SELECTED PROBLEMS
OF
NUTRACEUTICAL
AND
FUNCTIONAL FOOD

edited by
Tadeusz Trziszka,
Łukasz Bobak,
Małgorzata Kaźmierska

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Wrocław 2011

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PREFACE

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

The term "nutraceuticals" is a combination of two words "nutrition" and "pharmaceutical". It was first used in 1989 by S. De Felice – chairman of the American Foundation for Innovation in Medicine, whose primary aim was to accelerate medical discovery by creating a more productive clinical research community. The term was quickly accepted in many biomedical studies. By definition nutraceuticals are substances that may be considered as food or food compounds beneficial for human health and helping prevent diseases of civilisation. Nutraceuticals are not drugs but rather food products. They contain biologically active substances that may strengthen, weaken, or modify physiological and metabolic functions of the human body and at the same time may play a positive role in the prevention of many chronic and lifestyle diseases.

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

Taking the above data into account, a multiple author monograph was written in 15 subchapters and the following two chapters:

– Chapter 1. Bioactive substances for human health
– Chapter 2. Quality and safety in functional food production

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

Tadeusz Trziszka Łukasz Bobak Małgorzata Kaźmierska

Wrocław, 2011

CHAPTER 1

BIOACTIVE SUBSTANCES FOR HUMAN HEALTH

1

EVALUATION OF IMMUNOGLOBULIN AND LYSOZYME CONCENTRATION IN MILK ENRICHED WITH CAROTENOIDS

Introduction

There has been increasing interest in recent years in using feed management to control the composition of the fat-soluble micronutrients, carotenoids and vitamin E, in their role as antioxidants or carotenoids, and in their role as vitamin A precursors that directly influence the nutritional quality of the end products [Calderon 2007].

Since animals cannot synthesize carotenoids and animal feed is generally poor in carotenoids, about 30–120 ppm of total carotenoids, was added to animal feed to improve animal health, enhance meat color and quality, and increase vitamin A levels in milk and meat. [Ananda, Vadlani 2010]. Carotenoids can become one of the most popular feed enriching sources used for cows, which are also able to influence the sensory characteristics of products, not only directly by conferring a yellow colour, but also indirectly via their antioxidant properties [Barrefors et al. 1995]. Carotenoids are involved in the nutritional and sensory characteristics of dairy products, and are potential biomarkers for traceability of cow feeding management. [Noziere et al. 2006]. Moreover, together with Vitamin E and polyphenols, carotenoids are natural antioxidants in ruminant diets. They play a role in cell communication and immune function by protecting cells against free radical attack [van den Berg et al. 2000]. It has been demonstrated that carotenoids and retinol are able to reduce mastitis in dairy cows [Chew 1995], although the effect of β-carotene was not systematic [Folman et al. 1987, Oldham et al. 1991]. Carotenoids also have a positive role in fertility independent of the role of retinol [Hurley, Doane 1989]. In addition to their role in cow health, higher carotenoids concentrations in milk contribute to an improvement in the nutritional value of dairy products and, possible, higher concentration of antimicrobial proteins.

Despite these various roles of carotenoids in cows' feed and increasing immunity of cows, little attention has been paid to their influence on concentration of antimicrobial proteins in milk. The major antimicrobial proteins in milk are lysozyme, lactoferrin (Lf), lactoperoxidase (LP), and immunoglobulins [Mullan 2010].

Immunoglobulins (Igs), together with lysozyme, lactoferrin and lactoperoxidase form the very important antimicrobial system of bovine lacteal secretions. Igs are antibodies that are synthesized by mammals in response to antigenic or immunogenic stimuli such as bacteria and viruses, and thus provide protection against microbial infections.

Bovine serum and lacteal secretions contain three major classes of Igs: IgG, IgM and IgA. The bovine IgG molecule occurs predominantly in two subclasses: IgG1 and IgG2. The concentration of the various bovine Igs in serum and in lacteal secretions varies according to the breed, age, health status, and stage of lactation of the animal [Butler

1994, Larson 1992, McFadden et al. 1997). In colostrum, Igs make up 70±80% of the total protein content, whereas in mature milk, Igs account for only 1±2 % of the protein [Larson 1992].

The immunological function mediated by the Igs depends on the Ig class. IgG antibodies have a multitude of functions, the most important of which is possibly the activation of complement-mediated bacteriolytic reactions. Another vital function of Igs is their ability to augment the recognition and phagocytosis of bacteria by leucocytes (opsonisation). Igs also are able to prevent the adhesion of microbes to surfaces, inhibit bacterial metabolism, agglutinate bacteria, and neutralise toxins and viruses. IgM antibodies, although produced in smaller amounts than IgG, are considerably more efficient than IgG with regard to most of the above activities, especially complementmediated lysis. IgA, in contrast, does not fix complement or opposing bacteria, but agglutinates antigens, neutralises viruses and bacterial toxins, and prevents the adhesion of enteropathogenic bacteria to mucosal epithelial cells. [Zinkernagel et al. 1972, Hilpert et al. 1987].

Lysozyme is present in secretions such as saliva, egg white, milk and blood. Egg white lysozyme and human milk lysozyme are similar proteins [Farkey 2002]. Lysozyme cleaves the glycosidic bond between N-acetylmuramic acid and N-acetyglucosamine in bacterial peptidoglycans, which constitute the major part of the bacterial cell wall of gram-positive bacteria [Mullan 2010].

Lysozyme may have an antibacterial role in milk, serum and avian eggs. It has also been suggested that lysozyme may have an indirect effect on the defence systems of hosts as an immunomodulator through the stimulation of the immune system by break down products from the hydrolysis of peptidoglycan [Mullan 2010]. This hypothesis has been supported by the findings that feeding infants lysozyme-enriched formulas results in an increased level of secretory IgA in faeces [Lodinové, Jouja 1976].

As reviewed before, there are many factors that have effect on the concentration of immunoglobulins and lysozyme in milk. But there is luck of information about feed enriched with carotenoids influence on immunoglobulins and lysozyme concentration in milk, therefore the **aim** of the present study was to evaluate influence of feed with different content of carotenoids on lysozyme and the immunoglobulin concentrations in milk.

Materials and methods

27 bulk milk samples were analyzed. The samples were collected from afternoon milking. Each cows' group milk samples were immediately cooled to 4–8°C and transported to the laboratory, arriving at a temperature not exceeding 8°C.

Milk samples were obtained from conventional dairy herd in Latvia. Individual milk samples were taken from 15 healthy cows that were selected and divided into 3 groups. There were 2 experimental and 1 control groups of 5 cows in each. Stage of lactation (for all cows that was the beginning of lactation – 1st–3d Month), cow breed (Holstein, Latvian Brown and crossed) and lactation number (e.g. 1.–5.) were as similar as possible in all groups.

The basic feed (see Tab. 1) was equal of all groups, e.g. silage was fed to ad libitum and rapeseed animal meal – 2 kg per cow per day. The characterization of feed enriched with carotenoids in analysed groups' is given in a Table 1.

Table 1

The characterisation of feed

Characteristics of feed		Groups		
		Control (1st group)	Experimental (2nd group)	Experimental (3rd group)
Feed, per cow per day		silage – to ad libitum, rapeseeds animal meal – 2 kg		
Source of carotenoids, 100g per cow per day		rapeseeds oil	rapeseeds oil + carrots*	red palm oil "Carotino"
Content of carotenoids, mg per cow per day	β-carotene	207.0	1090.0	225.0
	α-carotene	–	221.0	22.0
	lycopene	–	–	0.8
	Sum	225.0	1325.0	275.0

*7 kg per cow per day

The extraction of total lipids was performed by the method of Hara and Radin [Hara, Radin 1978]. Carotenoid concentrations in feed were determined by HPLC using the technique consisted in a Waters Alliance 2695 HPLC with photodiode array detector monitoring between 280 and 600 nm, using a 150 x 4.6 mm, RP C18 column and Empower Pro softwear. The flow rate was 2 mL per min. and the mobile phase consisted of acetonitrile (70%), methanol-acetate ammonium 50 mM (15%), dichloromethane (10%) and water (5%). Concentration of carotenoids was calculated by using external standards (purity>95%) (Sigma-Aldrich).

The concentrations of immunoglobulins (IgA, IgG, IgM) and lysozyme were determined by turbodimetric method [Грант 1973]. The somatic cell count was determined by "Somacount 300" to exclude the possibility to analyse milk obtained from mastitis cows; in all milk samples the somatic cell count was till 400 000 ml^{-1}.

Parameters were detected for three duplications; the mean value of parameters was calculated. Descriptitive statistics were carried out to determine the differences of IgA, IgG, IgM and lysozyme concentration in different milk samples by Microsoft Windows for SPSS software packages.

The concentrations of immunoglobulins and lysozyme were determined in all groups according the following scheme (see Tab. 2).

Table 2

Sampling scheme

Samplings		
1st sampling	2nd sampling	3rd sampling
no additional supplements were administered to the herd for two week period prior to the sampling	35 days after start of feed supplementation with carotenoids	one week after the interruption of feed supplementation with carotenoids

Results and discussion

As was mentioned previously, the concentration of immunoglobulins and lysozyme in milk is influenced by a variety of factors, including feed, age, health status and stage of lactation.

Carotenoids, as well as feed enriched with carotenoids, influence cow's immune function [van den Berg et al. 2000], as result the concentration of immunoglobulins and lysozyme in milk can be affected.

The concentration of **IgG** in the experiment beginning in all analysed groups was significantly different ($p<0,05$). 35 days after start of feed supplementation with carotenoids the concentration of IgG in all groups, excluding the 3rd group, decreased (Fig. 1). Research results can be explained with period (April), when milk samples were obtained. According to the data from the literature [Heck et al. 2009], the protein content, and, as result, the concentration of IgG independently from with or without feed supplementation with carotenoids decreased. One week after the interruption of feed supplementation with carotenoids slightly higher concentration of IgG was in the 2nd and 3rd groups – 0,50±0,05 g l^{-1}, but significant difference among the analysed groups was not established. It means that feed enriched with carotenoids doesn't influence IgG concentration in milk. In all analysed samples the concentration of IgG was according to the data from literature – 0,15–0,80 g l^{-1} [Marnila 2002].

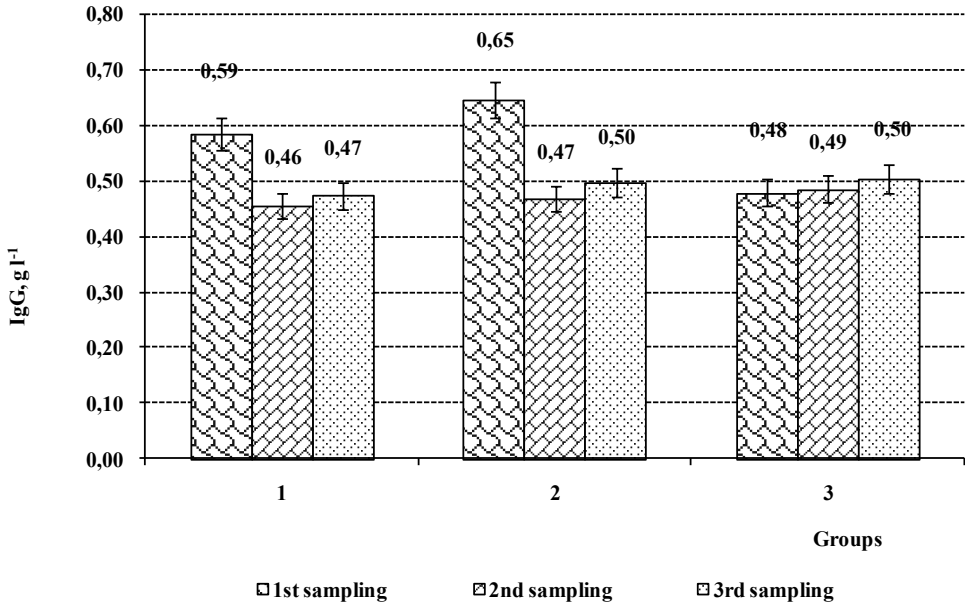

Fig. 1. The dynamics of the IgG concentrations in milk

In the beginning of the experiment the concentration of **IgA** in analysed groups was similar. Slightly higher concentration was in the 1st and 2nd groups, it was 0,14±0,001 g l^{-1}, then follow the 3rd group – 0,13±0,001 g l^{-1} (Fig. 2). In the beginning of the experiment in all analysed groups IgA concentration was according to the data from literature – 0,13 g l^{-1} [McFadden 1997]. 35 days after the start of feed supplementation with carotenoids the concentration of IgA in all groups slightly decreased. The explanation can be the same as in the case with IgG concentration. In this period (April), when milk samples were obtained, the protein content, and as result the concentration of IgA, was decreased, accordingly to the literature data [Heck et al. 2009].\

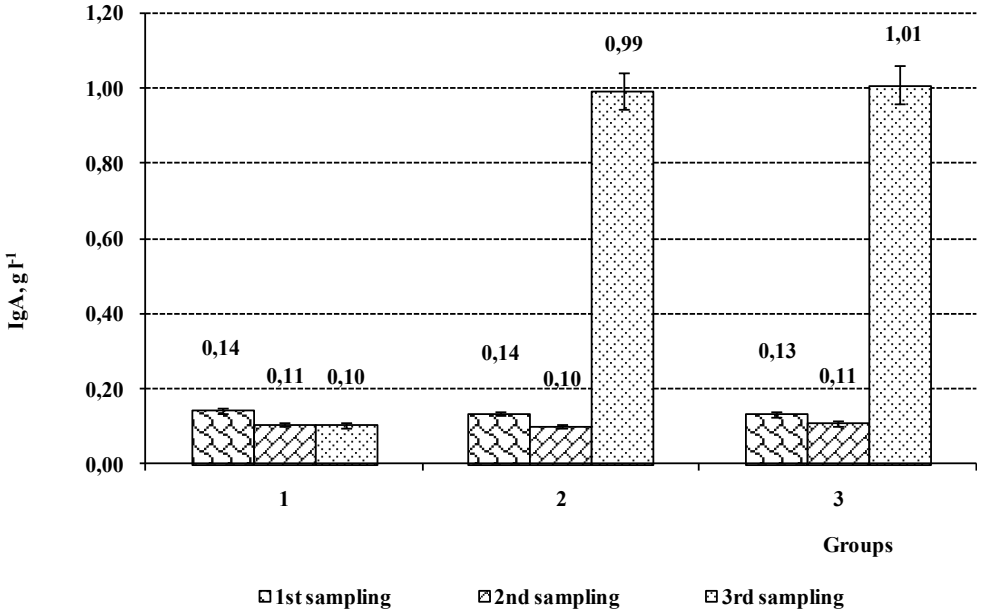

Fig. 2. The dynamics of the IgA concentrations in milk

One week after the interruption of feed enrichment with carotenoids the highest concentration of IgA was in the 3rd and 2nd groups – accordingly 1,01±0,10 g $l^{-1}$and 0,99±0,09 g l^{-1}, significantly lower ($p<0,05$) concentration of IgA was in the 1st group – 0,10±0,01 g l^{-1}. Compared with the experimental groups in control group it continued to decrease and the concentration of IgA was 10 times lower, than in the 2nd and 3rd groups. At the same time, in the experimental groups the concentration of IgA increased and was significantly higher ($p<0,05$), in comparison with the data from literature; the possible explanation is feed enrichment with carotenoids. There was no significant difference established in IgA concentration increase dependence on source of enriched feed.

In the beginning of the experiment the concentration of **IgM** in analysed groups was different. Slightly higher concentration was in the 3rd – 1,38±0,01 g l^{-1}, then follow the 1st group – 1,37±0,02 g l^{-1}, but the lowest IgM concentration was in the 3rd group – 1,35±0,02 g l^{-1} (Fig. 3). 35 days after the start of feed supplementation with carotenoids the concentration of IgM in all groups increased, significant difference among groups was not established.

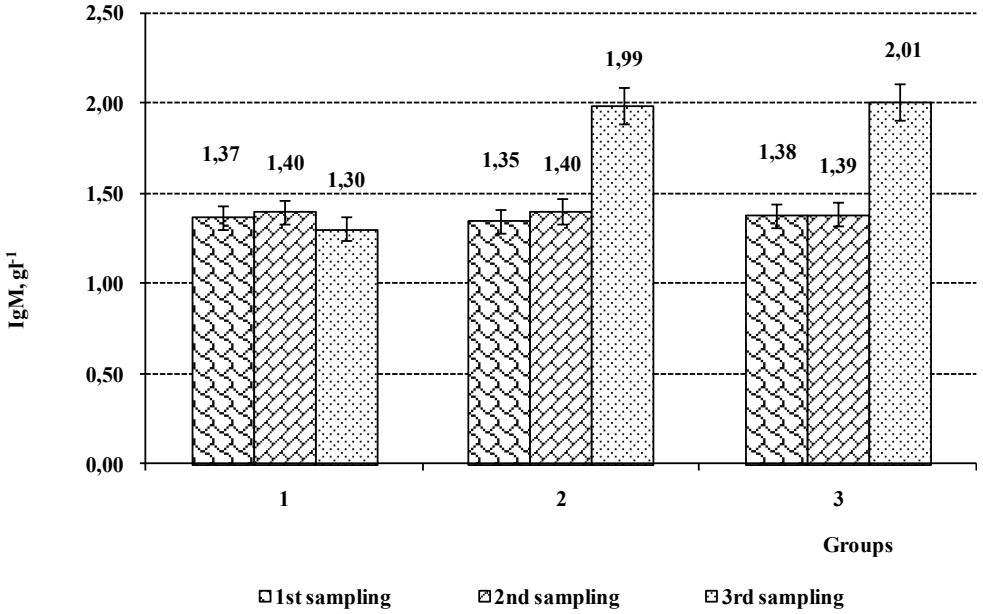

Fig. 3. The dynamics of the IgM concentrations in milk

One week after the interruption of feed enrichment with carotenoids the highest concentration of IgM was in the 3rd and 2nd groups, accordingly 2,01±0,02 g $l^{-1}$and 1,99±0,02 g l^{-1}. Significantly lower IgM concentration was determined in the 1st group – 1,30±0,01 g l^{-1}. In the 2nd and 3rd groups the concentration of IgG increased accordingly for 0,59 g $l^{-1}$and 0,62 g l^{-1}; the possible explanation is supplying the feed enriched with carotenoids.

The concentration of IgM in all analysed milk samples was significantly higher in comparison with the data from literature 0,04–1,00 g l^{-1} [Marnila 2002].

Increased concentration of immunoglobulins in milk due to supplying feed enriched with carotenoids could be evaluated as positive, because in past years the interest for cows' immunization with aim to maximize antibodies concentration in milk has increased [Korhonen et al. 2000].

In the beginning of the experiment the concentration of **lysozyme** in analysed groups was significantly different ($p<0.05$); the highest concentration was in the 1st group – 0,62±0,06 mg l^{-1}, then follow the 2nd group – 0,41±0,04 mg l^{-1}, significantly lower lysozyme concentration was in the 3rd group – 0,11±0,01 mg l^{-1} (Fig. 4).

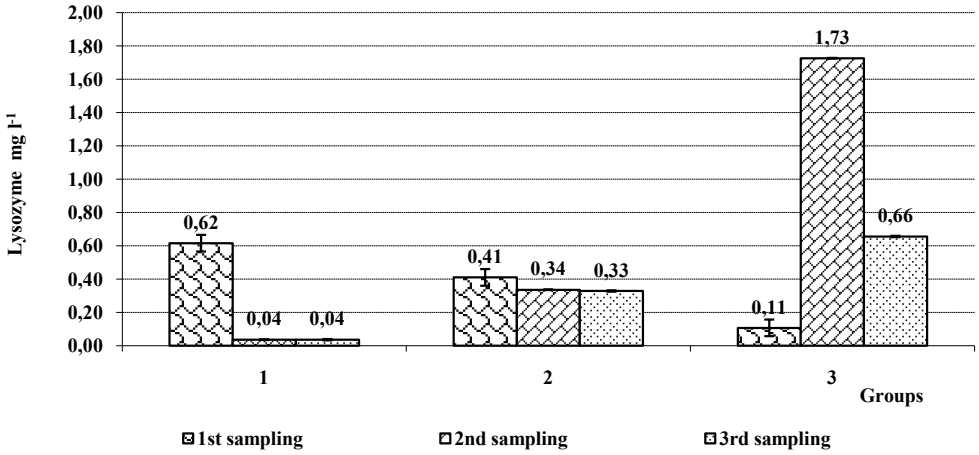

Fig. 4. The dynamics of the lysozyme concentrations in milk

35 days after the start of feed supplementation with carotenoids the concentration of lysozyme in all groups was very different: in the 1st group it significantly decreased ($p<0.05$), but in the 2nd group decrease was not significant ($p>0.05$), at the same time in the 3rd group significant increase was established. It could be connected with composition of carotenoids in red palm oil, where, besides α and β carotenes, such strong antioxidant as lycopene is included.

One week after the interruption of feed supplementation with carotenoids the results were contradictory: the highest concentration of lysozyme was in the 3rd group, accordingly 0,66±0,07 mg l^{-1}, then follow the 2nd – 0,33±0,03 mg l^{-1}. Significantly lower lysozyme concentration was determined in the 1st group -0,04±0,003 mg l^{-1} ($p<0.005$).

Research results show, that in the 2nd group the feed enriched with carotenoids have effect on lysozyme concentration in milk; compared with the 1st – control group, significant decrease of lysozyme was not established (see Fig. 4). Significant, but not extended effect was in the 3rd group – just after the interruption of feed supplementation with carotenoids, the concentration of lysozyme decreased. Significantly higher concentration of lysozyme seven days after the interruption of feed enrichment was established in both experimental groups.

During the experiment in all analysed groups the concentration of lysozyme (mg l^{-1}) was according to the data from literature 0–3,00 mg l^{-1} [Walstra et al. 1999].

Research shows different results. 35 days after feed supplementation in case of regarding immunoglobulins the concentration of IgA, IgG and IgM in the 2nd and 3rd groups was not significantly different from the control group, but significantly higher concentration of IgA and IgM was established one week after the interruption of feed enrichment with carotenoids. In this case the composition of carotenoids had not significant influence on immunoglobulin concentration in milk.

In case of lysozyme, 35 days after the start of feed enrichment with carotenoids and one week after interruption the concentration of lysozyme in the 2nd and 3rd groups was significantly different from the control group.

Conclusions

1. Feed enriched with carotenoids has accumulative effect on IgA and IgM concentrations in milk. The significant increase of IgA and IgM concentration was established one week after the interruption of feed supplementation with carotenoids. There was no significant difference established in IgA and IgM concentration dependence on source of enriched feed.
2. In both experimental groups 35 days after the start of feed supplementation with carotenoids and one week after the interruption of feed enrichment, concentration of lysozyme was significantly higher as in control group.
3. Feed enriched with carotenoids used in the 2nd and 3rd groups differently influences lysozyme concentration in milk, in the 3rd group it was significantly higher ($p<0.005$). It could be connected with enriched feed composition – a strong antioxidant – lycopene was presented in the 3rd groups feed.

References

Ananda N., Vadlani P.V., 2010. Production and optimization of carotenoid-enriched dried distiller's grains with solubles by Phaffia rhodozyma and Sporobolomyces roseus fermentation of whole stillage. Journal of Industrial Microbiology and Biotechnology, nr 11, 1183–1192.

Barrefors P., Granelli K., Appelqvist L.-A., Bjoerck L., 1995. Chemical Characterization of raw milk samples with and without oxidative off-flavor. Journal of Dairy Science, nr 78, 2691–2699.

Butler J.E., 1994. Passive immunity and immunoglobulin diversity. Indigenous Antimicrobial Agents of Milk – Recent Developments. IDF Special Issue 9404, nr 4, 14–50.

Calderon F., Chauveau-Duriot B., Pradel P., Martin B., Graulet B., Doreau M., Noziere P., 2007. Variations in Carotinoids, Vitamins A and E, and Color in Cow's Plasmaa and Milk Following a Shift from Hay Diet to Diets Containing Increasing Levels of Carotenoids and Vitamin E. Journal of Dairy Science, nr 12, 5651–5664.

Chew B.P., 1995. Antioxidant vitamins affect food animal immunity and health. Journal of Nutrition, nr 125, 1804S–1808S.

Farkey N.Y., 2002. Other Enzymes. Encyclopedia of Dairy Sciences ed. H. Roginski, J.W. Fuquay, P.F. Fox. Academic Press, Amsterdam, 946–947.

Folman Y., Ascarelli I., Kraus D., Barash H., 1987. Adverse effect of beta-carotene in diet on fertility of dairy cows. Journal of Dairy Science, nr 70, 357–366.

Hara A., Radin N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent, Analytical Biochemistry, nr 90, 420–426.

Heck J.M., Valenberg H.J.F., Dijkstra J., Hooijdonk A.C.M., 2009. Seasonal variation in the dutch bovine raw milk composition. Journal of Dairy Science, nr 10, 4745–4755.

Hilpert H., Brussow H., Mietens C., Sidoti J., Lerner L., Werchau H., 1987. Use of bovine milk concentrate containing antibody to rotavirus to treat gastroenteritis in infants. Journal of Infection Diseases, nr 156, 158–166.

Hurley W.L., Doane R.M., 1989. Recent developments in the roles of vitamins and minerals in reproduction. Journal of Dairy Science, nr 72, 784–804.

Korhonen H., Marnila P., Gill H.S., 2000. Bovine milk antibodies for health. British Journal of Nutrition, nr 1, S135–S146.
Larson B.L., 1992. Immunoglobulins of the mammary secretions (ed. by P. Fox) Advanced Dairy Chemistry 1 – Proteins. – London: Elsevier Science Publishers, 231–254.
Lodinové R., Jouja V., 1976. Antibody production of the mammary gland in mothers after oral colonizatin of their infants with E.coli 083. Paediatrica, nr 10, 878.
Marnila P., Korhonen H., 2002. Immunoglobulins. Encyclopedia of Dairy Sciences ed. H. Roginski, J.W. Fuquay, P. F. Fox. Academic Press, Amsterdam, 1950–1956.
McFadden T.B., Besser T.E., Barrington G.M., 1997. Regulation of Immunoglobulin Transfer into Mammary Secretion of Ruminants. Milk Composition, Production and Biotechnology. ed. by Welch ... [et al.]. CAB International, New Zealand, 133–151.
Mullan W.M.A., 2010. Major antimicrobial proteins in milk – Lactoperoxidase system. [Online]. Available from: http://www.dairyscience.info/exploitation-of-anti-microbial-proteins/52-antimicrobial-proteins.html?start=3 . Accessed: 11 August, 2010.
Noziere P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M., 2006. Carotenoids for ruminants: From forages to dairy products. Animal Feed Science and Technology, nr 131, 418–450.
Oldham E.R., Eberthart R.J., Muller L.D., 1991. Effects of Feed Enriched with Carotenoidsal Vitamin A or Beta-carotene During the Dry Period and Early Lactation on Udder Health. Journal of Dairy Science, nr 74, 3775–3781.
van den Berg G.J, Faulks R., Fernando Granado H., Hirschberg J., Olmedilla B., Sandmann G., Southon S, Stahl W., 2000. The Potential for the Improvement of Carotenoids Levels in Foods and Animal Models. European Journal Clinic Nutrition, nr 3, S32–S37.
Walstra P., Geurts T.J., Noomen A., Jellema A., van Boekel M.A.J.S., 1999. Dairy Technology. Principlies of Milk Properties and Processing. Marcel Dekker, New York, 709–727.
Zinkernagel R.M., Hilper H., Gerber H., 1972. The digestion of colostral bovine immunoglobulins in infants. Experientia, nr 28, 741.
Грант Х., 1973. Турбодиметрический способ количественного опредиление лизоцима с применением спектрофотометра СФУА, Лабораторное дело. (Turbodimetric method for determination of lysozyme by means of spectrophotometer SFUA, A Laboratory manual), Х. Грант, Л.И. Яворковский, И.А. Блумберга (ред.), 300–304 (In Russian).

2

THE CONTENT OF ANTIOXIDANT COMPOUNDS IN THE FRUIT OF SELECTED BERRY SPECIES FROM ORGANIC AND CONVENTIONAL PRODUCTION SYSTEMS

Introduction

For a long time there has been a big interest in products rich in bioactive substances, which through their numerous functions in the human body influence the health improvement. Among the plant products, berry fruit stand out in terms of their abundance of plant antioxidants. They are an excellent source of vitamin C, phenolic acids and flavonoids, among which special attention should be paid to highly concentrated anthocyanins. Flavonoids, as natural antioxidants, play a positive role in preventing cardiovascular diseases, since they effectively counteract the LDL oxidation [Borowska 2003]. By inhibiting phosphodiesterase and cyclooxygenase activities, they reduce platelet aggregation more effectively than aspirin, which is crucial in the prevention of atherosclerosis [Szajdek and Borowska 2004]. Together with vitamin C, flavonoids are involved in creating cross-linkages between polypeptide chains of collagen fibres, thereby strengthening the blood vessels. They also exhibit anti-tumour activity, involving the ability to capture free radicals and neutralize damaged cells caused by free radicals and molecular oxygen and peroxides, which supports the fact that these compounds should be supplied in the diet every day [Czeczot 2000]. Particularly noteworthy is a group of flavonoid compounds, which are the anthocyanins. In fruit, they are located in the outer layers of hypoderm, and in cells they are present in vacuoles in the form of pellets. The studies conducted in southern France showed 5-fold lower mortality from heart diseases among people living in that area, because of the increased consumption of fruit and vegetables rich in flavonoids, especially in anthocyanins [Szajdek and Borowska 2004]. Anthocyanins are also characterized by antibacterial effect, so that they effectively fight bacteria, such as E. Coli, which is the main cause of gastro-intestinal disorders. These compounds also have anti-inflammatory properties, therefore blackcurrant drinks soothe sore throats. Anthocyanins present in fruit also have a beneficial effect on the capillaries of eyes and accelerate the regeneration of rhodopsin which improves the ability to see. It was found that anthocyanins also increase the flexibility of blood vessels [Szustakowska-Chojnacka 2007].

Thanks to antioxidant properties, phenolic acids exert antiatherosclerotic, antitumour and antibacterial activities. They eliminate reactive oxygen species, sweep away free radicals, and cause the chelation of metal ions and the inhibition of enzymes from the group of oxidases. Thanks to the aforementioned, the human body is protected against oxidative stress and the development of lifestyle diseases. In relation to ferulic, caffeic and chlorogenic acids there has been demonstrated the ability to reduce and deactivate mutagenic and carcinogenic substances, such as aflatoxin B_1. On the other hand, gallic and caffeic acids prevent creating of mutagenic nitrosamines [Klusek et al. 2004, Surma-Zadora and Cieślik 2007].

Numerous scientific reports confirm that organic fruit and vegetables contain more antioxidant compounds compared to the crops from conventional farming, which testifies to their higher biological value. Thus, one can assume that regular consumption of organic products is one way to reduce the incidence of certain diseases and improve general health status of the body.

As a result of mineral fertilizing, in the conventional crops plant yielding rises along with the increase in the amount of water in plant cells, resulting in the decrease in dry matter content in raw materials [Kunachowicz et al. 1993, Rembiałkowska 2000, Szkatulska 1997]. As with the increase of dry matter there also grows the nutrient content, it can be assumed that organic farming is a way to preserve and enhance the nutritional value of fruit and vegetables. In conventional agriculture, with increasing yields, levels of vitamins and minerals often fall and this phenomenon is referred to as 'dilution effect' [Benbrook 2005].

Furthermore, the prohibition on the use of synthetic fertilizers and pesticides in organic production favours the start of plants' own defence systems against pathogens, involving among others the production of secondary metabolites, which include phenolic compounds that are also valuable natural antioxidants. The production of these compounds by the plants is also affected by the nitrogen content in soil. In the soil with a lower availability of nitrogen, which occurs in organic system where nitrogen is supplied to the soil as organic fertilizers, the plants produce primarily carbon-containing compounds, including simple and complex sugars and plant secondary metabolites. However, in the environment where the availability of nitrogen is greater, as in conventional production by the use of nitrogen fertilizers, which are readily soluble in water, the plants produce mainly nitrogenous compounds such as amino acids, proteins, and alkaloids [Bourne and Prescott 2002, Brandt and Mølgaard 2001].

Despite numerous studies conducted by scientists around the world, the knowledge about differences in the content of biologically active compounds in raw materials from organic and conventional farming is not sufficient. Hence, there is the great need for further research on the content of these compounds in different plant raw materials.

The aim of this paper was to compare the contents of antioxidant compounds, such as vitamin C, flavonoids, anthocyanins and phenolic acids in selected species of berry fruit from organic and conventional production.

Research material and methodics

The experiment was carried out in 2010, in the Laboratory of the Chair of Organic Food of the Warsaw University of Life Sciences (SGGW).

The studies included four species of berry plants (raspberry, blackberry, blueberry and wild strawberry). The selection of research material was based on their high health value and popularity among consumers. The raw material was represented by the fruit harvested at the usable ripeness phase, from certified organic plantations and from conventional plantations. Organic fruit were purchased in organic food stores, while the conventional ones were bought in the stores that sell conventional fruit.

The volume of laboratory samples was 1 kg. There were determined: dry matter content by gravimetric method under PN-R-04013:1988, the content of vitamin C by titration method according to PN-A-75101–11:1990 and the content of phenolic compounds by high performance liquid chromatography (HPLC) with the identification of phenolic compounds according to standards by Fluca and Sigma Aldrich companies (own method). In order to avoid losses in the content of biologically active compounds in the plant material, immediately after harvesting the fruit were frozen at -80^{o}C, and then subjected to freeze-drying, followed by analytical studies.

The statistical analysis of the results was performed using the computer program STATGRAPHICS 5.1. For the calculations there was used two-factor variance analysis, with the application of Tukey's test. The level of significance of the obtained results was 95% (α = 0.05), which means that the practical alpha (p-value) must take a value below α = 0.05, so that the difference is statistically significant.

Among the factors studied, there was the cultivation method (organic and conventional) and fruit species. The value of p-value coefficient is given in figures and tables. If the calculated coefficient was not statistically significant, the result of statistical analysis was determined as n.s. (not statistically significant). In addition, there were calculated standard deviations (s.d.) for the sample tested. Each analysis was performed in triplicate. Percentage differences showing the changes in the tested ingredient content of the plant material were calculated under the Worthington's formula [2001]: [(org – conv)/conv x 100%].

Results and discussion

In the berries studied there were no differences in dry matter content between the raw materials from organic and conventional production (Tab. 1). The results correspond to the results obtained by Kazimierczak et al. [2008] concerning the black currant fruit from organic and conventional crops. However, higher dry matter content in the organic raw materials compared to the conventional ones was obtained in the studies on onion [Hallmann and Rembiałkowska 2006], apples [Rembiałkowska et al. 2004], red beets [Sikora et al. 2008] and fresh carrot juice [Sikora et al. 2009].

Table 1

The content of dry matter and vitamin C in the selected fruit from organic and conventional production systems

Cultivation method	Fruit species	Dry matter g 100 g^{-1} of product	Vitamin C mg 100 g^{-1} of product
organic	blueberry	13.87	31.91
	wild strawberry	18.22	36.86
	raspberry	12.02	38.67
	blackberry	14.14	34.56
	mean*	**14.56±2.38**	**35.50±2.69**
conventional	blueberry	15.09	20.86
	wild strawberry	17.61	62.60
	raspberry	13.68	38.84
	blackberry	11.25	33.04
	mean	**14.41±2.43**	**38.84±15.86**
org/conv difference **		+1.0	-8.6
p-value			
cultivation		n.s.	<0.0001
species		<0.0001	<0.0001
cultivation x species		<0.0001	<0.0001

* mean ± standard deviation

** calculated according to the formula [(org-conv)/conv]*100% under Worthington [2001]

Source: Own studies

As a result of own research, there was found a significant effect of the cultivation method on the content of vitamin C in the fruit tested. Organic fruit contained lower level of the vitamin than conventional berries (Tab. 1). Contradictory results, in favour of organic raw materials, were obtained in the studies on three varieties of black currant [Kazimierczak et al. 2008], onion [Hallmann and Rembiałkowska 2006], pepper and tomato [Hallmann et al. 2006], potatoes [Rembiałkowska 2000] and beet roots [Sikora 2008]. However, similar as own research, the studies on carrot [Rembiałkowska and Hallmann 2007], and carrot and green peas [Bourne and Prescott 2002] from different cultivation systems gave the results in favour of conventional raw materials.

