]	Softening of dough [FU]	Quality number [mm]
0	59.4 d	4.6 b	5.0 a	100 b	64 a
5	61.2 c	3.0 c	5.0 a	115 ab	61 a
10	63.1 b	4.5 b	5.3 a	130 a	62 a
15	66.0 a	5.6 a	2.8 b	110 ab	66 a

Explanatory notes:
a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$.

With the increase of apple pomace content in the samples, bread volume has decreased from 597 cm^3 in the control sample to 345 cm^3 (15% pomace content) (Table 4), which has been caused by weakening gluten by the addition of pomace, being a source of dietary fiber, to the flour. A similar dependence has been noted by Ayadi et al. [2009]; Korus and Achramowicz [2004]; Masoodi and Chauhan [1998]; Masoodi et al. [2002]; Sudha et al. [2007a] and Wang et al. [2002]. With the increase of the apple pomace content, bread overbake has risen from 47.2% (control sample) to 57.2% in the sample with a maximum dietary fiber content (Table 4), which is caused by higher water absorption of mixtures containing pomace. The assessment of porosity of the crumb according to an 8-point Dallmann scale has ranged from 2.5 to 7.0 points (Table 4). The increase of apple pomace content in the samples has effected in the improvement of porosity of the crumb in the breads, raising the number of points assigned from 2.5–3.5 (for the control bread sample and the one with a 5% content) to 6–7 (for breads baked with a 10 or 15% content of pomace).

Table 4

Means values of quality properties of wheat bread depending on the content level of apple pomace

Content level of apple pomace [%]	Bread volume [cm^3/100g flour]	Overbake [%]	Porosity of the crumb acc. to Dallmann scale
0	597 a	47.2 c	2.5 b
5	507 b	51.9 b	3.5 b
10	410 c	55.6 a	6.0 a
15	345 d	57.2 a	7.0 a

Explanatory notes:
a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$;

The apple pomace used in this research was characterized with a relatively low content of NDF fiber, ADF fiber and hemicelluloses in comparison with the results obtained by Nawirska and Uklańska [2008]. Designation results of the fiber fractions contained in the mixtures are shown in Table 5. What has been found of the examined mixtures of flour and pomace is that they included a high content of hemicelluloses and NDF fractions. In the samples with a 15% content of pomace the highest content of both fractions (NDF – 54.79%, hemicelluloses – 52.05%) has been noted, and in the one with a 5% content, the lowest (NDF – 48.34%, hemicelluloses – 45.18%).

Table 5

Means values of dietary fibre fractions in wheat flour depending on the content level of apple pomace

Content level of apple pomace [%]	NDF [g/100g DM]	ADF [g/100g DM]	Hemicellulose [g/100g DM]
0	49.27 c	2.55 a	46.72 c
5	48.34 c	3.15 a	45.18 d
10	51.05 b	2.34 a	48.71 b
15	54.79 a	2.74 a	52.05 a

Explanatory notes:
a, b, c, d, e – mean values in the columns, denoted by different letters, differ statistically significant at level of $\alpha \leq 0.05$;
NDF- neutral dietary fibre (cellulose+hemicellulose+lignin)
ADF-acid nutritive fibre (cellulose+hemicellulose).

Conclusions

The increasing content of apple pomace in the mixtures has resulted in the rise of the maximum viscosity of gelatinized dough, water absorption of flour, bread overbake and the improvement of crumb porosity, as well as it has enriched the bread with a pleasant fruit smell and taste, at the same time reducing flour quality and bread volume indexes.

For the sample with a 15% content of pomace, the highest final gelatinization temperature and time of gelatinization, highest maximum viscosity of gelatinized dough and dough development time have been obtained, as well as the shortest dough stability.

Supplementing the dough with apple pomace has resulted in increasing the content of dietary fiber in the mixtures.

References

Ajila C.M., Leelavathi K., Prasada Rao U.J.S., 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. J. Cereal Sci., 48, 319–326.

American Association of Cereal Chemists (AACC), 2001. The definition of dietary fiber. A report. Cereal Foods World, 46(3), 112–129.

Anil M., 2007. Using of hazelnut testa as a source of dietary fiber in breadmaking. J. Food Eng., 80, 61–67.
Anioła J., Piotrowska E., Walczak K., Górecka D., 2008. Zastosowanie mikronizowanych preparatów wysokobłonnikowych w wyrobach ciastkarskich. Żywność. Nauka. Technologia. Jakość, 4 (59), 103–110.
Ayadi M.A., Abdelmaksoud W., Ennouri M., Attia H., 2009. Cladodes from Opuntia ficus indica as a source of dietary fiber: Effect on dough characteristics and cake making. Industrial Crops and Products, 30, 40–47.
Chau C.F., Hwang Y.L., 2003. Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of Citrus sinensis. J. Agric. Food Chem., 51, 2615–2618.
Chen H., Rubenthaler G.L., Schanus E.G., 1988. Effect of apple fiber and cellulose on the physical properties of wheat flour. J. Food Sci., 53, 304–305.
Fronc A., Nawirska A., 1994. Potential uses of waste products from fruit processing. Ochrona Środowiska, 2, 31–32.
Goristein S., Zachwieja Z., Folta M., Barton H., Piotrowicz J., Zembr M., Weisz M., Trakhtenberg S., Martin-Belloso O., 2001. Comparative content of dietary fiber, total phenolics, and minerals in persimmons and apple. J. Agric. Food Chem., 49, 952–957.
Guillon F., Champ M., 2000. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Res. Int., 33, 233–245.
Kołodziejczyk K., Markowski J., Kosmala M., Król B., Płocharski W., 2007. Apple pomace as a potential source of nutraceutical products. Pol. J. Food Nutr. Sci., 57, 4(B), 291–295.
Korus J., Achremowicz B., 2004. Zastosowanie preparatów błonnikowych różnego pochodzenia jako dodatków do wypieku chlebów bezglutenowych. Żywność. Nauka. Technologia. Jakość, 1(38), 65–73.
Masoodi F.A., Chauhan G.S., 1998. Use of apple pomace as a source of dietary fiber in wheat bread. J. Food Proc. Pres., 22(4), 255–263.
Masoodi F.A., Sharma B., Chauhan G.S., 2002. Use of apple pomace as a source of dietary fiber in cakes. Plant Food for Human Nutrition, 57, 121–128.
McQueen R.E., Nicholson J.W.G., 1979. Modification of the neutral-detergent fiber procedure for cereals and vegetables by using amylase. J. AOAC, 62,3, 676–680.
Mielcarz M., 2004. Żywieniowe i technologiczne aspekty zastosowania błonników pokarmowych do produkcji wyrobów piekarskich i ciastkarskich. Przegl. Piek., 8, 7–9.
Nawirska A., Uklańska C., 2008. Waste products from fruit and vegetable processing as a potential sources for food enrichment in dietary fibre, 7,2, 35–42.
Ohr L.M., 2004. Fortyfying with fiber. Nutraceuticals&Functional Foods, 58(2), 71–75.
PN-75/A-04018. Produkty rolno-spożywcze. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko.
PN-77/A-74041. Oznaczanie ilości i jakości glutenu.
PN-ISO 3093, 1996. Zboża. Oznaczanie liczby opadania.
PN-ISO 5529, 1998. Oznaczanie wskaźnika sedymentacyjnego. Test Zeleny'ego.
PN-ISO 5530-1, 1999. Fizyczne właściwości ciasta. Oznaczanie wodochłonności i właściwości reologicznych za pomocą farinografu.
PN-ISO 7973, 2001. Ziarno zbóż i przetwory zbożowe. Oznaczanie lepkości mąki. Metoda z zastosowaniem amylografu.
Rosell C.M., Rojas J.A., Benedito de Barber C., 2001. Influence of hydrocolloids on dough rheology and bread quality. Food Hydrocolloids, 15, 75–81.
Rupasinghe H.P.V., Wang L., Huber G.M., Pitts N.L., 2008. Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. Food Chem., 107, 1217–1224.

Sudha M.L., Baskaran V., Leelavathi K., 2007a. Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characeristics and cake making. Food Chem., 104, 686–692.

Sudha M.L., Vetrimani R., Leelavathi K., 2007b. Influence of fibr from different cereals on the rheological characetristics of wheat flour dough and on biscuit quality. Food Chem., 100, 1365–1370.

Wang J., Rosell C.M., Benedito de Barber C., 2002. Effect of the addition of different fibres on wheat dough performance and bread quality. Food Chem., 79, 221–226.

Van Soest P.J., 1963. Use of detergents in the analysis of fibrous feeds. J. AOAC, 13,5,825–835.

Vergara-Valencia N., Granados-Pérez E., Agama-Acevedo E., Towar J., Ruales J., Bello-Pérez L., 2007. Fibre concentrate from mango fruit: Characterization, associated antioxidant capacity and application as a bakery product ingredient. LWT, 40,722–729.

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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 [Day et al., 2006; Obuchowski, 1997]. 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 [Gallagher et al., 2004; Miedwiediew, 1999 Wang et al., 1999]. 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 ingre-

dients. 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 [Alamprese1 et al., 2007; BeMiller, 2008; Gallagher et al., 2004]. 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^{o}C; a temperature of the 2 zone 120^{o}C; a temperature of the head 95 ^{o}C; corotating screw revolutions = 50/min. Pasta samples were dried at 60^{o}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

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	–

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 colorimeter (CR 400 Chroma-Metter, Minolta, Japan) (AACC 14-22).

Pasta samples were also subjected to sensory evaluation test for color, 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 color 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 color. Pasta color is a result of yellow pigments content in raw material or additives used. Pigments contained in wheat, which determine the color 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, 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 color 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 color and smell improved.

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 color of product. Extruded pasta (cooking extrusion) before cooking were characterized by unattractive brown color. After cooking the color 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.

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

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

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 color 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.

Conclusions

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 color 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.

References

Alamprese C., Casiraghi E., Pagani M.A., 2007. Development of gluten-free fresh egg pasta analogues containing buckwheat. J. Eur. Food Res. Tech., 225, 2, 205–213.