In interpreting the findings cited, it must be borne in mind that different levels of vitamin C in the analysed products may result from several factors influencing the content of this compound in plants. Vitamin C is unstable antioxidant, sensitive to elevated temperature and light, and thus the conditions of climate and weather, storage and the manner of product processing [Ziemlański 2002].

Berries from organic farming produced more phenolic acids compared with conventional fruit, and the fruit of highbush blueberry were the richest in these compounds. Similar results were obtained in terms of total flavonoids and anthocyanins. Richer in these compounds have proved to be organic berries. In relation to other species, wild strawberries included significantly more flavonoids, while the highest content of anthocyanins was characteristic for blueberry fruit (Tab. 2).

Table 2

The content of total phenolic acids, flavonoids and anthocyanins in the fruit of selected berry species from organic and conventional production systems

Cultivation method	Fruit species	Phenolic acids	Flavonoids	Anthocyanins
		mg 100 g^{-1} of product		
organic	blueberry	60.72	7.96	590.94
	wild strawberry	3.03	13.94	42.73
	raspberry	9.86	6.36	101.67
	blackberry	3.62	9.89	435.46
	mean*	**19.31±25.15**	**9.54±2.97**	**292.70±238.41**
conventional	blueberry	54.96	6.86	348.18
	wild strawberry	2.64	10.63	85.20
	raspberry	4.55	11.04	64.52
	blackberry	1.21	8.03	296.56
	mean*	**15.84±23.63**	**9.14±1.83**	**198.62±131.33**
org/conv difference **		**+21.9**	**+4.4**	**+47.4**
p-value				
cultivation		<0.0001	0,0019	<0.0001
species		<0.0001	<0.0001	<0.0001
cultivation x species		0.0002	<0.0001	<0.0001

* mean ± standard deviation
** calculated according to the formula [(org-conv)/conv]*100% under Worthington [2001]
Source: Own studies

The results of various raw materials from different production systems, including black currant and its products [Kazimierczak et al. 2008], peaches [Carbonaro et al. 2002], grapes

[Levite et al. 2000], onions [Hallmann and Rembiałkowska 2006], tomatoes [Rembiałkowska et al. 2003] and red beets [Sikora et al. 2008] confirmed the higher content of phenolic compounds in organic raw materials compared to conventional ones. In addition, Kazimierczak et al. [2008], while examining different varieties of black currant from organic and conventional production systems, confirmed the impact of organic cultivation system on the accumulation of anthocyanins in berries.

In order to determine which of the berries have the most extensive and diverse composition the studied plant material was subjected to qualitative analysis. The qualitative analysis of flavonoid compounds helped to identify the following substances: rutin, quercetin, myricetin, kaempferol, D-quercetin glycoside, D-kaempferol glycoside, luteolin and apigenin. The largest number of detected flavonoids was typical for wild strawberry and highbush blueberry, which included six of the eight identified compounds (Fig. 1).

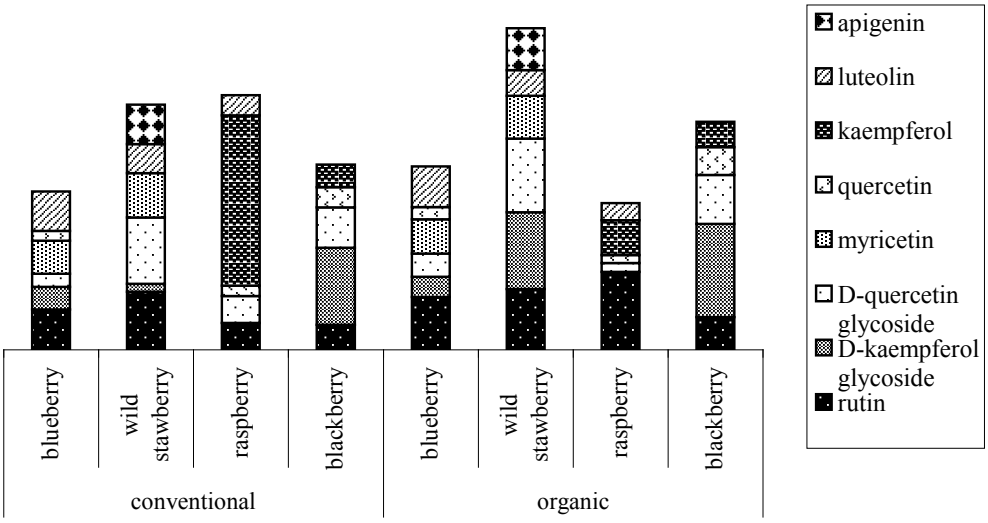

Fig. 1. The qualitative analysis of flavonoids determined in organic and conventional berry fruit (in mg 100 g $^{-1}$ of fresh matter)

Among the group of phenolic acids in the fruit tested, there were detected: chlorogenic, sinapic and cinnamic acids. Only highbush blueberries contained in their composition all of the identified acids; raspberries contained chlorogenic and sinapic acids, whereas in the wild strawberry and blackberry fruit phenolic acids were represented only by chlorogenic acid (Fig. 2).

Several of the identified compounds were not detected in some samples, which prevented a statistical analysis of the results with regard to the effects of cultivation method and plant species on the content of individual substances.

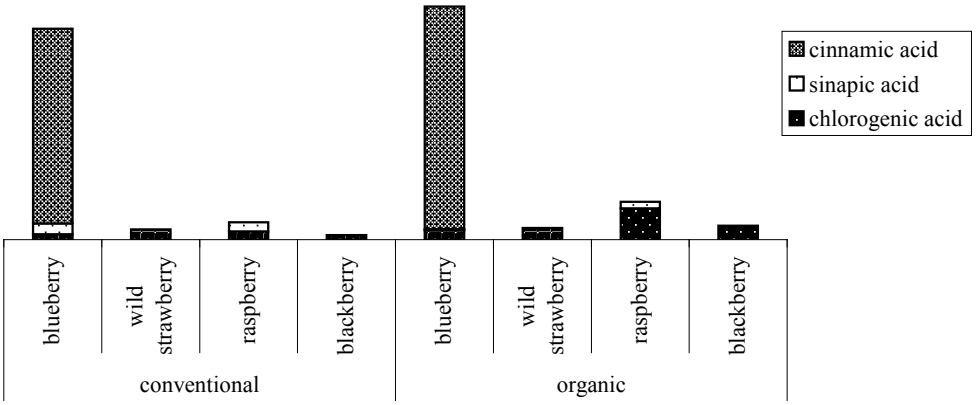

Fig. 2. The qualitative analysis of phenolic acids determined in organic and conventional berry fruit (in mg 100 g^{-1} of fresh matter)

Conclusions

1. Berries from the organic cultivation contained significantly more phenolic acids, flavonoids and anthocyanins, while the fruit from the conventional cultivation were characterized by higher content of vitamin C.
2. The origin of the fruit – either from organic or conventional production system – did not significantly affect the content of dry matter.
3. Regardless of the production system, which the fruit came from, among the species tested the highest content of phenolic acids and anthocyanins was typical for highbush blueberry, and the species containing the highest level of vitamin C and flavonoids was wild strawberry, the fruit of which contained the highest level of dry matter at the same time.
4. As a result of qualitative analysis of phenolic compounds in the fruit, it was found that the most diverse composition of the compounds of the group of flavonoids and phenolic acids was typical for highbush blueberry fruit.
5. Higher contents of most biologically active compounds tested, having antioxidant properties, were found in organic berries, so they can represent a significant source of antioxidants in the diet and thus contribute to the health promotion.

References

Benbrook Ch., 2005. Elevating Antioxidant Levels in Food through Organic Farming and Food Processing. An Organic Center State of Science Review; http://organic.insightd.net/reportfiles/Antioxidant_SSR.pdf; Internet 2.05.2011.

Bourne D., Prescott J., 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Critical Rev. in Food Sci. and Nut. 42 (1), 1–34.

Carbonaro M., Mattera M., Nicoli S., Bergamo P., Cappelloni M., 2002. Modulation of antioxidant compounds in organic vs. conventional fruit (peach Prunus persica L., and pear Pyrus communis L.) J. Agric. Food Chem. 50 (19), 9–11.

Czeczot H., 2000. Flawonoidy – naturalne antyoksydanty w naszej diecie. Żyw. Czł. Metab., XXVII (4), 197–201.

Hallmann E., Rembiałkowska E., 2006. Zawartość związków antyoksydacyjnych w wybranych odmianach cebuli z produkcji ekologicznej i konwencjonalnej. Journal of research and applications in agricultural engeneering, 51 (2), 42–46.

Hallmann E., Rembiałkowska E., Kaproń L., 2005. Zawartość związków bioaktywnych w pomidorach i papryce z uprawy ekologicznej i konwencjonalnej, (w:) Wybrane zagadnienia ekologiczne we współczesnym rolnictwie. Monografia PIMR T II Poznań.

Kazimierczak R., Hallmann E., Rusaczonek A., Rembiałkowska E., 2008. Antioxidant content in black currants from organic and conventional cultivation, EJPAU 11(2), #28.

Klusek T., Mścisz A., Gorecki P., Mrozikiewicz P.M., 2004. Substancje o aktywności farmakologicznej pochodzenia ziołowego w żywności funkcjonalnej. Żyw. Czł. Metab., XXXI, suplement 2, cz. I, 441–447.

Kunachowicz H., 1993. Zawartość niektórych składników odżywczych i zanieczyszczeń chemicznych w wybranych warzywach pochodzących z uprawy ekologicznej i konwencjonalnej. Żyw. Czł. Metab., XX, 4, 189–193.

Levite D., Adrian M., Tamm L., 2000. Preliminary results of resveratrol in wine of organic and conventional vineyards. Conference Proceedings, 256–257.

Rembiałkowska E., 2000. Zdrowotna i sensoryczna jakość ziemniaków oraz wybranych warzyw z gospodarstw ekologicznych, Wyd. SGGW Warszawa.

Rembiałkowska E., Adamczyk M., Hallmann E., 2004. Porównanie wybranych cech wartości odżywczej jabłek z produkcji ekologicznej i konwencjonalnej. Bromat. Chem Toksykol. Suppl., 201–207.

Rembiałkowska E., Hallmann E., Wasiak-Zys G., 2003. Jakość odżywcza i sensoryczna pomidorów z uprawy ekologicznej i konwencjonalnej. Żyw. Czł. Metab., 30 (3/4), 893–899.

Sikora M., Hallmann E., Rembiałkowska E., 2008. Porównanie zawartości składników odżywczych w korzeniach buraków ćwikłowych pochodzących z produkcji ekologicznej i konwencjonalnej. Wybrane zagadnienia ekologiczne we współczesnym rolnictwie: Monografia 5. Przemysłowy Instytut Maszyn Rolniczych, 141–145.

Sikora M., Hallmann E., Rembiałkowska E., Lipowski J., Marszałek K., 2009. Ocena wartości odżywczej i sensorycznej soków marchwiowych z produkcji ekologicznej i konwencjonalnej. Wybrane zagadnienia ekologiczne we współczesnym rolnictwie: Monografia 6. Przemysłowy Instytut Maszyn Rolniczych, 83–90.

Surma-Zadora M., Cieślik E. 2007. Żyw. Czł. Metab., XXXIV, nr 6, 1648–1656.

Szajdek A., Borowska J., 2004. Właściwości przeciwutleniające żywności pochodzenia roślinnego. Żywność. Nauka. Technologia. Jakość, 4 (44), 36–39.

Szkatulska A.,1997; Ekologiczne owoce i warzywa. Zdrowa żywność, zdrowy styl życia, 1 (35), 31–33.

Szustakowska-Chojnacka M., 2007. 100 roślin w twojej kuchni, PZWL, Warszawa, 37–42.

Ziemlański S., 2002: Witaminy antyoksydacyjne, [w:] Gawęcki J. (red.): Witaminy. Wyd. Akademia Rolnicza im. Augusta Cieszkowskiego w Poznaniu, Poznań, 71–77.

3

EVALUATION OF TOTAL PHENOLS AND ANTIRADICAL ACTIVITY OF GROUND-ELDER, CHICKWEED, GOOSEFOOT AND DANDELION GROWN IN LATVIA

Introduction

Worldwide it is of great interest to find new and safe antioxidants from natural sources. A large number of plants have been screened as a source of new phenolic antioxidants for alimentary, cosmetic and pharmaceutical use [Duthie and Crosier 2000]. Plants provide abundant natural antioxidants, which are vitally important for human health [Naczk and Shahidi 2006]. They prevent certain types of chemical damage caused by free radicals increase, which comes from various sources, including pesticides, smoking, etc. Some scientists believe that the destruction of free radicals may contribute to the fight with cancer, heart disease and stroke [Forristal 2002]. Analysis shows different antioxidant activity for each plant type, stimulated by the antioxidant components, such as α-tocopherol, β-carotene, vitamin C, selenium and phenolic compounds [Ismail 2004]. Extensive studies on functions and the role of polyphenols in humans began in the last century and are continued today [Rappoport 2003]. Polyphenols is a large, important and diverse class of antioxidants, beneficial to both plants and humans. Phenolic compounds commonly found in plants are biologically active substances having antiseptic, vitamin activity, expression, etc. properties [Rappoport 2003, Daayf 2008]. It is known that they phenolic compounds are very effective antioxidants [Shahidi 1992, Tapeiro 2002, Shahidi 2004].

Also in recent studies contradicting data exist about the correlation between the antioxidant activity and the polyphenolic content of plants and it is possible to identify opposite views:

1. linear correlation exists between antioxidant capacity and total polyphenols content [Diaz-Mula et al. 2011];
2. no significant correlation exists between phenolic content and antioxidant activity, considering that other compounds may be responsible for scavenge radicals [Gao et al. 2011].

Climatic conditions are critical for various compound formation in plants. Surface flavonoids are more synthesized in plants growing wild in dry conditions, to prevent plant from ultraviolet radiation [Grayer 1996]. Taking into account the current findings it is hard to identify reasons for synthesis of phenols for each plant family [Manach 2004].

Latvian flora is rich in wide range of medical plants, but some of them are recognized as weed. As most popular weeds found very commonly are ground-elder *Aegopodium podagratia* L., dandelion *Taraxacum officinale Wigg.* L., goosefoot *Chenopodium ambrosioides* L. and chickweed *Stellaria media* L. Only few studies are found about possibilities of weeds use as valuable additive to food, for example, as natural antioxidants.

The aim of current research was to determine total phenols and antiradical activity of several weeds (ground-elder, chickweed, goosefoot and dandelion) growing in Latvia.

Material and methods

Materials

Ground-elder (*Aegopodium podagraria* L.), chickweed (*Stellaria media* L.), goosefoot (*Chenopodium album* L.*)* and dandelion leaves and flowers (*Taraxacum officinale* L.) were collected at Jelgava (latitude 56°39' N, longitude 23°42' E) during the May 2011 with 15 day intervals: 2 May (I); 16 May (II) and 30 May (III).

The description of the samples is presented in Table 1.

Three hundred grams of each plant were collected.

Table 1

Characterization of collected plants

Plant	Date of collection	Abbrev. of vegetative stages	Plant height, cm	Other comments
Ground-elder *Aegopodium podagratia* L.	02.05.2011	I	7–12	without inflorescence
	16.05.2011	II	11–14	without inflorescences
	30.05.2011	III	12–15	without inflorescences
Dandelion leaves *Taraxacum officinale* Wigg.L.	02.05.2011	I	7–8	–
	16.05.2011	II	15–18	–
	30.05.2011	III	16–20	–
Dandelion flowers *Taraxacum officinale* Wigg.L.	02.05.2011	I	7–8	–
	16.05.2011	II	15–18	–
	30.05.2011	III	16–20	–
Goosefoot *Chenopodium ambrosioides* L.	02.05.2011	I	4–6	without inflorescences
	16.05.2011	II	7–9	without inflorescences
	30.05.2011	III	8–12	without inflorescences
Chickweed *Stellaria media L.*	02.05.2011	I	3–5	without inflorescences
	16.05.2011	II	6–13	with inflorescences
	30.05.2011	III	11–16	with inflorescences

Chemicals

Gallic acid, Folin-Ciocalteous phenol reagent, 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents (Na_2CO_3, ethanol) used in the research were obtained from Acros Organic, (USA).

Preparation of extracts from weeds

Fresh plants were homogenized and five grams of sample were extracted with 50 ml 80% ethanol solution in water. After 1 hour extraction using mixing, samples were filtered (paper No. 89). Extracts were prepared in duplicate.

Determination of total phenolic content (TPC)

The TPC of the plant extract was determined according to the Folin-Ciocalteu spectrophotometric method [Singleton et al. 1999] with some modifications. To 0.5 ml of extract 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) was added and, after 3 minutes 2 ml of Na_2CO_3 (75 g l^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). Total phenols were expressed as gallic acid equivalents (GAE).

Determination of DPPH radical scavenging activity

Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) radical as outlined by Yu et al. [2003]. The antioxidant reaction was initiated by transferring 0.5 ml of plant extract into a sample cavity containing 3.5 ml of freshly prepared DPPH$^{\bullet}$ methanol solution (0.004 g DPPH$^{\bullet}$ to 100 ml methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using a spectrophotometer JENWAY 6300. Inhibition of DPPH$^{\bullet}$ in percent (I%) of each extract sample was calculated from the decrease of absorbance according to relationship:
where

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}.} \times 100,$$

A_{blank} – absorbance of control reaction (methanol-water with DPPH$^{\bullet}$);
A_{sample} – absorbance of the tested samples.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity [Zhao et al. 2008].

Statistical analysis

Analysis of variance was performed by ANOVA procedure and $p<0.05$ was considered statistically significant. A linear correlation analysis was performed with the software SPSS 14.00 for Windows.

Results and discussion

Phenolic composition of plants is affected by different factors. In this study comparison of phenolic compounds of five plants depending on vegetative stage were determined. The content of total phenols varied from 571 to 2261 mg GAE 100 g^{-1} (Fig. 1). M. Škerget et al. [2005] in their studies found that plant material contains different amount of total phenols: laurel – 99.7 g GAE kg^{-1}, oregano – 186 g GAE kg^{-1}, olive tree leaves– 144 g GAE kg^{-1}. Comparing to the analysed samples, it is possible to conclude that weeds contain lower amounts of phenols.

The content of phenols differed significantly depending on vegetative stage and the highest content was determined at stage I (in younger plants) for ground-elder, dandelion leaves

and flowers. Diverse biological activities of dandelion, such as anti-angiogenic, anti-inflammatory, and anti-nociceptive activities were estimated in mice and murine macrophage cell line [Jeon et al. 2008]. Dandelion leaf is also known to be an effective hydrogen peroxide scavenger, because of its high polyphenol content [Hagymasi et al. 2000]. For goosefoot decrease in TPC was observed in stage III, whereas no significant differences between stages for chickweed were observed. I. H. Sellami et al. [2009] established that phenolic compounds of marjoram increased comparing early and late vegetative stage, but decreased during budding and flowering stages. The highest content of total phenols in ground-elder in stage I was detected, followed by dandelion leaves at stage I and goosefoot at stages I and II. Whereas the lowest content of total phenols in ground-elder at stage I and dandelion leaves at stage II and III were detected. Park et al. [2011] found total phenol content of dried dandelion obtained from Korea 0.14–0.17 mg g^{-1} DW.

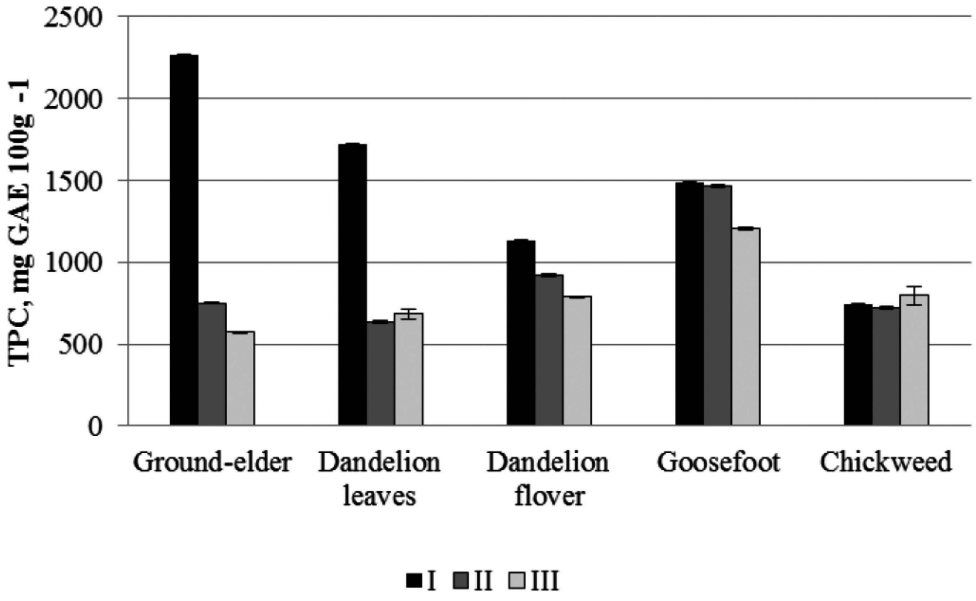

Fig. 1. Content of total phenols of ground-elder, chickweed, goosefoot and dandelion

The scavenging activity on DPPH• radicals has been widely used to determine the free radical-scavenging activity. DPPH• is a stable free radical that is dissolved in methanol and its colour shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the colour from the DPPH• assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation [Moreira et al. 2008]

Also antiradical activity of the plants differed significantly depending on vegetative stage and the highest content was determined at stage I (in younger plants) for ground-elder, dandelion leaves and flowers (Fig. 2). Goosefoot the highest activity reached at stage III, but chickweed at stage II. Antiradical activity varied from 5.1–91.3% (with the same dilution of

extract). Kumar et al. [2007] reported that goosefoot essential oil shows $ABTS^{+}$ activity and at 2000, 2500 and 3000 mg ml^{-1}, the activity increased to 82.30, 89.03 and 95.66%, respectively.

There was moderate positive correlation (r=0.75) established between plant phenol content and the ability scavenging $DPPH^{\bullet}$ radicals. Antioxidant activity is measured using various methods and the majority of methods show positive correlation between total phenol content and antioxidant activity, for example, in methods iron reducing (R^2=0.8871, p<0.001), delay of lipid peroxidation (R^2=0.7327, p<0.01), bond specific (R^2=0.6041, p<0.05) and bond non-specific (R^2=0.6589, p<0.01) binding of hidroxy groups. In the study of I. Hinneburg [2006] correlation between TPC and $DPPH^{\cdot}$ scavenging as well as between TPS and iron reducing was not established. In Hungary research on phenolic content and antioxidant properties of clove, cinnamon, marjoram, oregano, hyssop, rosmarin and other herbs were studied, and these studies proved close linear correlation between phenolic content in herbs and their ability to delay oxidation (r=0.9302). Research results of A. Lugasi et al. [1996] show importance of phenolic compounds in formation of herb antioxidant properties.

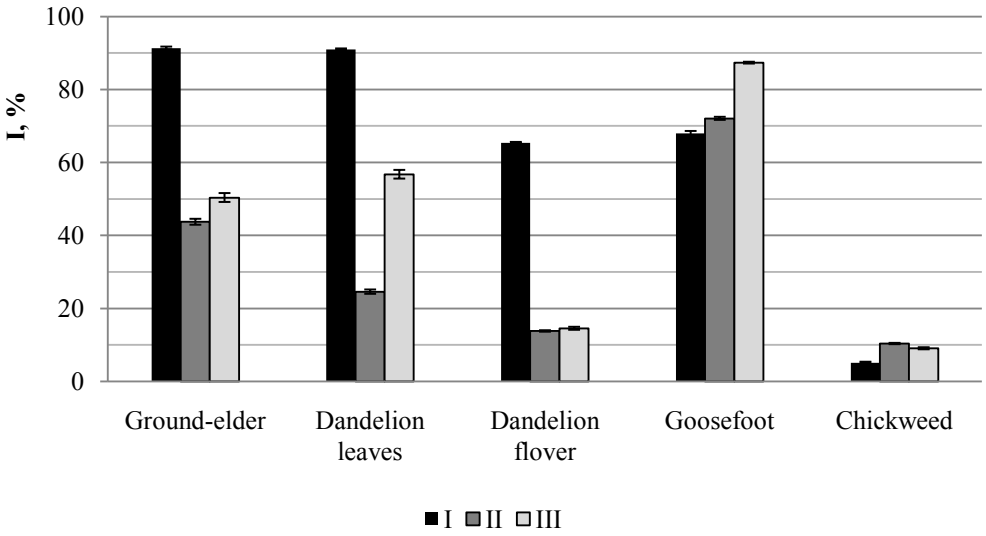

Fig. 2. DPPH scavening activity of ground-elder, chickweed, goosefoot and dandelion

Conclusions

This study confirms that weeds contain significant amounts of phenolic compounds that shows antiradical activity. The results obtained in present work showed that the highest content of phenolic compounds is in younger plants. Moderate positive correlation was observed between phenol content and antiradical activity. Further experiments are necessary to evaluate antioxidant activity of weed extracts in food matrixes.

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References

Daayf F., Lattanzio V, 2008. Recent Advances in Polyohenol Research [1 ed.] Wiley-Blackwell, 437 p.

Díaz-Mula H.M., Zapata P.J., Guillén F., Valverde J.M., Valero D., Serrano M., 2011. Modified atmosphere packaging of yellow and purple plum cultivars. 2. Effect on bioactive compounds and antioxidant activity. Postharvest Biology and Technology, vol. 61, 110–116.

Duthie G., Crosier A., 2000. Plant-derived phenolic antioxidants. Current Opinion in Clinical Nutrition and Metabolic Care, 3, 447–451.

Forristal A., Wang L.J., Shiow Y., 2002. The Antioxidant Herbs. World & I, vol. 17, 122 p.

Gao C.Y., Lu Y.H., Tian C.R., Xu J.G., Guo X.P., Zhou R., Hao G., 2011. Main nutrients, phenolics, antioxidant activity, DNA damage protective effect and microstructure of Sphallerocarpus gracilis root at different harvest time. Food Chemistry, vol.127, 615–622.

Grayer R.J., Bryan S.E., Veitch N.C., Goldstone F.J., Paton A., Wollenweber G., 1996. External flavones in sweet basil, *Ocimum basilicum*, and related taxa. Phytochemistry, vol. 43, 1033–1039.

Jeon H.J., Kang H.J., Jung H.J., Kang Y.S., Lim C.J., Kim Y.M., Park E.H., 2008. Anti-inflammatory activity of Taraxacum officinale. Journal of Ethnopharmacology, 115, 82–88.

Hagymasi K., Blazovics A., Lugasi A., Kristo S., Feher J., Kery A., 2000. In vitro antioxidant evaluation of dandelion (*Taraxacum officinale* WEB.) water extracts. Acta Alimentaria, 29, 1–7.

Hinneburg I., Dorman H.J.D., Hiltunen R., 2006. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry, vol. 97, 122–129.

Ismail A., Marjan Z.M., Foong C.W., 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry, vol. 87, 581–586.

Kumar R., Mishra A.K., Dubey N.K., Tripathi Y.B., 2007. Evaluation of Chenopodium ambrosioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. International Journal of Food Microbiology, vol. 115, 159–164.

Lugasi A., Dworschak E., Hovari J., 1996. Antioxidant property of polyphenolic compounds of culinary herbs and medical plants. Agri-Food Quality, An Interdisciplinary Approach. Cambridge, 546 p.

Manach C., Scabert A., Morand C., Remesy C., Jimenez L., 2004. Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition, vol.79, 727–747.

Naczk M., Shahidi F., 2006. Phenolic in cereals, fruit and vegetables: Occurrence, extraction and analysis. Jornal of Pharmaceutical and Biomedical Analysis, 1523–1542.

Park C.M., Park J.Y., Noh K.H., Shin J.H., Song J.S., 2011. *Taraxacum officinale* Weber extracts inhibit LPS-induced oxidative stress and nitric oxide production via the NF-κB modulation in RAW 264.7 cells. Journal of Ethnopharmacology, vol. 133, 834–842.

Rappoport Z., 2003. The chemistry of phenols, Wiley-Interscience, vol. 2, 1667 p.

Sellami I.H., Maamouri E., Chahed T., Wannes W.A., Kchouk M.E., Marzouk B., 2009. Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.). Industrial Crops and Products, vol. 30, 2009, 395–402.

Shahidi F., Naczk M., 2004. Phenolics in food and nutraceuticals, CRC Press, Boca Raton, 403 p.

Shahidi F., Wanasundara P.K.J.P.D., 1992. Phenolic antioxidants. CRC Critical reviews in Food Science and Nutrition, 67–103.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, vol. 29, 152–178.

Škerget M, Kotnik P., Hadolin M., Rižner Hraš A., Simonič M., Knez Ž., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry, vol. 89, 191–198.

Tapeiro H., Tew K.D., Nguyen Ba G., Mathe G., 2002. Polyphenols: Do they play a role in the prevention of human pathologies? Biomedicine and Pharmacotherapy, 56, 200–207.

Yu L., Haley S., Perret J., Harris M., Wilson J., Haley S., 2003. Antioxidant properties of bran extracts from Akron wheat grown at different locations. Journal of Agriculture and Food Chemistry, 51, 1566–1570.

Zhao H., Fan W., Dong J., Lu J., Chen J., Shan L., Lin Y., Kong W., 2008. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry, 107, 296–304.

4

BIOLOGICAL PROPERTIES OF LUTEIN AND ZEAXANTHIN AND THEIR ROLE IN PROTECTING HUMAN HEALTH

Introduction

There is a growing body of evidence that lutein and zeaxanthin, a non pro-vitamin A carotenoid pigment naturally found in plants and other animal product especially in egg yolk, plays an important role in human health. Carotenoids are fat soluble compounds that are associated with lipid fractions. Carotenoids are the most numerous and widespread group of pigments in nature. They have a long and interesting history. Studies on these pigments were started at the beginning of the 19th century, when the crystalline yellow pigment carotene was first isolated in 1831 by Wackenroder from carrots and the yellow pigments of autumn leaves were named as xanthophylls by Berzelius in 1837 [Tee 1992]. Nowadays more than 600 carotenoids have benn isolated from natural sources [Armstrong 1997]. They are responsible for the beautiful colours of many birds (flamingo, canary), insects (ladybird) and marine animals (salmon, crustaceans), as well as the colours of many flowers (marigold, narcissus), fruits (pineapple, citrus fruits, paprika) and falling leaves. These pigments provide different colours from the light yellow to dark red and when complexed with proteins they can produce green and blue colorations [Surai et al. 2001]. The colour of a given carotenoid is due to the absorption of light (typically 400–500 nm wavelengths) and is determined by the number of conjugates double bonds within the hydrocarbon backbone [Armstrong 1997].

From a chemical point of view carotenoids are polyisoprenoid compound and can be divided into two main groups: carotenes or hydrocarbon carotenoids only composed of carbon and hydrogen atoms and xanthophylls that are oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto epoxy, methoxy or carboxylic acid groups. Carotenoids are known to exist in different geometric forms (cis and trans isomers). These forms may be interconverted by light, thermal energy or chemical reactions. The vast majority of carotenoids are in the all-trans configuration [Rice-Evans et al. 1997]. Their structural characteristic is a conjugated double bond system, which influences their chemical, biochemical and physical properties[Rodriguez-Bernaldo de Quiros and Costa 2006]. However the most interesting in these compounds are their physical and biological function. Carotenoids are essential components in oxygenic photosynthetic organism functioning for example in light-harvesting reactions. Furthermore, these compound protect against potentially damaging combination of oxygen, light and photosynthesizing molecules by quenching both the triplet excited states of photosensitizers and singlet excited oxygen molecules [Armstrong 1997]

Lutein and zeaxathin are referred to xanthophylls. They are found in dark green, leafy vegetables such as spinach and kale and in some animal products such as egg yolk. Although these carotenoids are similar in structure to α- and β-carotene, they do not have provita-

min A activity [Ma and Lin 2009]. They are not essential nutrients for human health, but they display biological activities that have attracted great attention on the prevention and reversal of certain serious eye diseases [AREDS Research Group]. Furthermore, researches involving cell cultures, animal models and human studies has been directed to the potential role of lutein and zeaxanthin in protecting against several chronic diseases, particularly age-related macular degeneration (AMD) and cataract, cancer at various sites and heart disease and stroke [Ribaya-Mercado and Blumberg 2004]. The following review summarizes the background information about lutein and zeaxanthin, especially about biological properties, dietary sources, production diet supplements, bioavailability of these compound and protecting against different diseases.

Chemical structure of lutein and zeaxanthin

Carotenoids are based upon the same C40 isprenoid skeleton, which is modified by cyclisation, addition, elimination, rearrangement and substitution [Rice-Evans et al. 1997]. This C40 skeleton is synthesized by tail-to-tail linkage of two C20 geranylgaeranyl molecules [Oliver and Palou 2000]. Lutein and zeaxanthin are oxygenated carotenoids that also consist of 40-carbon compounds with nine conjugated double bonds in polyene chain. Their structures are characterized by the presence of two hydroxyl groups at the terminal rings of the molecule on the basic $C_{40}H_{56}$ carotene structure, and thus are referred to as xanthophylls.

Zeaxanthin (Fig. 1) is a stereoisomer of lutein (Fig. 2), differing only in the location of one double bond in one of the hydroxyl group. The chemical name of lutein is [(3R,3'R,6'R)-beta,epsilon-caroten-3,3'-diol] but chemical name of zexanthin is [(3R,3'R)-beta,beta-caroten-3,3'-diol]. Chemical formula and molecular weight of both compounds is the same. Lutein can exists in 8 isoforms and it is related with presence 3 asymmetric carbon atom, but zeaxanthin can exist in 3 stereoisomeric forms.

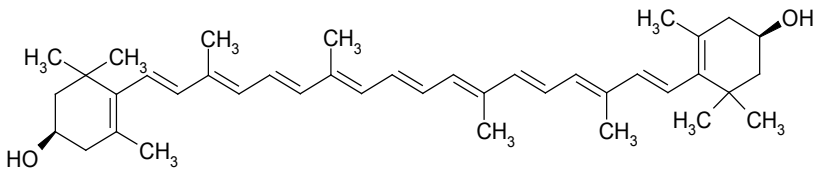

Fig. 1. Molecular structure of zeaxanthin

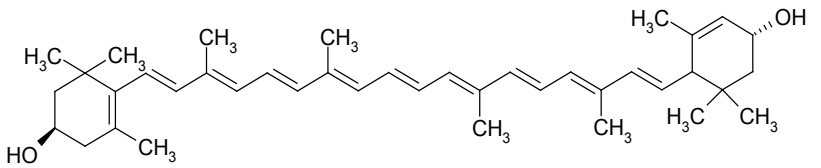

Fig. 2. Molecular structure of lutein

The hydroxyl groups are believed to provide unique biological function of lutein and zeaxanthin. The presence of substituted terminal β-rings in the molecule, however, also con-

fers a higher polarity, which determines, in part, distinctive characteristic during absorption, transport, metabolism and deposition in tissues. The hydrophilic properties allow them to react with singlet oxygen generated in water phase more efficiently than nonpolar carotenoids [Ma and Lin 2009]. In addition, the relatively higher polarity partly determines distinctive characteristic during their metabolism, light absorption, capture and stabilization in tissues and potential orientations in a bilayer membrane [Krinsky et al. 2002].

Dietary sources of lutein and zeaxanthin

Man is not capable of synthesizing carotenoids *de novo* and, thus, their presence in human is entirely of dietary origin, although is capable of modifying some of them to some extent [Granado et al. 1996]. Lutein and zeaxanthin are present in a wide variety of fruit and vegetables. Particularly lutein and zeaxanthin content is given in Table 1.