BeMiller J.N., 2008. Hydrocolloids, in: Arendt E.K., Dal Bello F. Gluten-Free Cereals Products and Beverages, Food Sci. Technol., International Series, 203–216.

Catassi C., Fasano A., 2008. Celiac disease. In: Arendt, E., K., Dal Bello, F. Gluten-Free Cereal Products and Beverages, Food Sci. Technol., International Series, 203–216, 1–22.

Chaisawang M., Suphantharica M., 2006. Pasting and rheological properties of native and anionic tapioca starches as modified by guar gum and xanthan gum. Food Hydrocol, 20, 641–649.
Chaisawang M., Suphantharica M., 2005. Effects of guar gum and xanthan gum additions on physical and rheological properties of cationic tapioca starch. Carbohydrate Polymers, 61, 3, 288–295.
Chen Z., Schols H.A., Voragen A.G.J., 2003. Starch granule size strongly determines starch noodle processing and noodle quality. J. Food Sci., 68, 5, 1584–1589.
Ciclitira P.J., Johnson M.W., Dewar D.H., Ellis H.J., 2005. The pathogenesis of coeliac disease. Mol. Asp. Med., 26, 6, 421–458.
Day L., Augustin M.A., Batey I.L., Wrigley C.W., 2006. Wheat-gluten uses and industry needs. Trends Food Sci. Technol., 17, 2, 82–90.
Eliasson A.C. 1986. Viscoelastic behavior during the gelatinization of starch. II. Effects of emulsifiers. J. Texture Studies, 17, 357–375.
Funami T., Kataoka Y., Omoto T., Goto Y., Asai I., Nishinari K., 2005. Effects of non-ionic polysaccharides on the gelatization and retrogradation behaviuor of wheat starch. Food Hydrocol., 19,1–13.
Gallagher E., Gormley T.R., Arendt E.K., 2004. Recent advances in the formulation of gluten-free cereal-based products. Trends Food Sci. Technol., 15, 143–152.
Gąsiorowski H., 2006. Corn. 6 Corn lipids. Przegl. Zboż-Młyn., 6, 18–24.
Huang J.C., Knight S., Goad C., 2001, Model prediction for sensory attributes of nongluten pasta. J. Food Qual., 24, 495–511.
Kaur L., Sigh J., Sigh N., 2005. Effect of glycerol monostearate on the physico-chemical, thermal, rheological and noodle making properties of corn and potato starches. Food Hydrocol., 19, 839–849.
Koning F., Schuppan D., Cerf-Bensussan N., Sollid L.M., 2005. Pathomechanisms in celiac disease. Best Pract. Res. Clin. Gastroenterol., 19, 3, 373–387.
Kovacs E., Vamos-Kardos E., Kiss-Laszlavik M. Pallaqi E., 1992. Effect of emulsifiers on the properties of pasta products. Acta Alim., 21, 205–217.
Lee A.R., Ng D.L., Zivin J., Green P.H.R., 2007. Economic burden of a gluten-free diet J. Hum. Nutr. Diet, 20, 423–430.
Makowska A., 2008. Effect of production technology on color and determination of yellow pigments in pasta. ABiD, 3, 60–65.
Miedwiediew G.M., 1999. Production of pasta from non-gluten starch raw materials. Przegl. Zboż-Młyn., 2, 6–7.
Nagano T., Tamaki E., Funami T., 2008. Influence of guar gum on granule morphologies and rheological properties of maize starch. Carbohydrate Polymers, 72, 1, 95–101.
Obuchowski W., 1997. The technology of industrial pasta production. Wyd. AR, Poznań, 12–13.
Schreurs E., Seibel W., Menger A., Pfeilsticker K., 1986. Effect of cooking water on cooking quality of pasta in relation to raw material quality III. Starch gelatinization behavior of pasta suspensions using different types of cooking water. Getreide Mehl u.-Brot. 40, 219–223.
Shiau S.Y., 2004. Effects of emulsifiers on dough rheological properties and the texture of extruded noodles, J. Texture Studies, 35, 93–110.
Sudhakar V., Singhal R.S., Kulkarni P.R., 1995. Studies on starch-hydrocolloid interactions: effect of salts. Food Chem., 53, 405–408.
Tan H.Z., Li Z.G., Tan B., 2009. Starch noodles: History, classification, materials, processing, structure, nutrition, quality evaluating and improving, Food Res. Inter., 42, 551–576.
Thompson T., 2001. Wheat starch, gliadin and the gluten-free diet, J. Am. Diet Assoc., 101, 1456–1459.
Wang N., Bhirud P.R., Sosulski F.W., Tyler R.T., 1999. Pasta-like product from pea flour by twin-screw extrusion, J. Food Sci., 64, 4, 671–678.
Wójtowicz A., 2007. Application of extrusion cooking in precooked pasta production, in: Mościcki L, Mitrus M, Wójtowicz A.: Extrusion cooking technique in farm and food industry. PWRiL, 118–136.
Zobel H.F., 1988. Starch crystal formation and their industrial importance. Starch, 40, 1–7.

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THE POSSIBILITY OF WHOLE EGG REDUCTION IN SPONGE CAKE PREPARED WITH DIFFERENT ADDITIVES

Introduction

The almost unique foaming, emulsifying, and heat coagulation properties of egg proteins confer them a very important functional role in the definition of cake physicochemical characteristics, such as volume and texture. This makes it extremely difficult to replace eggs in cakes successfully by a different source of proteins, even by the use of several types of additives, such as hydrocolloids [Ashwini et al., 2009].

High-quality cakes should have various attributes, including high volume, uniform crumb structure, tenderness, shelflife and tolerance to staling. These attributes depend on the balanced formulas, aeration of cake batters, stability of fluid batters in the early stage of baking, and thermal-setting stage. The quality of a finished cake can be influenced by the addition of such substances as hydrocolloids and other polysaccharides that affect these properties. The polysaccharides are used in food production as processing aids, to provide dietary fibre or to improve food texture, retard starch retrogradation, moisture retention and enhance the overall quality of the products during storage. Hydrocolloids also modify the pasting properties of starch. These starch properties, that include gelatinization temperature, paste viscosity and retrogradation of the starch, affect final quality of cakes and staling behaviour of baking products [Gomez et al., 2007].

Egg white proteins play a significant role in foam formation in cakes. Large volumes of air are easily dispersed into the batter before baking. Heating egg foam expands the air bubbles trapped in cake batter, and the volume of cake increases immediately after the coagulation of egg proteins during baking. For foam-type cakes, the whipping properties of eggs usually determine the final volume, cell structure and tenderness of the products. The better the whipping properties of eggs, the higher is the acceptability of cake appearance and texture [Arunepanlop et al., 1996].

Sponge cake is a foam-type cake because air bubbles are incorporated into batters prior to baking and foam is stabilized upon coagulation of foaming agent during baking. Dispersion of air bubbles in cake batters is usually generated by whipping egg white or whole egg proteins in the formulations [Celik et al., 2007].

Replacing egg white proteins with dairy proteins influences the textural and physical properties of cakes, especially when substitution is high with whey protein isolates cakes have undesired characteristics. Therefore, usually egg white proteins are partially replaced with whey protein isolate and additionally food hydrocolloids such as xanthan gum, hydroxylmethyl cellulose and/or additional carbohydrate raw materials like maltodextrins to improve appearance, texture and sensory properties of angel cakes [Arunepanlop et al., 1996].

The aim of the study was to recognize the possibility of reduction by 20% of whole egg addition to model sponge egg produced with wheat flour. Egg component was replaced by water, whey proteins, mono/didiglicerides, maltodextrins, potato starch and/or wheat flour was partially substituted by corn flour (25%).

Materials and methods

Sponge cakes were prepared at laboratory conditions by mixing (Hobart equipment) selected ingredients according to following formula for control sample: wheat flour 500 33% (containing 9.8% protein, 1.8% fat and 76.1% carbohydrates); fresh whole egg mass 33%; saccharose 33% and baking powder 1%. During the first stage of dough preparation whole egg mass was whipped with sugar for 5 minutes. To the prepared foam flour and baking powder were added and mixed for 3 minutes. Baking process was performed in electric oven at 180ºC for 48 minutes. The study was performed in two experiments; one with egg mass of perceived as lighter and yellow (average colour parameters L*=76.5, a*=3.4, b*=56.8) and second with whole egg perceived as darker and orange (L*=75.9, a*=4.3, b*=49.2). Other experimental cakes were manufactured by egg component replacement (20% of egg volume) with water (6.6% in whole dough), with whey proteins isolate (containing over 81% of proteins) solution, commercial mono/didiglicerides, maltodextrines (consist of 97% carbohydrates), potato starch (81% of carbohydrates) (Tab. 1). However, the last experimental variant was prepared according to control formula but wheat flour was partially (25%) substituted by corn flour (containing 7.3% protein and 1.6% fat).

Table 1

Design of the experiment

Ingredient	Wheat flour	Corn flour	Whole egg	Water	Whey proteins	Malto-dextrins	Emul-gator	Potato starch	Saccharose/ baking powder
Control	33	–	33	–	–	–	–	–	33/1
20% egg replacement	33	–	26.4	6.6	–	–	–	–	33/1
20% egg replacement whey proteins	33	–	26.4	5.3	1.3	–	–	–	33/1
20% egg replacement E471	33	–	26.4	6.4	–	–	0.2	–	33/1
20% H_2O replacement maltodextrin	33	–	26.4	5.3	–	1.3	–	–	33/1
20% H_2O replacement potato starch	33	–	26.4	5.3	–	–	–	1.3	33/1
Corn flour	24.7	8.3	33	–	–	–	–	–	33/1

The cake volume was measured by the volumetric rapeseed displacement method. Colour (L*, a*, b*) of the crumb were determined using Minolta chromameter. Sensory analysis of the cakes including colour and appearance of the crust, colour, structure and porosity of the crumb, and taste and odour of fresh cake was carried out according to the designed scale using 1 to 5 scores (Tab. 2). A panel of 7 assessors judged sponge cakes using descriptive analysis methodology. Panellist were selected, screened and recruited from the employees of the Wroclaw University of Environmental and Life Sciences. The definition of terms for each product type was created by assessors during preparation session.