Table 1

Carotenoid concentrations in egg products, fruits, vegetables and selected foods (μg/100 g) [Murillo et al. 2010, Perry et al. 2009]

Product	All Lutein (trans and cis)	All Zeaxanthin (trans and cis)
Egg (yolk+white), cooked	273	252
Egg yolk, cooked	744	686
Egg (yolk+white), raw	336	319
Egg yolk, raw	917	870
Apple, red delicious with skin	15	0
Broccoli,cooked	772	0
Kale, cooked	8 884	0
Lettuce, romaine	3 824	0
Orange juice	33	26
Parsley	4 326	0
Scallions, cooked in oil	0	2 488
Spinach, cooked	13 504	0
Tomato, raw	32	0
Mandarin	2.0	2.1
Apricot	0.6	0.3
Kiwi	0.7	0
Corn flour	2.1±0.2	9.4±0.7

Lutein concentration is particularly high in leafy green vegetables such as spinach, collards, lettuce and parsley and kale. Green leafy vegetables are the richest sources of lutein but contained small or no amounts of zeaxanthin [Perry et al. 2009]. Dietary sources of zeaxanthin include yellow corn, orange pepper, orange juice, honeydew and mango [Sajilata et al. 2008]. Zeaxanthin is also major carotenoid in cold-pressed marionberry, boysenberry, red raspberry and blueberry seed oils, followed by β-carotene, lutein and cryptoxanthin [Parry et al. 2005].

Furthermore, lutein and zeaxanthin are also present in some animal products, such as egg yolk. Lutein and zeaxanthin content in egg yolk is not so high as in leafy green vegetables but

egg yolk is a good source of these compounds because of their high bioavailability [Chung et al. 2004]. The daily lutein and zeaxanthin intake in the industrialized world is quite low, averaging between 1–2.5 mg/day in the USA and between 1.56–3.25 mg/day in Eurpoe [O'Neill et al. 2001]. Although there is no recommended daily intake for lutein and zeaxanthin, many intervention trials support the intake of at least 10 mg lutein for increasing plasma concentration, macular pigment accumulation, improvements in visual function and protection of the skin from environmental hazards [Parisi et al. 2008, Kvansakul et al. 2006].

Lutein and zeaxanthin production

A. LUTEIN AND ZEAXANTHIN PRODUCTION FROM MARIGOLD (*Tagetes erecta)*

Lutein is usually produced as a bulk compound from marigold oleoresin (*Tagetes erecta)*, which is has been used in traditional folk medicine for a long time [Breithaupt and Schlatterer 2005]. Marigold (*Tagetes erecta)*, belonging to the *Asteraceae* family, is a well known ornamental plant widespread all over the world with numerous species. In recent years, the interest in natural substances has been contributed to the re-evaluation of the *Tagetes* genus. This petals of marigold are rich in lutein and lutein fatty acid esters which, on the whole, represent over 90% of the pigments identified in this plant. The lutein content in marigold petals is variable and ranged from 17 up to 570 mg/100 g [Piccaglia et al. 1998]. Zeaxanthin esters typically occurs as a minor compounds and its content in marigold petal is about 5% of whole carotenoids content [Breithaupt and Schlatterer 2005]. The result is that the production of marigold petals is a labor-intensive land-demanding process that is currently feasible in developing economies.

Lutein is largely consumed as a food colorant and its sales amount to 150 000 000$ in the US only [Fernandez-Sevilla et al. 2009]. Currently, lutein is obtained from the peals of marigold after an extraction process which yields oleoresins with varying concentration of lutein that range from 5 to 50%, mostly in the diaester form which is roughly a half if free lutein is considered. These concentrates are the product most commonly used for the formulation of supplements. Lutein can be further purified by processes that involve saponification, further concentration and a final recrystallisation to its crystalline form [Khachik 2007]. Crystaline lutein is difficult to handle and is commonly sold as suspensions of the carotenoids in corn or sunflower oils. Pure free crystalline lutein is also available, most commonly in the form of microcapsules [Fernandez-Sevilla et al. 2010]. Lutein has also been produced synthetically, but at prices that cannot compete with marigold.

B. BIOTECHNOLOGICAL PRODUCTION OF LUTEIN

In recent years, biotechnological processes have become an attractive alternative to "naturally grown" xanthophylls: for example, the green microalga *Chlorella ptorothecoides* was investigated as a potential lutein source [Cisneros et al. 2004]. Microalgae have been considered as potential sources of lutein for several reasons:

1. its high lutein content (0.5–.12% dry weight) compared to marigold petals
2. petals do not have to be separated and whole microalgal biomass is processed
3. a homogenous biomass is produced at a constant rate regardless of time and weather, so it lends itself better precisely designed extraction process
4. valuable by-products that can be used to produce protein hydrolysates, other pigments and even valuable lipids depending on strain [Fernadez-Sevilla et al. 2010]

The production of lutein from microalgae should use quite less labor than marigold but, on the other hand, would demand extensive technology and a precisely designed process that should not only be effective in recovering the lutein but also provide appropriate valorization of the by-products, allowing to compensate for the higher cost of microalgal biomass compared to marigold petals. Two main factor make a microalga a good lutein producer: the lutein content and biomass productivity. Other factors such as the presence of a cell wall or the content of other carotenoids may be also a consideration [Fernadez-Sevilla et al. 2010].

Bioavailability of lutein and zeaxanthin

The intake of lutein and zeaxanthin in Europe is between 1.5–3.25 mg/day [O'Neill et al. 2001]. The content of zeaxanthin in the diet is usually much lower than either β-carotene or lutein and one recent estimate based on National Health and Nutrition Examination Survey data has suggested that the lutein:zeaxanthin ratio in the US diet is 5:1 [Hartmann et al. 2004]. Because of the low amounts of zeaxanthin in the diet, there are far fewer studies that have assessed its bioavailability than there are of lutein. However, as the structure of two xanthophylls carotenoids is very similar, studies on lutein bioavailability may assist in understanding zeaxanthin bioavailability [Thurnham 2007].

The presence of lutein and zeaxanthin in human blood and tissues is a result of the ingestion of food sources of xanthohylls [Johnson et al. 2010]. The bioavailability of carotenoids is determined by characteristics of the food source and interactions with the other dietary constituents. Studies with β-carotene and lycopene have shown that association with lipid matrix increases bioavailability of these carotenoids. Localization within a plant (eg, in chloroplast or chromoplast) and compound that interfere with intestinal micelle formation can decrease carotenoid bioavailability. To some extent, the inhibitory effect of the plant matrix can be overcome by cooking to break down the plant cell well and by decreasing food particle size. Mineral oil can also decreasing carotenoid absorption [Handelmann et al. 1999].

Recent reports indicated that egg yolk is highly bioavailable source of lutein, increasing serum lutein concentrations 110–350 nmol/L for each milligram of lutein ingested [Surai et al. 2000, Handelmann et al. 1999]. For comparison studies using vegetables as a source of lutein reported increases of 20–40 nmol/L for each milligram of lutein ingested. This studies indicated that egg lutein is several times more bioavailable than plant lutein. In egg yolks lutein is located in the digestible lipid matrix, which is composed of cholesterol, triacylglycerols and phospholipids. Lutein is dispersed in this matrix along with the other fat-soluble micronutrients such as vitamins A, D and E.[Handelmann et al. 1999]. In egg some part of lutein is esterified with fatty acids. The cholesterol content of the egg yolks may enhance the bioavailability of lutein from egg yolks.

Lutein and zeaxanthin and disease protection

The macular pigment is composed principally of three isomeric carotenoids: lutein, zeaxanthin and meso-zeaxanthin. They represent roughly 36, 18 and 18% of the total carotenoid of the retina [Landrum and Bone 2001]. Zeaxanthin is the dominant component in the central region of the retina and lutein is distributed throughout the retina. The retina is composed of photoreceptive cells (rods and cones) that transducer light into a neural signal. This signal

is then further processed the brain. Before that light is converted, it passes through the inner of the retina that contains the oxygenated carotenoids, lutein and zeaxanthin [Stringham et al. 2010]. In the human retina and macula it is identified specific xanthophylls-binding proteins. The human retina is particularly vulnerable to oxidative damage because of its high proportion of easily peroxidisable long-chain polyunsaturated fatty acids. High enegry short-wavelenght visible light and high metabolic activity also promote the generation of reactive oxygen species (ROS) which are highly reactive and readily react with the lipid, protein and nucleic acids in the macula, thereby resulting in irreversible damage to various cell structures. It is generally believedthat cumulative oxidative damage is in part responsible for the pathogenesis age-related macular degeneration [Beatty et al. 2000].

The antioxidant properties of carotenoids have been proven mainly based on their abilities to quench singlet oxygen, scavenge free radicals, inhibit peroxidation of membrane phospoholipids and reduce lipofuscin formation. Since lutein and zeaxanthin are the main carotenoids accumulating in the macula and lens, they are thought to play a unique role in the protection against light-initiated oxidative damage [Ma and Lin 2009].

Excessive light exposure leads to retinal damage and increases the rate of photoreceptor apoptosis and there is an exponential rise in the retinal injury with decreasing wavelength [Roca et al. 2004]. The spectrum of lutein and zeaxanthin includes a broad absorption band, with a peak at 450nm roughly, and therefore these carotenoids can absorb and attenuate the damaging blue light before it reaches the photoreceptors. It has been estimated that macular carotenoids reduce the amount of blue light reaching the macula by much as 40%. In human retina, lutein and zeaxanthin exist in the highest concentrations in the photoreceptor axon layer and the inner plexiform layer of the fovea, which is also consistent with their roles as optical filters [Ma and Lin 2009].

A. AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in people over the age of 65 in industrialized countries. Risk factors for the AMD include advancing age, family history of AMD and cardiovascular risk factors such as hypertension and cigarette smoking, higher body mass index [Seddon et al. 2010]. Both genetic and environmental factors are evident as contributing to the risk of AMD. Ocular exposure to sunlight has been linked to AMD. Photooxidation of polyunsaturated lipids might well impair the normal cycle of lipid biochemistry in the retinal pigment epithelium during photoreceptor phagocytosis, leading to the damaging buildup of drusen which characterizes AMD [Landrum and Bone 2000]. Two types of AMD exist: early (or dry) and late (or wet). Early AMD is characterized clinically by yellowish deposits known as soft drusen accumulation, patchy atrophy and pigmentary abnormalities in the retinal pigment epithelial and Bruch's membrane, while late-stage manifestations encompass choroidal neurovascularisation, subretinal haemorrhage detachment of pigment epithelial and retinal scarring. There is a growing body of evidence implicating oxidative stress blue light damage in this process. As the major components of macular pigment, it comes as no surprise that lutein and zeaxanthin have the beneficial effects on preventing the onset and progression of age-related macular degeneration [Ma and Lin 2009].

B. CATARACT

Cataract is a common cause of vision impairment in the elderly and the most common cause of blindness worldwide [Foster and Johnson 1990]. Cataracts is an opacification of the lens

in the eye which obstructs the passage of light, often resulting in impaired vision or blindness. In the United States, the potentially blinding effect of cataract among the elderly is dramatically reduced because cataract surgery is readily available, effective and safe. The prevalence of cataract increases with age from less than 5% in persons under 65 years of age to approximately 50% in those 75 years of age and older. Exposure to ultraviolet light may contribute to the progression of cataract formation. Oxidative damage to lens cell membranes is considered an important factor in the initiation and progression of age-related cataract and increased lipid peroxidation products have been detected in lens and aqueous humor of patients with cataracts [Ribaya-Mercado and Blumberg 2004].

It has been postulated that dietary antioxidants, especially lutein and zeaxanthin, play crucial role in the prevention of the oxidation of lens proteins and the formation of cataract. Increasing intake of foods rich in lutein and zeaxanthin such as spinach, kale was most consistently associated with the lower risk of cataract, while cataract was not strongly associated with consumption of carotene-rich foods [Ma and Lin 2009].

C. CANCER

Nutritional factors are widely believed to be critical in carcinogenesis. Furthermore evidence from epidemiologic studies indicates that diets high in fruits and vegetables are associated with a lower risk of numerous cancer. It has been estimated that up to 70% of all cancer is attributed to diet [Steinmetz and Potter 1996]. Xanthophylls may posses antimutagenic and anticarcinogenic properties and play role in the health of body tissues other than the eye as suggested by research studies related to carcinogenesis and the risk for cancer The mechanism for a potential protective role of xanthophylls against carcinogenesis may include selective modulation of apoptosis, inhibition of angiogenesis, enhancement of gap junctional intercellular communication, induction of cell differentiation, prevention of oxidative damage and modulation of the immune system [Ribaya-Mercado et al. 2004]. Oxidative metabolites of lutein, thought to arise from lutein's antioxidant mechanism of action, have been isolated and characterized from extracts of human serum and plasma [Khachik et al. 1995].

D. HEART DISEASE

A growing body of experimental evidence and observational studies suggest that lutein and zeaxanthin may play role in the prevention of coronary heart disease and stroke. It has been estimated that luteinis highly effective in reducing low-density lipoproteins and inhibiting inflammatory response of monocytes to lipoproteins trapped in artery wall [Ribaya-Mercado et al. 2004].

Conclusions

The xanthophylls, lutein and zeaxanthin, have specific distribution patterns in human tissue especially in the retina and macula. The presence of these xanthohylls is thought to provide a special function in these tissues. A growing body of evidence suggests that lutein and zeaxanthin may contribute to the protection against several age-related diseases, including cataract, age-related macular degeneration, heart disease and some forms of cancer. For this reason it is worth noting that diet rich of fruits, vegetables and eggs (because of its high bioavailability) could protect us against described diseases and maintain human health.

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References

AREDS Research Group, 2007. The relationship of dietary carotenoid and vitamin A, E and C intake with age-related macular degeneration in a case-control study. AREDS Report no. 22. Archives of Ophthalmology, 125, 1225–1232.

Armstrong G.A., 1997: Genetics of eubacterial carotenoid biosynthesis: a colorful tale. Annual Review of Microbiology, 51, 629–659.

Beatty S., Koh H., Phil M., Henson D., Boulton M., 2000. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Survey of Ophthalmology, 45, 115–134.

Breithaupt D.E., Schlatterer J., 2005. Lutein and zeaxanthin in new dietary supplements – analysis and quantification. European Food Research and Technology, 220, 648–652.

Chung H., Rasmussen H.M., Johnson E.J., 2004. Lutein Bioavailability Is Higher from Lutein-Enriched Eggs than from Supplements and Spinach in Men. The Journal of Nutrition, 134, 1887–1893.

Cisneros M., Benavides J., Brenes C.H., Rito-Palomares M., 2004. Recovery in aquenous two-phase systems of lutein produced by the green microalga *Chlorella protothecoides.* Journal of Chromatography B, 807(1), 105–110.

Fernandez-Sevilla J.M., Fernandez F.G. A., Grima E.M., 2010. Biotechnological production of lutein and its applications. Applied Microbiology and Biotechnology, 86, 27–40.

Foster A., Gordon G.J., 1990. Magnitude and causes of blindness in the developing world. International Ophthalmology, 14(3), 135–140.

Foster A., Johnson G.J., 1990. Magnitude and causes of blindness in the developing world. International Ophthalmology, 14, 135–140.

Granado F., Olmedilla B., Blanco I., Rojas-Hidalgo E., 1996. Major fruit and vegetables contributors to the main serum carotenoids in Spanish diet. European Journal of Clinical Nutrition, 50, 246–250.

Handelman G.J., Nightingale Z.D., Lichtenstein A.H., Schaefer E.J., Blumberg J.B., 1999. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. The American Journal of Clinical Nutrition, 70, 247–251.

Hartmann D., Thurmann P.A., Spitzer V., Schalch W., Manner B., Cohn W., 2004. Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. American Journal of Clinical Nutrition, 79(3), 410–417.

Johnson E.J., Maras J.E., Rasmussen H.M., Tucker K.T., 2010. Intake of Lutein and Zeaxanthin Differ with Age, Sex, and Ethnicity. Journal of the American Dietetic Association , 110, 1357–1362.

Khachik F., 2007. Process for extraction and purification of lutein, zeaxanthin and rare carotenoids from marigold flowers and plants. US Patent 7, 173, 145.

KhachikF., Beecher G.R., Smith Jr J.C., 1995. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. Journal of Cellular Biochemistry, 22, 234–246.

Krinsky N.I., Landrum J.T., Bone R.A., 2003. Biologic mechanism of the protective role of lutein and zeaxanthin in the eye. Annual Review of Nutrition, 23, 171–201.

Kvansakul J., Rodriquez-Carmona M., Edgar D.F., Barker F.M., Kopcke W., Schalch W., Barbur J.L., 2006. Supplementation with the carotenoids lutein and zeaxanthin improves human visual performance. Ophthalmic and Physiological Optics, 26(4), 362–371.

Landrum J.T., Bone R.A., 2001. Lutein, Zeaxanthin, and the Macular Pigment. Archives of Biochemistry and Biophysics, 385(1), 28–40.
Ma L., Lin X.M., 2009. Effects of lutein and zeaxanthin on aspects of eye health. Journal of the Science of Food and Agriculture, 90, 2–12.
Murillo E., Melendez-Martinez A.J., Portugal F., 2010. Screening of vegetables and fruits from Panama for rich sources of lutein and zeaxanthin. Food Chemistry, 122, 167–172.
O'Neill M.E., Carroll Y., Corridan B., Olmedilla B., Granado F., Blanco I., 2001. A European carotenoid database to assess carotenoid intakes and its use in five-country comparative study. The British Journal of Nutrition, 85, 499–507.
Oliver J., Palou A., 2000. Chromatographic determination of carotenoids in foods. Journal of Chromatography, 881, 543–555.
Parisi V.T. M., Gallinaro G., Varano M., Saviano S., Piermarocchi S., 2008. Carotenoids and antioxidants in age-related maculopathy Italian study: multifocal electroretinogram modifications after 1 year. Ophthalmology, 115 (2), 128–135.
Parry J., LanS., Luther M., Kequan Z., Yurawecz M.P., WhittakerP., Liangli Y., 2005. Fatty acid compositionand antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. Journal of the Science of Food and Agriculture, 53(3), 566–573.
Perry A., Rasmussen H., Johnson E.J., 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. Journal of Food Composition and Analysis, 22, 9–15.
Piccaglia R., Marotti M., Grandi S., 1998. Lutein and lutein ester content in different types of *Tagetes patula* and *Tagetes erecta.* Industrial Crops and Products, 8, 45–51.
Ribaya-Mercado J.D., Blumberg J.B., 2004. Lutein and Zeaxanthin and Their Potential Roles in Disease Prevention. Journal of the American College of Nutrition, 23(6), 567S-587S
Rice-Evans C.A., Samson J., Bramley P.M., Holloway D.E., 1997. Why do we expect carotenoids to be antioxidants *in vivo*? Free Radical Research, 26, 381–398.
Roca A., Shin K.J., Liu X., Simon M.I., Chen J., 2004. Comparative analysis of transcriptional profiles between two apoptotic pathways of light-induced retinal degeneration. Neuroscience, 129, 779–790.
Rodriguez-Bernaldo de Quiros A., Costa H.S., 2006. Analysis of carotenoids in vegetable and plasma samples: A review. Journal of Food Composition and Analysis, 19, 97–111.
Sajilata M.G., Singhai R.S., Kamat M.Y., 2008. The Carotenoid Pigment Zeaxanthin – A Review. Comprehensive Reviews in Food Science and Food Safety, 7, 29–49.
Seddon J.M., Reynolds R., Rosner B., 2010. Associations of smoking, body mass index, dietary lutein, and the LIPC genetic variant rs10468017 with advanced age-related macular degeneration. Molecular Vision, 16, 2412–2424.
Steinmetz K.A., Potter J.D., 1996. Vegetables, gruit, and cancer prevention: A review. Journal of the American Dietetic Association, 96, 1027–1039.
Stringham J.M., Bovier E.R., Wong J.C., Hammond B.R., 2010. The Influence of Dietary Lutein and Zeaxanthin on Visual Preformance. Journal of Food Science, 75(1), R24-R29.
Surai P.F., MacPherson A., Speake B.K., Sparks N.H., 2000. Designer egg evaluation in a controlled trial. European Journal of Clinical Nutrition, 54, 298–305.
Surai P.F., Speake B.K., Sparks N.H. C., 2001. Carotenoids in Avian Nutrition and Embryonic Development. 1.Absporption, Availability and Levels in Plasma and Egg Yolk. Journal of Poultry Science, 28, 1–27.
Tee E.S., 1992. Carotenoids and retinoids in human nutrition. Critical Reviews in Food Science and Nutrition, 31, 103–163
Thurnham D.I., 2007. Macular zeaxanthins and lutein – a review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease. Nutrition Reseach Reviews, 20, 163–179.

5

EGG YOLK OF GREENLEG PARTRIDGE LAYERS AS A SOURCE OF THE PHOSPHOLIPIDS

Introduction

The egg means the beginning of the life. It has all the substances needed to create new life: lipids, proteins, vitamins, minerals. Egg yolk is also an excellent natural emulsifier, widely used in food industry. These functional properties partly are shaped by the phospholipids contained in egg yolk. Approximately 50% of yolk is dry matter. After spray drying, the powder contains about 60% of lipids, which consist of neutral lipids (65%), phospholipids (31%), and cholesterol (3%). Pure egg yolk phospholipids have found their use in cosmetics and pharmaceutical applications, especially in emulsion and liposome formulation [Aro 2009].

Phospholipids belong to the group of amphipathic compounds built from alcohol chain linked by ester bonds with fatty acids in sn1 and sn2 position and the rest of phosphoric acid (V) esterified in sn3 position linked by ester bonds with choline in PC and with ethanolamine in PE.

They are found in all living cells, whether of animal and plant origin. In humans and in animals, the phospholipids are concentrated in the vital organs, such as the brain, liver, and kidney; in vegetables, their highest content was determined in the seeds, nuts, and grains [Bragagnolo 2003]. Phospholipids are participants in metabolic processes and major components of plasma membrane and organelle membranes that maintain the integrity of the cell or organelles by creating a semi-impermeable barrier from their outside environment. [Szuhaj 2005]. Phospholipids include: phosphatidylcholine (lecitin), phosphatidylethanolamine (cephalin), phosphatidylinositol and phosphatidylserine, rich in polyunsaturated fatty acids, essential for the proper functioning of the human body [Gładkowski 2009, Hayat 2009] (Fig. 1).

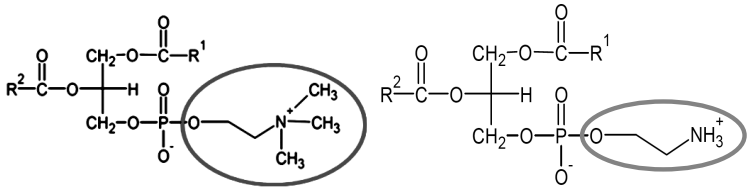


Fig. 1. Chemical structure of a phospholipid – phosphatidylcholine (lecitin, PC), phosphatidyloetanoloamine (cephalin, PE)

Biologically active polyunsaturated fatty acids (PUFA) of the omega–3 and omega-6 families are beneficial to human health [Gładkowski 2011, Hayat 2009]. PUFAs are components of cell membranes and precursors of other very significant cell ingredients like eicosanoids [Garcia-Rebollar 2008]. They have positive effect on the cardiovascular system by lowering cholesterol level, decreasing triacylglycerol synthesis, inhibiting platelet aggregation and lowering blood pressure [Sinanoglou 2011]. The PUFAs protect skin from atopic dermatitis, psoriasis, acne and skin allergies [Fekete 2010].

The omega-3 fatty acids: docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are orthomolecular, conditionally essential nutrients that enhance quality of life and lower the risk of premature death. They function exclusively via cell membranes, in which they are anchored by phospholipid molecules. DHA is proven essential to pre- and postnatal brain development, whereas EPA seems more influential on behavior and mood. Both, DHA and EPA generate neuroprotective metabolites [Parris 2007].

Egg yolk is one of the richest sources of omega-3 polyunsaturated acids, and their fatty acid composition can be easily changed by modifications in the feeding of laying hens [Shapira 2008, Hayat 2009]. Studies have showed that, by adding natural sources of omega-3 PUFAs to hen feed, a significant enrichment of egg yolk lipids with these acids is observed [Cherian 2008, Kouba 2011].

Recently Trziszka et al [2011] reported the application of Humokarbowit and Humobentofet enriched with fish oil and linseed oil for feeding Lohmann Brown laying hens. In phase III of the laying period, they observed an overall increase in the content of egg yolk fat and more valuable fatty acid profile of total lipids of egg yolk.

The difference in composition and content of phospholipids fraction isolated from enriched and not enriched Greenleg Partridge egg yolk is demonstrated. The enriched eggs were obtained by proper nutrition of hens.

Materials and methods

The eggs were obtained from "Tronina" firm. The control group of Greenleg Partridge hens was treated with standard compound feed stuffs. The experimental group was fed with supplemented fodder composed of, i.e. Humobentofet, linseed oil, seafood preparations rich in DHA, EPA and ALA.

Egg yolk was homogenized and subjected to fractionation. The egg yolk was stored at -80±1°C and then lyophilized. The lyophilisate was mixed with ethanol (1:3 w/v) and shaken, and the resulting mixture was centrifuged (15 000 rpm, 15 min.). The alcohol contained in the supernatant was distilled using a vacuum evaporator (p=140 mbar). Phospholipid precipitate obtained after the evaporation of alcohol was suspended in hexane and precipitated with cold acetone (4°C), (acetone, hexane ÷ 1/3 v/v). The acetone precipitation procedure was optimized earlier in laboratory [Gładkowski 2010].This procedure allowed to determine the purity of phospholipids (which consist residual amounts of TAG and cholesterol) expressed as the content of substances insoluble in acetone [Gładkowski et al. 2009, Gładkowski et al. 2011].

The phospholipid fatty acid profile assay (GC/MS):

The phospholipid fractions were dried and converted to fatty acids methyl esters (FAME) as follows: 50 mg of phospholipids were dissolved in 4 ml of 0.5 M methanolic NaOH solution and heated under reflux for 2 min. After that, 4 ml of BF_3 – MeOH complex was added and

the mixtures were heated again under reflux for next 2 min. After cooling, the mixtures were extracted with 6 ml of hexane and the organic layers were washed with saturated NaCl solution. Hexane extracts were dried over anhydrous magnesium sulphate and analyzed directly by gas chromatography.

Conditions of chromatographic analysis (GC/MS): gas chromatograph mass spectrometer 6890N coupled with Agilent GC5973 was used to determine the fatty acid profile. The column used – HP 5, the flow of carrier gas (He) at the level of 1.0 ml/min. Injector temperature was set at 230°C, and the detector at 240°C. Temperature program was established in the following time frame: 100°C / hold 0.5 min. than 3°C /min to 180°C, hold 17 min and 5°C /min to 210°C, hold 45min. The content of fatty acids in phospholipid fraction is presented in Figure 1.

The content of basic groups of phospholipids assay (HPLC):

The content of basic groups of phospholipid fractions was measured with High Performance Liquid Chromatography (HPLC). 10 mg samples were dissolved in 1 ml of isopropanol and analyzed directly by liquid chromatography.

Conditions of chromatographic analysis (HPLC): HPLC system Agilent HP1200 was used to define the content of phosphatidylocholine (PC) and phosphatidylethanolamine (PE) in the extracts obtained. For this purpose, 1% solution of extract of phospholipids in isopropyl alcohol was prepared and after filtration was subjected to chromatographic analysis. Eluent was prepared with acetonitrile, methanol and phosphoric acid (100/10/1.8 v/v/v) and the flow was set at 2.5 ml/min. The column LiChrospher ® 100 Diol 250x4.6 mm 5 µm thermostated at at 55°C. The content of basic groups of phospholipid fractions is presented in Figure 2.

The phospholipid fraction content assay

The phospholipid content (Ct) expressed the percentage of phospholipids in dry egg yolk (after acetone precipitation) (Fig. 3).

The acid value of phospholipid fraction

Acid value (AV) is one of the most characteristic values in lipid chemical analysis. It describes the mass of potassium hydroxide in milliters that is required to neutralize one gram of lipids (mg KOH/g of sample) [Nieuwenhuyzen 2008]. During the time triglycerides are converted into fatty acids and glycerol, causing an increase in acid number, so that value is also the a measure of freshness.

Statistical analysis

The resulting figures were compared using statistical methods, computer program STATISTICA Version 8.0 (Statsoft). The scheme involved a two-way ANOVA with Duncan test at the alpha 0.05.

Results and discussion

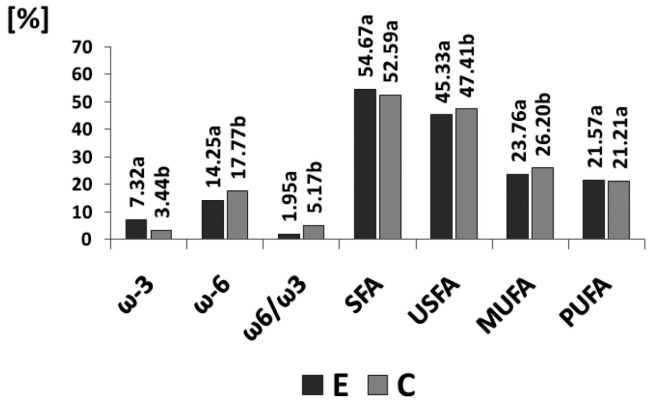
The phospholipids are the main structural component of biological membranes. The fatty acids built in phopsholipids have an important structural role in brain (DHA), are crucial for the brain growth (AA) and function (EPA). The egg yolk is one of the richest source of omega-3

polyunsaturated fatty acids, because of their composition it can be modified by feeding of laying hens [Hayat 2009, Shapira 2008]. It is possible to extract the egg phospholipid fraction from the hens kept in the ecological system.

The study allowed for the preparation of procedures for the isolation of phospholipids and assess their value.

Fatty acids profile

The phospholipids extracted from enriched eggs were characterized with high content of omega 3 fatty acids – 7.32% in experimental group and 3.44% in control one (Fig. 2). The lower amount of omega 6 fatty acids is observed in experimental group (14.25%) in compare to control one (17.77%).

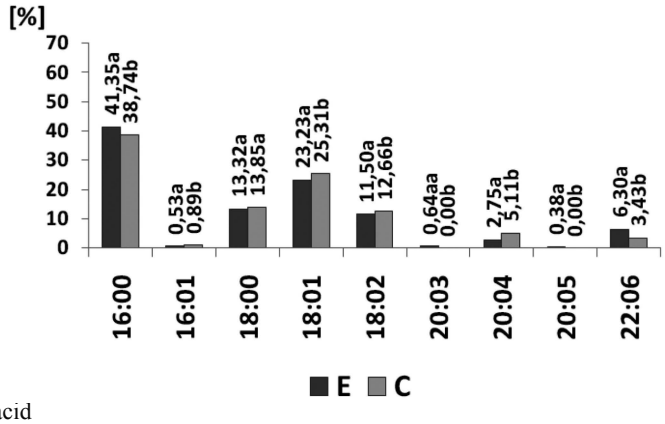


SFA – saturated fatty acids
USFA – unsaturated fatty acids
MUFA – monounsaturated fatty acids
PUFA – polyunsaturated fatty acids
ω-3 – omega-3 fatty acids
ω-6 – omega-6 fatty acids
ω-6/**ω**-3 – omega-6/omega-3 fatty acids ratio

Fig. 2. Fatty acids composition in phospholipid fraction from the experimental (E) and control (C) group of Greenleg Partridge egg yolk [%]

The omega-6 to omega-3 fatty acids ratio is very important for human health. The imbalance is responsible for many chronic heart conditions. Simopolous and De Meester [2009] concluded from anthropological, epidemiological and molecular-level studies that humans evolved with a diet of a ratio of omega-6/omega-3 fatty acids close to 1:1, whereas in today's western diets the ratio ranges from 15:1–17:1 [Simopoulos 2006, Simopoulos and De Meester 2009]. Samman [2009] in his research has observed the ratio 2,2. Gładkowski et al. [2011] has shown lower ratio in experimental group (2.3) than in control one (8.1) in Lohmann Brown eggs. The omega 6 to 3 ratio is 3 times lower in experimental group (1.95) than in control group (5.17).

EPA content is observed only in experimental group (0.38) (Fig. 3), and the DHA content in experimental group is two times higher (6.3) than in control one (3.43).



C16:0 palmitic acid
C18:0 stearic acid
C18:1 oleic acid **ω**-9 Δ^{9}
C18:2 linoleic acid LA 18:2 **ω**-9 $\Delta^{9,12}$
C18:3 α-linoleic acid ALA18:3 **ω**-3 $\Delta^{9,12,15}$
C20:3 eicosatrienoic acid 20:4 **ω**-6 $\Delta^{11,14,17}$
C20:4 arachidonic acid AA 20:4 **ω**-6 $\Delta^{5,8,11,14}$
C20:5 eicosapentaenoic acid EPA 20:5 **ω**-3 $\Delta^{5,8,11,14,17}$
C22:6 docosahexaenoic acid DHA 22:6 **ω**-3 $\Delta^{4,7,10,13,16,19}$
E – experimental group
C – control group
a,b – statistically consistent groups with the level of significance at p=0.05

Fig. 3. Fatty acids profile of egg yolk phospholipid fraction isolated from experimental (E) and control (C) group of Greenleg Partridge [%]

The production of PUFA enriched eggs were intensively studied in recent years, but the unfortunately the Greenleg Partridge eggs are not described as often as Lohmann Brown ones. Gładkowski et al. [2011] has analyzed phospholipids extracted from Lohmann Brown egg yolks and has shown that the content of DHA and **α-linolenic acid** (18:3) in experimental group (4.72%, 3.52%, respectively) was higher than in control one (2.68%; 0%), but EPA content was not observed at all. In 2009 Gładkowski in standard Greenleg Partridge eggs indicated high DHA amount in phospholipid fraction of egg yolk – 4.5% in cephalin and 1.3% in lecithin, but 0% of EPA and **α-linolenic acid content**. Samman [2009] has presented 2.05% of DHA and 4.52% of AA. This phospholipid fraction did not contain EPA.

Two main phospholipid fractions are phosphatidylcholine, (lecithin, PC) and phosphatidylethanolamine (cephalin, PE). The percentage difference of these fractions between experimental and control group was stastistically significant.The higher content of phosphatidylcholine (PC) (68%) and lower content of phosphatidylethanolamine (PE) (32%) was observed in experimental group (Fig. 4).

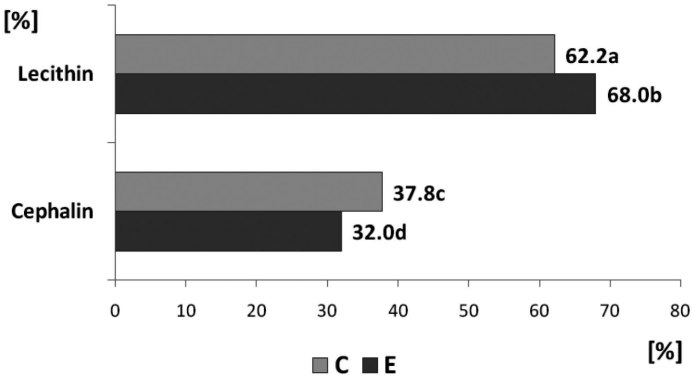


Fig. 4. The content of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the phospholipid fraction isolated from dry egg yolk [%]

The PC:PE ratio 3:1 was presented by Trziszka [2000] in phospholipid fraction, similarly to Huopalahti [2007], who indicate 2.5:1 ratio. Gładkowski et al. [2009] declared in Lohmann Brown phospholipids the ratio 8:1. In this research the ratio is much lower than in other studies – 2.1:1.

The percentage contents of phospolipid fraction in dry egg yolk (content, Ct) is shown in Fig. 5. The difference between experimental and control group was not statistically significant- both scores were close to 20.5%.

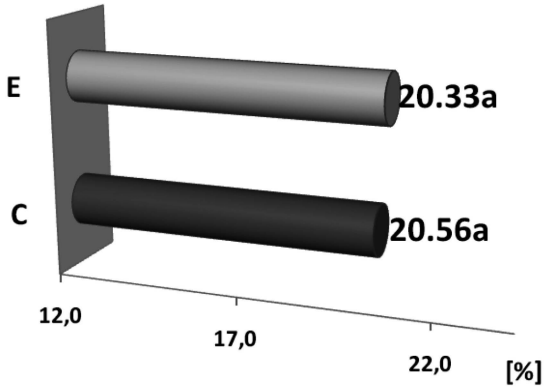


Fig. 5. The phospholipid content from dry egg yolk in experimental and control hens groups [%]

The level of phospholipid fraction in dry egg yolk is showed in data by Huopalahti [2007] – 20% in dry egg yolk. Trziszka [2000] also indicates 20% of phospholipid fraction.

The acid values differences between experimental and control groups of phospholipids was not stastistically significant. The acid value in experimental group was 24 mg/g and in control it was 24.5 mg/g. The acid value is defined by the National Soybean Processors Association [1986–1987] and Food Chemicals Codex [1996]. According to them, the acid values of phospholipids measured in this research confirm the good quality of extracted fractions (the quality limit is 34 mg/g) [Food Chemicals Codex, 4th ed., National Academy Press, Washington].

Results presented in this paper indicate that the supplementation of laying hen feeds with humic-fat preparations is an effective way of producing n-3 PUFA-enriched eggs with a balanced n-6/n-3 ratio. A significant decrease in n-6/n-3 ratio was achieved. Especially, the enrichment of egg yolk with n-3 PUFA is nutritionally valuable, because the production of lecithin from egg yolk, as a food supplement.

Conclusions

The study showed, a statistically significant effect of enrichment of feed given to laying hens on fatty acid profile labeled in the structure of phospholipids. Reduced fatty acid ratio n6/n3 increase the nutritional value of eggs and allows apply this kind of eggs by nutraceutica production. The experiment proof that in enriched eggs decrease level of cephalin and increase lecithin.

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References

Bragagnolo N., Rodriguez-Amaya D.B., 2003. Comparision of the cholesterol content of Brazilian chicken and quail eggs, Journal of Food Composition an Analysis, 16, 147–153.

Cherian G., 2008. Egg quality and yolk polyunsaturated fatty acid status in relation to broiler breeder hen age and dietary n-3 oils., Poultry Science, 87, 1131–1137.

Fekete K., Marosvolgyi T., Jakobik V., Decsi T., 2009. Methods of assesment of n-3 long chain polyunsaturated fatty acid status in humans: a systematic review, Am. J. Clin. Nutr., 89, 2070S–84S.

Food Chemicals Codex, 4th ed., 1996. National Academy Press, Washington, D.C., 220–221.

Garcia-Rebollar P., Cachaldora P., Alvarez C., De Blas C., Mendes J., 2008. Effect of the combined supplementation of diets with increasing levels of fish and linsees oils on yolk fat composition and senioral quality of eggs in laying hens, Animal Feed Science and Technology, 140, 337–348.

Gładkowski W., Chojnacka A., Kiełbowicz G., Pisarski B., Trziszka T., Wawrzeńczyk C., 2009. Charakterystyka frakcji fosfolipidowych izolowanych z żółtek jaj pochodzących od kur Lohmann Brown i zielononóżki kuropatwianej, Przemysł Chemiczny, 88/5.

Gładkowski W., Kiełbowicz G., Chojnacka A., Gil M., Trziszka T., Dobrzański Z., Wawrzeńczyk C., 2011. Fatty acid composition of egg yolk phospholipid fractions following feed supplementation of Lohmann Brown hens with humic-fat preparations, Food Chemistry, 126, 1013–1018.

Gładkowski W., Kiełbowicz G., Chojnacka A., Pisarski B., Wawrzeńczyk Cz., 2010. Isolation of phospholipids from lyophilized hen egg yolks, Przemysł Chemiczny, 89 (4).

Hayat Z., Cherian G., Pasha T.N., Khattak F.M., Jabbar M.A., 2009. Efect of feeding flax and two types of antioxidants on egg production, egg quality, and lipid composition of eggs., J., Appl. Poult. Res., 18 541–551.

Huopalahti R., 2007. Bioactive egg compounds Springer.
Kouba M., Mourot J., 2011. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids, Biochimie, 93, 13–17.
Parris M., Kidd, 2007. Omega-3 DHA end EPA for Cognition, Behavior, and Mood: Clinical Findings and Structural-Functional Synergies with Cell Membrane Phospholipids, Alternative Medicine Review, Volume 12, Number 3, 207–227.
Samman S., Kung F.P., Carter M.L., Foster M.J., Ahmad Z.I., Phuyal J.L., Petocz P., 2009. Fatty acid composition of certified oranic, convential and omega-3 eggs, Food Chemistry, 116, 911–914.
Shapira N., Weill P, Loewenbach R., 2008. Egg fortification with n-3 polyunsaturated fatty acids (PUFA): nutritional benefits versus high n-6 PUFA western diets, and consumer acceptance, Isr Med. Assoc. J. Apr., 10 (4), 262–5.
Simopolous A.P., 2009. Evolutionary aspects of the dietary omega-6: omega-3 fatty acid ratio: medical implications, World Rev Nutr Diet., 100, 1–21.
Simopoulos A.P, De Meester F., 2009. A balanced omega-6/omega-3 fatty acid ratio, cholesterol and coronary heart disease, Karger Publishers.
Simopoulos AP., 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases., Biomed Pharmacother, 60, 502–507.
Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedical Pharmacotherapy, 56, 365–379.
Sinanoglou V.J., Strati I.F., Miniadis-Meimaroglou S., 2011. Lipid, fatty acid and carotenoid of edible egg yolks from avian spacies: a comparative study, Food Chemistry, 124, 971–977.
Trziszka T., 2000. Budowa i skład chemiczny jaja, [w:] Jajczarstwo, nauka, technologia, praktyka. Praca zbiorowa, Wydawnictwo AR we Wrocławiu.
Trziszka T., Dobrzański Z., Kaźmierska M., Tronina Ł., Skiba, M., 2011. The effects of supplementation of dietary humic preparations on the egg quality parameters in Lohmann Brown hens. Archiv fur Geflugelk, 75 (2), 84–90.
Trziszka T., Dobrzański Z., 2004. Wykorzystanie surowca jajczarskiego do produkcji nutraceutyków i preparatów biomedycznych, cz. I., Polskie Drobiarstwo, 4, 2–4.
Trziszka T., Dobrzański Z., 2004. Wykorzystanie surowca jajczarskiego do produkcji nutraceutyków i preparatów biomedycznych, cz. II., Polskie Drobiarstwo, 5, 10–11.
Willem van Nieuwenhuyzen and Mabel C. Tomás, 2008. Update on vegetable lecithin and phospholipid technologies, Eur. J. Lipid Sci. Technol., 110, 472–486.
Food Chemicals Codex, 4th ed., 1996. National Academy Press, Washington, D.C., 220–221.

6

HEALTH PROMOTING INGREDIENTS OF THE CHICKEN EGG

Introduction

Since ancient times the chicken egg is a valuable food for humans. On the one hand it contains many nutrients which can cover human requirements and on the other hand it contains many ingredients with health-promoting effects. The last point may be less aware to the consumer. The basic nutrients like protein (amino acids) and energy (fatty acids) are located in the yolk and are required by the embryo for growth. Further specific ingredients like minerals, trace minerals and vitamins are also important factors in the development of the embryo.

In general, the chicken egg can be separated in three parts which differ distinctly both in their composition, in their role in embryogenesis and in their meaning for human nutrition. The egg yolk supplies the nutrient for the growing embryo, the albumen is the protective barrier against pathogenic microorganisms and the shell is packing material and regulates water and gas exchange. For human nutrition the shell does not play any role. Ingredients important for human nutrition are located in the albumen and in the yolk. The amino acid profile of the chicken egg matches exactly human requirements for amino acids, therefore, the biological value of 100 was assigned to the egg. Furthermore, egg yolk contains considerable amounts of essential polyunsaturated fatty acids (PUFA) which are precursors of biological effectors, and vitamins, minerals, trace elements and further components like lecithin and globulins with nutritive physiological meaning. On the other side egg yolk cholesterol and some egg white proteins may also bear health risks for some humans.

Due to its nutritive value the chicken egg is an important food for humans in most countries of the World. Egg consumption varies considerably between countries both due to consumption habits and to production facilities. In Europe in 2009 average annual per capita egg consumption amounted to 13.5 kg (EU-27) and to 12.7 kg in Poland and in Germany, respectively [MEG 2010]. In the World egg production and consumption is continuously increasing, whereas, in Europe the level is mainly constant. The highest increase in production and consumption is observed in China. Increasing egg consumption is mainly reflecting the increase in prosperity and the demand for an improved nutrition of the people. In contrast, in Australia, in North American and in European countries the consumer is more and more interested in food not only covering nutritive physiological demands but also delivering health promoting substances. The chicken egg is a suitable tool in both providing basic nutrients and health promoting substances. The last aspect was picked up for the development of functional eggs. Per definition functional eggs contain specific ingredients in a higher amount resulting in additional benefits for human health and well-being. The increase (enrichment) of ingredients is achieved by applying specific nutrition programs to the laying hens.

The objective of the present paper is to summarize the nutritive physiological meaning and the health promoting properties of the chicken egg, to show specific benefits of functional eggs and to touch also probable hazards arising from egg consumption.

Composition of chicken egg

A standard egg of 60 g contains 24–28% yolk and 62–66% egg white [Grashorn 2010]. The proportion of the shell varies between 9 and 11%. Yolk proportion is increasing with the age of the hen in the expense of egg white. The yolk contains more protein than egg white and is the only source of fat. In fresh eggs egg white is like gelatin and is turning to liquid during storage. This is caused by resolution of the complex between ovomucin and lysozyme due to the increase of pH [Ternes et al. 1994]. The egg yolk consists of several circular layers of yolk material which have a different composition and the germ disk is found on top of the yolk.

Nutritive value

The dry matter content of the egg is about 34%. In the whole egg the fat is about 10%, whereas, in the yolk it is about 33%. The protein content is 12% and 17% in the albumen and in the yolk, respectively. The shell is the main source of minerals (Tab. 1).

Table 1

Nutrient composition of the egg [after Worm 1988]

	Whole egg	**Shell**	**Albumen**	**Yolk**
Weight [g]	58	6	33	19
Water [%]	65.6	1.6	87.9	48.7
Dry matter [%]	34.4	98.4	12.1	51.3
Protein [%]	12.1	3.3	10.6	16.6
Fat [%]	10.5	Traces	Traces	32.6
Carbohydrates [%]	0.9	–	0.9	1.0
Minerals [%]	10.9	95.1	0.6	1.1

The amino acids profile of the protein directly reflects human requirements. Therefore, the chicken egg was assigned the biological significance of 100 [D-A-CH 2000]. One egg of 60 g covers 50–60% of human amino acids requirements (Tab. 2). Lysine and threonine are the dominating amino acids. The bioavailability of protein and amino acids is about 65% for raw and 95% for cooked eggs, respectively [Seuss-Baum 2007].

Table 2

Contents of essential amino acids in the edible egg
[mg/egg; egg weight 60 g, edible part 54 g; Seuss-Baum 2007]

	Whole egg	**Albumen**	**Yolk**
Isoleucine	130	221	157
Leucine	302	470	356
Lysine	475	751	562
Methionine+Cystine	356	632	443
Phenylalanine+ Tyrosine	362	356	346
Threonine	551	767	621
Tryptophan	254	850	319
Valine	92	130	103

The egg is also a rich source of vitamins with most vitamins found in the yolk. Panthotenic acid, vitamin E (α-tocopherol), vitamin B_1 and B_2 are the predominant vitamins. The vitamin content of the egg can be influenced by feeding of hens, especially for lipophilic vitamins like α-tocopherol. The chicken egg can cover up to 30% of recommended daily intake of vitamins in humans (Tab. 3). There are big differences in the bioavailability of different vitamins in the egg. Low availability is reported for vitamin B_{12}, biotin and partly vitamin E [Seuss-Baum 2007], whereas, high ones for A, B_2 and B_6. As for proteins, bioavailability of vitamins may be improved by heat treatment (cooking) of eggs [Seuss-Baum 2007].

Table 3

Contents of selected vitamins in the edible egg and% of covering human RDI
[recommended daily intake; egg weight 60 g; D-A-CH 2000; Heseker and Heseker, 1999]

Vitamin	**Unit**	**Content/egg**	**RDI**	**% of RDI**
Vit. B_2 (Riboflavin)	[µg]	162	1 300	12
Vit. B_6 (Pyrodoxin)	[µg]	65	1 350	5
Vit. B_{12}	[µg]	1	3	33
Pantothenic acid	[µg]	1 200	6 000	20
Biotin	[µg]	10	45	22
Vit. K	[µg]	25	70	36
Vit. A (Retinol)	[µg]	160	900	18
Vit. E (α-Tocopherol)	[µg]	700	13 000	5

Further nutrients to be mentioned are minerals and trace elements. Especially trace elements are of interest as they are involved in many metabolic processes as catalysts. Important trace elements are iron, iodine and selenium [Tab. 4]. The egg contributes also significantly to the covering of human requirements. Bioavailability of trace elements is higher in the natural egg than in the food [Seuss-Baum 2007].

Table 4.

Contents of selected minerals and trace elements in the edible egg and% of covering human RDI
[egg weight 60 g; D-A-CH 2000; Heseker and Heseker 1999]

	Unit	**Content/egg**	**RDI**	**RDI [%]**
Calcium	[mg]	30	1 000	3
Phosphorus	[mg]	90	700	13
Magnesium	[mg]	6,5	325	2
Sodium	[mg]	80	550	15
Iron	[mg]	1.8	12.5	14
Iodine	[µg]	22	200	11
Selenium	[µg]	15	45	33
Zinc	[µg]	800	8 500	9

The most interesting group of nutrients is the fatty acids as they are important precursors of several biological modulators. The fatty acids profile of the egg yolk directly reflects the fatty acids profile of the laying hens' diet. In general, eggs are rich in polyunsaturated fatty acids (PUFA) which are essential to humans (Tab. 5). The relation between omega-6 (n-6) and omega-3 (n-3) fatty acids amounts to 7.1:1 in a standard egg which corresponds fairly well to the relation recommended by D-A-CH [2000]. In contrast to the relation of n-6/n-3 no

RDIs are given for the contents of EPA and/or DHA by D-A-CH [2000] which are of specific interest for their health promoting effects. This point will be raised again in the next chapter.

Table 5

Fatty acids profiles of standard egg [egg weight 60 g; soybean oil in diet; Steinhilber 2003]

Fatty acid	Abbreviation	Formula	mg/egg
Palmitic acid	PA	C16:0	106
Oleic acid	OA	C18:1	1 920
Linoleic acid	LA	C18:2n6	1 064
Linolenic acid	LNA	C18:3n3	71
Arachidonic acid	AA	C20:4n6	112
Eicosapentaenoic acid	EPA	C20:5n3	Traces
Docosahexaenoic acid	DHA	C22:6n3	94
n-6/n-3			7.1

The last group of nutrients is the carotenoids which are responsible for the typical yolk coloration. Carotenoids are supplemented to laying hens' diets mainly for their pigmenting ability, but they are also useful in the human metabolism as antioxidants and, partly, as vitamin A precursors. Also, this point will be raised again in the next chapter.

Health-promoting ingredients

Egg white

Most health-promoting ingredients are found in the egg yolk, but there are several albumen proteins and peptides which are of some interest. Most ingredients of the albumen show clear antimicrobial activity and by this protect the content of the egg (and the developing embryo) from potentially harmful micro organisms [Ternes et al. 1994]. Ovalbumin is the dominating protein in egg white and belongs to the family of serpins [Davis and Reeves 2002]. The proteins of this family act as proteinase inhibitors and by this control coagulation of enzymes. Lysozyme and cystatine are further proteins with strong antimicrobial activities. A quite interesting group is the bioactive peptides. Ovokinin is a vasorelaxing peptide which can lower blood pressure [Fujita et al. 1995]. The active form of these proteins and peptides can be achieved by hydrolysis [Lopez-Fandino et al. 2007]. By this process a peptide with angiotensin-converting enzyme (ACE) inhibition properties is built which is able to control cardiovascular diseases, especially hypertension [Davalos et al. 2004]. The combination of ACE inhibition and strong antioxidative properties make egg white peptides an efficient tool for the control of hypertension in humans. Another interesting group is the globulins which show immune-stimulating properties besides anti-oxidative capacity [Narahari 2003a]. There are some more substances included in albumen which can reduce multiplication of cancer-inducing viruses. For an effective application of these ingredients in human nutrition isolation and purification is necessary.

Egg albumen acts further as an antidot by counteracting some toxins and irritants. The albumen can protect the mucous membranes in the stomach and in the intestine. Furthermore, egg white proteins have a high water holding capacity, by this counteracting enteritis. Therefore, the egg is believed to be a good drug against gastritis, enteritis, diarrhea, dysentery and dehydration [Narahari 2003a].

Yolk

The yolk contains several lipid fractions showing health-promoting effects. As indicated in the chapter on nutritive value of the egg the fatty acids have be mentioned as one quite important class of nutrients for human consumption. The basic fatty acids of PUFA are essential to humans and have to be ingested with the food. Linoleic (LA) and linolenic (LNA) acids are the basic fatty acids of the n-6 and n-3 groups, respectively. Both fatty acids are transformed to longer and more unsaturated types in the liver by using the same enzymes [Doenecke et al. 2005]. LA is the precursor of AA and LNA of EPA which are again the precursors of the eicosanoids. Eicosanoids are biological modulators like prostaglandins, leukotrienes and thromboxanes. Eicosanoids built from AA favor cardiovascular diseases, inflammation processes, cancer and loss of cognition [Farrell 1995], whereas, eicosanoids built from EPA counteract these effects. Positive effects on brain function and visual acuity are reported [Davis and Reeves, 2002]. For an optimal regulation of this feedback system the relation between n-6 and n-3 derived eicosanoids is importance. As the available amounts of AA and EPA depend on the amounts of these fatty acids in the food it is of importance to adjust an optimal relation between n-6 and n-3 in the food. This is considered by the D-A-CH reference values [2000] which recommend a relation of about 5.5:1 (n-6/n-3). As due to consumer behavior in many industrialized countries the relation between n-6 and n-3 distinctly exceeds the recommended relation due to high intake of n-6 fatty acids with the food. Therefore, eggs enriched with omega-3 fatty acids have been developed and are marketed as functional eggs. These eggs have significantly higher contents of n-3 fatty acids and show proven positive effects on human health and well-being (Tab. 6). The content of DHA in the enriched eggs is two to three times higher than in the conventional egg. Based on the suggestion of Simopoulos et al. [2000] that the daily intake of EPA and DHA should be at least 650 mg one omega-egg can cover 14–33% of this recommended intake. But, it has to be considered that this recommendation is based on high intake levels of n-6 fatty acids.

Table 6

Contents of DHA in enriched egg brands (mg/54 g edible egg)

Egg name	**DHA**	**Reference**
Conventional egg	94	Steinhilber [2003]
Designer egg	175	Narahari [2003b]
Omega egg	132	Omega-DHA-Company
Columbus egg	100	De Meester et al. [2000]
'Fish' egg	215	Van Elswyck et al. [1995]
Vi-Omega-3 egg	120	Yannakopoulos [2007]

Another fatty acid which has specific health promoting properties is conjugated linoleic acid (CLA). It is quite well documented that CLA acts anti-oxidative, anti-cancerous, anti-atherosclerotic and reduces both fatness and plasma-cholesterol [Aletor et al. 2003]. The main source of CLA is milk products. The RDI for CLA is 0.1% of daily food intake, i.e. 2–2.4 g/day. According to Shang et al. [2004] the content of CLA can be increased to 1.1 g/egg when the feed is supplemented with 6% CLA. Thus, one egg can cover 50% of the RDI.

Lecithin is a phospholipid fraction of egg yolk and is a well known emulsifier. Lecithin (phosphatidylcholin) comprises about 70% of phospholipids. In egg yolk lecithin is com-

bined with long-chain PUFA, mainly AA and DHA. In contrast to other egg ingredients lecithin is well tolerated and generally recognized as safe (GRAS) [Rossi 2007]. Cholin is a precursor of the neurotransmitter acetylcholin and has in combination with the high content of n-3 fatty acids positive effects against age-related alterations of memory and learning [Rossi 2007]. Furthermore, phosphatidylcholin is transformed to phosphatidylserin which improves brain functioning in Alzheimer's patients. Currently, for this purpose mainly lecithin from soy-beans is used which is less effective.

Immunglobulins are a further interesting group of ingredients which may be used for immunotherapy and immunoprophylaxis. Egg yolk is rich in immunglobulin Y (IgY). One hen can produce 17–35 g of total IgY antibodys per year of which 1 to 10% can be antigen-specific [Schade et al. 2007]. IgY has already been used effectively in treatment of intestinal infections in children, for protection against *Helicobacter pylori* (proposed cause of gastric ulcer), treatment of colitis and celiac diseases, treatment of cystic fibrosis, treatment of poisoning and as prophylaxis in dental caries [Schade et al. 2007].

Further interesting ingredients of the yolk are lipoprotein YLP-p17,5 which acts as a growth stimulator in infants, salicylic acid which has anti-inflammatory effects, taurin which prohibits the formation of plagues in atherosclerosis, and chromium which reduces plasma cholesterol and blood glucose, and improves insulin secretion.

Oxidation is a normal process in tissues for producing energy for metabolic processes. Lipids, mainly fatty acids, are the major source for oxidative processes [Doenecke et al. 2005]. Oxidation requires oxygen and is enhanced by temperature. Besides the provision of energy several metabolites are built which may be harmful to the tissues like peroxids and free radicals. Therefore, living tissues have a sophisticated defense system to control and neutralize these molecules [Surai 2003]. The first level of the oxidative defense is the control of the building of peroxids and free radicals and the second one is the scavenging and neutralizing of these molecules, what is done by antioxidants. Due to the fact that the chicken egg provides the environment for the developing embryo it contains many antioxidants to protect the embryo against peroxids and free radicals coming from the metabolic processes. Although, egg white contains ovotransferrin, an iron-binding protein with a strong antioxidative capacity, most antioxidants are located in the yolk, like e.g. phosvitin, α-tocopherol (vitamin E), and carotenoids.

Phosvitin comprises about 4% of the yolk and has strong iron-binding capacity. About 95% of iron in the egg yolk is bound to phosvitin. Due to the strong iron-binding capacity of phosvitin this yolk protein is an efficient antioxidant [Guerin-Dubiard et al. 2007, Ternes et al. 1994].

The egg yolk contains considerable amounts of vitamin E which is easily transferred from the diet to the yolk by lipoprotein-rich triglycerides. Bioavailability of vitamin E from egg yolk ranges between 15 and 65% [Seuss-Baum 2007]. Increasing the amount of vitamin E in the yolk is especially indicated for n-3 fatty acids enriched eggs to combat the higher liability for oxidative processes.

The role of carotenoids as antioxidants is less considered. Like fatty acids carotenoids in egg yolk directly reflect the content of these substances in the diet of the hen. The carotenoid content of the egg yolk varies between 500 und 800 µg/egg depending on color intensity (Tab. 7). In general, content of carotenoids does not vary between organic and conventional eggs [Schlatterer and Breithaupt 2006]. Bioavailability of carotenoids varies largely between 10 and 60% [Seuss-Baum 2007].

Table 7

Contents of carotenoids in fresh eggs
(2.5 mg/kg native xanthophylls, 3 mg/kg ester of β-apo-8-carotenoic acid,
4 mg/kg canthaxanthin; value 13 of DSM yolk color fan)

Pigments	[µg/egg]
Lutein	37
Zeaxanthin	6
Ester of β-apo-8-carotenoic acid	205
Canthaxanthin	206
Total pigments	454

Ingredients with potential health risks

Some egg white proteins besides cholesterol may bear some risks for consumers. Like in milk (lactose) some globulins from egg white (e.g. lysozyme) cause heavy allergic reactions, especially in children. The reason may be an abnormal reaction of the mucosal immune system towards dietary antigens [Mine and Yang 2007]. The highest allergenicity is reported for lysozyme, followed by ovomucin, ovalbumin and ovomucoid [Mine 2003]. As there are no solutions for people suffering from allergenic reactions to egg proteins affected consumers have to avoid the intake of eggs [Mine and Yang 2007].

Cholesterol is probably the ingredient of the egg with the most negative image. In earlier studies it was found that the cholesterol content in the food influences blood cholesterol level in humans and that there exists a correlation between cholesterol intake and the incidence of artheriosclerosis. This led to a condemnation of eggs in human food. Although, it was proven meanwhile that dietary cholesterol does not really influence blood cholesterol in humans and that there does not exist a proven relationship between dietary cholesterol and the incidence of cardio-vascular diseases some human nutritionists still recommend to have a low egg consumption. In the USA 'cholesterol-free' food is still a fast seller. Nonetheless, it is generally acknowledged that cholesterol is an important precursor of many biological regulators like hormones, bile acids and vitamins and is a constituent of cell membranes and tallow which functions as protection of the skin [Ternes et al. 1994]. Furthermore, it is proven that dietary cholesterol content does not increase blood content in healthy humans considerably [Seuss--Baum 2007]. When assessing the blood cholesterol level of humans it has to be considered that the relation between HDL and LDL/VLDL cholesterol is of interest rather than the total cholesterol content. Another interesting point in dealing with cholesterol content of eggs is the fact that the content of eggs produced from today's laying hens is distinctly lower than some decades ago (Tab. 8). Today, one egg contains only 70% of the 1970s' egg.

Table 8
Annual egg production, yolk proportion and cholesterol content of the egg today and 40 years ago [white shelled eggs; Ternes et al. 1994, Grashorn 2010]

	1970	**2010**
Egg production [pieces/a]	250	300–320
Yolk proportion [%]	32	26–28
Cholesterol [mg]	270	190

According to the recommended daily intake (RDI) of cholesterol in humans of 300 mg [D-A-CH 2000], one egg supplies about 63% of this intake. But, it has to be considered that healthy consumers may have higher intakes.

Eggs as nutraceuticals

The high content of healthy and health-promoting substances in the chicken egg has stimulated attempts to use the egg as a bioreactor for the production of the desired substances. This is different from the idea of the functional egg which is intended as a complete food for humans with additional, positive effects. The nutraceutic egg is used to produce the desired substances which are extracted, concentrated and purified for further use in food processing [Aro 2007]. This principle is already applied for producing lysozyme, cystatin and lecithin and may be extended to further bioactive peptides. But, this is a more industrial approach of using beneficial substances of the egg.

Conclusions

In conclusion, the 'normal' and the 'functional' (enriched) chicken egg is a rich source of nutrients and of health-promoting substances for human nutrition, although the existence of allergenic proteins and cholesterol may exclude it for some consumers. Some ingredients also have remedy effects which predestined the chicken eggs to be incorporated in the Ayurveda concept. The use of eggs as bioreactor for producing health-promoting substances to be used in medicine and feed processing is a future issue which will gain further interest.

References

Aro H., 2007. Extraction of several egg compounds at a pilot scale. in Huopalathi R, López-Fandino R, Anton M, Schade R: Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.

Aletor V.A., Eder K., Becker K., Paulicks B.R., Roth F.X., Roth-Maier D.A., 2003. The effects of conjugated linoleic acids or an a-glucosidase inhibitor on tissue lipid concentrations and fatty acid composition of broiler chicks fed a low-protein diet. Poultry Science, 82, 796–804.

D-A-CH, 2000. Referenzwerte für die Nährstoffzufuhr. 1st edition, Umschau Verlag Frankfurt.

Dávalos A., Miguel M., Bartolomé B., Lopez-Fandino R., 2004. Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. Journal of Food Protein 67, 1939–1944.

Davis C., Reeves R., 2002. High value opportunities from the chicken egg. A report for the Rural Industries Research and Development Corporation. RIRDC Publication No 02/094.
De Meester F., Lignian J., Phillipin L., Thiry J.F., Dejardin P., Ramirez P., Remacle C., Erpicum T., Stannard J., Wijnia J., Ohi M., Sim S.S., Shapira N., Merkle J.A., Ball H.R., 2000. Egg yolk phospholipids as a balanced dietary source of vitamin F. XXI World Poultry Congress, Montreal, Canada.
Doenecke D., Koolman J., Fuchs G., Gerok W., 2005. Karlsons Biochemie und Pathobiochemie. Thieme Verlag Stuttgart.
Farrell D.J., 1995. The hearty egg is good for you. World Poultry Misset, 11 (4), 27–29.
Fujita H., Usui H., Kurahashi K., Yoshikawa M., 1995. Isolation and characterization of ovokinin, a bradykinin B1 agonist peptide derived from ovalbumin. Peptides, 16, 785–790.
Grashorn M.A., 2010. Faustzahlen zur Eiqualität. in Geflügeljahrbuch 2011. Verlag Eugen Ulmer Stuttgart, 235–249.
Guerin-Dubiard C., Castellani O., Anton M., 2007. Egg compounds with antioxidant and mineral binding properties, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R.: Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.
Hargis P.S., Van Elswyk M.E., 1993. Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. World's Poultry Science Journal, 49, 251–264.
Heseker B., Heseker H., 1999. Nährstoffe in Lebensmitteln. Umschau-Zeitschrift-Verlag Frankfurt a.M.
Lopez-Fandino R., Recio I., Ramos M., 2007. Egg-protein-derived peptides with antihypertension activity, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R.: Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.
MEG, 2009. Marktbilanz Eier und Geflügel 2010. Verlag Eugen Ulmer Stuttgart.
Mine Y., Yang M., 2007. Concepts of hyperallergenicity, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R.: Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.
Mine Y., Zhang J.W., 2002. Comparative studies on antigenicity and allergenicity of native and denaturated egg white proteins. Journal of Agriculture and Food Chemistry, 50, 2679–2683.
Narahari D., 2003a. Health-promoting and therapeutic uses of egg. Poultry International, 42(10), 45–47.
Narahari D., 2003b. Production of health promoting functional eggs. 10th European Symposium on the Quality of Eggs and Egg Products, St. Brieuc, France.
Rossi M., 2007. Use of lecithin and lecithin fractions, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R., Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.
Schade R., Zhang X.Y., Tersolo H.R., 2007. Use of IgY antibodies in human and veterinary medicine, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R., Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.
Schlatterer J., Breithaupt D.E., 2006. Xanthophylls in commercial egg yolks: quantification and identification by HPLC and LC-(APC)MS using a C30 phase. Journal of Agriculture and Food Chemistry, 54, 2267–2273.
Seuss-Baum I., 2007. Nutritional evaluation of egg compounds, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R.: Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.
Shang X.G., Wang F.L., Li D.F., Yin J.D., Li J.Y., 2004. Effects of dietary conjugated linoleic acid on the productivity of laying hens and egg quality during refrigerated storage. Poultry Science, 83, 1688–1695.
Simopoulos A.P., 2000. Human requirements for n-3 polyunsaturated fatty acids. Poultry Science, 79, 961–970.
Steinhilber S.: Einfluss von genetischem Typ, Legeabschnitt und Futterfett auf die Anreicherung von Hühnereiern mit Omega-3-Fettsäuren und die Auswirkungen auf die Produktqualität. PhD thesis University Hohenheim, 2003.

Surai P.F., 2003. Natural antioxidants in avian nutrition and reproduction. Nottingham University Press, Nottingham, UK.
Ternes W., Acker L., Scholtyssek S., 1994. Ei und Eiprodukte. Paul Parey Verlag Stuttgart.
Van Elswyck M., 1994. Looking ahead: will eggs become a dietary alternative to fish? Poultry International, 33(14), 82–88.
Worm N., 1988. Die ernährungsphysiologische Bedeutung des Eies. notabene medici, (9), 510.
Yannakopoulos A.L., 2007. Egg enrichment in omega-3 fatty acids, [in:] Huopalathi R., López-Fandino R., Anton M., Schade R.: Bioactive egg compounds. Springer-Verlag Berlin Heidelberg.

CHAPTER 2

QUALITY AND SAFETY IN FUNCTIONAL FOOD PRODUCTION

1

QUALITY AND SAFETY OF THE ORGANICALLY PRODUCED FOOD

Introduction

Organic methods in farming are considered as environment friendly, mainly due to a fundamental principle of harmonious cooperation with the nature and the lack of chemization. There is already a lot of evidence that the condition of environment, soil and groundwater is improving as a result of organic farming implementation [Haas et al. 2000]. Organic farming is also regarded as the system improving the crops quality. Consumers expect from the organic products a higher health and nutritional quality, whereas there are many studies comparing the crops quality obtained from organic system and others, such as integrated or conventional ones. The regulations which specify the conditions of organic plant and animal production are very strict, and adhering to them should result in high crops quality. A similar situation concerns organic food processing. Although currently conventional processing allows several hundreds of different type of food additives (colourings, fixing agents, improvers, etc.), organic processing admits only several dozens of such additives and all of them belong to natural substances. It puts the organic food producers in particularly difficult position – they have to aim at preserving product durability without the use of chemical agents. However, it is fundamentally important for health of consumers who search for healthy food more often.

The production by the means of organic methods is regulated in Europe by the Council Regulation (EC) No. 834/2007 as of June 28, 2007 on organic production and marking of organic products, repealing the Regulation (EEC) No. 2092/91, and the Commission Regulation (EC) No. 889/2008 as of September 5, 2008 establishing particular principles of implementing the Regulation (EC) No. 834/2007 on organic production and marking of organic products with regard to organic production, marking and control. Quoting the Regulation No. 834/2007, the organic production is defined as 'an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes'. Moreover 'organic farming should primarily rely on renewable resources within locally organized agricultural systems. In order to minimize the use of non-renewable resources, wastes and by-products of plant and animal origin should be recycled to return nutrients to the land.'

Currently, organic food is becoming more and more popular all over the world, and the European organic products market has been developing very intensively since the early '90. According to the report on organic farming, prepared by the Swiss FiBL Institute – the Research Institute of Organic Agriculture [FiBL 2009], in 2008 the value of the European organic food market amounted to 18 billion euros and still has a vast and unused potential. An unquestion-

able leader on the organic food market is Germany with the outlet of 5.58 billion euros. The organic food market is very well-developed in the UK, France and Italy as well [FiBL 2009].

Sudden growth of the organic products market results from, among others, decreasing consumers' confidence in the conventional food dominating on the market, produced by intensive methods, with the use of synthetic fertilisers, chemical agents for plants protection and growth regulators, and concerning processing – the use of preservatives, synthetic flavouring and aromatic substances. An intensive farming system has a huge negative impact not only on environment, but also on the food quality and safety. The recent crisis concerning the food safety on different stages of the whole agricultural and food chain has induced consumers to look for safer and more authentic food, contributing to the increase in the organic products demand as well. The last decade famous food scandals (e.g. BSE, FMD, and food contamination by dioxins or bacteria causing food poisoning) have resulted in the consumers' turn toward safer and better controlled production methods, which organic production still belongs to.

Under the Regulation No. 834/2007, an organic product must be marked by an identification number (code) and the European organic farming logo (the number and logo are attached after a certification process). The certification refers to agricultural farms and companies, but the Regulation does not impose an obligation of controlling a product's origin on itself. The Regulation No. 834/207 establishes that the aim of organic production methods is to receive high quality food products. However, the Regulation does not specify a definition of high quality.

The quality of food products is a very complex issue and a subject of many debates, the result of which are various definitions of this term. A definition of food quality is still developing and changing. At first, the interest mainly concerned the quality represented by quantity/measurable parameters, and now the growing attention is being paid to comprehensive/holistic approach towards the quality problem. 'Holistic model of the quality assessment' was adopted by Meier-Ploeger and Vogtmann [1991]; according to the justification, while conducting the assessment of the organic food quality there should be considered its all aspects and possible viewpoints, applying (also corresponding to consumer's expectations which should be met by a high quality organic product). Ecological criteria of the food quality are divided into analytical and holistic criteria groups. First of them have long been known, however, the second group is significantly younger and connected with development of organic awareness and farming. The analytical criteria group includes: technological, nutritional and sensory values, whereas the holistic criteria group contains: authenticity, biological value, ethical aspects and holistic methods of the food quality assessment.