Collected results were statistically analysed using Statistica ver. 8.0 by analysis of variance at $p>0.05$

Table 2

Sensory analysis of the cakes according to the designed scale

Score	1	2	3	4	5
Crust colour and apperance	dark or light brown, cracked	light brown, slightly cracked	brown, slightly cracked	dark or light brown, without cracks	brown without cracks
Crumb colour	light creamy	light yellow	creamy yellow	creamy	yellow
Crumb structure	spreading	tight, crumbled, poorly connected with skin	improperly connected with skin	good connected with skin	perfectly connected with skin
Crumb porosity	big pores in the whole cake	big pores in the middle part	small pores non-uniformly distributed	small pores less visible	small pores uniformly distributed
Taste	almost non-detectable	slightly detectable	sweet, floury	medium sweet typical for sponge cake	delicate medium sweet typical for sponge cake
Odour	intensive, not typical for sponge cake	low-intensive, not typical for sponge cake	low-intensive, eggy, typical for sponge cake	semi-intensive, eggy, typical for sponge cake	intensive, eggy, typical for sponge cake

Results and discussion

Replacement of eggs with water (20%) did not decrease cake volume (Tab. 3, 4). Celik et al. [2007] also observed no effect of eggs content lowering by 25% in sponge cake formulations on the discussed parameter but physical characteristics of baked products was lowered when decrease in egg content replaced by water was 50% and 75%. Substitution of eggs with whey proteins or emulgator E471 also has not been caused an increase in discussed parameter. Whey proteins were used in sponge cake formulation e.g. made from the egg white foam, if 25% of egg white was replaced by whey proteins only slight effect was observed however at 50% of substitution the cake volume was lesser even 40% [Morr et al., 2003]. In our own studies only maltodextrin application resulted in statistically significant higher cake volume, especially when lighter whole egg was used in formulation.

Replacement of whole egg by 20% of water with or without additives did not effect crust colour and appearance when light/yellow whole egg was applied. Although, there was a tendency to drop in scores if only water was added. In case of darker and more orange whole egg usage lowering of judgment for variant 20% H_2O was statistically significant but the lowest score was attributed to sponge cake made with whey proteins, while addition of corn flour caused low insignificant increase of the score (Fig. 1).

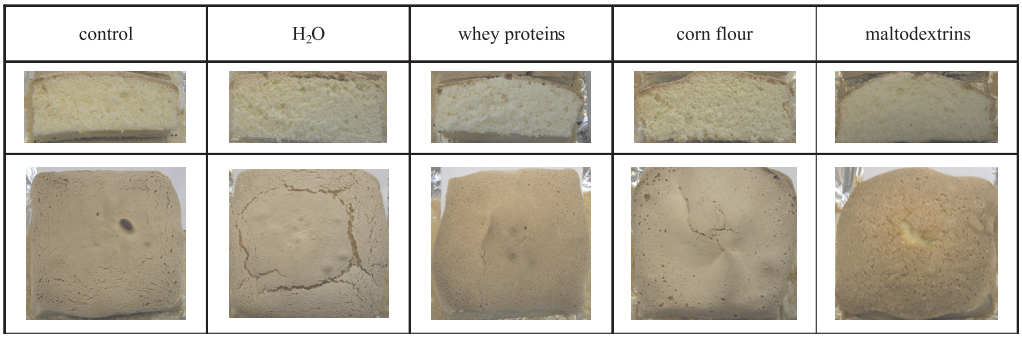


Fig. 1. Cross-section and surface of sponge cakes

Replacement of 20% of whole egg by water more evidently decreased crumb colour especially when lighter and more yellow raw material was applied. Controversial results were obtained for sponge cakes formulated with lowered egg content but with addition of whey proteins. The usage of lighter raw material caused increase in colour acceptance but if darker whole egg was used lowering of crumb colour of sponge cake was noticed. Such differentiation was observed also for variant with maltodextrin (higher judgment for lighter raw material and no effect in case of darker one). Replacement of water with addition of ingredients and/or wheat with corn flour improved crumb structure. Only when lighter whole eggs were replaced with water worsening of crumb structure (Fig.1), and lower score for this parameter were judged.

Emulsifiers are usually applied in the baking industry as dough strengtheners (such as-sodium stearoyl-2-lactylate and diacetyl tartaric acid esters of monodiglycerides) and crumb-softeners (for example monoacylglycerols and glycerol monostearate) [Goméz et al. 2004, Kohajdova et al. 2009]. In own studies crumb structure was improved when water with monodigicerides was added to the dough instead part of lighter egg mass.

The main destabilizing mechanism for the cake batter is gas diffusion from small to large bubbles [Hicsasmaz et al., 2003] . Lowering of egg content generally led to better porosity of analysed sponge cakes, especially when darker raw material was used. The best porosity was observed for variant with 20% of water and whey proteins or maltodextrin added as well as if corn flour or potato starch were used in formulations. Taste of sponge cakes was not affected by changes in formulations if darker and more orange raw material was applied. Also odour was not influenced by used additives. Although, potato starch addition lowered the acceptance of odour.

The kind of raw material with different colour characteristics highly influenced instrumental parameters of cake colour. In case of lighter more yellow raw material the L* value of

sponge cake ranged from 90.1 (variant with water and emulgator) to 94.2 (variant with water and whey proteins). While the control was characterized by average lightness of 91.3. Replacement of eggs with water generally lowered b* (yellowness) value except for variant with maltodextrins. In experiment with darker and more orange whole egg L* value was ranging from 78.4 (with corn flour) to 83.2 (water and whey proteins) and control was on the level of 80.6. Variant with whey proteins showed also the lowest b* value 20.8 in comparison to control (24.0). Due to expectation replacement of wheat with corn flour increased yellowness and lowered lightness of sponge cake.

Table 3

Physicochemical and sensory characteristics of sponge cake made with light/yellow whole egg mass

Variant	Volume (cm^3)	Crust colour	Crumb colour	Crumb structure	Crumb porosity	Taste	Odour	Colour		
								L	a	b
Control	680^{a}	3.5^{a}	3.3^{bc}	3.2^{b}	4.0^{a}	3.3^{a}	4.0^{b}	91.3^{b}	-1.9^{ab}	44.6^{b}
20% of H_2O	670^{a}	3.0^{a}	2.3^{a}	2.3^{a}	4.3^{a}	3.5^{a}	4.0^{b}	94.1^{c}	-2.6^{d}	43.3^{ab}
Whey proteins 20%	638^{a}	3.2^{a}	4.0^{c}	3.7^{bc}	5.1^{b}	4.6^{c}	4.0^{b}	94.2^{c}	-1.8^{a}	42.4^{a}
Emulgator 20%	702^{a}	3.6^{a}	3.0^{ab}	4.0^{c}	4.1^{a}	4.1^{b}	3.0^{a}	90.1^{a}	-2.1^{bc}	42.8^{a}
Maltodextrin 20%	801^{b}	$3.^{a}$	4.0^{c}	4.0^{c}	4.3^{a}	3.7^{ab}	4.1^{b}	93.3^{c}	-2.4^{cd}	44.4^{b}

a,b,c,…, – the same letters determine not statistically significant differences at $p>0.05$, $n = 4$.

Table 4

Physicochemical and sensory characteristics of sponge cake made with dark/orange whole egg mass

Attributes	Volume (cm^3)	Crust colour	Crumb colour	Crumb structure	Crumb porosity	Taste	Odour	Colour		
								L	a	b
Control	674^{a}	4.5^{d}	3.7^{c}	2.6^{a}	3.6^{a}	4.0^{a}	4.0^{c}	80.6^{b}	-3.1^{ab}	24.0^{cd}
20% of H_2O	683^{a}	3.8^{bc}	3.5^{bc}	3.3^{b}	4.8^{b}	4.0^{a}	4.0^{c}	80.9^{b}	-3.0^{ab}	21.4^{ab}
Whey proteins 20%	687^{a}	3.0^{a}	2.7^{a}	3.7^{b}	4.3^{ab}	4.2^{a}	4.5^{d}	83.2^{e}	-2.8^{a}	20.8^{a}
Emulgator 20%	678^{a}	4.0^{c}	3.0^{ab}	3.2^{b}	3.7^{a}	4.1^{a}	3.3^{b}	81.1^{bc}	-3.3^{bc}	21.2^{ab}
Maltodextrin 20%	714^{a}	4.5^{d}	3.5^{bc}	3.3^{b}	4.2^{ab}	4.2^{a}	4.0^{c}	81.9^{d}	-3.4^{c}	22.9^{bc}
Potato starch 20%	683^{a}	3.5^{b}	3.3^{bc}	3.7^{b}	$4.^{ab}$	4.0^{a}	2.6^{a}	81.8^{cd}	-3.3^{bc}	21.3^{ab}
Corn flour	700^{a}	4.7^{d}	5.0^{d}	3.6^{b}	4.3^{ab}	4.0^{a}	4.0^{c}	78.4^{a}	-2.9^{a}	25.5^{d}

a,b,c,…, – the same letters determine not statistically significant differences at $p>0.05$, $n = 4$.

Conclusions

It can be concluded that replacement of 20% the amount of added whole egg by water did not effect cakes volume but led to lowering of some texture/quality attributes. The most effective additive in sponge cake mixtures with reduced eggs content by 20% was maltodextrine causing increase in cake volume and improve crumb structure. Addition of whey proteins increased lightness and decreased yellowness of the cake. The substitution of 25% of wheat with corn flour without lowering of whole egg addition resulted in higher yellowness and lower lightness. In addition to that sensory analysis revealed that colour of crust and crumb as well as crumb structure were improved. Whole egg addition in sponge cake formulations could be lowered by 20% without significant deterioration of the quality of final product when maltodextrin was used as an ingredient.

References

Arunepanlop B., Morr C.V., Karleskind D., Laye I., 1996. Partial replacement of egg white proteins with whey proteins in angel food cakes. J. Food Sci., 61, 1085–1093.

Ashwini A., Jyotsna R., Indrani D., 2009. Effect of hydrocolloids and emulsifiers on the rheological, microstructural and quality characteristics of eggless cake. Food Hydrocolloids, 23, 700–707.

Celik I., Yılmaz Y., Isık F., Ustun O., 2007. Effect of soapwort extract on physical and sensory properties of sponge cakes and rheological properties of sponge cake batters. Food Chem., 101, 907–911.

Gomez M., Ronda F., Caballero P.A., Blanco C.A., Rosell C.M., 2007. Functionality of different hydrocolloids on the quality and shelf-life of yellow layer cakes. Food Hydrocolloids, 21, 167–173.

Gómez M., Oliete B. , Pando V. , Ronda F. Caballero P.A., 2004. Effect of fermentation conditions on bread staling kinetics. Europ. Food Res. Technol., 226,1379–1387.

Hicsasmaz Z., Yazgan Y., Bozoglu F., Katnas Z., 2003. Effect of polydextrose-substitution on the cell structure of the high-ratio cake system. Lebensm.-Wiss. U.-Technol., 36, 441–450.

Kohajdova Z., Karovicova J, Schmidt S., 2009. Significance of emulsifiers and hydrocolloids in bakery industry. Acta Chim. Slovac., 2, 46–61.

Morr C.V., Hoffmann W., Buchheim W., 2003. Use of applied air pressure to improve the baking properties of whey protein isolates in angel food cakes. Lebensm.-Wiss. U.-Technol., 36, 83–90.

34

QUALITY CHANGES OF CONFECTIONERY PRODUCTS DURING FROZEN STORAGE TIME

Introduction

Quality changes in bread and confectionery products mainly are related to the starch retrogradation process and moisture migration from or to product. Research has proved that loss of moisture is not the only reason for bread and confectionery product staling. It is found that bread staling is caused by retrogradation of starch polymers, crystallization of amylase and amylopectin, when gelatinized starch is changed into its initial crystalline structure, as a result bread staling happens during its storage. In bread staling process temperature (the fastest changes happens at 0°C), size, shape and concentration of starch molecules play important role. Several researchers have found that retrogradation happens only to amylopectin but not amylase [Hug-Iten et al., 2003; Bai and Chinachoti, 2000; Kulp and Joseph, 2000; Irwin and Paul, 1998]. In order to reduce retrogradation rate and subsequently increase product storage time, it is posible use freezing. Mackey et al. Proved that it is possible to store frozen dough products up to 6 months (at -23– -18°C temperature) without significant changes in sensory attributes and volume of the product [Mackey et al., 1952].

The economic and demographic evolution of the foodservice and in-store baking industries will provide an ample foundation for growth of the frozen baked goods industry in Latvia in the years to come. Whether marketed through foodservice or food retailing outlets, frozen confectionery provide an important venue for achieving cost efficiencies in the face of increased competition and fragmenting markets. With time, product quality will improve as the industry climbs the technological learning curve. The success of the frozen confectionery industry remains first and foremost economical driver [Kulp et al., 1998].

Materials and methods

The frozen products were made in an industrial enterprise and after freezing were delivered to the Faculty of Food Technology for analysis and evaluation of product quality changes during its storage.

Sensory properties (flavour, taste and appearance), moisture content and microbiological contamination were determined for three types of samples – fillings, frozen and baked confectionery products. Plate counting method was used for microbiological assays. The samples (dilutions 1:100; 1:1000) for the investigation were taken on the 1st, 30th, 60th and 90th days. Enterobactery was detected on VRBD agar (1:10; 1:100) in two replications. Counting

of colonies formed and calculating the number of CFUs was accomplished by Acolyte colony counter. Moisture content in samples (fillings, frozen and baked products) was determined using moisture balance XM 120 Precisa. For determination of moisture content approximately 5 g of sample is placed in the moisture balance chamber for heating at 105°C until constant weight is reached.

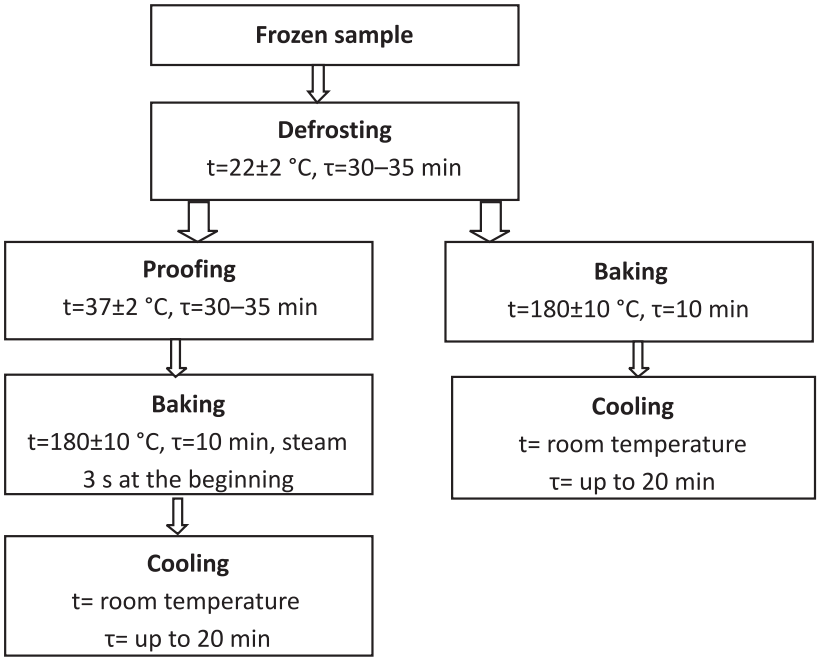


Fig. 1. The scheme for preparation of confectionery products

Delivered frozen products were stored in a freezer at temperature of -18±2°C. Experiments were designed according to the scheme presented in Figure 1.

Partially baked frozen pies after defrosting were baked at 180±10°C in a convection type oven without steam injection. Product proofing was done in the proofer chamber (SVEBA DAHLEN), and baking in a rotational type oven (SVEBA DAHLEN).

Results and discussion

In order to control the microorganism development, the fillings used for confectionery product preparation were tested prior to the filling device and after the filler (Fig. 2).

Latvian Republic guidelines "Good Hygiene and Manufacturing practice guidelines for confectionery and culinary production enterprises" determine the permissible level of total plate count in confectionery products at $5x10^4$ or 50000 cfu per gram.

Total plate count in curd, spicy, apple and cream fillings prior to filling into confectionery products was detected at 2100–37967 cfu g^{-1}, which comply with requirements set in the regulatory documents. Although the total plate counts in fillings after the filler device and in

meat pie filling exceeded the permissible limit about 1.3–1.6 times. The result demonstrates that the producers should pay more attention to hygiene of production equipment, because it might be a source of microbiological contamination. More strict requirements should be set to raw materials and production hygiene in meat pie production.

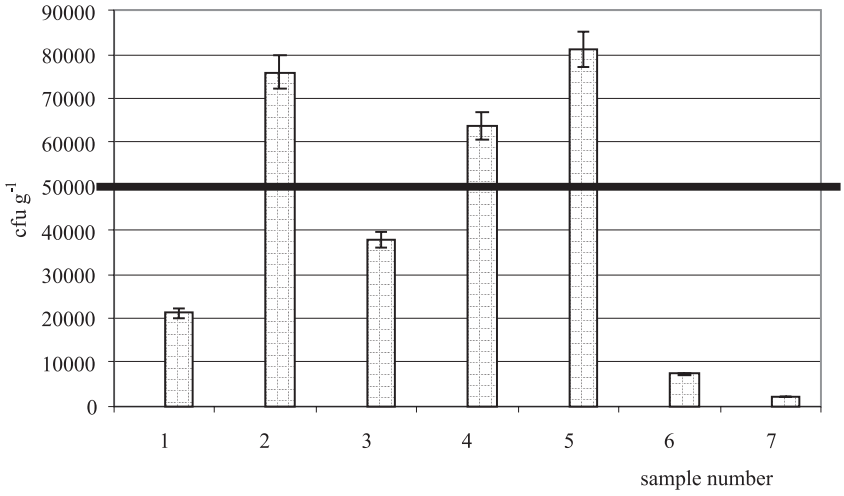


Fig. 2. Total plate count in the fillings used for the studied confectionery product preparation: 1 – curd filling prior to the filler device; 2 – curd filling after the filler device; 3 – spicy filling prior to the filler; 4 – spicy filling after the filler; 5 – filling for meat pies; 6 –filling for apple pies, 7 – boiled cream filling

The results of total plate count assay during frozen storage and subsequently baked products are presented in Table 1.

Table 1

Total plate count changes in frozen and baked products during storage

Sample no.	Total plate count, cfu g^{-1}							
	immediately after freezing		after 1 month		after 2 months		after 3 months	
	frozen	baked	frozen	baked	frozen	baked	frozen	baked
1M*	160967	6183	5360	5130	2765	2145	0	0
2M	181500	2703	34600	6025	1455	5840	441.5	0
3M	152913	3213	12100	5210	1735	4045	52.5	343
4M	123233	3413	9825	2176	4300	2585	290.5	0
5M	131767	3250	6380	2410	985	2620	81.5	0

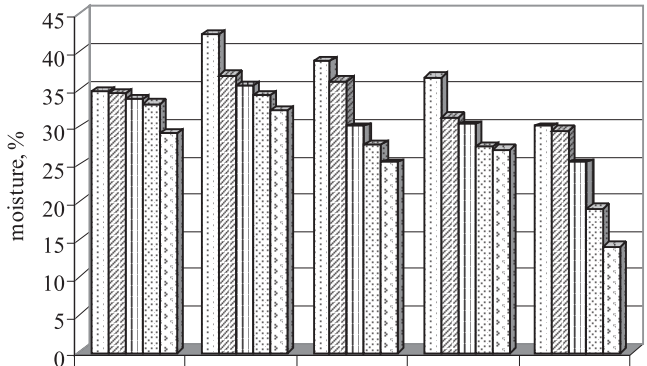
* 1M – Spicy pies, 2M – Meat pies, 3M – Bun with a boiled cream filling "Labrit", 4M – Pastry with curd, 5M – Pastry with apple filling.