Technological value. A technological value of food products refers to their distinctive features in the light of requirements of different interest groups. For specific participants of food production chain, producers, processors, distributors and consumers, the most important may be completely different discriminants, dependant on a specific purpose that a given product is to be designed for. Among technological aspects is storage capacity; the feature critical from processing viewpoint is e.g. juice production capacity, and for a consumer the technological value is presented as, for example, the use while cooking. The higher technological value of organic plant products is determined, e.g. by higher dry matter content, thanks to which organic products have better storage quality [Bulling 1987, Rembiałkowska 2000]. Samaras [1978] showed in his studies that the main influence on the amount of mass losses after vegetable storage period has a type of material which plants are fertilized with. All root vegetables (carrots, kohlrabi, beets and potatoes), tested by Samaras [1978], which had been fertilised

with organic manure, were characterized by much lower storage losses. Higher storage losses of vegetables grown with mineral fertilizer may be associated with a higher content of water absorbed by plant together with readily soluble mineral compounds. When mineral fertilizers were used, the average storage losses amounted to 46.4% of initial mass, and with the application of organic manure – 28.9% of initial mass [Samaras 1978]. Potatoes turned out to be the least sensitive to storage losses [Samaras 1978]. Bulling [1987] made a list of studies comparing the differences in the storage losses between organic and conventional cultivation of vegetables and fruit. Mean values for raw materials tested in 53 studies showed 10% lower losses for the benefit of organic farming. In case of organic cereal, the content of gluten (necessary in baking processes) is lower and consequently it is obligatory to develop appropriate baking technology during the organic flour bread production.

Among unfavourable properties of organic meat there should be mentioned an increased TBARS concentration, which indicates intensive fat oxidation processes f.e. in organic poultry [Castellini et al. 2002] and pork [Lopez-Bote et al. 1998, Nilzen et al. 2001, Warnants et al. 1999]. Fisher et al. [2000] stated that both in organic and conventional farming there can be obtained a similar slaughter yield, but at more favourable quality properties of organic mutton. Three sheep breeds were submitted to the study, thanks to which it was proved that appropriate breed selection is responsible for gaining desirable yield. Pork carcasses from organic farms have a higher fat-free body mass [Bee et al. 2004, Sundrum and Acosta 2003], that is why they are higher estimated at wholesale pricing – compared to conventional carcasses, they had a higher mass of loin and gammon [Sundrum and Acosta 2003]. Combes et al. [2003a, b] compared organic and conventional meat of the rabbit of similar weight but different age. Carcass weight ratio to the weight of the rear carcass was higher, and the fat content was lower in organic rabbits; cooking losses were the lowest in the limb of organic doe-rabbit, and the highest in conventional rabbits. Organic beef was characterized by higher fat-free body mass compared to conventional beef [Fisher et al. 2000, Hansson et al. 2000].

The chemical composition of milk is an indicator of quality, not only for its consumers, but also processors. High protein content is important in the production of cheese, while the high fat content is essential in butter manufacturing. Beta-carotene increases the intensity of the butter colour, so it is also considered in the assessment of technological quality. For many dairy products, the important role is played by the antioxidant level, which guarantees avoiding of undesirable losses caused by excessive oxidation of fats [Abrahamsen et al. 2008]. The degree of saturation of fatty acids, in turn, affects the hardness, texture and flavour of dairy products, especially cheese and butter [Chen et al. 2004]. The presence of saturated fatty acids of long chains increases the hardness of butter, while the milk with a high content of unsaturated fatty acids gives softer products (e.g. better spreadability of butter) [Butler et al. 2008].

Sensory quality. A sensory quality includes such product's features which are assessed by a human by the means of special tests as described below and the organs of taste, smell, touch, sight and hearing. Among these criteria, a crucial role is played by the look of raw materials and finished products (colour, size, freshness, firmness and cleanness) as well as other important organoleptic properties, such as taste, smell and texture. The sensory quality is highly significant with regard to selection process while shopping for food. A sensory assessment of food products has two areas of application. The first study area is the assessment of the level of consumers' desirability, acceptance and preferences with respect to organic products, carried out by the means of so called 'consumer testing'. In case of consumer testing, products are assessed by unqualified people, the role of whom is to identify their preferences

with respect to the studied product. The second sensory study area is product's assessment based on defined criteria and by specially trained people (so called 'sensory panel'), and the results obtained are submitted to statistical analysis. There is a possibility of the use of numerous methods, depending on the study target. In tests conducted with consumers involved as well as by a qualified panel, organic vegetables and fruit are frequently better assessed in terms of their sensory properties [Rembiałkowska 2000].

There have been also carried out the studies on food preferences, performed on rats. The results indicate a higher quality, including better sensory properties, of organic raw materials [Maeder 1993, Velimirov 2001, 2002]. Rats that received both organic and conventional food definitely preferred organic carrots (81% of animals), and organic wheat (68%). Smaller differences were noted with the choice of apples and red beets from organic farming, which were selected more eagerly by more than a half (58%) of the rats tested.

Fat is the main carrier of flavour, so that differences in sensory evaluation of organic and conventional beef are mainly caused by different fatty acid composition. This evaluation is also influenced by the content of intramuscular fat, which was higher f.e. in the organic beef [Woodward and Fernandez 1999]. Organic lamb showed a better quality in terms of juiciness, tastiness and general acceptability [Angood et al. 2008]. A study of Castellini et al. [2002] where a sensory panel had taken part revealed that the organic chicken breast meat was juicier, and generally tasted better than the meat from the same part of conventional chickens. Combes et al. [2003b] compared organic and conventional meat of the rabbit of similar weight but different age also in terms of sensory quality. A trained sensory panel was able to distinguish, using the triangle test, organic meat from conventional one (83.3% of correct answers). The results of the evaluation of central part of the meat, conducted by the trained sensory panel, showed that organic rabbit meat was more tender than conventional one. An organoleptic assessment of pork shows that organic pork is less tender than conventional one [Danielsen et al. 1999]. It may result from lower daily mass gains of porkers from organic farming; therefore, while slaughtering their meat presents a lower proteolytic potential [Sather et al. 1997].

The studies of sensory quality of milk clearly show the advantage of conventional milk. The research by Zadoks [1989] confirm this fact, showing a greater raw material acceptability among consumers. A specific odour of cow's milk is primarily responsible for this assessment – it is much more intense in the case of organic milk. Modern consumers are not accustomed to such properties, preferring 'carton' milk of neutral smell, commonly available on the market. Currently, there is also a tendency to consume milk with reduced fat content. Organic milk has a higher fat percentage, which considering the fact that fat is the main carrier of flavour significantly affects its organoleptic characteristics.

Nutritional value. A nutritional value may be interpreted as a minimum content of impurities in food (residues of pesticides, nitrates, heavy metals, etc.) at optimum content of valuable components (vitamins, mineral elements, proteins, etc.).

Nitrates and nitrites. Lots of data prove a significantly higher content of nitrates and nitrites in conventional crops compared to organic ones. It results from the fact that the plants fertilised with synthetic, easy-dissolvable nitrogen fertilisers absorb a lot of these compounds by a root system, and it results in the nitrates accumulation in leaves and other plant organs. In organic system there are used organic fertilisers (compost, manure), which also include nitrogen, but it is organically-bound. When they come into soil, they are decomposed by the soil micro-organisms and edaphon, and there is created humus consisting of organic and mineral compounds. When it is necessary, nitrogen compounds are absorbed by plant from

the humus in a specific quantity, therefore, there is marginal possibility of excessive nitrates accumulation in plant organs [Vogtmann 1985]. It is critical for human health, since nitrates are transformed into nitrites which may cause a dangerous disease called methaeminoglobinaemia of newborn babies, small children and the elderly [Mirvish 1993]. Moreover, nitrites may react with amines, creating nitrosamines – carcinogens and mutagens, which cause alimentary canal tumours and leukaemia [Szponar and Kierzkowska 1990]. This process is dangerous not only for small children, but also for adults, regardless of the age. Basing on different data, the nitrates content in organic and conventional crops has been compared. Assuming the nitrates content in conventional crops as 100%, on average 48% of the content is found in conventional raw materials [Kunachowicz et al. 1993, Leszczyńska 1996, Rembiałkowska 1998, 2000, Rutkowska 1999, Hajslova et al. 2005, Guadagnin et al 2005]. Basing on the data, there can be stated that organic methods allow the reduction of nitrates and nitrites absorbed by a human body by approx. 50%. The results present relatively highest levels of nitrates in beetroots compared to other vegetables. Unfortunately, beetroots tend to accumulate nitrates in roots, therefore – despite high nutritional value – a production method should be particularly considered, and it is desirable to choose organic vegetables. However, it confirms a general rule that leafy vegetables accumulate more nitrates, and they are followed by root vegetables and potatoes respectively [Leszczyńska 1996, Rembiałkowska 1998, 2000, Rutkowska 1999].

Pesticides. Pesticides are used to increase the crops profitability, protecting them against insects feeding, fungi diseases, mycotoxins and bacterial infections. However, the applied chemical compounds do not only affect the target organisms – their residues in plants accumulate and move along food chain, penetrating a consumer's body. In order to reduce a negative impact of pesticides on human health, there has been introduced so called Maximum Residue Level/Limit – MRL which can be found in food. MRL is usually established by testing pesticides on rats. It is believed that the pesticides consumption below MRL is not risky for health. However, even in low concentrations, pesticides are known for or supposed to be a reason of many diseases and health problems, including inborn defects or tumours [BMA 1992, Howard 2005]. A fundamental problem consists in the fact that MRL for pesticides is generally established by the means of testing of specific remedies (separately) on rats for relatively short period of time. There is hardly anything known about the effects of consumption of hundreds of different pesticides during a life cycle. According to the great English toxicologist, Professor Howard [2005] from the University of Liverpool, the most recommended method of protection is avoiding of any pesticides consumption, particularly in case of pregnant and breastfeeding women, and small children under 3. A serious risk to consumers' health is represented by chronic poisoning, which is caused by a long-term organism exposure to low dose of the toxic compound. Therefore, pesticides impair the immune system and negatively affect the nervous system operation. They also contribute to development of tumour diseases. Many of them have similar chemical structure as human hormones, and consequently, they influence the decrease in fertility both of men and women [Howard 2005]. An example of such impact is nonylphenol effect, an active substance of several pesticides. Its chemical structure is very similar to major woman's reproductive hormone, i.e. oestrogen. The women who consume the products including nonylphenol may have a disturbed reproductive cycle, since the compound partly dislodges oestrogen from metabolic processes [Odum et al. 1997]. There are still used lots of compounds which impact on the human immune and hormonal systems has been proved [Ansar Ahmed 2000]. Howard [2005] pays attention to the changes of man's sperm quantity as a result of the pesticide appliance in plant

cultivation. Since the 50-ies of 20th century, i.e. thereabout since chemical agents for plant protection were introduced to the market, there has been observed a downward tendency in quantity and quality of the sperm of the European residents. The problem appears in such countries as: Germany, France, Italy, the UK, Sweden or Greece. The infertility problem concerns about 15–20% of all pairs who want to have a baby. With regard to growing scale of the occurrence, the WHO has acknowledged infertility as a social disease [WHO 2010].

Every person has higher or lower amount of synthetic compounds in his/her body, including pesticides. Gilman et al. [1997] compared the content of some pesticides in plasma of the women from the subarctic zone. The study results irrefutably prove the fact that chemical compounds, which are used for cultivated plant protection, accumulate in a human body. The results indicate that although the DDT use has been prohibited since the 70-ies of 20th century, considerable amounts of DDT derivatives are still found in women's plasma. Besides, surprisingly high levels of the derivatives are confirmed in the plasma of women who live in – by all appearances – pollution-free countries, like Greenland or Iceland. The mechanisms of toxic effects of every of those substances are known more or less profoundly, but in a human body they form a mix which may develop completely new properties. So far, a method of examining the mix toxicity has not been invented, so the mix effects have not been sufficiently recognized yet.

In order to define the level of pesticide poisoning risk to children, Pennycook et al. [2004] assessed it on the basis of the consumption of apples and pears by the youngest residents of the UK. As a result, it was determined what number of 1.5–4.5-year children is daily exposed to consumption of such pesticide quantity which exceeds a maximum permissible dose (ARD – acute reference dose). There were considered dithiocarbamates, phosmet and carbendazim. Depending on the type of chemical compound and the year when the crops were harvested, the number amounted to 10–226 children a day. It emphasises how the raw material contamination with pesticides is divergent. In utmost cases, its level exceeded the ARD almost by six times.

Organic farming is an alternative to the risk of pesticide residues in crops. It is a legally protected system, which assumes, among others, a complete rejection of the use of chemical agents for plant protection. In the framework of organic farming, there are only allowed some natural plant protection methods, e.g. the use of natural enemies of pests or allelopathy. Thanks to that, the level of organic crop contamination is considerably lower compared to conventional raw materials, commonly available on the market. This fact is borne out by the results of different authors' studies.

In 1994–1999, in the USA, Baker et al. [2002] submitted to analysis the content of pesticides in fruit and vegetables from three production systems – conventional, integrated (in-between organic and conventional ones) and organic. The percentage of organic raw materials with the confirmed pesticide residues was about three times lower than in conventional fruit and vegetables, and about twofold lower than in integrated raw materials. The analysis was conducted by the means of three testing programmes, and each of them proved a significantly lower percentage of contaminated raw materials among organic fruit and vegetables. Among conventional raw materials, the greatest number of samples including pesticide residues was found in such vegetables as celery and spinach, and among fruit – in pears and apples. However, the number of organic samples was too low to attribute a precise risk level depending on fruit and vegetable species. Similar large-scale studies (excluding integrated production) were carried out in 1995–2001, in Belgium [AFSCA-FAVV 2001]. According to their results, the percentage of pesticide-contaminated organic crops amounted to 12%, whereas in case of

conventional farming – 49%. The studies conducted in Sweden, in 2002–2003 [The Swedish 2003, 2004] confirmed this tendency, acknowledging the pesticide residues in case of 3% of organic raw materials, 11% of integrated ones and 44% for conventional production. The studies carried out in Poland provided surprising results due to the fact that the highest percentage rate of pesticide-contaminated raw materials belonged to integrated cultivation, i.e. 47% (2005) and 48% (2006). Conventional farming, however, presented an intermediate state between the two remaining farming systems – 28% of raw materials in 2005 and 21% in 2006 contained the residues of chemical agents for plant protection. In organic farming, the percentage of contaminated crops reached the level of 5% (2005) and 7% (2006), i.e. it was the lowest among all three systems. However, the confirmed residues of chemical agents for plant protection indicated the cases of forbidden use [Gnusowski and Nowacka 2007], which points out still defective control system, maintained by certifying units. According to the latest studies results [Gnusowski et al. 2009], the residues were found in 4.4% of examined organic raw materials.

There were conducted some studies which allowed researchers to state what amount of chemical agents for plant protection is absorbed by a human body depending on a type of diet. One of first studies concerning that subject was undertaken by Aubert [1987] who analysed the content of chlorinated hydrocarbons in the milk of the women on different diets. Diet differences consisted in the percentage of organic products in total food consumed. The study unequivocally showed that the more organic products were consumed by a woman, the lower content of chlorinated hydrocarbons (components of numerous pesticides) was found in the milk which she fed a child with. Similar studies were carried out in the USA, but they concerned nursery school children, and a comparison was based on the content of organophosphorus pesticide metabolites in urine [Curl et al. 2003]. Before the samples were taken, children from one group had only eaten certified organic products, and children from another group – market conventional products. Significant differences were observed in case of dimethyl metabolites – their concentration in urine of the children eating conventional food was nearly 6 times higher compared to the organic group children. The percentage of the samples with dimethyl metabolites was also considerably higher in case of the children on conventional diet. In turn, Lu et al. [2006] conducted the study on one group of school children, but it included three separate stages. During first three days the participants consumed conventional food, for following five days – organic food, and for next seven days they were on conventional diet again. The urine samples were taken two times (in the morning and evening) on every day of the study, and they were analysed in relation to the content of organophosphorus pesticide metabolites. According to the study, the average contents of MDA (malathion dicarboxylic acid, metabolite of malathion) and TCPY (3,5,6-trichloro-2-pyridinol, metabolite of chlorpyrifos) in urine decreased to undetectable level just after switching into organic diet, and they remained at the same level until coming back to conventional diet. Malathion and chlorpyrifos belong to the pesticides which are most commonly used in modern farming. The levels of other organophosphorus pesticide metabolites were also lower during second, i.e. 'organic' stage of the study, but they were not detected often enough to make differences statistically significant [Lu et al. 2006]. It should be underlined that the analysed organophosphorus pesticides are typical of very high toxicity towards children – they have teratogenic effects, impairing foetal development, and contribute to central as well as peripheral nervous system disorders, and they can cause ADHD [Abdel Rasoul et al. 2008].

The above data show that organic products consumption may fundamentally reduce the risk of absorbing pesticides surplus with food, and consequently facilitate the improvement of public health.

Heavy metals. Heavy metals, such as cadmium, lead, arsenic, mercury and zinc enter the food chain from numerous sources: industry, transportation, municipal waste and farming. For example, mineral nitrogen fertilisers used in conventional farming may bring cadmium into plant crops, but also metal industry and transportation cause the soil and crops cadmium contamination. Therefore, the studies do not show distinct differences in the heavy metals content between organic and conventional raw materials. Some results point out a higher level of heavy metals in conventional raw materials, while others show opposite outcomes [Rembiałkowska 2000]. The problem to be sort out is whether organic farming methods (composting, increase of the organic matter content in soil, soil pH growth, etc.) may decrease the heavy metals absorption by crop plants.

Contamination with natural fertilisers and zoonotic bacteria. Composted animal manure is most often used fertiliser in organic farming. It is known that composting considerably reduces the pathogen level, however, the composted manure is not entirely bacteria-free. Contamination with faecal pathogens (in particular, *E. coli*) represents a potential risk of foodborne diseases, if a product contains a sufficient level of vital pathogens [Kouba 2003]. Composting not always destroys spore forms, e.g. *Clostridium sp*. Another problem is associated with animal infections (zoonoses), which can be transmitted from animals to people by food consumption. Compared to a conventional breeding practice, a broad access to outdoor run, supported in organic breeding, exposes animals to soil disease-producing bacteria to higher degree. Moreover, the presence of rats, mice and birds increases the risk of *Salmonella* or *Campylobacter* infections to animals, particularly in poultry production. The report presented in 2001 by the EU experts [Kouba 2003] points out that organic farming – compared to conventional one – brings to higher rate of *Salmonella* infections in case of eggs, poultry and pork. However, it was not borne out by other research. It should be stated that the previous studies have not given an unequivocal answer whether organic breeding system creates real problems of foodborne diseases [Kouba 2003].

A good indicator of the quality of milk, as well as an indicator of health of cows, is somatic cell count – SCC. Their high content indicates the presence of udder inflammation as well as reduces the quality of such milk. The study results in terms of somatic cell count in milk from organic production are varied. The studies conducted in Denmark showed no differences in this respect between conventional and organic raw materials, whereby the longer the cows were bred in organic system, the lower the SCC indicator was [Bennedsgaard et al. 2002]. The Norwegian studies [Hardeng and Edge 2001] also showed no differences in the SCC, but in the organic herd there were less frequent udder inflammations. In turn, the studies by Tikofsky et al. [2003], conducted in New York, showed that milk from organic cows contained fewer somatic cells compared to conventional herds. It is believed that lower milk productivity of organic cows can result in less frequent occurrence of mastitis, because the frequency of metabolic disorders, such as ketosis and milk fever, is much lower in organic herds [Hardeng and Edge 2001, Vaarst and Enevoldsen 1997]. Karwowska [1999] conducted a study of microbiological quality of milk from Polish organic and conventional farms. She marked the number of bacteria of greatest concern to consumers. The experience showed that the most pathogenic groups of bacteria: *Streptococcus agalactiae* (causing loss of cows' milk) and *Streptococcus aureus* (responsible for milk coagulation) occurred in greater amounts in conventional milk [Karwowska 1999]. The comparisons of microbiological indicators in milk samples from both production systems were also made in the Netherlands [Zadoks 1989]. The milk of cows from conventional breeding contained significantly higher amounts of the bacteria resistant to high temperatures, butyric acid bacteria, aerobic bacteria

and the bacteria responsible for mastitis (*Mastitis streptococci*). However, the organic milk contained significantly higher amounts of coli group bacteria.

Mycotoxins are secondary metabolites of fungi, which occur in nature, while there are favorable conditions like high temperature, humidity and organic matter. The main fungal species producing mycotoxins are Fusarium, Aspergillus and Penicillium [Kouba 2003]. In recent years, more and more attention is being paid to the toxins, which are metabolic products of fungi, growing on many common agricultural products and processed food. They are called mycotoxins. Some of them are very dangerous because they have a toxic effect on human, animals and plants. Ingestion of even small quantities of mycotoxins can cause serious, sometimes irreversible changes in the body, leading to cancer and other serious diseases. Mycotoxins have strong toxic properties, and they can get into the body not only through food but also through the respiration system and the skin [Stępien et al. 2007, Budak 1998]. Major food commodities affected are cereals, nuts, dried fruit, coffee, cocoa, spices, oil seeds, dried peas and beans and fruit, particularly apples. Mycotoxins may be also found in beer and wine, because of the use of contaminated barley, other cereals and grapes in their production. Mycotoxins also enter the human food chain via meat or other animal products such as eggs, milk and cheese as the result of livestock eating contaminated feed. It is very difficult to remove a mycotoxin once formed; this means that the best method of control is prevention [Chełkowski 1985, 1994]. Currently, several hundred mycotoxins are known. The discovery of mycotoxins (60s of the twentieth century) is the explanation for a series of diseases which were unexplained.

Moulds are visible, if grows on the surface of the product, but we should be aware that when they are inside they can be difficult to discern. The same situation is with mycotoxins, they cannot be seen. They are stored in the mycelium, and then it can be secreted into the food and feed. Removing spoiled part of the product does not solve the problem, since mycotoxins penetrate into the product and remain toxic even after the heat treatment [Stępien et al. 2007]. Formation of mycotoxins may be closely related to the so-called harvest diseases of cereal grains, oilseeds, fruits and vegetables. Another important factor causing mycotoxins formation is improper storage of plant products after harvest. Since several years, in European countries, is growing problem of fusarium ear rot disease, which paralyze mainly crops of wheat, barley, maize and it contributes to the reduction of yield, grain quality deterioration, and also leads to infection of these crops by mycotoxins. Major mycotoxins posing food safety and health risk are: aflatoxin, ochratoxin, patulin, deoxyniwalenol, zearalenone (www.mycotoxin.org).

Nowadays more and more studies and researches are performed on this subject because consumers are more aware of food safety issue. The case of mycotoxins in organic products cause big discussion all over the world, because in organic farming there is prohibition of use of fungicides. However studies show that occurrence of mycotoxins in the organic products, in comparison with conventional is on the same level or in some cases even lower. Very important is to find an answer if the production method has an influence on mycotoxins growth. In most cases organic products are less contaminated than conventional one [Spadaro et al. 2006, Versari et al. 2007, Shollenberger et al. 2002]. Even if the amount of mycotoxins in organic products is bigger the difference is very small and does not exceed an admissible limit [Gottschalk 2007, Jestoi 2004, Pussemier 2004, Maeder et al. 2007]. As an important example is winter wheat, which is the most common cereal consumed in European countries (mostly in form of bread and pasta) and can be infested with mycotoxins, so it is why so many researches are carried to ensure its safety. The results show a lower degree of grain damage

by Fusarium fungi and lower concentrations of mycotoxins in grain from organic farming. Environmental factors have equally large impact on mycotoxins content as use of highly resistant varieties [Wieczyńska 2010].

Vitamins, phenolic compounds and mineral compounds. A nutritional value of food mainly depends on appropriate content of the compounds necessary for proper human body operation. The content of phytocompounds in plant products is a subject of great interest in modern food science. Growing number of evidence indicates that secondary plant metabolites play a critical function for human health and may be significant from nutritional point of view [Lundegårdh and Mårtensson 2003].

The variation in composition between organic and conventional products is dependent on differences in production practice typical for organic and conventional crops. On organic farms, the crops are grown without the use of synthetic plant protection products and readily soluble mineral fertilizers. Due to the exclusion of the use of chemical protection products in organic farming, activation of natural mechanisms of plant defence system against diseases and pests takes place. Natural protective substances in plants are so called secondary metabolites, which also represent an essential element of daily human diet. In organic farming, in the place of readily soluble mineral fertilizers, there are applied natural animal manures, green manures, compost and a varied crop rotation, which leads to optimal soil biological activity.

Plant metabolites in the form of phenolic compounds are of particular interest because of their potential antioxidant activity and other healthy properties, including the properties that may prevent cancer [Brandt and Mølgaard 2001]. The content of the secondary substances from the phenolic compound group in plant products is therefore of great interest, and there are being developed more and more scientific studies comparing their contents in organic and conventional products. Secondary plant metabolites are substances naturally synthesized by the plant, but usually they do not take direct part in the creation of its cells. They are typically produced as a plant reaction to various external stimuli, acting as regulators of physiological changes in the event of an attack of pests or other stress factors [Brandt and Mølgaard 2001]. For a human, they are an important source of antioxidant compounds, so called antioxidants, which protect the body against the influence of many external factors and limit the spread of lifestyle diseases [Di Renzo et al. 2007]. Plant secondary metabolites can be divided into compounds containing no nitrogen: phenolic compounds, such as phenolic acids, flavonoids (there are six classes of compounds among flavonoids: flavones, flavonols, flavanones, flavanols, isoflavones, anthocyanidins) and terpenoids (e.g. tetraterpenes: carotenoids, xanthophylls), and nitrogen-containing compounds (alkaloids, amines, non-protein amino acids, glycosides, glucosinolates). The main area of interest are flavonoids, which constitute a large group of several thousand different compounds, they play an important role in healthcare, performing many functions in the human body [Bidlack 1998]. Flavonoids have strong antioxidant and metal chelating activity; they influence the neutralization of free radicals, e.g. by inhibiting the development of cancer; they counteract atherosclerosis, strengthen blood vessel walls and thus prevent the formation of microbleedings and stroke; they reduce blood clot formation and thus decrease the risk of stroke; they act as protectors in relation to vitamin C, increasing its effectiveness; they support the immune system, preventing some bacterial and viral infections; some flavonoids have astringent properties, soothing irritation of mucous membranes [Bidlack 1998]. In most studies comparing organic and conventional raw materials in terms of content of secondary metabolites, there are measured total polyphenols, without distinction between individual compounds belonging to this group. To express the

content of polyphenols in the plant there can be used a conversion unit, such as tannic acid [Carbonaro et al. 2002]. The content of flavonoids or flavonols alone can be expressed as an equivalent of the amount of quercetin [Rembiałkowska et al. 2003a, Young et al. 2005, Hallmann and Rembiałkowska 2006]. However, in organic and conventional strawberries Anttonen et al. [2006] compared the content of individual substances: quercetin and kaempferol belonging to flavonols. As a separate group of polyphenolic compounds, there are also tested anthocyanins in plant products [Rembiałkowska et al. 2003a, 2004, Hallmann and Rembiałkowska 2006, Tarozzi et al. 2006]. Polyphenol content is compared by some authors in terms of dry matter of the product, but in most cases as the content in the fresh matter of the product. But apart from that, in terms of the polyphenol content, all analyzed studies – except for the one [Anttonen et al. 2006] which showed a lower level of one substance (kaempferol) in organic strawberries compared to conventional ones – indicate a significant advantage of organic fruit (apples, apple juice, apple sauce, peaches, pears, blackberries, strawberries, frozen strawberries, red oranges) [Weibel et al. 2000, Carbonaro and Mattera 2001, Carbonaro et al. 2002, Asami et al. 2003, Rembiałkowska et al. 2003a, 2004, 2006, Weibel et al. 2004, Anttonen et al. 2006, Tarozzi et al. 2006].

Also in the case of the studies comparing organic and conventional vegetables, most of them give the total polyphenol content, without division into individual substances. To express the content of polyphenols in the plant there can be used a conversion unit, such as tannic acid [Carbonaro et al. 2002]. The content of flavonoids or flavonols alone can be expressed as an equivalent of the amount of quercetin, and the anthocyanin content – as the equivalent of delphinidin. According to all comparative studies listed in Table 2, organic vegetables (frozen corn, tomatoes, cabbage, Chinese cabbage Pac Choi, lettuce, red pepper and onion) contain significantly more polyphenols than conventional ones [Asami et al. 2003, Rembiałkowska et al. 2003b, Young et al. 2005, Hallmann et al. 2005, Hallmann and Rembiałkowska 2006].

Another group of secondary metabolites of plants, characterized by strong antioxidant properties, are carotenoids. In environment, there are over 600 pigments called carotenoids, which give the plants yellow, orange and red colour. Carotenoids are also found in green leafy vegetables, but their colouring is masked by green chlorophyll. The best-known carotenoid is beta-carotene found in many orange and yellow fruit as well as green leafy vegetables. Lycopene gives tomatoes vivid red colour. Lutein and zeaxanthin stain corn in yellow. Carotenoids play an important role for human health. They impact on lowering blood cholesterol levels, and thus favourably affect the heart. They assist in enhancing the immune system of the body, particularly beta-carotene which stimulates the increase in the number of lymphocytes. They also exhibit anti-tumour activity, mainly due to their antioxidant properties and neutralization of free radicals [Stracke et al. 2008]. According to the foreign studies comparing the content of total carotenoids in organic and conventional vegetables, the biggest differences in favour of organic raw material were demonstrated for pepper [Perez-Lopez et al. 2007]. Slightly higher (by 1.13%) was also the mean content of lycopene in organic tomatoes [Caris-Veyrat et al. 2004, Toor et al. 2006, Rickman Pieper and Barrett 2009, Juroszek et al. 2009]. According to the research by Abele [1987], the content of beta-carotene in organic carrots was higher. However, Warman and Havard [1997] showed lower contents of beta-carotene in organic carrots. Caris-Veyrat et al. [2004] presented over 40% more beta-carotene in organic tomatoes. In the studies carried out in the Chair of Organic Food at the Warsaw University of Life Sciences/Poland in organic vegetables there was demonstrated a higher content of beta-carotene in tomatoes and peppers [Rembiałkowska et al.

2003b, Hallmann et al. 2005, 2007], lutein in peppers [Hallmann et al. 2005, 2007] and total carotenoids in peppers [Hallmann et al. 2007, 2008, Hallmann and Rembiałkowska 2007a, 2008a]. However, in the case of lycopene, its higher content in organic raw material was only found in tomato juice [Hallmann and Rembiałkowska 2008b], whereas the lycopene level was lower in organic peppers and tomatoes [Hallmann et al. 2005, 2007, Rembiałkowska et al. 2005, Hallmann and Rembiałkowska 2007a,b, 2008a, b].

The group of powerful antioxidants also includes vitamin C, which in the human body also plays a fundamental role for several metabolic functions. First of all, since it ensures the proper functioning of the immune system. It also interacts in the biosynthesis of collagen, accelerates the process of wound healing and bone coalescence. In addition, it participates in the metabolism of fats, cholesterol and bile acids, regenerates vitamin E and other low-molecular antioxidants, such as glutathione, and it has a stabilizing effect in relation to the flavonoids. It is characteristic of bacteriostatic and even bactericidal properties against some pathogens. It facilitates the assimilation of non-haem iron and it is involved in the production of red blood cells. Vitamin C inhibits the formation of carcinogenic nitrosamines in the body, thus reducing the negative impact of nitrates on the human body [Mirvish 1993]. Besides the two studies in which there was obtained a lower content of vitamin C in organic frozen corn [Asami et al. 2003] and organic tomatoes [Rembiałkowska et al. 2003b], for the majority of plants it was possible to demonstrate that an organic raw material is characterized by a higher content of vitamin C: spinach [Schuphan 1974, Vogtmann et al. 1984], celery [Schuphan 1974, Leclerc et al. 1991], Savoy cabbage [Schuphan 1974], white cabbage [Rembiałkowska 1998, 2000], lettuce [Schuphan 1974], leek [Lairon et al. 1984], potatoes [Schuphan 1974, Petterson 1978, Fischer and Richter 1984, Rembiałkowska and Rutkowska 1996, Rembiałkowska 2000, Hajslova et al. 2005], Swiss chard [Moreira et al. 2003], onion [Hallmann and Rembiałkowska 2006], tomatoes [Rembiałkowska et al. 2003b, 2005, Hallmann et al. 2005], pepper [Hallmann et al. 2005, 2007a], apples [Rembiałkowska et al. 2003a] and oranges [Rapisarda et al. 2005]. According to the above mentioned studies, the content of vitamin C is on average 30% higher for organic raw materials, and the biggest differences in the content of this vitamin in favour of organic vegetables have been demonstrated for onion.

A list of studies comparing the content of mineral components, made by Worthington [2001], indicates higher contents of mineral components (iron, magnesium and phosphorus) in organic vegetables compared to conventional ones. According to Worthington [2001], a possible cause of a higher content of mineral elements in organic raw materials is related to the higher content of microorganisms in organically cultivated soil. These microorganisms produce many compounds that help plants by introducing substances, such as citrate, binding with the soil minerals, which thus become more readily available to plant roots.

Hansen et al. [2000] and Nilzen et al. [2001] compared a chemical composition of the pork of outdoor run animals with the meat of porkers raised only in farm buildings. In the first group animals' meat there was found a higher concentration of vitamin E. Higher level of iron was found in the organic chicken [Castellini et al. 2002].

Differences in the contents of individual vitamins in milk have not been clearly ascertained. The Swedish studies [Emanuelson and Fall 2007], which included winter feeding of organic and conventional herds, showed no significant differences in the amount of vitamins contained in cow's milk. The experiment, however, was based on very similar feed rations (a mixture of clover and grass with large quantities of concentrated fodder), which resulted

in similar performance in both groups. Bergamo et al. [2003] and Butler et al. [2008] found differences in the amount of fat-soluble vitamins, but they resulted from the use of grazing pasture, which took place in organic and extensive farming, in contrast to the conventional system. This comparison does not concern as much farming systems as the method of animals' feeding. However, there were carried out studies which pointed out higher levels of vitamin C in organic milk [Lund and Algers 2003]. A similar situation occurs in the case of mineral substance content in milk. Organic production implies abandonment of the use of mineral supplements, making the content of these components generally higher in conventional milk. It is borne out by the studies by Coonan et al. [2002], indicating the shortage of copper, selenium, zinc, iodine and molybdenum in organic milk. As the reason of this state, Kuusela and Okker [2007] indicate low trace element abundance of soils from the area of organic farms. The use of synthetic fertilizers, enriching the soil in specific macro- and microelements, is not allowed in organic system. As a result, plant raw materials, which have grown on such soils, form the fodders poor in these components, resulting in their shortages in milk. However, there were carried out studies which found higher calcium content in the raw material produced in organic farming [Lund and Algers 2003, Zadoks 1989]. Antioxidants, especially vitamin E and carotenoids, represent another argument for the consumption of milk from organic production. Their contents, found in organic cows' milk, were higher, which again results from the fact that feeding of these cows is based on green pasture forage [Nielsen et al. 2004, Butler et al. 2008]. This is confirmed by the results of research conducted under the QLIF program (Quality Low Input Food), according to which the level of antioxidants in organic milk was almost double compared to conventional milk [QLIF 2008].

Total sugars. Higher sugar content means not only better technological quality, as in the case of sugar beet, but also better taste of fruit and vegetables, i.e. higher sensory value. In tests performed with the participation of both consumers and the trained panel, vegetables and fruit from organic production are often rated better in terms of their sensory properties [Rembiałkowska 2000]. The studies clearly show a higher content of total sugars in organic vegetables and fruit, such as carrot, sugar beet, red beet, potatoes, spinach, kale, cherries, red currant and apples [Zadoks 1989, Rembiałkowska 1998, Rembiałkowska et al. 2004, 2005, Stertz et al. 2005, Hallmann and Rembiałkowska 2006, Hallmann et al. 2007].