Total plate count in all samples immediately after freezing exceeded the permissible level (50000 cfu g^{-1}) approximately 2.4–3.6 times, while after baking none of the samples had total plate count exceeding the limit (Table 1). The highest total plate count immediately after freezing was found in spicy pies (160967±23291 cfu g^{-1}) and meat pies (181500±1384 cfu g^{-1}), which is closely related to the above described results demonstrating that the highest contamination was observed in meat fillings especially (Fig. 2). The microbiological assay shows that the producers have to pay more attention to hygiene of equipment and environment in order to avoid recontamination of product.

After a month of confectionery product storage at 18±2°C in polypropylene (PP) bags total plate count was within permissible limits and after four months storage the microbial contamination was not detected in the studied samples. It allows to draw a conclusion that a part of microorganisms is inactivated in the baking process and after four month storage at 18±2°C their activity is stopped, therefore this temperature is the optimum temperature for frozen confectionery product storage.

During the storage of confectionery products the presence of *Coli* forming bacteria was observed. Analysis of fillings used for confectionery product preparation showed that only apple filling was free of *Coli* forming bacteria. Immediately after freezing some products was still contaminated with *Coli* forming bacteria namely in meat pies it reached 6947 cfu g^{-1}, but in pastry with curd filling - 113 cfu g^{-1}. While after a month storage *Coli* forming bacteria was not detected in any sample.

In the study moisture changes in confectionery products were evaluated during four month frozen storage, the results are presented in Figure 3.



	meat pie	bun with cream	pastry with apple	pastry with curd	spicy pie
0	34.6	42.44	38.78	36.6	30.1
1	34.41	36.8	36.18	31.29	29.5
2	33.7	35.5	30.05	30.29	25.27
3	33.2	34.2	27.69	27.32	19.24
4	29.13	32.1	25.23	26.98	14.11

Fig. 3. Moisture content changes in ready-to-bake products during four month frozen storage

The highest moisture loss (15.99%) was observed during the storage of partially baked spicy products, while the smallest moisture loss was found in meat pies. The differences in moisture loss could be explained by various properties and structure of the fillings. The fillings having higher moisture content might easier release the moisture during the storage, thus giving higher moisture reduction.

Confectionery products baked after two month frozen storage despite the observed moisture los still had acceptable sensory attributes – appearance and taste. After 3 and 4 months storage darker spots were formed on the surface of baked products. These spots might be formed due to ice crystal presence on the product surface, which after defrosting and in the beginning of baking melted giving more wet place, which resulted in appearance defect after baking. As a result the conclusion can be made that frozen confectionery products can be stored in PP bags up to 2 months, because during this time no significant changes happen to sensory properties of the baked product.

Conclusions

Total plate counts in fillings after the filler device and in meat pie filling exceeded the permissible limit about 1.3–1.6 times. The result demonstrates that the producers should pay more attention to hygiene of production equipment, because it might be a source of microbiological contamination.

Part of microorganisms is inactivated in the baking process and after four month storage at 18±2°C their activity is stopped, therefore this temperature is the optimum temperature for frozen confectionery product storage.

Frozen confectionery products can be stored in PP bags up to 2 months, because during this time no significant changes happen to sensory properties of the baked product.

References

Lainez E., Vergara F., Barcenas M.E., 2008. Quality and microbial stability of partially baked bread during refrigerated storage. J. Food Eng., 89, 414–418.

Kulp K., Joseph G., 2000. Handbook of cereal science and technology. Second Ed., New York, Basel: Marcel Dekker, pp. 790.

Irwin A.T., Paul R., 1998. Food storage stability. CRC Press LLC, 539.

Baik M.Y., Chinachoti P., 2000. Moisture redistribution and phase transitions during bread staling. Cereal Chem., 77, 484–488.

Hug-Iten S., Escher F., Conde-Petit B., 2003 Staling of bread: role of amylase and amilopectin and influence of starch-degrading enzymes. Cereal Chem., 80 (6), 654–661.

Kulp K., Lorenz K., Brümmer J., 1998. Frozen and refrigerated doughs and batters. St. Paul, Am. Assoc. Cereal Chemists, 280.

Mackey A.O., Jones P., Dunn J., 1952. The effect of ingredient variations on the quality of white and chocolate batters baked prior to freezing or baked from frozen batters. Food Res. 17, 216–224.

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PACKAGING OF HALF-FINISHED FRESH VEGETABLE PRODUCTS

Introduction

The competence in food preparation technology is the necessary prerequisite for innovative food and service development, but the knowledge of food safety assurance empowers a food service manager to validate the technological processes from the viewpoint of food safety. The successful development in the catering sector includes an innovative development of food and services. Food quality and safety issues depend on the quality of raw stuffs and half-finished products.

Quality of fresh vegetables is influenced by several factors:

– internal factors (chemical content of food products, active acidity pH of the medium, water activity a_w, contamination level of raw material with microorganisms);
– external factors (pre-treatment and processing technologies, storage temperature, type and material of packaging, atmosphere in the package).

Good quality is one of the main problems of every vegetable storage stage. The important problem is to describe cooling of vegetables by air ventilation. Using basic laws of physics, we obtain the process mathematical model. Natural losses characterize losses of moisture which unfavourably influence the storage ability of live organisms. If duration of cooling vegetables is shorter, there are less losses of mass in production, but larger air expenditure is necessary which in its turn causes additional expenses [Aboltins et al., 2007].

Investigations show that storage in the place of growing decreases losses for 18% in average, but by using combination of natural and artificial cold the economic effect is much higher. Temperature is an essential factor in decreasing intensity of breathing. However, taking into consideration characteristics of product being cooled, cooling will be inefficient.

The most significant factor, characterising where and what food products consumer buys, is the safety and quality of the product which is provided by adequate packaging.

Packaging by use of modified atmosphere medium – MAP or vacuum is considered as potential methods in storage of food products without using preservatives [Hotchkiiss, 1999].

Investigations confirm that vegetables are very sensitive to changes of microclimate parameters; their high deterioration is related to the great evaporation ability, continuous transfer of physiological heat and high reaction against moisture losses.

Materials and Methods

Four different types of vegetable salad mixes from the farm "Ezerkaulini" are used in the research:

- red cabbage salad mix;
- kale salad mix;
- lettuce salad mix;
- cauliflower-celery salad mix.

Fresh vegetable salad mixes prepared with the aim to estimate their quality and storage possibilities in the commercial network where their storage temperature changes from +2 to +6°C, with the shelf-life of 7 days.

Mixes are packed in a polypropylene box 190×140×50 mm, surrounded by a polypropylene bag of the size 260x190 mm, thickness 38 micrometers, applying gas mix: O_2 10% : CO_2 10% : N_2 80%. Polypropylene packaging is mechanically more durable and safeguards the packaged product against mechanical deformation, as well as its thermo stability from -30°C to +140...+160°C insures warming up of ready-made packed foodstuff for nutrition without removing it from the package. Net weight of the package is 300±0.5g. All salad mixes are stored for 11 days at two different temperatures +2±1°C and +6±1°C, experiments carried out in three repetitions.

The following items are determined during storage:

1. Mass changes by using electronic scales *ACCULAB IV-600*;
2. Changes in active acidity pH level of the medium by using *INOLAB SELECTA pH 720 pH-meter*;
3. Determination of breathing intensity influence on the composition of protective gasses in the package during storage by using the analyzer OXYBABY ®V O_2/CO_2;
4. Colour changes by using the "ColorTecPCM/PSM" equipment in colour system CIE L* a*b*;
5. Content of ascorbic acid (vitamin C) in salads (mg l00g $^{-1}$) is determined by iodine method (T-138-15-01:2002);
6. The total number of micro-organisms is determined according to *LVS ISO 4833:2003* with the method of dilution and sowings on Petri dishes

Research is carried out at the research laboratories of Microbiology and Packaging material qualities at the Faculty of Food Technology, Latvia University of Agriculture.

We consider that the production has been lodged in a pile with its height *H (m)* and it is ventilated through air channels from the bottom. During ventilation there are 4 unknown functions within the layer of production: vegetable temperature $\Theta(x,t)$ and moisture $W(x,t)$, temperature of the ventilated air $T(x,t)$ and the moisture of this air in the layer $d(x,t)$.

For the mathematical modelling of the process partial differential equation system (1)-(4) was used relating vegetable temperature $\Theta(x,t)$ and moisture $W(x,t)$ to ventilated air temperature $T(x,t)$ and moisture $d(x,t)$ in the layer during ventilation (*Aboltins et al., 1997*.

$$\frac{\partial W}{\partial t} = k\left(W_p - W\right) \qquad t > 0,\ x > 0 \tag{1}$$

$$\frac{\partial d}{\partial t} + a_1 \frac{\partial d}{\partial x} = \frac{k}{a_2}\left(W - W_p\right) \qquad t > 0,\ x > 0 \tag{2}$$

$$\frac{\partial \Theta}{\partial t} = c_1 (T - \Theta) + c_2 (W_p - W) \qquad t > 0,\ x > 0 \tag{3}$$

$$\frac{\partial T}{\partial t} + a_1 \frac{\partial T}{\partial x} = c_0 (\Theta - T) \qquad t > 0,\ x > 0 \tag{4}$$

Thermal capacity coefficient for vegetables c_d was chosen in the form

$$c_d = 4.19 - 0.028 \cdot n_s, \tag{5}$$

where: n_s – amount of dry matter (%).

Results and disscussion

Transfer characteristics dependence of moisture and temperature, hidden condensation/evaporation heat, nonlinearity of sorption isotherm, emergence of heat and moisture during breathing process is some of the factors, which make problems of moisture and heat transfer mutually nonlinear and therefore cannot be solved analytically.

Coherence is obtained in the temperature range -2…+5°C characterising dependence of dew-point temperature T_r on air relative moisture φ (d.b.) and temperature T (°C) [Aboltins et al., 2007].

$T_r = (13.1 + T) \cdot \varphi - 13.1$

Emergence of moisture drop-type phase on the surface of production should be prevented because it promotes microbiological processes which cause deterioration of production.

In order to eliminate mass losses and preserve vegetables 2 steps should be observed:

– vegetables should be harvested, handled to storehouses and cooled fast,
– heat from vegetables in the breathing process should be deferred during storage.

The 1st step (pre-cooling) should be carried out very fast, because every delayed hour causes mass losses of production decreasing its nutritional value. Using the cooled air with the temperature 0°C for ventilation and assuming that production temperature should drop to 6°C, losses of potato and carrot mass Δm (%) are presented depending on the initial and final temperature of production difference $\Delta\Theta$ (°C) and cooling time (Fig. 1 and 2).

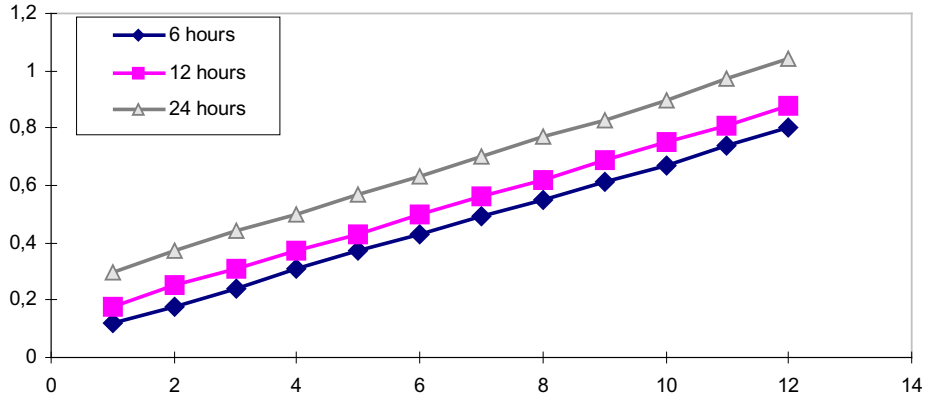

Fig.1. Potato mass losses Δm, cooling them to 6°C at the temperature difference ΔΘ

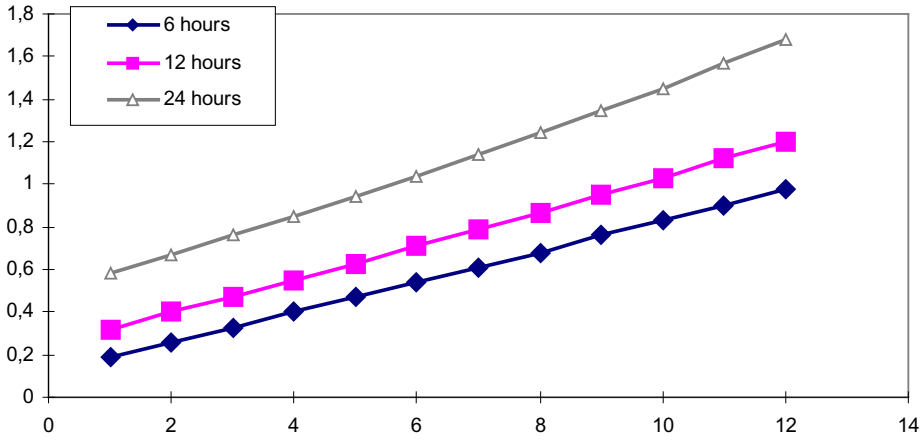

Fig. 2. Carrot mass losses Δm, cooling them to 6°C at the temperature difference ΔΘ

The other range of the issue is to find out production mass losses during the 2nd stage, when the vegetable temperature has to be decreased to 0°C. It should be emphasized that the evaporation heat amount, particularly of carrots and beets, depends very much on the store-house temperature (Fig. 3).

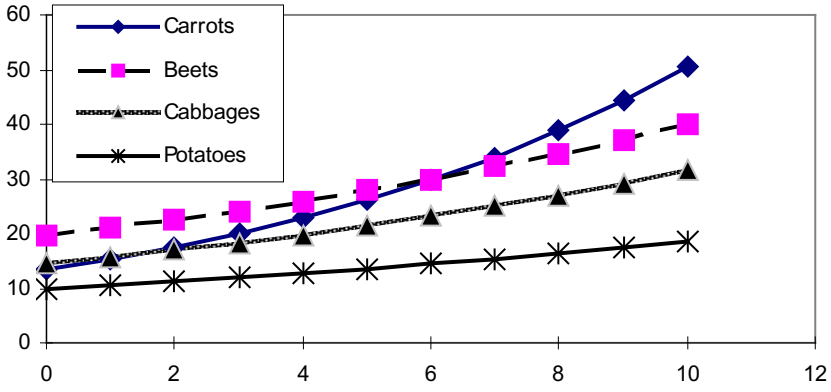

Fig. 3. Dependence of vegetable breathing heat *q (W/t)* on their temperature *T* (°C)

Taking into consideration that ventilation takes place in winter with air temperature -10°C during three ventilation periods: 15 min., 30 min. and 1 h, depending on temperature difference coherence among mass losses of carrots is obtained (Fig. 4). It is very similar with potatoes.

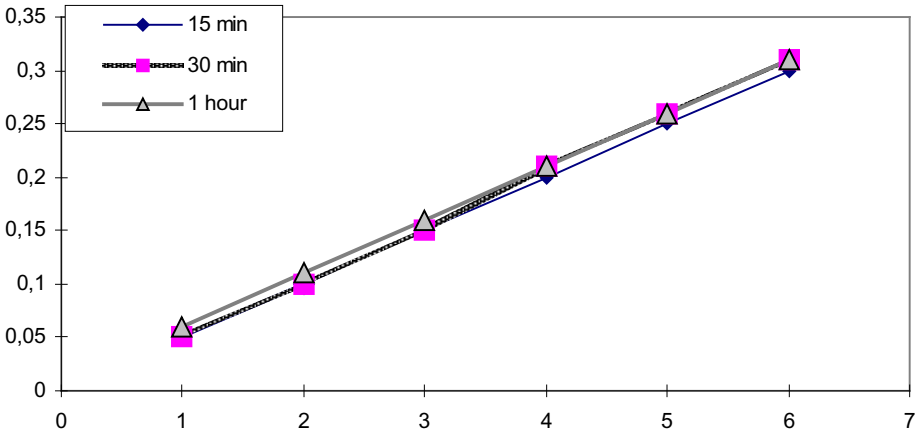

Fig. 4. Carrot mass losses Δm, cooling them to 0°C at the temperature difference ΔΘ

Evaluating the technological process of making salad according to the requirements of HACCP system principles, as the possible stages in emergence of potential risk reasons turn out to be pre-treatment of vegetables, their storage before and after packaging and the very procedure of packaging. In all these stages of technological process the most important is the microbiological risk reason, but in the process of vegetable pre-treatment also the physical risk reason.

In order to prevent emergence of potential risk reasons, principles of good hygiene practice and principles of good production practice have to be strictly observed. Therefore attention was paid to the quality of drinking water used in the pre-treatment process, air pollution in the cooling chamber of the product, harmlessness of the packaging material and the very process of packaging.

As the storage process of packaged vegetables is one of the most important, changes of physical and microbiological quality indices are determined just in this stage of technological.

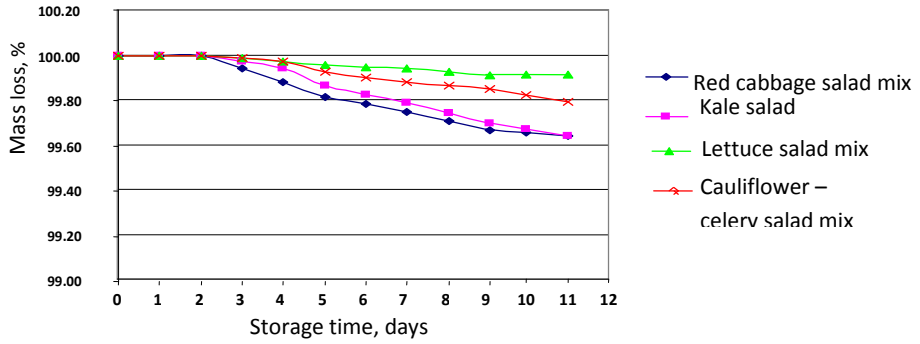

Fig. 5. Changes of mass in salads during storage at the temperature 2±1°C process

Water vapour migration through the chosen packaging material is insignificant; during storage it provides slight mass loss. The packaged mass almost does not change till the third day of storage, mass loss ranges between 0.01–0.03% in comparison with the initial gross weight of the package.

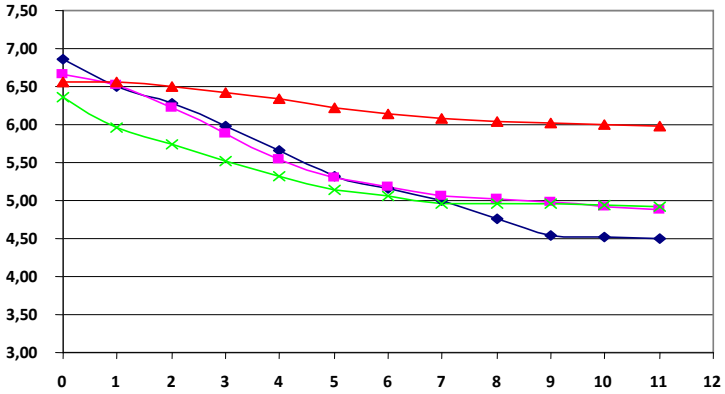

Fig. 6. Changes in pH level during storage at the temperature 2±1°C

The active acidity pH of vegetable cell sap essentially affects micro-organisms cell metabolism because bio-catalytic agents – enzymes of metabolism have pH interval of certain activity. In sour medium, characteristic to the prepared salad mixes, cell sap prevents development of proteolytic bacteria, but facilitates development of yeast and mould. Changes in active acidity pH indicate the development of micro-organisms and activity in the package during storage.