Fat. The composition of fatty acids is a very important factor conditioning a nutritional value. Ruminant meat is especially valuable, since numerous studies have shown that the ratio of polyunsaturated fatty acids n-6:n-3 is much lower compared to other kinds of meat. It results from a high concentration of linolenic acid (18:3), the high content of which can be found in grass [Wood et al. 1999, 2003]. Originally, meat consumed by a man was naturally rich in n-3 acids, which are beneficial in terms of anti-inflammatory properties, reducing the risk of heart attack [Bucher et al. 2002], breast, prostate and colorectal cancer [Augustsson et al. 2003, Deckere 1999]. However, the content of n-6 acids (increasing the risk of atherosclerosis lesions and development of cancerous lesions) was lower than in currently available meat of breeding animals – in a human diet, the proportion of n-6:n-3 acids amounted to approx. 1:1. Nowadays, due to industrial production of animal fodders (based on numerous grains including n-6 acids), the proportion comes to 30:1 [Berrisch-Hempen 1995]. N-6 fatty acids appear in quantities significantly higher than n-3 acids [Enser et al. 1998]. This has been confirmed by the results of the studies by Marmer et al. [1984] and Matthes and Pastushenko [1999], when a diet of animals was switched from a pasture system into grain mix. These changes caused decrease in the content of polyunsaturated fatty acids and consequently increase in the ratio of acids n-6:n-3. Angood et al. [2008] compared the composition

of fatty acids pool and a nutritional quality of organic and conventional lamb, offered on the British market. The study presented significant differences, i.e. organic meat had a higher content of n-3 polyunsaturated fatty acids. Both kinds of meat, however, showed a favourable proportion of n-6:n-3 acids quantity [Angood et al. 2008]. Castellini et al. [2002] analysed quality parameters of poultry from organic and conventional farms. Significant differences were found especially in the amount of n-3 acids, including docosahexaenoic acid (DHA), the level of which was two times higher than in conventional meat. Most likely, it is caused by the grass present in the animals' diet.

In the study of Pastushenko et al. [2000] was found a lower concentration of saturated fatty acids (22,4%) had been ascertained in organic beef compared to conventional one (40%). The studies on pork indicate an advantage of organic pork in terms of high level of polyunsaturated fatty acids and a lower content of saturated ones [Hansen et al. 2000, Nilzen et al. 2001]. Analyses of chemical composition of poultry systems showed a higher content of saturated and polyunsaturated fatty acids as well as lower content of monounsaturated ones in organic meat [Castellini et al. 2002]. Pla et al. [2007] analysed a composition of fatty acids in rabbit meat from organic and conventional breeding systems. Meat of organic rabbits contained a lower level of monounsaturated fatty acids and a higher content of polyunsaturated ones. The concentration of saturated fatty acids turned out to be similar for both types of meat.

According to research carried out so far, organically produced milk has a higher nutritional value comparing to conventional milk. The most important compounds taken into account are antioxidants and fatty-acid composition. Organic milk contains higher levels of conjugated linoleic acid (CLA), polyunsaturated fatty-acids and had a favourable n-6:n-3 ratio [Kusche and Baars 2007, Butler et al. 2008, Bloksma et al. 2008]. Therefore, organically produced milk has probably a beneficial impact on human health [Connor 2000, Parodi 2003], because CLA and n-3 fatty-acids reduce risk of cancer and type 2 diabetes, as well as stimulate the immune system [Pariza 2003, Lock and Bauman 2004, Wahle et al. 2004]. The fatty-acid profile in milk depends on a number of factors, such as feeding regime, outdoor grazing (especially in summertime), content of concentrates and silage in animal diet [Kusche 2009]. Besides the elevated levels of CLA and essential n-3 fatty-acids (e.g. alpha-linolenic acid), there are higher amounts of fat-soluble antioxidants in organic milk. Butler et al. [2008] proved that such milk contains more alpha-tocopherol and carotenoids than conventionally produced milk.

Proteins. A number of experiments analysed in review papers [Rembiałkowska 2000, Worthington 2001] indicate that the amount of total proteins is lower in organic crops than conventional ones, but the protein quality – measured by the content of basic amino acids – is higher in organic crops. According to Worthington [2001], nitrogen from every type of fertilisers influences the amount and quality of the plant-produced protein. A great amount of nitrogen available for a plant increases protein production, however, it reduces carbohydrates production. Furthermore, proteins produced in response of high nitrogen level contain lower quantities of some basic amino acids, e.g. lysine, and therefore they represent a lower nutritional value for people.

Authenticity. An authenticity criterion of food product may be interpreted in two ways. Fist of all, the food authenticity is understood as a possibility of traceability of a product, i.e. possibility to check whether the characteristics of the product examined actually correspond with the features which are attributed to; for example, the studies carried out in order to check if the products offered on the market as organic ones actually come from organic production [Kahl et al. 2010]. Therefore, there have been efforts made in search of the methods which would allow tracking all 'product's biography' in fast and simple way, and as a result they

would become an efficient tool of controlling the products offered on the market. Under the second concept, which is closer to the author of the present expertise, authenticity may be understood as a counterbalance to the growing tendency of food globalisation. More and more people are looking for safe food, locally produced by a producer they know. Nowadays, food products are remotely transported – from a production area, they pass a processing stage and reach a sales point. As a result, there have been applied such food production and processing methods which make it possible, but consequently, products lose their authenticity. For that reason, consumers are intensively searching for the minimally processed products, from familiar and safe sources, e.g. bought locally and directly from a farmer. In the USA, an average distance of food transportation from production area to consumption place equals to 2,000 km [Wilkins and Gussow 1997]. On the one hand, there are scientific studies which show that it is possible to satisfy nutritional needs of the consumers from New York state, mainly basing on the food locally produced, but on the other hand, the state local farming had almost been ceased, although most New York consumers assessed local varieties of vegetables and fruit as best looking, and of better taste and smell [Wilkins and Gussow 1997]. An example of active objection against the food globalisation may be the establishment of 'slow food' movement which supports production of the food alternative to 'fast food'.

Biological value. A biological value of food identifies how food influences human and animal health, and it is a following criterion which derives from holistic approach towards the food quality and the belief that it is not sufficient to know chemical composition of food in order to determine dependence between the food consumed and human and animal health. With respect to the above, health is regarded not only as no diseases, but also as well-being, fertility and vitality. An analysis of food composition is really crucial, but it does not give a complete answer, because, among others, it is not possible to determine the content of all potentially active substances (insufficient knowledge and technical and financial capacity). Furthermore, great role is also played by the awareness that analytical methods concern only one specific aspect of the food quality. However, it should be taken into account that whole product means more than a total of its separate components; a product has effect on a human body as a whole (proportions and interactions between particular substances), and its impact on health may be entirely assessed only by conducting the research on entire product's influence on a living organism. So far there have been conducted several studies concerning the issue, but only on lab animals – mainly mice, rats and rabbits. Due to many limitations of formal, logistics and economic nature, there is no studies which assess organic food direct impact on human health. According to the research, there is a tendency towards more favourable parameters of fertility and immunity of small mammals fed with organic pasture compared to conventionally fed animals. Nevertheless, it is obligatory to carry out further thorough studies in this field [Williams 2002, Padel 2005].

Ethical value. An ethical value of the food quality is made up of three aspects: **environment impact aspect, social and economic aspect and breeding animal welfare**. Organic farmers are obliged to maintain the environment's condition as good as possible. Every form of human activity, including farming, has a negative impact on the natural environment changes. Farming, and particularly its intensive method, is one of economy branches which along with industry contribute to environment degradation. The application of organic production methods significantly protects against the agriculture pressure put on different environment aspects. A comparative list of the organic and conventional farming impact on environment was prepared by Tyburski and Żakowska-Biemans [2007]. The authors take notice of the fact that organic farming is less energy-intensive, which is highly important par-

ticularly nowadays when the attention is paid to the world's energy crisis jeopardy; organic farming reaches lower energy consumption, because, among others, it does not use artificial fertilisers and pesticides, the production of which requires high energy inputs. At the same time, high energy consumption leads to high greenhouse gas emissions, which farming greatly contributes to. Therefore, organic plant production considerably influences the reduction of greenhouse gas emissions. Besides, conventional farming leads to water eutrophication and contamination with, among others, pesticide residues, whereas organic farming protects ground and surface water. Natural environment diversity resulting from landscape spacious complexity in organic farming plays also three important roles: ecological, production and aesthetic and health functions. **The ecological function** consists in maintaining biodiversity and homeostasis, i.e. balance/optimum species number. Organic farms create an existence basis for many plant and animal species, not only those designed for production purposes, but also accompanying species. **The production function** is based on prophylaxis, i.e. the use of prevention, not control, protecting plants against weeds, pests and plant diseases. It is possible thanks to maintenance of biological balance, i.e. homeostasis of whole landscape. **The aesthetic and health function** of organic farming results from the fact that people are an integral part of environment and they can only exist owing to harmonious co-existence with nature. A contact with the nature is a basic condition of mental health, sense of own life as well as other creatures' lives. Moreover, mental health is a foundation of physical health. In this way, by enriching agricultural landscape, organic farming positively influences human health and improves the tourism advantages of agricultural areas. **Social and economic aspects.** The selection of agricultural products produced, processed and sold under social equality and justice conditions is more and more popular among the EU consumers. The principles of fair trade with developing countries are really crucial. By boycotting companies which do not follow social and economic principles, consumers may have an impact on decrease in social inequalities; currently, the inequalities are real in production, processing and sales of agricultural products, particularly in tropical countries. Consumers have an opportunity of choice, due to a broad access to the information on the companies dealing with food trade; in order to notify consumers, there are published special informational brochures. You can find out from such leaflets if a company participates in e.g. tropical trees cut-down. **Animal welfare.** Currently, ecologically-aware consumers are more and more convinced that animal breeding methods are also important while making a decision on a product purchase. A reason is animal suffering, e.g. very unfavourable breeding conditions, not adjusted to the animals needs (crowd, aggression, and diseases). The food ethical value is becoming more important criterion, since consumers more often pay attention to environment, social and humanitarian aspects. High ethical advantages of organic food result form the fact that its production allows maintaining biodiversity which is vanishing on the Earth; besides it supports fair production and distribution chain (fair trade), and cares about farm animal welfare. All of these is contrary to serious lack of ethics, resulting from the production intensification, agriculture chemization and monoculture, terrible working conditions of conventional farmers and dire suffering of animals on industrial fattening farms, introduced by the aggressive lobby related to intensive plant and animal production.

Holistic methods of food quality assessment. There are being continued searches for fast and reliable methods which would show quality differences between organic and conventional food in a short period of time, and therefore, they could be applied in the food identification and detection of false food products present on the market. The studies on organic food with the use of holistic criteria are not as numerous as those applying analytical methods, however, they arouse great interest, because – as mentioned – it is believed that a sole comparison

of food chemical composition is not sufficient to explain its potential impact on human and animal health. The use of holistic methods is the food quality concept complementary to other methods of the quality assessment and they represent one aspect of such a complex occurrence as the food quality. Similarly to biological value, the holistic methods of the quality assessment also attempt to answer the question on the quality of food interpreted not as a set of chemical compounds, but as a whole, which cannot be described only by the chemical composition analysis. At current development status of holistic methods it may be stated that they have a potential to distinguish product origins, but it is not ascertained which quality aspect regarding the impact on human health they cover, and dependence between human health and those methods has not been defined yet.

Copper chloride biocrystallization belongs to the most widespread imaging methods. It was developed by Ehrenfrid Pfeiffer in the '20-ies of last century. Biocrystallograms are created as a result of submitting a mix of the examined sample and copper chloride to crystallisation process, and like in case of every imaging method they are characteristic of each sample examined. For preparing food samples (juices, extracts), there are not used any chemical agents, but a gentle processing method in order to minimally integrate into the product matrix. In 2001, there was established an initiative associating three centres from Germany, the Netherlands and Denmark, the aim of which is joint cooperation on the biocrystallization method (and on imaging chromatography, as described below). The effect of the cooperation is, among others, a complex habilitation thesis by Kahl [2006], concerning the standardisation and validation of crystallisation method. Thanks to testing various parameters under ISO 17 500 standard, it is possible to state that this method may be successfully used in every lab for the food quality studies [Busscher et al. 2010]. Biocrystallograms are traditionally assessed, based on different morphological features and with the use of such techniques as indexing or ranking, which were submitted to standardisation under ISO standards applied in sensory analysis [Huber et al. 2010]. Recently there has been tested a computer analysis of biocrystallogram texture. The use of this biocrystallogram assessment technique enables to provide the information contained in image of statistical analysis.

Vertical Capillary Dynamolysis was developed in the '20-ies of last century by Kolisko [Kolisko and Kolisko 1923]. Although this method relies on the chromatography occurrence, it is not aimed at separating specific substances included in a sample, as it happens in case of traditional chromatography. As for all holistic methods, the purpose of this method is a total depiction of living organisms' capacities to form structures and maintain a high level of matter arrangement. The most popular version of the method under WALA company. It results in images of colourful forms, typical for the sample examined. Balzer-Graf [Balzer-Graf and Balzer 1991], who has been dealing with the research on imaging chromatography method for over 20 years, states that forms creation, typical for a product, is the reflection and result of entire plant's 'biography'. The method under WALA was documented and standardised by Załęcka [2006]. The most important factors which may have an impact on the method were described. The method was validated using two varieties of wheat and two samples of carrot juice, differing in processing procedure. Moreover, a visual chromatogram assessment was submitted to standardisation. Under ISO standards, applied in products sensory assessment, a statistical presentation of the results obtained was possible [Załęcka et al. 2010].

Radial Capillary Dynamolysis was developed in 1953 by Pfeiffer and it was to serve as a quality test for examining soils, composts and biological substrates [Pfeiffer 1984].

Drop picture method used to assess the water quality and it is performed in the Institute of Flow Sciences in Switzerland (Institut fuer Stroemungswissenschaften).

For several years the researchers from the FiBL institute (the Research Institute of Organic Agriculture) in Switzerland have been conducting studies on Kirlian photography method, i.e. gas discharge visualisation (GDV) (http://www.foodaktuell.ch/printeditorial. php?id=404).

For measurement of P-value (electrical efficiency) of food products there are taken into consideration three electrochemical parameters which are associated with the food quality and organisms' health state [Hoffman 1991].

Since 1978 Fritz Albert Popp has been dealing with dependences between biophoton emission and the food quality. Biophotons are described as very weak but long-lasting visible radiation (light quantums) of the spectrum from 200 to 800 nm, emitted by living organisms. [Popp 1991].

Animal experiments. Research concerning the impact of complete fodder on animal health has a specific character. Usually experiments are based on the investigation of results of a single compound. Conducting animal experiments is aimed at using laboratory organisms as models for humans. The fodder has to be the only factor differing the experimental groups. The used crops should originate from strictly defined farming systems, with similar climatic and soil conditions. Such proper planning of comparative study enables the correct conclusions. There are several parameters and indicators taken into account when comparing the results of such animal experiments. The most frequent ones are fertility and reproduction rates (number of pregnant females, condition of female reproductive organs, etc.), as well as condition of the newborns (birth mortality, body weight gain, survival rate) and physiological parameters (biomarkers analysis in blood and tissues, blood haematology). Comparative studies are usually performed on rats, rabbits, mice and chickens. The relation between the fodder origin and fertility of rabbits was a subject of few comparative studies. Aehnelt and Hahn [1973] and Staiger [1986] confirmed the enhanced fertility rates as a result of organic crops consumption. However, Alter [1978] and Meinecke [1982] performed similar research, which showed no significant differences in fertility and state of generative organs between analysed groups of rabbits. Among the four studies mentioned above only one conducted by Staiger [1986] involved more than one generation of animals. This research confirmed the decreasing fertility of rabbits fed conventionally, whereas no changes in organic group were observed. Another experiment concerning fertility of rats has been conducted by Jensen [2004]. This Danish comparative study included analyses of epididymis weight, sperm density and testicle histopathology; the results showed no differences. Italian research conducted by Paci et al. [2003] regarded the reproductive performance of local rabbit breeds from conventional and organic husbandry. According to the result, the rearing system significantly affected the birth mortality and the length of gestation. The females grown in organic farm with access to open air exhibited a shorter gestation length than those housed in conventional system. The organic system and delivery-season (and probably the interaction between these two factors) influenced the birth mortality. Litters of females reared at open air in organic husbandry exhibited lower percentages of mortality than ones housed in rabbitry. According to the results of research carried out in Denmark by Lauridsen et al. [2005], organic fodder stimulates the immunological reactivity caused by an antigen as the secondary immune response. Rats fed organically exhibited an elevated level of Immunoglobulins G and alpha-tocopherol in their blood serum. Furthermore, they performed decreased total body fat content and much more relaxed behaviour. Research regarding the immune status of chickens was conducted by Huber et al. [2009]. The study involved two generations of animals, fed on fodder from organic and conventional sources. The chickens fed organically exhibited an increased immune responsivity, as well as stronger "catch-up groeth" after a challenge. However chicken fed on conventionally fodder showed a higher weight gain comparing to another

group. Ren et al. [2001] compared in vitro the antimutagenic and antioxidative effect of green vegetables from different cultivation systems. The results showed that organic spinach, Welsh onion, green pepper, Chinese cabbage and qing-gen-cai exhibit significantly higher antioxidative properties than ones grown conventionally. Furthermore, increased resistance to mutagenicity in case of organic vegetables was observed as well. Olsson et al. [2006] carried out in vitro studies and proved decreased colon- and breast cancer cells proliferation and stimulated repair of bacterial DNA on organic plant materials comparing to conventional ones. The study compared an effect of organically vs. conventionally grown strawberries and anticarcinogenic activity of their secondary metabolites.

Human health. Human health is dependent on many factors. According to American Centre of Diseases Control [Pasowicz 1996], the predominant factors are connected with the lifestyle (54%); in that very important is the psychological status and nutrition pattern; environmental conditions (21%) are also influencing the food quality, and it is important to consume foods with the minimal level of the contaminants; genetic background (15%); medical service (10%). People eating organic food usually perform a different lifestyle than conventional consumers. Factors such as living conditions, nutritional pattern, eating habits and sport are as important for human health as the quality of consumed food products. Therefore the certain comparative assessment of condition of people eating food from different production systems is very difficult to conduct. There are several observational studies showing, that people on organic diet evaluate their health status better than others [Rembiałkowska et al. 2008]. Studies concerning the long term impact of organic food products on human health are very difficult to establish. There are several factors obstructing the concluding. The predominant complication is bioavailability – the way a compound becomes resorbed by a human body. Moreover, every single organism reacts differently on a food product and it's impossible to predict this reaction. Thus little information on correlation between organic diet and consumers' health status is available. However, a few efforts have been made so far. There are several types of comparative studies conducted on humans. The 'intervention studies' include a group of people, where the only diversified element is the diet. The set of other factors should be stable, therefore people performing the same lifestyle under the same conditions are especially preferred (e.g. in prisons, orphanages or convents). Moreover, it should be a blinded study, where a volunteer doesn't know the kind of applied diet. Besides, a 'cross-over' study is possible, when different test phases of experiment are performed consecutively. The results are obtained by analyzing the biomarkers, showing the potential health responses. The 'observational' study includes a larger group of people reviewed with questionnaires. The volunteers report themselves, so study is not fully controlled. The diet is one of a number of factors taken into account. Research performed in Italy involved 10 healthy men consuming organic and conventional products for a period of 2 weeks [Di Renzo et al. 2007]. After the organic diet, an increased plasma antioxidative activity was observed. However, statistical analysis was not complete and no conclusion on significant differences could be made. Moreover, the second phase of experiment was based on more mature crops, because they were harvested later. Nonetheless, the analysis of antioxidative properties of vegetables, fruits, wine and milk used confirmed the better nutritional quality of organic samples [Di Renzo et al. 2007]. Another study included a controlled cross-over dietary intervention on a group of 16 people fed organically and conventionally in a design for 2x3 weeks [Grinder-Pedersen et al. 2003]. The measured parameters were excreted flavonoid levels, as well as a content of selected oxidative defence markers in blood plasma. Significant differences occurred in urinary excretion of kaempherol and quercetin – they were much higher after a period of organic diet.

Content of analysed markers in plasma was similar during the whole experiment – nonetheless, protein oxidation and plasma antioxidant capacity were higher after organic food consumption. The vegetables and fruits used in both types of diet were distributed from similar geographical regions, but variations of some plant products given during different periods were not the same. Therefore, it is not clear, whether the final result was an effect of different production methods or different varieties of plant foods [Grinder-Pedersen et al. 2003]. Study named PARSIFAL involved about 14000 children from 5 European countries. The experiment compared the health status of children eating biodynamic and organic food (according to their antroposophic lifestyle) and group consuming mass-produced food, commonly available on the conventional food market [Alfven et al. 2006]. Children from antroposophic group exhibited less allergies and lower body weight than ones from another group. Study conducted in the Netherlands (KOALA Birth Cohort Study) involved 2 700 newborns and their mothers. As a result of intake of organic dairy products there was a diminished eczema risk in children [Kummeling et al. 2008]. According to Rist et al. [2007], consumption of such products was also associated with an elevated CLA content in breast milk of mothers.

Conclusions

Due to increasing anthropogenic environmental pollution, more and more people suffer from so called lifestyle diseases, which include, e.g. allergies, diabetes, obesity, atherosclerosis, cancer. However, one of the reasons for the development of lifestyle diseases is also the improper lifestyle, and abnormal eating habits. In addition, the nutritional value of food continues to deteriorate. For best results, adherence to a healthy diet should be linked with the consumption of the highest quality food. The results of the studies comparing the quality of organic and conventional food indicate the possibility of applying them in the prevention of lifestyle diseases

It should be stated that organic crops are for sure safer than conventional ones in terms of nitrate contents. Organic food consumption significantly reduces the body exposure to pesticide poisoning. Being aware of how negative effects are connected with excessive pesticide related risks, every attempt to reduce and eliminate this danger should be undertaken and supported. Mycotoxins are dangerous to humans, animals, plants and microorganisms. These toxins may occur naturally in row material, but also during process, transport and storage. It is suggested that the mycotoxins contamination of products from organic farming can be higher because of the ban of the use of fungicides. Many studies have shown that this thinking is not correct. However, data are often so divergent that it is already carrying out many tests to get a clear answer.

The nutritional value of food depends largely on the appropriate content of the compounds necessary for the proper functioning of the human body. According to the studies analyzed in this chapter, the content of nutrients specific to a given raw material is in most cases higher when it comes from organic farming. This includes compounds belonging to the powerful antioxidants: vitamin C, phenolic compounds, carotenoids, sugars and dry matter. The latter two components contribute, on the one hand, to the improvement of technological quality and the reduction of storage losses, and on the other hand, they significantly increase the palatability of organic fruit and vegetables, and make the flavour more intense than in the case of conventional raw materials; thanks to that fact, consumers assess the flavour of organic

raw materials also as a more typical and characteristic of the plant, which is also confirmed by the studies on the food preferences carried out on animals.

Meat from organic farms is characterized by positive qualitative properties, such as the favourable ratio of fatty acids and low total fat content. Better sensory evaluation of organic meat is conditioned by a higher intramuscular fat content than in conventional meat. The adverse properties of organic meat include the lower total mass of carcasses (lower daily mass gains) and the worse storage quality (high TBARS indicator). A key factor influencing the content of bioactive substances in meat is animals' feeding – a nutritional value of meat is much higher if fresh green pasture forage dominates in feed rations.

Organic milk has a beneficial fatty acid composition (including a high content of CLA), high levels of vitamins and antioxidants, acting as an important part of human health prophylaxis. Due to the ban on the use of mineral supplements and fertilizers in organic farming, milk from such production may be characterized by the deficiency of specific macro-and microelements. The milk of animals from organic breeding system is worse assessed by consumers due to the specific organoleptic characteristics, especially the odour. A key factor determining the quality of animal products is the animals' feeding – the use of seasonal pasture grazing and the reduction of concentrated fodder in feed rations positively influence the content of bioactive substances in milk; differences in the nutritional value of milk from different production systems become blurred in the winter season, when pasture cows' feeding is impossible.

The health effects of organic vs. conventional foods have been investigated in several studies. In vitro analyses indicated better repair of bacterial DNA and decrease of cancer cells proliferation on organic vs. conventional plant materials. Animal studies indicated better fertility indexes and increased immune parameters in organically fed animals. The effects of organic foods on human health are still not well known. However, according to PARSIFAL study children representing antrophosophic lifestyle, including biodynamic and organic food, had less allergies and lower body weight, while KOALA study associated consumption of organic dairy products with lower eczema risk in children. To conclude, the overall number of studies comparing the quality and safety of organic vs. conventional foods is growing rapidly. It is also possible to observe increasing interest in investigating the health effects of organic food consumption. Results indicating higher nutritional quality and safety of organic foods in terms of many measured compounds, as well as the results of in vitro and animal dietary intervention studies, showing the positive impact of organic foods on reproductive and immune status of animals, are promising. The first experiments investigating health impact of organic foods on humans brought contradictory results; they are still insufficient to formulate the clear conclusions. Therefore, the most essential problem – the impact of the organic food consumption on human health – still needs to be investigated in the coming years.

Organic methods in farming and processing can significantly improve the quality of agricultural products compared with conventional methods, which are based on the intensification and use of chemicals. Therefore consumption of organic products should be recommended among all consumers.

References

Abdel Rasoul G.M., Abou Salem M.E., Mechael A.A., Hendy O.M., Rohlman D.S., Ismail A.A., 2008. Effects of occupational pesticide exposure on children applying pesticides. Neurotoxicology, no 29 (5), 833–838.

Abele U., 1987. Produkqualität und Düngung – mineralisch, organisch, biologisch-dynamisch. Angewendte Wissenschaft (Schriftenreihe des Bundesministers für Ernährung, Landwirtschaft und Forsten), Heft Nr. 345.

Abrahamsen R.K., Borge G.I., Harstad O.M., Haug A., Wetlesen A., 2008. Milk quality – a future approach from a researcher‘s point of view. Journal of Animal and Feed Sciences, no 16, Suppl. 1, 209–226.

Aehnelt E., Hahn J., 1973. Fertility of animals – a means to test organic food and feed stuff? Tierarztliche Umschau, Nr 28, 155–160.

AFSCA-FAVV 2001. Chemical safety of organically produced foodstuffs. Report from the scientific committee of the Belgian federal agency for the safety of the food chain (AFSCA-FAVV), Brussels.

Alfven T., Braun-Fahrlander C., Brunekreef B., von Mutius E., Riedler J., Scheynius A., van Hage M., Wickman M., Benz M.R., Budde J., Michels K.B., Schram D., Ublagger E., Waser M., Pershagen G., 2006. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle – the PARSIFAL study. Allergy, no 61(4), 414–421.

Alter G., 1978. Über den Einfluß von Fütterungsfaktoren auf das Fruchtbarkeitsgeschehen. Den Ascorbinsäuregehalt und den histologischen Aufbau der Ovarien von weiblichen Kaninchen. Tierärztliche Hochschule Hannover.

Angood K.M. Wood J.D., Nute G.R., Whittington F.M., Hughes S.I., Sheard P.R., 2008. A comparison of organic and conventionally-produced lamb purchased from three major UK supermarkets: Price, eating quality and fatty acid composition. Meat Sci., no 78 (3), 176–184.

Ansar Ahmed S., 2000. The immune system as a potential target for environmental estrogens (endocrine disrupters): a new emerging field. Toxicology, no 156, 191–206.

Anttonen M.J., Hoppula K.I., Nestby R., Verheul M.J., Karjalainen R.O., 2006. Influence of fertilization, mulch color, early forcing, fruit order, planting date, shading, growing environment, and genotype on the contents of selected phenolics in strawberry (Fragaria × ananassa Duch.) fruits. J. Agric. Food. Chem., no 54, 2614–2620

Asami D.K., Hong Y.-J., Barrett D.M., Mitchell A.E., 2003. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. J. Agric. Food Chem., no 51, 1237–1241.

Aubert C., 1987. Pollution du lait maternel, une enquete de Terre vivante. Les Quatre Saisons du Jardinage, no 42, 33–39.

Augustsson K., Michaud D.S., Rimm E.B., Leitzmann M.F., Stampfer M.J., Willett W.C., Giovannucci E., 2003. A prospective study of intake of fish and marine fatty acids and prostate cancer. Cancer Epid. Biomark. Prev., no 12, 64–67.

Baker B.P., Benbrook Ch.M., Groth III E., Benbrook K.L., 2002. Pesticide residues in conventional, IPM-grown and organic foods: Insights from three U.S. data sets. Food Addit. Contam., no 19(5), 427–446.

Balzer-Graf U., Balzer F.M., 1991. Steigbild und Kupferchloridkristallisation – Spiegel der Vitalqualitaet von Lebensmitteln, [in:] Lebensmittelqualitaet – ganzheitliche Methoden und Konzepte (Eds. Meier-Ploeger A., Vogtmann H.), Mueller, Karlsruhe.

Bee G., Guex G., Herzog W., 2004. Free-range rearing of pigs during the winter: Adaptations in muscle fiber characteristics and effects on adipose tissue composition and meat quality traits. J. Anim. Sci., no 82, 1206–1218.

Bennedsgaard T.W., Thamborg S.M., Vaarst M., Enevoldsen C., 2003: Eleven years of organic dairy production in Denmark: herd health and production related to time of conversion and compared to conventional production. Livestock Prod Sci., no 80, 121–131.

Bergamo P., Fedele E., Iannibeli L., Marzillo G., 2003. Fat-soluble vitamins contents and fatty acid composition in organic and conventional Italian dairy products. Food Chemistry, no 82, 625–631.
Berrisch-Hempen D., 1995. Fettsäurezusammensetzung von Wildfleisch – Vergleich zum Fleisch schlachtbarer Haustiere. Fleischwirtschaft, Nr 75, 809–813.
Bidlack W., 1998. Functional Foods. Biochemical and Processing Aspects. G. Mazza, ed. Lancaster, Technomic Publishing Co., pp 437.
Bloksma J., Adriaansen-Tennekes R., Huber M., van de Vijver L.P.L., Baars T., de Wit J., 2008. Comparison of organic and conventional raw milk quality in the Netherlands, Biol. Agric. Hort., no 26, 69–83.
BMA 1992. The BMA guide to pesticides, chemicals and health. Report of science and education. British Medical Association, UK.
Brandt K., Molgaard J.P., 2001. Organic agriculture: does it enhance or reduce the nutritional value of plant foods? J. Sci. Food Agric., no 81, 924–931.
Bucher H.C., Hengstler P., Schindler Ch., Meier G., 2002. n-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am. J. Med., no 112, 298–304.
Budak A., 1998. Mikotoksyny, [w:] Zarys mikologii lekarskiej. (Ed. Baran E.), Wrocław, 533–539
Bulling W, 1987: Qualitaetsvergleich von ‚biologisch' und ‚konventionell' erzeugten Feldfruchten. Regierungsprasidium, Stuttgart.
Busscher N., Kal. J., Andersen J-O., Huber M., Mergardt G., Doesburg P., Paulsen M., Ploeger A., 2010. Standardization of the Biocrystallization Method for Carrot Samples. Biological Agriculture and Horticulture, Great Britain, no 27, 1–23.
Butler G., Nielsen J.H., Slots T., Seal Ch., Eyre M.D., Sanderson R., Leifert C., 2008. Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. J. Sci. Food Agric., no 88, 1431–1441.
Carbonaro M. and Mattera M., 2001. Polyphenoloxidase activity and polyphenol levels in organically and conventionally grown peach (Prunus persica L., cv. Regina bianca) and pear (Pyrus communis L., cv. Williams). Food Chemistry, no 72, 419–424.
Carbonaro M., Mattera M., Nicoli S., Bergamo P., Cappelloni M., 2002. Modulation of antioxidant compounds in organic vs. conventional fruit (peach Prunus persica L., and pear Pyrus communis L.). J. Agric. Food Chem., no 50 (19), 9–11.
Caris-Veyrat C., Amiot M.J., Tyssandier V., Grasselly D., Buret M., Mikolajczak M., Guilland J-C., Bouteloup-Demange C., Borel P., 2004. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomato and derived purees, consequence on antioxidant plasma status in humans. J. Agric. Food Chem., no 52, 6503–6509.
Castellini C., Mugnai C. and Dal Bosco A., 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci., no 60, 219–225.
Chełkowski J , 1994. Significance of Fusarium metabolites in interaction between a cereal plant and a pathogen. Genet. Pol., no 35B, 137–142.
Chełkowski J., 1985. Mikotoksyny, wytwarzające je grzyby i mikotoksykozy. Wyd. SGGW, Warszawa.
Chen S., Bobe G., Zimmerman S., Hammond E.G., Luhman C.M., Boylston T.D., 2004. Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. J. Agric. Food. Chem., no 52, 3422–3428.
Combes S., Lebas F., Lebreton L., Martin T., Jehl N., Cauquil L., Darche B., Corboeuf M.A, 2003a. Comparison lapin "Bio"/lapin standard: Caractéristique des carcasses et composition chimique de 6 muscles de la cuisse. Proc. 10emes Journées de la Recherche Cunicole, Paris, 133–136
Combes S., Lebas F., Juin H., Lebreton L., Martin T., Jehl N., Cauquil L., Darche B., Corboeuf M.A, 2003b. Comparaison lapin "Bio"/lapin standard: Analyses sensorielles et tendrete mecanique de la viande. 10emes Journées de la Recherche Cunicole, Paris, 137–140.

Commision Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products with regard to organic production, labeing of organic products and control.

Connor W.E., 2000. Importance of n-3 fatty acids in health and diseases. American Journal of Clinical Nutrition., no 7, 171–175.

Coonan C., Freestone-Smith C., Allen J., Wilde D., 2002. Determination of the major mineral and trace element balance of dairy cows in organic production systems [in:] (Eds. Kyriazakis, Zervas), Proceeding of Organic Meat and Milk from Ruminants, Athens, October 4–6, EAAP Publication, no. 106, 181–183.

Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.

Curl C.L., Fenske R.A., Elgethun K., 2003. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Environ. Health Perspect., no 111(3), 377–382.

Danielsen V., Hansen L.L., Möller F., Bejerholm C., Nielsen S., 1999. Production results and sensory meat quality of pigs fed different amounts of concentrate ad lib. Clover grass or clover grass silage. Proc. Seminar no. 303: Ecological Animal Husbandry in the Nordic Countries. Denmark, 79–86.

Deckere De E.A., 1999. Possible beneficial effect of fish and fish n-3 polyunsaturated fatty acids in breast and colorectal cancer. Europ. J. Cancer Prev., 213–221.

Di Renzo L., Di Pierro D., Bigioni M., Sodi V., Galvano F., Cianci R., 2007. Is antioxidant plasma status in humans a consequence of the antioxidant food content influence? European review for medical and pharmacological Sciences, 185–192.

Emanuelson U., Fall N., 2007. Vitamins and selenium in bulk tank milk of organic and conventional dairy farms. EAAP 2007, Dublin, Ireland.

Enser M., Hallett K.G., Hewett B., Fursey G.A.J., Wood J.D., Harrington G., 1998. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Sci., no 49, 329–341.

FiBL – Research Institute of Organic Agriculture 2009: Organic Farming in Europe – A Brief Overview. Prepared for the European Organic Congress „Organic Food and Farming in times of Climate Change, Biodiversity loss and Global Food Crisis", Frick/Brussels, December 1.

Fisher A.V., Enser M., Richardson R.I., Wood J.D., Nute G.R., Kurt E., Sinclair L.A., Wilkinson R.G., 2000. Fatty acid composition and eating quality of lamb types derived from four diverse breed x production system. Meat Sci., no 55, 141–147.

Fischer A., Richter C., 1984. Influence of organic and mineral fertilizers on yield and quality of potatoes [in:] The Importance of Biological Agriculture in a World of Diminishing Resources (Eds. Vogtmann H., Boehncke E., Fricke I.). Witzenhausen: Veriagsgruppe Weiland, 236–248.

Gilman A.P., Van Oostdam J.C., Hansen J.C., Odland J.O., Tchachtchine V., Walker J., Lagerkvist B.J., Olafsdottir K., Weber J.P., Bjerregaard P., Klopov V., 1997. The AMAP International Symposium on Environmental Pollution in the Arctic. Tromso, Norway, June 1–5, 57–58.

Gnusowski B., Nowacka A., 2007. Pozostałości środków ochrony roślin w polskich płodach rolnych pochodzących z różnych systemów gospodarowania. Fragmenta Agronomia, no 3 (95), 121–125.

Gnusowski B., Nowacka A., Walorczyk S., Łozowicka B., Szpyrka E., Sadło S., 2009. Badania pozostałości środków chemicznej ochrony roślin w płodach rolnych, roślinnych produktach spożywczych i paszach pochodzących z produkcji ekologicznej w roku 2008. Progress in Plant Protection, no 49 (4), 1857–1863.

Gottschalk C., Barthel J., Engelhardt G., Bauer J., Meyer K., 2007. Occurrence of type A trichothecenes in conventionally and organically produced oats and oat products. Mol. Nutr. Food Res., no 51, 1547–1553.