By carrying out research it was ascertained that aerobic spore formers – bacteria of *Bacillus* family, were dominating. (These bacteria split organic compounds. The presence of *E. coli* form bacteria was not found in any of the prepared salad samples. The total number of bacteria starts to increase much faster after 24 hour long storage and during storage increases twice [Blija et al., 2008].

On the basis of worked out functional analysis of storage model, it is possible to optimize regulation possibilities of heat-moisture processes for a particular kind of production. Taking into consideration ensuring of heat transfer from the product, it will be possible to carry out successful storage of vegetables. It enables to perfect technological process of storage. Optimized and experimentally verified model will help to explain and predict physical processes in vegetables during storage.

Environmental temperature has an essential impact on number of products bacteria packed in modified medium because by increasing the temperature solubility of carbon dioxide decreases in the liquid stage of products wherewith it does not have so strong abilities to suppress micro-organisms.

Quality of products in modified atmosphere packaging is significantly influenced by temperature. The preferable storage temperature is in the range between 0 ± 1°C and +4 ± 1°C.

The natural freshness of vegetables and storage period are influenced by characteristic properties of the product as well as external factors. Thus the external factors influencing quality are the following: temperature; sanitary and hygienic conditions; gas atmosphere; production methods. The factors mentioned have crucial significance during the production and storage of the product.

On the basis of worked out functional analysis of storage model, it is possible to optimize regulation possibilities of heat-moisture processes for a particular kind of production. Taking into consideration ensuring of heat transfer from the product, it will be possible to carry out successful storage of vegetables. It enables to perfect technological process of storage. Optimized and experimentally verified model will help to explain and predict physical processes in vegetables during storage.

Conclusions

The packing used provides minimal mass losses. Optimized and experimentally verified model will help to explain and predict physical processes in vegetables during storage. Numerical quantity of pH decreases during storage what ascertains the fact that splitting carbohydrates takes place and organic acids are formed. The packaging material used for lettuce salad mix is suitable for storing salad up to 3 days because during storage changes in content of protective gasses O_2 are minimal, as well as changes in total sum of micro-organisms taking place after 72 h storage period are admissible: 10 – 6.2% and CO_2 10 – 21.6%. The total number of bacteria increases 2.2 times, when salads are stored for 72 hours at the temperature 2°C, but 3.2 times, if stored at 6°C.

References

Aboltins A., Skrupskis I., Iljins U., 2000. Influence of technological factors on maintenance of fruit and vegetable production. 21st Century Together With Science and Practice. Reports. Jelgava, Latvia, 60–66.

Blija A., Skudra G., Skrupskis I., Kozule V., Skrupska D., 2008. Half-finished vegetable products of high readiness. 3rd Baltic Conf. Food Science and Technology FOODBALT 2008 Jelgava, Latvia, 20–24.

Hotchkiiss J.H., 1999. Modified atmosphere food packaging of poultry and related products: Controlled / Modified Atmosphere vacuum Packaging of Food, 349.

Zerihun Desta T., Van Brecht A., Meyers J., Baelmans M., Berckmans D., 2003. CFD model validation for a ventilated installation. Acta Horticulturae, 599, 405–411.

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FATTY ACIDS AND CHOLESTEROL PROFILE IN EGG YOLK FROM LAYING HENS HOUSED IN ECOLOGICAL CONDITIONS

Introduction

The organic agriculture is growing in popularity [Yussefi, 2006], its production systems are consequently included to farming [Dabbert et al., 2001; Śmiechowska, 2002; Żakowska-Biemas, 2003] that is why consumers have positive judgement of its products and associate them with health [Zhao et al., 2007] and environmental benefits [Sołtysiak, 1995; Stawicka, 2004]. The designed egg production technology has been further improved and several bioactive compounds are dietary incorporated into eggs (Rudnicka and Dobrzański 2000; Surai and Sparks, 2001). However the ecological eggs are noticed to have similar [Cherian et al., 2002] or higher [Hidalgo et al., 2008] amounts of saturated fats in comparison to eggs from other production systems.

Eggs are one of primary sources of cholesterol in the diet of high developed societies, and its amount is developing on the level of 200–250 mg in yolk. Since contents of cholesterol are a basic characteristics of the dietary quality of eggs, this issue is controversial. Possibilities of lowering the cholesterol amount in eggs on the dietary is limited. In addition to that enrichment eggs into n-3 acids sometimes causes the increase in the cholesterol amount about 5–10%. Many research focused on the possibility to reduce cholesterol level and to change fatty acid contents of egg yolk [Ayerza and Coates, 2000; Grobas et al., 2001; Milinsk et al., 2003; Bragagnolo and Rodriguez-Amaya, 2003]. But decrease yolk cholesterol by genetic selection or by feeding modification seems to be still very difficult.

This study was undertaken to compare the egg components and yolk fatty acids composition of ecologically and commercially available laying hen eggs.

Materials and methods

Eggs of lying hens of the native race of "Zielononóżka kuropatwiana" (Greenleg Pertrage) ecologically housed were used in the experiment. The hens of two experimental groups were kept in free-range system and fed at least twice a day with the mixture containing: Group I – whole grain of wheat and maize, wheat bran, fish meal (under 2%), mineral premix (0,5%), limestone and as a complement of the diet a carrot, beetroot and red beet were given; Group II – wheat, lupine, forage salt, limestone, lucerne and also potatoes, white beet and beetroot. The control group was the eggs from hens of cross-breeding Lohmann Brown line housed on the litter. Birds were fed *ad libitum* with standard diet containing maize, wheat,

barley, soya-extracted meal, sunflower meal, limestone, triticale, rape meal, plant fats, dicalcium phosphate, forage salt, Met-Lys and other organic and mineral compounds.

The egg yolk was analyzed on fatty acids and cholesterol profile. Lipid extraction was performed according to Folch et al. [1957]. Fatty acids composition was determined by the gas chromatography method combined with mass spectroscopy GC/MS by Agilent Technologies 6890N SC System. Cholesterol content was determined by high performance liquid chromatography HPLC (Agilent 1100, column C18, λ=210).

The obtained results were elaborate with statistical analysis methods (Statistica ver. 7.0) with significance accepted at $p \leq 0.05$.

Results and discussion

The results of the quality research of eggs are presented in Table 1. The weight of whole eggs was significantly lower than those of LB layers. Also significant differences were observed in percentage of structural components of eggs (yolk and albumen) in all groups under investigation. The content of yolk in eggs laid by Greenleg Partrige were much higher as compared to that from Lohmann Brown hens. Albumen share was significantly lower in experimental eggs. It has been reported that diet, hen age, strain and other environmental factors influence the size and composition of the eggs [Schneideler et al., 1998]. Haugh units in case of fresh material differ in one experimental group, but it could be brought about inappropriate storage conditions. The colour of yolk in both GP groups was relatively light. Better value, from consumer's point of view, was observed in LB eggs.

Table 2 shows the composition of fatty acids, which is one of the important factors of nutritional value of egg yolk lipids. His distinctive feature is a relatively low share of saturated fatty acids which the high contests of monounsaturated and polyunsaturated acids is accompanying [Trziszka, 2000]. The content of lipids and fatty acids found in eggs can be modified genetically, but the essential influence has the age, housing and feeding of laying hens (especially of polyunsaturated fatty acids PUFA). The amount of fatty acids depending on the cross-breeding of laying hens is only changing slightly [Codony et al., 1995]. However in own studies the influence of the genetic differ among native race (Greenleg Pertrage) and the Lohmann Brown.

In the present examination the highest content of saturated fatty acids (SFA) (38.3%) was determined for eggs of Greenleg Pertrage housed in ecological conditions (Table 2). For two other groups the SFA content was tend at level of 35,7%. The higher SFA content in the yolk of the experimental group I results in lower share of monounsaturated fatty acids (MUFA), while in the control group it exceeds the 50% of all fatty acids. Higher percentage of stearic and palmitic acids reflected in higher percentage of saturated fat in organic eggs [Samman et al., 2009]. High content of SFA in eggs from GP layers confirm the study by Trziszka et al. [2004], however the storage decreased the contents of saturated fatty acids (especially palmitic acid), but increased the amount of unsaturated fatty acids (especially oleic acid).

Sum of contents of polyunsaturated fatty acids PUFA (n-6 + n-3) in yolk lipids of Greenleg Pertrage is higher (15.3%) than for the control group (13.4%). It causes an increase PUFA/SFA relationship from about 0.37 for hens held on the litter (Lohmann Brown), to 0.40 for

Greenleg from group I and 0.43 for the group II (Table 2). The ratio PUFA/SFA depends on changes in layers feeding and for commercial eggs should reach 0.32 [Codony et al., 1995).

The so-called perfect diet is characterized by a relation of n-6 to n-3 acids on more than 5:1 level, while in the diet of modern societies consuming 'fast food' products this level reaches 25:1. In commercial eggs the n-6/n-3 relationship is varying from 1:15 into 1:20 [Farell, 1995], and isn't so beneficial like in fat fishes (sardine, menhaden, halibut). It is possible to change it relatively easily with the hens feeding. Supplementation of linseed and fish oil in quail diets affected a significant increase of n-3 PUFA in yolk lipids (2.88%) compare to the control (0.67%) [Kaźmierska et al., 2007]. It is the most important, from the nutritional point of view, to determine the n-6/n-3 PUFA ratio. This, according to the British Nutrition Foundation (1992) should rated between 4:1 and 6:1. Results collected in the study showed that ratio n-6/n-3 PUFA in fresh enriched quail eggs equaled 4:1. Kaźmierska et al. (2005) already proved that free range quails produce eggs with low ratio between n-6/n-3 PUFA (5.6) and Koreleski et al. (1998) confirmed the possibility of modifying the ratio n-6/n-3 fatty acids families through supplementation of different sources of PUFA to quail diet, to the satisfactory low level i.e. 4.0. In own examinations for the control group (Lohmann) the value of n-6:n-3 was indicated on the level about 19:1, however in the yolk of eggs of Greenleg hens a relationship was found as 7:1 and 10:1. It shows beneficial effect of the hens diet of native races on composition of fatty acids.