Guadagnin S.G., Rath S., Reyes F.G.R., 2005. Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. Food Additives & Contaminants, 22 (12), 1203–1208.

Grinder-Pedersen L., Rasmussen S.E., Bugel S., Jorgensen L.V., Dragsted L.O., Gundersen V., Sandstrom B., 2003. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans, J. Agric. Food Chem., no 51, 5671–5676.

Haas G., Wetterich F., Köpke U., 2000. Life cycle assessment of intensive, extensified and organic grassland farms in southern Germany. Proceedings 13th International IFOAM Scientific Conference, 28–31 Sept., Basel, Switzerland, 157.

Hajslova J., Schulzova V., Slanina P., Janne K., Hellenas K.E., Andersson Ch., 2005. Quality of organically and conventionally grown potatoes: Four-year study of micronutrients, metals, secondary metabolites, enzymic browning and organoleptic properties. Food Addit. Contam., no 22, 514–534.

Hallmann E., Rembiałkowska E., 2006. Zawartość związków antyoksydacyjnych w wybranych odmianach cebuli z produkcji ekologicznej i konwencjonalnej. Journal of Research and Applications in Agricultural Engineering, no 51 (2), 42–46.

Hallmann E., Rembiałkowska E, 2007a. Zawartość związków bioaktywnych w owocach papryki z uprawy ekologicznej i konwencjonalnej. Żywienie Człowieka i Metabolizm, nr XXXIV, 538–543.

Hallmann E., Rembiałkowska E, 2007b. Comparison of the nutritive quality of tomato fruits from organic and conventional production in Poland. Proceedings of the 3rd International Congress of the European Integrated Project Quality Low Input Food (QLIF) (Eds U. Niggli et el.,), University of Hohenheim, Germany, March 20–23, 131–135.

Hallmann E., Rembiałkowska E, 2008a. The content of selected antioxidant compounds in bell pepper varieties from organic and conventional cultivation before and after freezing process. Proceedings of the Second Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR) (Eds. D. Neuhoff et al.). Modena, 18–20 June, vol. 2, 802–805.

Hallmann E., Rembiałkowska E., 2008b. Ocena wartości odżywczej i sensorycznej pomidorów oraz soku pomidorowego z produkcji ekologicznej i konwencjonalnej. Journal of Research and Applications in Agricultural Engineering, no 53/ 3, 88–95.

Hallmann E., Rembiałkowska E., Kaproń L., 2005. Zawartość związków bioaktywnych w pomidorach i papryce z uprawy ekologicznej i konwencjonalnej. Wybrane zagadnienia ekologiczne we współczesnym rolnictwie. Monografia, PIMR, Poznań, vol. 2, 258–263.

Hallmann E., Rembiałkowska E., Szafirowska A., Grudzień K., 2007. Znaczenie surowców z produkcji ekologicznej w profilaktyce zdrowotnej na przykładzie papryki z uprawy ekologicznej. Roczniki PZH, nr 58/1, 77–82.

Hallmann E., Sikora M., Rembiałkowska E., 2008. Porównanie zawartości związków przeciwutleniających w owocach papryki świeżej i mrożonej pochodzącej z uprawy ekologicznej i konwencjonalnej. Postępy Techniki Przetwórstwa Spożywczego, nr 18/1, 30–33.

Hansen L.L., Bejerholm C., Claudi M.C., Andersen H.J., 2000. Effects of organic feeding including roughage on pig performance, technological meat quality and the eating of the pork. Proc. 13-th IFOAM Sci. Conf., Basel, 288.

Hansson J., Hamilton C., Ekman T., Forslund K., 2000. Carcass quality in certified organic production compered with conventional livestock production. J. Vet. Med., no 47, 111–120.

Hardeng F., Edge V.L., 2001. Mastitis, ketosis and milk fever in 31 organic and 93 conventional Norwegian dairy herds. J. Dairy Sci., no 84, 2673.
Hoffman M., 1991. Elektro-chemische Merkmale zur Differenzierung von Lebensmitteln, [in:] Lebensmittelqualitaet – ganzheitliche Methoden und Konzepte. (Eds. Meier-Ploeger A., Vogtmann H.), Mueller, Karlsruhe.
Howard V., 2005. Pesticides and Health, [in:] A lecture at the Congress: "Organic Farming, Food Quality and Human Health". 5–6 January, Newcastle, UK.
http://www.foodaktuell.ch/printeditorial.php?id=404
Huber M., Andersen J-O., Kahl J., Busscher N., Doesburg P., Mergardt G., Kretschmer S., Załęcka A., Meelursarn A., Ploeger A., Nierop D., Vijver van de L., Baars E., 2010. Standardization and Validation of the Visual Evaluation of Biocrystallizations. Biological Agriculture and Horticulture, Great Britain, no 27, 25–40.
Huber M., van de Vijver L.P.L, Parmentier H., Savelkoul H., Coulier L., Wopereis S., Verheij E., van der Greef J., Nierop D. and Hoogenboom R.A.P., 2009. Effects of organically and conventionally produced feed on biomarkers of health in a chicken model. British Journal of Nutrition, 1–14.
ISO 17025, 2000. General requirements for the competence of testing and calibration laboratories. ISO, Geneva.
Jensen M.N., 2004. Organic diet and fertility – possible effects of diet on male reproductive parameters. M.Sc. Thesis, University of Southern Denmark, 34–47.
Jestoi M., Somma M.C., Kouva M., Veijalainen P., Rizzo A., Ritieni A., Peltonen K., 2004. Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets. Mol. Nutr. Food Res, no 48, 299–307.
Juroszek P., Lumpkin H.M., Yang R.-Y., Ledesma D.R., Ma C.-H., 2009. Fruit Quality and Bioactive Compounds with Antioxidant Activity of Tomatoes Grown On-Farm: Comparison of Organic and Conventional Management Systems. Journal of the Agricultural and Food Chemistry, no 57, 1188–1194.
Kahl J., 2006. Entwicklung, in-house Validierung und Anwendnung des ganzheitlichen Verfahrens Biokristallisation für die Unterscheidung von Weizen-, Möhren- und Apfelproben aus unterschiedlichem Anbau und Verarbeitungsschritten. Universität Kassel, Fachgebiet Ökologische Lebensmittelqualität und Ernährungskultur, Witzenhausen.
Kahl J., Busscher N., Ploeger A., 2010. Questions on the Validation of Holistic Methods of Testing Organic Food Quality. Biological Agriculture and Horticulture, no 27, 81–94.
Karwowska W., 1999. Ocena jakości mikrobiologicznej mleka w gospodarstwach ekologicznych i konwencjonalnych, [w:] Porównanie ekologicznych i konwencjonalnych gospodarstw rolnych w Polsce (Eds. Górny M.). Wydawnictwo SGGW, Warszawa.
Kolisko E., Kolisko L., 1923. Agriculture of Tomorrow. Printed by John Jennings, Gloucester.
Kouba M., 2003. Quality of organic animal products. Livestock Production Science, no 80, 33–40.
Kummeling I., Thijs C., Huber M., van de Vijver L.P., Snijders B.E., Penders J., Stelma F., van Ree R., van den Brandt P.A., Dagnelie P.C., 2008. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. Br. J. Nutr., no 99(3), 598–605.
Kunachowicz H., Rutkowska U., Nadolna I., Iwanow K., Karłowski K., Marzec Z., Baryłko- Pikielna N., Matuszewska I., 1993. Zawartość niektórych składników odżywczych i zanieczyszczeń chemicznych w wybranych warzywach pochodzących z uprawy ekologicznej i konwencjonalnej, Żywienie Człowieka i Metabolizm, 20, 339–350.
Kusche D., Baars T., 2007. Ökologische Milchqualität und gesundheitliche Fragen – Forschungsvorhaben am Fachgebiet Biologisch-dynamische Landwirtschaft [in:] Zwischen Tradition und Globalisierung (Ed. Zikeli et al.), Universität Hohenheim, Stuttgart. 465–468.

Kusche D., 2009. Organic milk and nutritional benefits for the consumer. Organic Food Quality and Health, Newsletter March, Edition No. 1/2009.
Kuusela E., Okker L., 2007. Influence of organic practices on selenium concentration of tank milk-a farm study. Journal of Animal and Feed Sciences, no 16, Suppl. I, 97–101.
Lairon D., Termine E., Gauthier S., Trouilloud M., Lafont H., Hauton J. Ch, 1984. Effects of Organic and Mineral Fertilization on the Contents of Vegetables in Minerals, Vitamin C and Nitrates, In: The Importance of Biological Agriculture in a World of Diminishing Resources (Eds. Vogtmann H. et al.). Proc. 5th IFOAM Conference, Verlagsgruppe, Witzenhausen.
Lauridsen C., Jorgensen H., Halekon U., Lars-Porskjer Ch., Brandt K., 2005. Organic diet enhanced the health of rats. Newsletter from Danish Research Centre for Organic Farming, no 1, 2–11.
Leclerc J., Miller M.L., Joliet E., Rocquelin G., 1991. Vitamin and Mineral Contents of Carrot and Celeriac Grown under Mineral or Organic Fertilization, Biological Agriculture and Horticulture, no 7, 339–348.
Leszczyńska T., 1996. Azotany i azotyny w warzywach pochodzących z upraw konwencjonalnych i ekologicznych, Bromat. Chemia Toksykol., nr 29/3, 289–293.
Lock A.L., Bauman D.E., 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids, no 39, 1197–1206.
Lopez-Bote C.J., Diestre A., Monfort J.M., 1998. Sustained utilization of the Iberian pig breed. Meat Sci., no 49, 17–27.
Lu Ch., Toepel, K., Irish R., Fenske R.A., Barr D.B., Bravo R., 2006. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ. Health Perspect., no 114(2), 260–263.
Lund V., Algers B., 2003. Research on animal health and welfare in organic farming – a literature review. Livest. Prod. Sci., no 80, 55–68.
Lundegardh B., Martensson A., 2003. Organically produced plant foods – evidence of health benefits. Acta Agric. Scand., Sect. B, Soil and Plant Sci., no 53, 3–15.
Maeder P., Hahn D., Dubois D., Gunst L., Alföldi T., Bergmann H., Oehme M., Amado R., Schneider H., Graf U., Velimirov A., Fließbach A., Niggli U., 2007. Wheat quality in organic and conventional farming: results of a 21 year field experiment, J. Sci. Food Agric., no 87, 1826–1835.
Maeder P., Pfiffner L., Niggli U., Balzer U., Balzer F., Plochberger K., Velimirov A., Besson J-M., 1993. Effect of three farming systems (bio-dynamic, bio-organic, conventional) on yield and quality of beetroot (Beta vulgaris L. var. esculenta L.) in a seven year crop rotation. Acta Hor., no 339, 10–31.
Maeder P., Pfiffner L., Niggli U., Balzer U., Balzer F., Plochberger K., Velimirov A., Besson J-M., 1993. Effect of three farming systems (bio-dynamic, bio-organic, conventional) on yield and quality of beetroot (Beta vulgaris L. var. esculenta L.) in a seven year crop rotation. Acta Hor., no 339, 10–31.
Marmer W.N., Maxwell R.J., Williams J.E., 1984. Effects of dietary regimen and tissue site on bovine fatty acid profiles. J. Animal Sci., no 59, 109–121.
Matthes H.D., Pastushenko V., 1999. Einfluß der landwirtschaftlichen Produktionsweise auf den Fettsäurengehalt des Fleisches. Ernährungs-Umschau, Nr 46, 335–338.
Meier-Ploeger, A., Vogtmann, H., 1991. Lebensmittelqualität einganzheitlicher Anspruch, [in:] ÖkologischeLandwirtschaft. Landbau mit Zukunft. Alternative Konzepte (Ed. Vogtmann. H.), C.F. Müller-Verlag, Karlsruhe, 175–210.
Meinecke B., 1982. Effect of conventionally and organically produced feed stuff on the fertility of rabbits. Zentrablatt für Veterinarmedizin, no 29, 5–15.
Mirvish S.S., 1993. Vitamin C inhibition of N-nitroso compounds formation. Am. J. Clin. Nutr., no 57, 598–599.

Moreira M.R., Roura S.I., Valle del C. E., 2003. Quality of Swiss chard produced by conventional and organic methods. Lebensm.-Wiss. U.-Technol., Nr 36, 135–141.
Nielsen J.H., Lund-Nielsen T., Skibsted L., 2004. Higher antioxidant content in organic milk than in conventional milk due to feeding strategy. DARCOFenews, Newsletter from Danish Research Centre for Organic Farming, 3 (http://www.darcof.dk/enews/sep04/milk.html)
Nilzen V., Babol J., Dutta P.C., Lundeheim N., Enfält A.C., Lundstrom K., 2001. Free range rearing of pigs with access to pasture grazing- effect on fatty acid composition and lipid oxidation products. Meat Sci., no 58, 267–275.
Odum J., Lefevre P.A., Tittensor S., Paton D., Routledge E.J., Beresford N.A., Sumpter J.P., Ashby J., 1997. The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenicity assay. Reg Toxicol Pharmacol, no 25, 176–188.
Olsson M.E., Andersson C.S., Oredsson S., Berglund R.H., Gustavsson K.E., 2006. Antioxidant levels and inhibition of cancer cell proliferation in vitro by extracts from organically and conventionally cultivated strawberries. J. Agric. Food Chem., no 54(4), 1248–1255.
Paci G., Lisi E., Maritan A., Baglacca M., 2003. Reproductive performance in a local rabbit population reared under organic and conventional system. Annali della Facolta di Medicina Veterinaria, no LVI/20, 115–126.
Padel S., 2005. Exploring the gap between attitudes and behaviour. British Food Journal, no 107 (8), 606–625.
Pariza M.W., 2003. The biological activities of conjugated linoleic acid. Advances in Conjugated Linoleic Acid Research (2nd edn), ed. by Christie WW, S´eb´edio JL and Adlof RO. AOCS Press, Champaign, IL, 12–20.
Parodi P.W., 2003. Conjugated linoleic acid in food. Advances in Conjugated Linoleic Acid Research (2nd edn), ed. by Christie WW, S´eb´edio JL and Adlof RO. AOCS Press, Champaign, IL, 101–122.
Pasowicz M., 1996. Od czego zależy stan zdrowia. Biuletyn Informacyjny Zarządu Głównego Polskiego Klubu Ekologicznego, nr 2, 6.
Pastuschenko V., Matthes H.-D., Hein T. and Holzer Z., 2000. Impact of cattle grazing on meat fatty acid composition in relation to human nutrition. Proceedings of the 13th International IFOAM Scientific Conference (eds. Alföldi T., Lockeretz W., Niggli U.), Basel, Switzerland, 293–296.
Pennycook F.R., Diamand E.M., Watterson A., Howard W., 2004. Modeling the dietary pesticide exposures of young children. International Journal of Occupational and Environmental Health, no 10(3), 304–309.
Pérez-López A.J., López-Nicolas J.M., Núnez-Delicado E., Amor F.M., Carbonell-Barrachina A.A., 2007. Effects of Agricultural Practices on Color, Carotenoids Composition, and Minerals Contents of Sweet Peppers, cv. Almuden, J. Agric. Food Chem., no 55, 8158–8164.
Petterson B.D., 1978. A Comparison between the Conventional and Biodynamic Farming Systems as Indicated by Yields and Quality [in:] International IFOAM Conference – Towards a Sustainable Agriculture – Sissach.
Pfeiffer E., 1984. Chromatography applied to quality testing. Bio-Dynamic Literature, Wyoming, Rhode Island.
Pla M., Hernandez P., Arino B., Ramirez J.A., Diaz I., 2007. Prediction of fatty acid kontent in Rabbit meat and discrimination between conventional and organic production systems by NIRS methodology. Food Chemistry, no 100, 165–170.
Popp F.A., 1991. Biophotonen – Analyse der Lebensmittelqualitaet In: Lebensmittelqualitaet – ganzheitliche Methoden und Konzepte (Eds. Meier-Ploeger A., Vogtmann H.), Mueller, Karlsruhe.

Pussemier L., Larondelle Y., Peteghem van C., Huyghebaert A., 2006. Chemical safety of conventionally and organically produced foodstuffs: a tentative comparison under Belgian conditions, Food Control, no 17, 14–21.

QLIF (Quality Low Input Food) 2008. www.qlif.org

Rapisarda P., Calabretta M.L., Romano G., Intrigliolo F., 2005. Nitrogen Metabolism Components as a Tool To Discriminate between Organic and Conventional Citrus Fruits. J. Agric. Food Chem., no 53(7), 2664–2669.

Rembiałkowska E., 1998. Badania porównawcze jakości zdrowotnej i odżywczej marchwi i białej kapusty z gospodarstw ekologicznych i konwencjonalnych. Rocz. AR Pozn. CCCIV, Ogrod., nr 27, 257–266.

Rembiałkowska E., 2000. Zdrowotna i sensoryczna jakość ziemniaków oraz wybranych warzyw z gospodarstw ekologicznych. Fundacja Rozwój SGGW, Warszawa.

Rembiałkowska E., Adamczyk M., Hallmann E, 2003a. Jakość sensoryczna i wybrane cechy jakości odżywczej jabłek z produkcji ekologicznej i konwencjonalnej. Bromat. Chem. Toksykol., nr 36, 33–40.

Rembiałkowska E., Hallmann E., Wasiak-Zys G., 2003b. Jakość odżywcza i sensoryczna pomidorów z uprawy ekologicznej i konwencjonalnej. Żywienie Człowieka i Metabolizm, nr 30, 893–899.

Rembiałkowska E., Hallmann E., Szafirowska A., 2005. Nutritive quality of tomato fruits from organic and conventional cultivation. Culinary Arts and Sciences V. Global and National Perspectives. (eds. Edwards, J.S.A., Kowrygo, B., Rejman, K.), 193–202.

Rembiałkowska E., Hallmann E., Adamczyk M., Lipowski J., Jasińska U., Owczarek L., 2006. Wpływ procesów technologicznych na zawartość polifenoli ogółem oraz potencjał przeciwutleniający przetworów (soku i kremogenu) uzyskanych z jabłek pochodzących z produkcji ekologicznej i konwencjonalnej. Żywność, Technologia Jakość, nr 1(46), Suplement, 121–126.

Rembiałkowska E., Kazimierczak R., Średnicka D., Bieńko K., Bielska M., 2008. Different aspects of organic and conventional food consumers lifestyle. New Medicine, no 1, 16–19.

Rembiałkowska E., Rutkowska B., 1996. Comparison of sensory, nutritional and storage quality of potatoes from ecological and conventional farms. In: Proc. of the 5th International Conf. "Quality for European Integration" (eds. Szafran M., Kozioł J., Małecka M.), Poznań, 382–385.

Rembiałkowska E., Wasiak-Zys G., Adamczyk M., 2004. Porównanie wybranych cech jakości jabłek oraz ich przetworów z produkcji ekologicznej i konwencjonalnej, [w:] Wybrane zagadnienia ekologiczne we współczesnym rolnictwie. Monografia (red. Z. Zbytek). Przemysłowy Instytutu Maszyn Rolniczych, Poznań, 143–155

Ren H., Endo H., Hayashi T., 2001. Antioxidative and antimutagenic activities and polyphenol content of pesticide-free and organically cultivated green vegetables using water-soluble chitosan as a soil modifier and leaf surface spray. J. Sci Food Agic., no 81, 1426–1432.

Rickman Pieper J., Barrett D. M., 2009. Effects of organic and conventional production systems on quality and nutritional parameters of processing tomatoes. J. Sci. Food. Agric., no 89, 177–194.

Rist L., Mueller A., Barthel C., Snijders B., Jansen M., Simoes-Wüst A.P., Huber M., Kummeling I., von Mandach U., Steinhart H., Thijs C., 2007. Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. Br. J. Nutr., no 97 (4), 735–743.

Rutkowska G., 1999. Badania zawartości azotanów i azotynów w warzywach uprawianych ekologicznie i konwencjonalnie. Przem. Spoż., nr 6, 47–49.

Samaras J., 1978. Nachernteverhalten unterschiedlich geduengter Gemusearten mit besonderer Berucksichtigung physiologischer und mikrobiologiscer Parameter. Schriftenreihe "Lebendige Erde" Darmstadt.

Sather A.P., Jones S.D.M., Schaefer A.L., Colyn J., Robertson W.M., 1997. Feedlot performance, carcass composition and meat quality of free-range reared pigs. Can. J. Anim. Sci., no 77 (2), 225–232.
Schollenberger M., Jara H.T., Suchy S., Drochner W., Müller H.-M, 2002. *Fusarium* toxins in wheat flour collected in area in southwest Germany. International Journal of Food Microbiology, 72 (1–3), 85–89.
Schuphan W., 1974. Nutritional value of crops as influenced by organic and inorganic fertilizer treatments- Qualitas Plantarum – Pl.Fds.hum.Nutr., no XIII (4), 333–358.
Spadaro D., Ciavorella A., Frati S., Garibaldi A., Gullino M.L, 2007. Incidence and level of patulin contamination in pure and mixed apple juices marketed in Italy. Food Control, 18 (9), 1098–1102
Staiger D., 1986. The influence of conventional vs. organic fodder on fertility, health condition and quality of rabbit meat. M.Sc. Thesis, University of Bonn, Germany, 35–61.
Stertz S.C., Santos R.M.I., Sossela De Freitas R.J., 2005. Qualidade nutricional e contaminantes da batata (solanum tuberosum l., solanaceae) convencional e orgânica na regio metropolitana de curitiba – paraná. B.CEPPA, Curitiba, vol. 23, no 2, jul./dez., 383.
Stępień M., Sokół-Leszczyńska B., Łuczak M, 2007. Mykotoksyny, produkty spożywcze a zdrowie człowieka. Post. Mikrobiol., nr 46 (2), 167–177
Stracke B.A., Ruefer C.E., Bub A., Briviba K., Seifert S., Kunz C., Watzl B., 2008. Bioavailability and nutritional effects of carotenoids from organically and conventionally produced carrots in healthy men, Br. J. Nutr. Forthcoming, 1–9.
Sundrum A., Acosta A.Y., 2003. Nutritional strategies to improve the sensory quality and food safety of pork while improving production efficiency within organic framework conditions. Report of EU-project, Improving Quality and Safety and Reduction of Costs in the European Organic and 'Low Input' Supply Chain, CT-2003 506358.
Szponar L., Kierzkowska E., 1990. Azotany i azotyny w środowisku oraz ich wpływ na zdrowie człowieka. Post. Hig. Med. Dośw., nr 44, 327–350.
Tarozzi A., Hrelia S., Angeloni C., Morroni F., Biagi P., Guardigli M., Cantelli-Forti G., Hrelia P., 2006. Antioxidant effectiveness of organically and non-organically grown red oranges in cell culture systems. Eur. J. Nutr., no 45, 152–158.
The Swedish Monitoring of Pesticide Residues in Food of Plant Origin, 2003. Livsmedelsverkets rapport no 12/2003.
The Swedish Monitoring of Pesticide Residues in Food of Plant Origin, 2004. EC and National Report, Livsmedelsverkets rapport nr 12/2004.
Tikofsky L.L., Barlow J.W., Santisteban C., Schukken Y.H., 2003. A comparison of antimicrobial susceptibility patterns for Staphylococcus aureus in organic and conventional dairy herds. Microb. Drug. Resist., no 9, Suppl 1, 39.
Toor R.K., Savage G.P. and Heeb A., 2006. Influence of different types of fertilizers on the major antioxidant components of tomatoes. J. Food Comp. Anal., no 19, 20–27.
Tyburski J., Żakowska-Biemans S., 2007. Wprowadzenie do rolnictwa ekologicznego. Wydawnictwo Szkoły Głównej Gospodarstwa Wiejskiego, Warszawa.
Vaarst M., Enevoldsen C., 1997. Patterns of clinical mastitis manifestations in Danish organic dairy herds. J. Dairy Res., no 64, 23.
Velimirov A., 2001. Ratten bevorzugen Biofutter. Oekologie & Landbau, no 117, 19–21.
Velimirov A., 2002. Integrative Qualitaetsmethoden im Zusammenhang mit der P-Wert_Bestimmung. Tagungsband 9. Internationale Tagung Elektrochemischer Qualitaetstest, 30.05.–01.06.2002, Institut fuer Gemuesebau und Blumenproduktion, Mendel-Universitaet.
Versari A., Parpinello G.P., Mattioli A.U., 2007. Survey of Patulin Contamination in Italian Apple Juices from Organic and Conventional Agriculture. Journal of Food Technology, no 5 (2), 143–146

Vogtmann H., Temperli A.T., Kunsch U., Eichenberger M., Ott P., 1984. Accumulation of nitrates in leafy vegetables grown under contrasting agricultural systems. Biol Agric Hort, no 2, 51–68.
Vogtmann H. 1985. Ökologischer Landbau – Landwirtschaft mit Zukunft. Pro Natur Verlag, Stuttgart, S. 159.
Wahle K.W.J., Heys S.D., Rotondo D., 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Rec. Prog Lipid Res., no 43, 553–587.
Warman P.R., Havard K. A., 1997. Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agriculture, Ecosystems and Environment, no 61, 155–162.
Warnants N., Oeckel van M.J., Boucque C.V., 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. J. Anim. Sci., no 77, 2478–2490.
Weibel F.P., Bickel R., Leuthold S., Alfoldi T., 2000. Are organically grown apples tastier and healthier? A comparative field study Rusing conventional and alternative methods to measure fruit quality. Acta. Hortic., no 7, 417–427.
Weibel F.P., Treutter D., Haseli A., Graf U., 2004. Sensory and healthrelated quality of organic apples: a comparative field study over Tyree years using conventional and holistic methods to assess fruit quality. 11th International Conference on Cultivation Technique and Phyotpathological Problems in Organic Fruit Growing, LVWO, Weinsberg, Germany, 185–195.
WHO, 2010. World Health Statistics 2010. WHO Statistical Information System. http://www.who.int/whosis/whostat/EN_WHS10_Full.pdf
Wieczyńska J., 2010. Środowiskowe uwarunkowania występowania mikotoksyn w pszenicy ekologicznej i konwencjonalnej. SGGW, Warsaw.
Wilkins J.L., Gussow J.D., 1997. Regional Dietary Guidance: Is the Northeast Nutritionally Complete? Agricultural Production and Nutrition. Proceedings of an International Conference, Boston, Massachusetts, Tufts University, Medford, MA 02155, March 19–21, 23–33.
Williams C.M., 2002. Nutritional quality of organic food: shades of grey or shades of green? Proceedings of the Nutrition Society, no 61, 19–24.
Wood J.D., Enser M., Fisher A.V., Nute G.R., Richardson R.I., Shed P.R., 1999. Manipulating meat quality and composition. Proc. Nutrition Society, no 58, 363–370.
Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M., 2003. Effects of fatty acids on meat quality: a review. Meat Sci., no 66, 21–32.
Woodward B.W., Fernandez M.I., 1999. Comparison of conventional and organic beef production systems II. Carcass characteristics. Livestock Prod. Sci., no 61, 225–231.
Worthington V., 2001. Nutritional Quality of Organic Versus Conventional Fruits, Vegetables, and Grains. The Journal of Alternative and Complementary Medicine, no 7/2, 161–173.
www.mycotoxin.org
Young J.E., Zhao X., Carey E.E., Welti R., Yang S-S., Wang W., 2005. Phytochemical phenolics in organically grown vegetables. Mol. Nutr. Food Res., no 49, 1136–1142.
Zadoks J.C., 1989. Development of Farming Systems. Evaluation of the five-year period 1980–1984. Pudoc, Wageningen.
Załęcka A., 2006. Entwicklung und Validierung der Steigbildmethode zur Differenzierung von ausgewählten Lebensmitteln aus verschiedenen Anbausystemen und Verarbeitungsprozessen. Universität Kassel, Fachgebiet Ökologische Lebensmittelqualität und Ernährungskultur, Witzenhausen.
Załęcka A., Kahl J., Doesburg P., Pyskow B., Huber M., Skjerbaek K., Ploeger A., 2010. Standardization of the Steigbild Method. Biological Agriculture and Horticulture, Great Britain, no 27, 41–57.

2

COMPARATIVE ANALYSIS OF FATTY ACIDS PROFILE AND CHOLESTEROL CONTENT OF EGGS YOLKS OF DIFFERENT BREEDS LAYING HENS HOUSED IN ECOLOGICAL CONDITIONS

Introduction

The egg has not been conceived by the hen to be fed to human beings, but its objective of supporting the embryo development for three weeks, makes it a good supply of the three main nutritional requirements: energy, protein and essential accessory factors (vitamins, carotenoids, minerals and certain fatty acids and proteins) [Cherian et al. 2002, Elkin 2006]. According to the World Health Organization the protein in egg has the highest true digestibility of major foods, and its nutritive value is also high because it contains the essential amino-acids in the required proportions. The egg has also been described as a low energy source of equilibrated proteins and easily digested fats. It represents an important source of phosphorus (together with milk is, the richest food in available phosphorus), iron and vitamins; however it is deficient in glucides, calcium and vitamin C. Although one third of the egg yolk are lipids, the egg as a whole is considered to be a low-calorie source of other nutrients. Moreover, from egg yolk lipids, just 1/3 are saturated fatty acids and eggs are rich in linolenic acid, essential in human nutrition. The lipids are present in the yolk in an emulsified form that favours their almost completely digestion by man (94–96%). Egg yolks contain the long-chain omega-3 fatty acid DHA, which is necessary for the brain and proper retinal function in the eye, and the long-chain omega-6 fatty acid arachidonic acid, which is required for the healthy skin, hair, libido, reproduction, growth and response to injury. These fatty acids are primarily needed by young children, pregnant and lactating women, and people with degenerative diseases involving oxidative stress, especially those of the nervous system such as Alzheimer's.

There is debate over whether egg yolk presents a health risk. Some research suggests dietary cholesterol increases the ratio of total to HDL cholesterol and, therefore, adversely affects the body's cholesterol profile [Weggemans et al. 2001], whereas other studies show that moderate consumption of eggs, up to one a day, does not appear to increase heart disease risk in healthy individuals [Hu et al. 1999]. Harold McGee argues that the cholesterol in the yolk is not what causes a problem, because fat (in particular, saturated) is much more likely to raise cholesterol levels than the actual consumption of cholesterol. A 2007 study of nearly 10,000 adults demonstrated no correlation between moderate (6 per week) egg consumption and cardiovascular diseaseor strokes except in the sub-population of diabetic patients that presented an increased risk of coronary heart disease [Qureshi et al. 2007]. Many reserches focused on the posibility to reduce cholesterol level and to change fatty acid contents of egg yolk [Grobas et al. 2001, Milinsk et al. 2003, Bragagnolo and Rodriguez-Amaya 2003].

Ecological production systems are growing in popularity, with an estimated. Consumers judge ecologic products positively and associate them with health [Zanoli and Naspetti 2002, Zhao et al. 2007] and environmental benefits [Shepherd et al. 2005, Zhao et al. 2007].

Although evidence available regarding the environmental impact of different agricultural methods is still in its infancy, it has been estimated that ecologic systems utilize less energy-intensive methods than do conventional practices, and therefore contribute a smaller share of greenhouse gases and have greater potential for carbon sequestration [Ziesemer 2007]. Organic eggs are reported to have similar [Cherian et al. 2002] or higher [Hidalgo et al. 2008] levels of saturated fat as compared with eggs from other production systems. Therefore, attention research on models of ecological eggs, based on the selection of a suitable genetic composition and customized diet in order to recruit the eggs with high nutritional and dietetic properties and good economic efficiency of their production. The results of previous research, as well as the recommendations arising from the regulations indicates that a regional breed animals are more useful to ecological farming. Also breed conservative hens (Greenleg Partridge, Yellowleg Partridge, Sussex, Rhode Island Red and others) and their hybrids can contribute to the acquisition, with good indicators of performance tests, the eggs of the respective nutritional and dietetic properties [Trziszka 2000].

The aim of the study was to analyze the content of fatty acids, and cholesterol in eggs collected from different breeds laying hens: Greenleg Partridge (Z-11), Yellowleg Partridge (Ż-33), Sussex (S-66), Rhode Island Red (R-11), kept under uniform environmental and feeding conditions in accordance with ecological requirements.

Materials and methods

The aim of the study was to analyze the content of fatty acids, and cholesterol in eggs collected from different breeds laying hens: Greenleg Partridge (Z-11), Yellowleg Partridge (Ż-33), Sussex (S-66), Rhode Island Red (R-11) maintained in the Rosocha Laying Hen Breeding Farm Ltd. Co., within the Experimental Station Grodziec Śląski of the National Research Institute of Animal Production. The laying birds were kept under uniform environmental and feeding conditions in accordance with ecological requirements (Commission Regulation (EC) No 889/2008 of 5 September 2008). The layers were at 38 weeks of age. The study was carried out an 20 eggs collected from each breed of layers. Lipids were extracted from homogeneous yolk samples with a mixture of methylene chloride:methanol. Cholesterol content was determined by GC following the AOCS Official Method Ch 6–91 procedure (1997). Briefly, extracted lipids (50 mg) were saponified with 1 M KOH in methanol for 18 h at room temperature, then water was added and the unsaponifiables were extracted three times with hexane:methyl *tert*-butyl ether (1:1, *v/v*). The solvent was evaporated under a stream of nitrogen. Dry residues were dissolved in 0.3 mL pyridine and silylated with 1 mL of Sylon BTZ (Supelco, Bellefonte, PA, USA). Derivatives of the sterols were separated on a 7890A Agilent Technologies (Wilmington, DE, USA) gas chromatograph equipped with a DB-35MS capillary column (25 m x 0.20 mm x 0.25 μm; J&W Scientific, Folsom, CA). A sample of 0.5 μL was injected in the splitless mode. The column temperature was held at 100ºC for 5 min, then programmed to 250ºC at 25ºC/min, held for 1 min, then further programmed to 290ºC at 3ºC/min and held for 20 min. The detector and injector temperature was set at 300ºC. Hydrogen was used as the carrier gas at a flow rate 1.5 mL/min. An internal standard, 5α-cholestane, was used for cholesterol quantification. Cholesterol was identified by a comparison of retention time of the standard. Fatty acids composition was determined after methylation by gas chromatography (ISO/FDIS 17059). Methyl esters of fatty acids (FAME) were prepared according to AOCS Method Ce 1k-07. Diluted FAME were separated

on a HP 5980 series II (Hewlett Packard, Palo Alto, USA) equipped with an Innowax capillary column (30 m x 0.20 mm x 0.20 µm) and flame ionization detector (FID). Hydrogen was used as the carrier gas at flow rate of 1,5 mL/min. The column temperature was programmed from 60ºC to 200ºC at 12ºC/min, and final temperature was held for 25 min. Detector and injector temperatures were set at 250ºC. Fatty acids were identified by comparison of the retention times with authentic standards and the results were reported as weight percentages after integration and calculation using ChemStation (Agilent Technologies).

Results and discussion

Fatty acids are an important nutrient of egg yolk. Mostly they are in glycerides and phospholipids form and less frequently in free form, lipoproteides etc. Table 1 show the composition of fatty acids in eggs yolks of different breeds laying hens housed in ecological conditions. The results of the study showed that percentage composition of saturated fatty acids (SFA) in lipids extracted from analyzed eggs ranged between 34,32 and 35,57%.

Table 1

Profile of fatty acids in eggs yolks of different breeds laying hens housed in ecological conditions

Fatty acid		Yellowleg Partridge	Greenleg Partridge	Sussex	Rhode Island Red
Myristic acid	C14:0	0.25	0.26	0.28	0.28
Palmitic acid	C16:0	25.19	25.31	25.61	24.73
Heptadecanoic acid	C17:0	0.16	0.16	0.16	0.15
Stearic acid	C18:0	9.70	9.20	9.40	9.16
Arachidic acid	C20:0	<LOD	<LOD	0.12	<LOD
Palmitoleic acid	C16:1	3.05	3.06	3.50	3.32
Heptadecenoic acid	C17:1	0.15	0.15	0.16	0.17
Oleic acid	C18:1	45.15	45.82	45.31	46.6
Eicisenoic acid	C20:1	0.27	0.28	0.24	0.29
Linoleic acid (n-6)	C18:2	11.23	10.94	10.90	10.40
Linoleic acid (n-3)	C18:3	0.58	0.59	0.51	0.64
Arachidonic acid (n-6)	C20:4	2.86	2.40	2.14	2.22
Eicosapentaenoic acid (n-9)	C20:5	0.15	0.21	0.25	0.30
Docosahexaenoic acid (n-3)	C22:6	1.39	1.30	1.40	1.26
SFA		35.3	34.93	35.57	34.32
MUFA		48.62	49.31	49.21	50.38
PUFA		16.21	15.44	15.2	14.82
PUFA/SFA		0.46	0.44	0.43	0.43

Palmitic and stearic saturated acids were observed in yolks from all breeds laying hens. The content of palmitic acid did not differ within experimental groups and was ca. 25%. Kaźmierska et al. [2005] showed a lower palmitic acid contend (ca. 23%) in eggs collected from free range hens.