α-linolenic acid ALA (18:3) and docosaheksaenoic acid DHA (22:6) are most biologically valuable n-3 acids [Codony et al., 1995; Farrell 1995]. Higher levels of ALA and DHA in omega-3 eggs contributed to a higher n-3 fats content and n-3:n-6 ratio [Gao and Charter 2000; Drymel 2002; Yannakopoulos et al. 2005; Samman et al., 2009]. In own examinations the content of ALA acid was indicated on the level of the 0.23% in the control and reaches 0.6–0.9% in the yolk of Greenleg hens, i.e. 2-3-times higher. It probably partially could result from feeding rich in this acid, but also was caused by free-range housing system.

Content of DHA acid in yolk lipids of eggs of Greenleg was about twice higher that in the control group (table 2). It can be stated that one egg of Greenleg hen is inserting about 1/3 requirement of this acid in the human diet, taking into consideration the proposal to eat at least 150 mg of DHA.

Presented studies showed that the genetic diversity and the system of the breeding did not influence the content of cholesterol. In eggs coming from hens households with environmental methods and in the control group 13 mg/g of cholesterol was determined, in addition a slightly higher concentration was noted for group II (about 0,5 mg/g) (table 3). There was found 219 mg of cholesterol in 1 egg yolk of Greenleg, while for control group it was 206 mg. It should be underline that higher amount of cholesterol results mainly from a large share of yolk in these eggs. This results were confirmed in the supplementation studies on quails [Kaźmierska et al., 2007], where cholesterol content in eggs from all groups was similar. Moreover, cholesterol level analysed in the experimental material was comparable to data published by Bragagnolo and Rodriguez-Amaya [2003], which found 12 mg cholesterol per g of egg yolk. Milink et al. (2003) reported that omega-3 oils addition to chicken diet does not change the cholesterol content on egg yolk. Decreasing yolk cholesterol by genetic selection or feeding modification is difficult to achieve, but some papers indicated such trends [Trziszka et al. 2004; Kaźmierska et al. 2005; Yannakopoulos et al., 2005].

Conclusions

Yolk lipids of eggs of Greenleg hens breed in ecological conditions have the higher nutritional value than those from commercial breeds because of the higher amount of n-3 acids, especially α-linoleic fatty acid (ALA) and docosahexaenoic fatty acid (DHA) and moreover they have lower value of the relationship of n-6/n-3 acids (7:1- 10:1) compared to control (19:1). It is possible to regard eggs from native breeds as functional food.

The content of cholesterol in the yolk of eggs from the ecological production is similar to commercial eggs.

Table 1

Physical parameters of fresh eggs

Parameters	Control group	Group I	Group II
Egg weight [g]	65,8 ^{c}	54,9 ^{a}	57,8 ^{b}
Yolk [%]	24,6 ^{a}	30,0 ^{b}	28,6 ^{b}
Albumen [%]	63,0 ^{b}	58,1 ^{a}	59,4 ^{a}
Shell [%]	12,4 ^{a}	11,9 ^{a}	12,0 ^{a}
Haugh units	84 ^{b}	77 ^{a}	87 ^{b}
Yolk colour (Roche scale)	13 ^{c}	8 ^{b}	6 ^{a}

Table 2

Profile of fatty acids in ecological (Greenleg Partrige) and conventional (Lohmann Brown) eggs

Fatty acid		Control group	Group I	Group II
Myristic acid	C14:0	0,29	0,33	0,31
Pentadecanoic acid	C15:0	0,04	0,05	0,07
Palmitic acid	C16:0	26,94	28,16	26,30
Heptadecanoic acid	C17:0	0,16	0,20	0,21
Stearic acid	C18:0	8,41	9,60	8,85
Palmitooleic acid	C16:1	3,21	3,33	2,82
Oleic acid	C18:1	46,81	43,60	45,32
Eicosenoic acid	C20:1	0,19	0,20	0,08
Linoleic acid (n-6)	C18:2	11,28	12,54	12,39
Linoleic acid (n-3)	C18:3	0,23	0,86	0,58
Eicozatrienoic acid (n-6)	C20:3	0,04	–	0,10
Arachidonic acid (n-6)	C20:4	1,44	0,99	1,55
Docosahexaenoic acid (n-3)	C22:6	0,43	0,95	0,76
SFA		35,84	38,34	35,74
MUFA		50,21	47,13	48,22
PUFA		13,42	15,34	15,38
PUFA/SFA		0,37	0,40	0,43
n6/n3 ratio		19,3	7,5	10,5

Table 3

Concentration of cholesterol in ecological (Greenleg Partrige) and conventional (Lohmann Brown) eggs

Cholesterol content	Control group	Group I	Group II
mg/1g of yolk	12,7	12,8	13,2
mg/1 egg yolk	205,7	212,0	218,6

References

Ayerza R., Coates W., 2000. Dietary levels of chia: Influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. Poultry Sci., 79, 724–739.

Bragagnolo N., Rodriguez-Amaya D.B., 2003. Comparison of the cholesterol content of Brazilian chicken and quail eggs. J. Food Comp. Anal., 16, 147–153.

Cherian G., Holsonbake T.B., Goeger M.P., 2002. Fatty acid composition and egg components of speciality eggs. Poultry Sci., 81, 30–33.

Codony R., Barroeta A., Grobas S., 1995. Fatty acids and cholesterol: recent improvements in egg nutritional value. Proceed. VI Europ. Symp. Quality Eggs and Egg Products. WPSA. Zaragoza, Spain, 361–373.

Dabbert S., Zanoli R., Lampkin N., 2001. Elements of a European Action Plan for Organic Farming. European Conference: Organic Food and Farming, May 2001, Copenhagen, Denmark.

Drymel W., 2002. Wpływ czynników genetycznych, żywieniowych i środowiskowych na kształtowanie jakości i przydatności technologicznej surowca jajczarskiego. Praca doktorska. Wydział Nauk o Żywności AR we Wrocławiu.

Farrell D. J., 1995. The problems and practicalities of producing an omega (n)-3 fortified egg. Proceed. VI Europ. Symp. Quality Eggs and Egg Products. WPSA. Zaragoza, Spain, 351–360.

Folch J., Lees M. Stanley S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497–509.

Gao Y.C., Charter E.A., 2000. Nutritionally important fatty acids in hen egg yolks from different sources. Poultry Sci., 79 (6), 921–924.

Hidalgo A., Rossi M., Clerici F., Ratti S., 2008. A market study on the quality characteristics of eggs from different housing systems. Food Chem., 106, 1031–1038.

Kaźmierska M., Jarosz B., Korzeniowska M., Trziszka T., Dobrzański Z., 2005. Comparative analysis of fatty acid profile and cholesterol content of egg yolks of different bird species. Pol. J. Food Nutr. Sci., 14/55, SI 1, 69–73.

Kaźmierska M., Korzeniowska M., Trziszka T., Jarosz B., 2007. The effect of fodder enrichment with PUFAs on quail eggs. Pol. J. Food Nutr. Sci., Vol. 57, No. 4(B), pp. 281–284.

Koreleski J., Kuchta M., Ryś R., Sieradzka A., 1998. Comparison of the influence of rapeseed oil and fish fat in laying hen nutrition on the level of polyunsaturated fatty acids in egg yolk. Rocz. Nauk. Zoot., 25, 91–102.

Milink M. C., Marakamou A. E., Gomes S. T. M., Matshusita M., de Souza N. E., 2003. Fatty acid profile of egg yolk lipids from hens fed chicks rich in n-3 fatty acids. Food Chem., 83, 287–292.

Rudnicka A., Dobrzański Z., 2000. The effect of humic and falty preparations on productivity and egg quality of molted Lohmann Brown hens. Proc. X Congr. ISAM, Maastricht, The Netherlands, 2–6 July 2000, 247–251.

Samman S., Kung F. P., Carter L.M., Foster M.J., Ahmad Z.I., Phuyal J.L., Petocz P., 2009. Fatty acid composition on certified organic, conventional and omega-3 eggs. Food Chem. 116, 911–914.

Schneideler S.E., Jaroni D., Froning G., 1998. Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. Poultry Sci., 77, 192–196.

Sołtysiak U., 1995: O kryteriach w rolnictwie ekologicznym, w: Sołtysiak U. (red.): Rolnictwo ekologiczne. Od producenta do konsumenta. Stowarzyszenie EKOLAND, Stifung, Leben&Umwelt, Warszawa, 11–22.

Stawicka J., Szymczak-Piątek M., Wieczorek J., 2004. Wybrane zagadnienia ekologiczne, Wyd. SGGW, Warszawa.

Śmiechowska M., 2002. Standaryzacja produkcji żywności w polskich gospodarstwach organicznych, w: Ślawska E. (red.): Jakość żywności a rolnictwo ekologiczne. Wydawnictwo Naukowe PTTŻ, Kraków.

Trziszka T., 2000. Jajczarstwo. Nauka Technologia Praktyka, Wydawnictwo Akademii Rolniczej we Wrocławiu.

Trziszka T., Dobrzański Z., Oziembłowski M., Jarmoluk A., Krasnowska G., 2004. An attempt to compare the quality of chicken eggs from cage system and ecological production. Arch. Geflügelk. 68 (6), 269–274.

Yannakopoulos A., Tserveni-Gousi A., Christaki E., 2005. Enchanced Egg Production In Practice: The Case of Bio-Omega-3 Egg. Intern. J. Poultry Sci., 4 (8), 531–535.

Yussefi M., 2006. Organic farming worldwide 2006. Overview and main statistics. In Willer H., Yussefi M. (Eds.), The world of organic agriculture statistics and emerging trends. Berlin: International Federation of Organic Agricultural Movements.

Zhao X., Chambers E., Matta Z., Loughin T.M., Carey E.E., 2007. Consumer sensory analysis of organically and conventionally grown vegetables. J. Food Sci., 72, 87–91.

Żakowska-Biemas S., Gutkowska K., 2003. Rynek żywności ekologicznej w Polsce i w krajach Unii Europejskiej. Wydawnictwo SGGW, Warszawa.

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