The total content of monoenic acids (MUFA) constituted from 48,62 to 50,38%. Lipids extracted from eggs collected from Rhode Island Red hens were characterized by the highest

level of monounsaturated fatty acids. The yolks from Sussex eggs had the highest content of palmitoleinic acid (3,50%), whereas in the yolks from Rhode Island Red oleinic acids was found to predominate (46,6%) (Tab. 1). The highest concentration of polyunsaturated fatty acids (PUFA) was observed in lipids from Yellowleg Partridge eggs (16,03%).

From the nutritional point of view, it is important to determine the n-6/n-3 ratio, which should account for 4–6:1. Results collected in our study showed the ratio n-6/n-3 PUFA in eggs yolks of different breeds laying hens between 7,15–6,64:1. It is more than 2 times lover than normally observed in eggs from hens housed on the litter feeding with standard diet [Skiba et al. 2009] (Fig. 1).

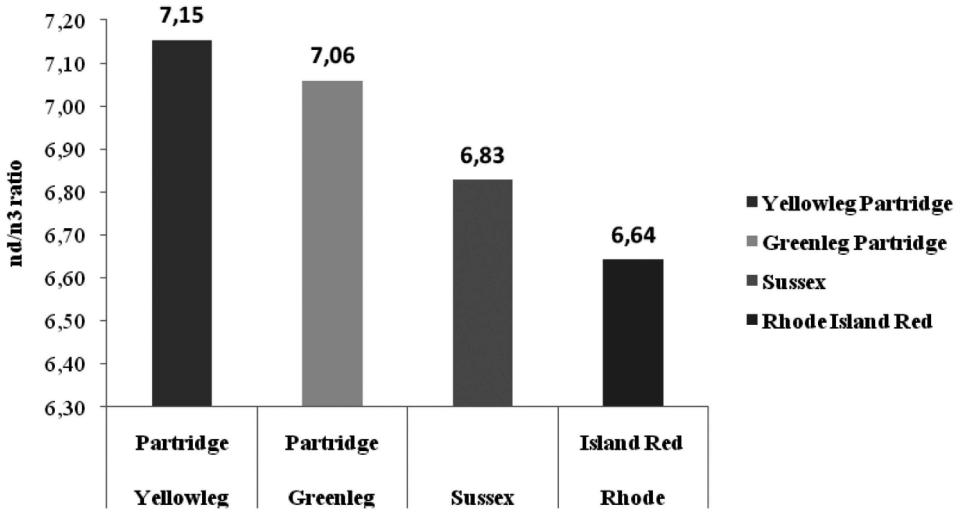


Fig. 1. Ratio between n-6/n-3 polyunsaturated fatty acids in eggs yolks of different breeds laying hens housed in ecological conditions

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 contributed to a higher n-3 fats content nad n-6/n-3 ratio [Samman et al. 2009]. In our examinations the content of ALA acid was similar (ca. 0,6%) in Rhode Island Red, Yellowleg Partridge and Greenleg Partridge eggs. Generally it is 2–3 times higher than normally observed in eggs from hens housed on the litter feeding with standard diet [Skiba et al. 2009].

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 PUFA). The amont of fatty acids dependind on the cross-breeding of laying hens changing slightly [Codony et al. 1995]. However in Skiba et al. [2009] studies the influence of the genetic differ among native race (Greenleg Pertrage) and the Lohmann Brown. Samman et al. [2009] show that little difference exists in the fatty acid composition of eggs that are produced by conventional or organic methods. Organic eggs have a small although statistically higher ($P < 0.05$) percentage of saturated fats than have conventional eggs, mainly due to higher amounts of palmitic and stearic acids. Within egg type, differences in the contents of saturated fat were observed in a sub-analysis of conventional eggs based on housing conditions. Eggs produced

by caged hens contained a significantly lower percentage of saturated fats, mainly due to lower levels of stearic acid, as compared to "barn-laid" or "free-range" eggs. The magnitude of the difference in saturated fat, when comparing conventional with organic eggs, and within conventional eggs, was small and similar to that reported previously [Hidalgo et al. 2008]. Cherian et al. [2002] reported no effect of organic production method on egg fatty acid composition; the lack of concordance may be related to the relatively smaller sample size used in that study. Samman et al. [2009] reports that omega-3 eggs have significantly lower levels of myristic and palmitic acids, resulting in a lower percentage of saturated fats, and a significantly higher percentage of ALA, DHA, and total omega-3 fatty acids. Correspondingly, the n-3:n-6 ratio was significantly higher in omega-3 eggs than in organic and conventional eggs. The total fat content in the egg yolks was significantly lower in the omega-3 eggs than in organic and conventional eggs. It has been reported previously that diet, hen age, strain and other environmental factors influence the size and composition of the eggs [Scheideler et al. 1998]. The manipulation of hens' diets to increase the levels of omega-3 fats in eggs has been demonstrated by incorporating fish oil [Navarro et al. 1972] or flax seed [Ferrier et al. 1995] in the feed. The levels of omega-3 in eggs can be naturally high in some "freerange" eggs [Simopoulos and Salem 1989] and confirm the influence of hens' diets on egg composition. In the Samman et al. [2009] studies "free-range" eggs contained similar quantities of fatty acids, including omega-3 fats, as do eggs from other conventional production systems. The effects of egg consumption on risk factors for metabolic disease and heart disease have been well studied. Large-scale studies in humans [Dawber et al. 1982, Hu et al. 1999] concluded that the consumption of one egg per day is unlikely to have an impact on cardiovascular disease risk in non-diabetic individuals. Egg consumption has been shown to promote satiety [Vander et al. 2005] and raise the concentration of high density lipoprotein-cholesterol in overweight subjects consuming a low-fat diet, contributing to a large decrease in the numbers of these subjects classified as having the metabolic syndrome [Mutungi et al. 2008].

Cholesterol content was analysed in our study using a standard GC/MS method. The highest content of cholesterol was determined in Yellowleg Partridge (14,66 mg/1g yolk) and Rhode Island Red (14,31 mg/1g yolk) egg yolk lipids, whereas the lowest in Greenleg Partridge (13,63 mg/1g yolk) and Sussex (13,90 mg/1g yolk) eggs (Tab. 2). Stepinska et al. [1993] showed a lower cholesterol content in the eggs of Greenleg Partridge hens when compared to those of Rhode Island Red and Leghorn hens. Niemiec and Świerczewska [1995] reported on the effect of hen genotype on the content of yolk lipid compounds.

Table 2

Concentration of cholesterol in eggs yolks of different breeds laying hens housed in ecological conditions

Cholesterol content	Yellowleg Partridge	Greenleg Partridge	Sussex	Rhode Island Red
mg/g of yolk	14.66	13.63	13.90	14.31
mg/g of egg yolk	240.79	229.03	231.02	232.39

Conclusions

Consumer preferences for foods derived by particular production methods may be driven by environmental factors perceived benefits to health or advice from some health professionals. The consumption of eco eggs is likely to make a significant contribution toward the recommended target intake of long chain polyunsaturated fatty acids. Yolk lipids of eggs from different breeds laying hens: Greenleg Partridge (Z-11), Yellowleg Partridge (Ż-33), Sussex (S-66), Rhode Island Red (R-11) kept under uniform environmental and feeding conditions in accordance with ecological requirements, have the higher nutritional value than those from commercial breeds. Eco eggs characterized high amount of n-3 acids, especially linoleic fatty acid (ALA) and docosahexaenoic acid (DHA) and moreover they haw low n-6/n-3 value 7,15–6,64:1.

References

Bragagnolo M., Rodriguez-Amaya D.B., 2003. Comparison of the cholesterol content of Brazilian chicken and quail eggs. J. Food Comp. Anal. 16 (2), 147–153.

Cherian G., Holsonbake T.B., Goeger M.P., 2002. Fatty acid composition and egg components of specialty eggs. Poult. Sci, 81, 30–33.

Cherian G., Holsonbake T.B., Goeger M.P., 2002. Fatty acid composition and egg components of speciality eggs. Poult. 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 Eggs Products. WPSA. Zaragoza, Spain, 361–373.

Dawber T.R., Nickerson R.J., Brand F.N., Pool J., 1982. Eggs, serum cholesterol, and coronary heart disease. Am. J Clin. Nutrit., 36, 617–625.

Elkin RG., 2006. Reducing shell egg cholesterol content. I. Overview, genetic approaches, and nutritional strategies. W. Poult. Sci. J., 62, 665–687.

Farrel D.J., 1995. The problems and practicalities of producing an omega (n)-3 fortified egg. Proceed. VI Europ. Symp. Quality Eggs and Eggs Products. WPSA. Zaragoza, Spain, 351–360.

Ferrier L.K., Caston L.J., Leeson S., Squires J., Weaver B.J., Holub B.J., 1995. Linolenic acid- and docosahexaenoic acid-enriched eggs from hens fed flaxseed: Influence on blood lipids and platelet phospholipid fatty acids in humans. Am. J. Clin. Nutrit., 62, 81–86.

Grobas S., Méndez J., Lázaro R., Blas C., Mateos G.G., 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poult. Sci., 80:1171–1179.

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.

Hu F.B., Stampfer M.J., Rimm E.B., 1999. A prospective study of egg consumption and risk of cardiovascular disease in men and women. JAMA, 281 (15), 1387–94.

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., 55, 69–73.

Milinsk M.C., Murakami A.E., Gomes S.T. M., Matsushita M., Souza N.E., 2003. Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. Food Chem., 83, 287–292.

Mutungi G., Ratliff J., Puglisi M., Torres-Gonzalez M., Vaishnav U., Leite J.O., 2008. Dietary cholesterol from eggs increases plasma HDL cholesterol in overweight men consuming a carbohydrate-restricted diet. J. Nutrit., 138, 272–276.

Navarro J.G., Saavedra J.C., Borie F.B., Caiozzi M.M., 1972. Influence of dietary fish meal on egg fatty acid composition. J. Sci. Food Agric., 23, 1287–1292.

Niemiec J., Świerczewska E., The effects of the genotype x feed interaction on the quality of eggs. 1995 in: Proceed. VI Europ. Symp. Quality Eggs and Eggs Products. WPSA. Zaragoza, Spain, 221–226.

Qureshi A.I., Suri F.K., Ahmed S., Nasar A., Divani A.A., Kirmani J.F., 2007. Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. Med. Sci. Monit., 13 (1), CR1–8.

Samman S., Kung F.P., Certer 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.

Scheideler S.E., Jaroni D., Froning G., 1998. Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. Poult. Sci., 77, 192–196.

Shepherd R., Magnusson, M., Sjödén, P.O., 2005. Determinants of consumer behavior related to organic foods. Ambio, 34, 352–358.

Simopoulos A.P., Salem N. Jr., 1989. N-3 fatty acids in eggs from range-fed Greek chickens. N. Engl. J. Med., 321, 1412.

Skiba M., Oziembłowski M., Kaźmierska M., 2009. Fatty acids and cholesterol profile in egg yolk from laying hens housed in ecological conditions. Food Technology Operations. UP Wrocław, 310–315.

Stepinska M., Niemiec J., Cywa-Benko K., Egg yolk cholesterol concentration in different hen strains. 1993, in: Proceed. V Europ. Symp. Qual. Eggs and Egg Products. WPSA Tours, France, 459–464.

Trziszka T., 2000, Jajczarstwo. Wyd. AR Wrocław, 151–211 (in Polish).

Vander Wal J.S., Marth J.M., Khosla P., Jen K.L. C., Dhurandhar N.V., 2005. Short-term effect of eggs on satiety in overweight and obese subjects. J. Am. Coll. Nutrit., 24, 510–515.

Weggemans R.M., Zock P.L., Katan M.B., 2001. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. Am. J. Clin. Nutr. 73 (5), 885–91.

Zanoli R., Naspetti S., 2002. Consumer motivations in the purchase of organic food: A means-end approach. Brit. Food J., 104, 643–653.

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, S87–91.

Ziesemer J., 2007. Energy use in organic food systems. Natural Resources Management and Environment Department, Food and Agricultural Organization of the United Nations. http://www.fao.org/docs/eims/upload/233069/energy-use-oa.pdf Accessed 28.03.08.

3

BREWER´S SPENT GRAIN AS A LOW-COST SOURCE FOR EFFECTIVE TOCOTRIENOL (VITAMIN E) EXTRACTION AND QUALITY ASPECTS OF TOCOTRIENOL RICH FUNCTIONAL FOOD

Introduction

The fat-soluble vitamin E is known to consist of eight tocochromanols: four tocopherols (T) isomers (α-, β-, γ-, δ-T) and four tocotrienols (T3) isomers (α-, β-, γ-, δ-T3). As depicted in Fig. 1, T3 are structurally equal to the corresponding T isomer, having the same chromanol moiety, but differ in the side chain, as they have three double bonds forming an isoprenoid structure. In recent years, interest in T3 has grown enormously as emerging evidence in literature suggests that they have unique health benefits which make them superior to T [2006]. For example, T3 – not T – have been reported (i) to lower serum and low density lipoprotein (LDL) cholesterol levels in animals and humans due to an inhibitory effect on hydroxymethylglutaryl coenzym A (HMG-CoA) reductase activity, (ii) to have powerful anticancer properties in cell culture and a variety of animal models (e. g. suppression of breast and melanoma tumours), and (iii) are able to cross the blood-brain barrier and possess neuroprotective properties in nanomolar concentrations. Moreover, T3 act efficiently as antioxidants in biological membranes [Serbinova et al. 1991] as well as in food systems [Seppanen et al. 2010]. Therefore, T3 may be of great use in food products, nutraceuticals and biomedical preparations.

So far, T3 preparations available on the market have originated mainly from palm oil, which is a product of large-scale plantations in Southeast Asia. In Europe, barley has been identified as one of the richest sources for T3. Barley *oil* reaches levels of T3 up to 1200 $mg \cdot kg^{-1}$, and it is supposed to have the highest T3 content of any natural oil [Moreau et al. 2007]. The composition of tocochromanols in the oils depends on their source. Whereas total tocochromanols from palm oil are dominated by γ-T3, barley oil is particularly rich in α-T3 (>47% of total tocochromanols) [Tiwari and Cummins 2009]. However, α-T3 has been shown to be up to three times better orally bioavailable in rats [Yap et al. 2003] and in humans [Ternes et al. 2011].

The low fat content in barley grains (about 2%) is the limiting factor for cost-effective T3 extraction. It has been proposed by Moreau et al. [2007] that the oil yields from the feedstocks should be as high as possible; for the oil extraction process to be economically feasible this must be at least 5–9%. Dried brewer´s spent grain (DBSG) is a low-cost by-product from the beer brewing process. After malting and brewing, the starchy endosperm is hydrolysed and extracted from the barley grain, and the remaining brewing residue is depleted in carbohydrates and enriched in fat (3–10% dry weight (dw)). This makes DBSG a promising feedstock for T3 extraction. Furthermore, it has been reported in literature that

barley-T3 are particularly located in the protein-rich aleuron layer and milling and sieving of barley grains may allow separation of this fraction. Therefore, our first aim was to evaluate DBSG as feedstock for T3 extraction and to optimise the feedstock by milling and sieving for subsequent solvent extraction, in order to develop a new technology for effective T3 extraction from DBSG.

Concerning their intended use in functional food, T3 are most appreciated because of their unique beneficial health properties. So far, these properties have only been associated with T3 which exhibit the chemically unchanged molecular structure. Therefore, effort is made to preserve the T3 in all stages of extraction and food processing, in order to maximise the potential health benefits.

T3 extracts are also appreciable as food ingredients because they act as antioxidants by preventing autocatalytic lipid peroxidation processes [Seppanen et al. 2010]. While acting as antioxidants in food systems, T3 become oxidised, forming oxidation products and one can observe a decrease of T3 levels [Wagner et al. 2001]. αT3 is the T3 isomer which degrades fastest in fats and oils [Kamal-Eldin and Appelqvist 1996], leading to a loss of antioxidant efficiency. Prior to their intended use as antioxidants, degradation processes of T3 may already occur in the feedstock for T3 extraction (DBSG) or during extraction procedure.

In contrast to oxidation products of T, those of T3 are not well investigated. Up until now, it has been commonly accepted that oxidation of T3 takes place at the chromanol moiety. The products formed are expected to be similar to T oxidation products since T and T3 have the same chromanol moiety. In the case of α-T, the most important products resulting from oxidation are αtocopherolquinone (α-TQ), α-tocopherolquinone epoxides [Rennick and Warner 2006], formyl-γ-tocopherol [Pirisi et al. 1998], and dimeric products [Kupczyk and Gogolewski 2003]. The physiological properties of T oxidation products have been the topic of numerous studies: e. g., after enzymatic reduction of α-TQ, the resulting α-tocopherolhydroquinone can act as a cellular antioxidant again [Siegel et al. 1997] and α-TQ is discussed as targeting multiple pathogenic factors in Alzheimer´s disease [Yang et al. 2010]. On the contrary, γ-TQ is a highly active agent which is involved in cytotoxicity, apoptosis and mutagenesis [Cornwell et al. 2003, Jones et al. 2002].

As T3 oxidation products only dimeric structures [Goh et al. 1992] and αtocotrienolquinone (α-T3Q) [Yoshida et al. 2003] are described. Further research is required to investigate T3 oxidation products and the kinetics of their formation more precisely. Knowledge of α-T3 oxidation products is a prerequisite to determine their importance in α-T3 rich raw materials, extracts or food regarding storage and processing, and to evaluate their physiological and technological properties. However, no analytical method for identifying and separating T3 oxidation products is available. Therefore, our second aim was to develop an analytical HPLC method for analysing α-T3 oxidation products, and to apply this method for evaluating quality aspects of α-T3 rich functional food.

Tocotrienols are minor constituents of plant oils and fats and therefore their potential oxidation products occur in comparatively low concentrations, too. Measurement of the analytes' content in lipids requires extraction and concentration prior to analysis by HPLC. The enrichment of analytes is complicated because of interfering matrix compounds and because the oxidation products are poorly thermostable and undergo a further degradation. In our study, we intended to enrich α-T3 oxidation products by molecular distillation. The extraction of tocopherols and tocotrienols by molecular distillation has been well investigated for vegetable oils and fats as well as for oil deodoriser distillates [Liu et al. 2008, Posada et al. 2007].

Study design

The first objective of this research was to determine whether DBSG can be separated into fractions enriched in fat and T3 by milling and sieving, and to characterise the fractions from sieves of different mesh sizes: 500-, 800-, 850-, and 1000-µm. Different charges of DBSG were milled and sieved with laboratory and industrial techniques. Oil yield, T and T3 levels and protein content of the separated sieving fractions were determined, in order to propose an optimised procedure for T3 extraction. Experimental details have been reported recently [Bohnsack et al. 2011].

We also aimed to estimate the importance of T3 degradation in DBSG during storage. Therefor, DBSG was processed according to the optimised extraction method (lab-scale) immediately after drying, and after a storage period of 4 and 11 months at room temperature in the dark. T and T3 levels in ethanolic extracts were compared as a function of storage time. Furthermore, the suitability of an extraction method commonly used in the pharmaceutical industry for plant drug extraction, i.e. maceration with 90% ethanol, should be tested. Knowing the effect of a large-scale maceration process (125 kg DBSG sieving fraction feedstuff) on T3 stability would be helpful for developing an effective solvent extraction method.

The second objective was to investigate the formation of oxidation products associated with α-T3 degradation. Our approach comprised: (i) autoxidation of α-T3 in a model system (n-hexane), (ii) developing an HPLC method for separating the oxidation products, (iii) determining the structure of the oxidation products, (iv) developing an isolation method for the oxidation products from a lipid matrix and (v) finally applying the findings to food systems (e. g. DBSG).

Results and discussion

Tocochromanols in sieving fractions of DBSG after lab-scale milling and sieving

Milled DBSG (n=3) was divided into sieving fractions with particle sizes <500 µm and 500–1000 µm. Parameters such as oil yield, tocochromanols (T and T3), and protein were determined in the DBSG sieving fractions. The results are presented in Table 1. Soxhlet extraction with 96% ethanol revealed oil yields from sieving fractions <500 µm which were significantly elevated compared to sieving fractions with larger particle sizes. Also, the levels of total tocochromanols were significantly enriched in the sieving fractions < 500 µm, approximately by a factor of two. The percentage of T3 on total tocochromanols was about 70%. We also conducted a spot-check on n-hexane extraction of sieving fractions with particle sizes <500 µm and found the oil yields were always lower (8.24–12.39% dw). In terms of the oil yield, Soxhlet extraction using 96% ethanol always yielded at least 45% more tocochromanols from the feedstuff (DBSG sieving fraction) than when using n-hexane. Therefore, extraction with 96% ethanol appears to be more promising for developing a large-scale extraction process.

We have shown that the DBSG sieving fraction with particle sizes <500 µm were significantly enriched in tocochromanol compared to samples >500 µm. The small particle size fraction also had significantly higher protein levels than samples >500 µm. It can be deduced that the tocochromanol content of DBSG sieving fraction is positively correlated to its protein content. This tallies with the results of Panfili et al. [2008], who found the highest

Table 1

Tocochromanols in oil extracted from sieving fractions of dried brewer´s spent grain (DBSG) after lab-scale milling and siev

	Particle size range of sieving fraction [µm]	Mass [%]	Protein [% dw]	Oil yield [% dw], Soxhlet extraction with 96% ethanol	Tocopherols [mg·kg^{-1}]				Tocotrienols [mg·kg^{-1}]				[mg·kg^{-1}]		
					α-T	β-T + coeluting matrix peak[a]	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total T (α-, γ- + δ-T)	Total T3	Total T + T3
DBSG (n=3)	500–1000	44.8	23.6	11.7	56.6	46.9	32.0	11.6	126.8	39.8	60.5	17.4	100.1	244.6	344.7
	< 500	26.0	30.9[a]	14.4[b]	152.9	76.5	88.9	15.0	310.1	85.8	118.6	37.4	256.8[b]	552.0[b]	808.8[b]
DBSG batch intended for plant-scale processing	No milling and sieving	100.0	25.72	10.81	133.1	75.1	68.8	4.5	323	91.2	141.7	35.5	206.4	591.9	798.3
	> 800	35.8	19.26	11.13	83.5	72.4	57.2	4.1	231.2	81.2	130.5	30.7	144.8	473.6	618.4
	500–800	25.8	20.58	9.63	73.6	73.7	71.3	4.4	207.7	86.7	141.1	33.2	149.3	468.7	618.0
	< 500	38.5	30.93[b]	13.70[b]	144.0	67.2	75.5	5.0	347.5	103.6	162.2	33.7	224.5[b]	647.0[b]	871.5[b]

taken from: Bohnsack C., Büsing A., Ternes W. and Drotleff A.M. [2011]

T, tocopherols; T, tocopherols; T3, tocotrienols, dw, dry weight [a] Calculated as β-T [b] Marked values are significantly higher than the corresponding value of the same sample´s fraction with larger particle size ($p > 0.001$)

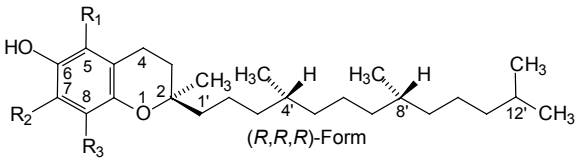
tocochromanol levels in successively removed pearling fractions of barley with the highest protein content.

In preparation for the intended up-scaling of the T3 extraction process, another DBSG batch was investigated (Tab. 1). The DBSG sample intended for plant-scale processing had the same production characteristics as the previous samples (n=3), but was produced and collected some months later than the others. However, the mass percentage of sieving fraction <500 µm (DBSG sample intended for plant-scale processing) was noticeably higher than that of the previous samples. Although the tocochromanol content of this fraction was in the same range as that of the previous samples, the sieving fractions >500 µm were less depleted in tocochromanols. This difference may have been due to variations in barley varieties and harvest times during the beer production year.

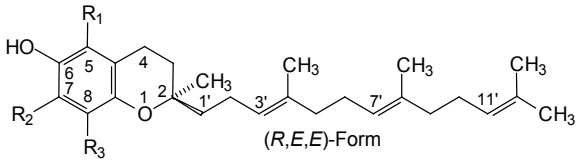
Tocochromanols in sieving fractions of DBSG after plant-scale milling and sieving

The results from lab-scale milling and sieving (Tab. 1) were in principle reproducible after plant-scale processing of a 1000-kg DBSG batch (Tab. 2). However, the mass percentage of the sieving fraction <500 µm was considerably lower (26.5%) than after lab-scale milling and sieving (38.5%) (Tab. 1), most likely due to a less intense plant-scale milling step. The tocochromanol content of the plant-scale sieving fractions of the DBSG batch showed the same trend as the lab-scale sieving fractions of this batch. The highest tocochromanol levels were found in the oil extracted from the <500 µm fraction (731.1 $mg \cdot kg^{-1}$), but oils from fractions with larger particle sizes were less depleted in tocochromanols than in lab-scale processed samples.

All in all, our experiment confirmed that milling and sieving of DBSG is a suitable method to increase oil yield and protein content from an unprocessed DBSG source. Further, tocochromanol levels were found to be highest in the fraction with the highest protein content and oil yield, the latter being essential for good production efficiency.



Tocopherols	R_1	R_2	R_3
α-T	CH_3	CH_3	CH_3
β-T	CH_3	H	CH_3
γ-T	H	CH_3	CH_3
δ-T	H	H	CH_3



Tocotrienols	R_1	R_2	R_3
α-T3	CH_3	CH_3	CH_3
β-T3	CH_3	H	CH_3
γ-T3	H	CH_3	CH_3
δ-T3	H	H	CH_3

Fig. 1. Structures of the tocochromanols (tocopherols and tocotrienols) and their isomers

Quality aspects: degradation of T3 in DBSG during storage

We observed a considerable decrease of DBSG tocochromanol levels during storage (Fig. 2). α-T3 was the isomer which degraded most dramatically within 4 months (85%), followed by α-T (79%), and γ-T3 (73%). After 11 months of storage, the degradation was 93% (α-T3), 86% (α-T), and 88% (γ-T3), respectively. The total tocochromanol content in oil from DBSG

Table 2

Tocochromanols in oils extracted from sieving fractions of dried brewer´s spent grain after plant-scale milling and sieving

Particle size range of sieving fraction [μm]	Mass [%]	Protein [% dw]	Oil yield [% dw], Soxleth extraction with 96% ethanol	Tocopherols [mg·kg^{-1} of oil extracted with 96% ethanol]				Tocotrienols [mg·kg^{-1} of oil extracted with 96% ethanol]				[mg·kg^{-1} of oil extracted with 96% ethanol]		
				α-T	β-T + coeluting matrix peak[a]	γ-T	Δ-T	α-T3	β-T3	γ-T3	δ-T3	Total T (α-, γ- + δ-T	Total T3	Total T + T3
> 800	38.3	16.02	7.35	85.4	76.3	58.5	8.2	188.8	77.3	119.0	43.1	152.9	428.2	581.1
500–800	35.2	25.73	11.38	116.9	59.1	62.0	6.6	248.2	85.0	143.2	62.4	185.6	538.8	724.4
< 500	26.5	33.30	14.03	119.4	54.2	63.3	5.9	252.9	84.7	136.1	68.8	188.6	542.5	731.1

taken from: Bohnsack C., Büsing A., Ternes W. and Drotleff A.M. [2011]
T, tocopherols; T, tocopherols; T3, tocotrienols, dw, dry weight [a] Calculated as β-T [b] Marked values are significantly higher than the corresponding value of the same sample´s fraction with larger particle size ($p > 0.001$)

Table 3

Tocochromanols in oils extracted from sieving fractions of dried brewer´s spent grain after plant-scale milling and sieving using different extraction methods and scales

Extraction method	Oil yield [% dw]	Colour of extracted oil	Tocopherols [mg·kg^{-1} of oil extracted with 96% ethanol]				Tocotrienols [mg·kg^{-1} of oil extracted with 96% ethanol]				[mg·kg^{-1} of oil extracted with 96% ethanol]		
			α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total T (α-, γ- + δ-T	Total T3	Total T + T3
Lab-scale Soxhleth	14.0	yellowish	119	54[a]	63	6	253	85	136	69	189	543	731
Lab-scale maceration[b] I (400 g)	12.1	yellowish	116	65[a]	67	9	248	82	116	71	192	517	709
Lab-scale maceration[b] II (400 g)	9.5	yellowish	103	57[a]	56	8	181	68	95	54	167	398	565
Plant-scale maceration[c] (125 kg)	5.2	dark brown	48	3	7	4	72	8	11	11	59	102	161

T, tocopherols; T, tocopherols; T3, tocotrienols, dw, dry weight [a] β-T + coeluting matrix peak , calculated as β-T
[b] One-step maceration was performed using 4 L 90% ethanol; extraction process lasted 4 h at room temperature
[c] One-step maceration was performed using 1000 L 96% ethanol; extraction process lasted 4 h at room temperature, overnight precipitation 14 h, and solvent evaporation 6 h (50°C, 100 mbar)

sieving fractions <500 µm prepared from freshly dried brewer's spent grain was 965 mg·kg^{-1}, this decreasing 74% within 4 months, and 84% within 11 months of storage. These findings tally with those of Piironen et al. [1988] who found a poor storage stability of T and T3 in wheat flour and rye meal: in 12 months at room temperature, the losses of α-T3 and α-T were about 80% [Piironen et al. 1988].

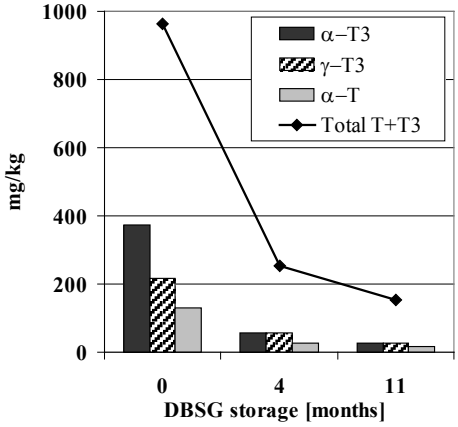


Fig. 2. Decrease of α-tocotrienol (α-T3), γ-tocotrienol (γ-T3), α-tocopherol (α-T), and total tocochromanol (T + T3) levels in oil extracted from DBSG sieving fractions <500 µm, in function of DBSG feedstuff storage (room temperature, exclusion of light)

Quality aspects: degradation of T3 during large-scale solvent extraction of a DBSG sieving fraction

Plant-scale drying of brewer´s spent grain is already in practice, and plant-scale milling and sieving of DBSG have proven by us to be suitable for optimising the feedstuff. However, large-scale solvent extraction of DBSG sieving fractions is still a challenge. The results from lab- and large-scale maceration tests are depicted in Table 3. Though lab-scale maceration resulted in a similar oil yield and tocochromanol content of the extracted oil compared to Soxhlet extraction, the large-scale maceration failed to reproduce this result: the oil yield was low and the T3 content decreased about 75%. On the basis of these results, maceration as practised in the pharmaceutical industry does not seem to be optimal for the solvent extraction process due to tocochromanol degradation.

Cost-effectiveness is essential for profitable T3 extraction and we calculated that competitive prices would be achievable when applying large-scale solvent extraction (10–100 t feedstuff) as commonly used for oil seed extraction. Pre-tests under real conditions are not possible at present, mainly because there is no company available which accepts commision orders in these mass ranges of feedstuff. Therefore, the suitability of the final step of the proposed T3 extraction procedure remains to be confirmed.

We found out that degradation of α-T3 and other tocochromanols is an important issue in DBSG processing and extraction. However, knowing that oxidation is the main reason for α-T3 degradation, information on oxidation products in DBSG extracts is lacking so far.

Determining the tocochromanol level at a single date of food processing or storage by use of the common analytical methods (HPLC with fluorescence detection), one gets no information on the proportion of tocochromanols which have already undergone degradation forming oxidation products. To consider food quality and safety concerns an analytical method is needed as a precondition for investigating α-T3 oxidation products.

Investigation of α-T3 oxidation products

Autoxidation of α-T3 in the model system n-hexane generated a mixture of α-T3 oxidation products. A newly developed HPLC method made it possible to separate α-T3, its most prominent oxidation products and the other T3 and T isomers within 60 min. Structure elucidation using spectroscopic methods revealed α-tocotrienol dimers and trimers and α-tocotrienolquinone dimers as oxidation products as well as the following so far unknown α-T3 oxidation products: 5formyl-γ-tocotrienol (5FγT3), 7-formyl-β-tocotrienol (7FβT3), and α-tocotrienolquinone (αT3Q) [Büsing and Ternes 2010].

Determining naturally occurring α-T3 oxidation products in oils and fats requires an isolation method from the lipid matrix. In our study, lard and wheat germ oil (containing α-T3 and its oxidation products) were molecularly distilled and distillates were HPLC analysed. 5FγT3, 7FβT3 and α-T3Q as well as α-T3 were enriched tenfold by molecular distillation from a lipid matrix, indicating an effective extraction of tocochromanols and oxidation products from lipid matrices for subsequent HPLC analysis [Büsing and Ternes 2010].

The newly developed molecular distillation method is at present applied to investigate the degradation kinetics of α-T3 and the formation of its oxidation products in lipid matrices by means of HPLC. The results will be published soon.

Conclusions

Milled DBSG sieving fractions <500 µm appear to be a suitable feedstock for economical extraction of T3-rich oil and may be useful e. g. in developing food additive or healthcare markets. In terms of tocochromanol extraction efficiency and to meet concerns on food quality and safety, efforts should be made to preserve the valuable vitamin E isomers and to prevent the generation of their oxidation products. The compounds 5FγT3, 7FβT3 and α-T3QSPD were described for the first time as resulting from α-T3 oxidation. Furthermore, the enrichment of analytes by molecular distillation allows the investigation of the most prominent α-T3 oxidation products in lipid matrices. α-T3 oxidation products may serve as indicators for elapsed or ongoing oxidation processes. The newly developed analytical HPLC method may be the basis for further studies on quality aspects of T3 rich functional food and on physiological properties of T3 oxidation products.

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References

Bohnsack C., Büsing A., Ternes W., Drotleff A.M., 2011. Tocotrienol levels in sieving fraction extracts of brewer's spent grain. European Food Research and Technology, nr 232, 563–573.

Büsing A., Ternes W., 2010. Trennung und Charakterisierung der Oxidationsprodukte von Tocotrienolen. Lebensmittelchemie, nr 64, 134.

Cornwell D.G., Kim S., Mazzer P.A., Jones K.H., Hatcher P.G., 2003. Electrophile tocopheryl quinones in apoptosis and mutagenesis: Thermochemolysis of thiol adducts with proteins and in cells. Lipids, nr 38, 973–979.

Goh S.H., Hew N.F., Lee M., 1992. Stereochemistry of Bichromanyl Dimers from Gamma-Tocopherol and Gamma-Tocotrienol. Tetrahedron Letters, nr 33, 4613–4616.

Jones K.H., Liu J.J., Roehm J.S., Eckel J.J., Eckel T.T., Stickrath C.R., Triola C.A., Jiang Z.C., Bartoli G.M., Cornwell D.G., 2002. gamma-Tocopheryl quinone stimulates apoptosis in drug-sensitive and multidrug-resistant cancer cells. Lipids, nr 37, 173–184.

Kamal-Eldin A., Appelqvist L.A., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, nr 31, 671–701.

Kupczyk B., Gogolewski M., 2003. Effect of menadione (vitamin K-3) addition on lipid oxidation and tocopherols content in plant oils. Nahrung-Food, nr 47, 11–16.

Liu D.H., Shi J., Posada L.R., Kakuda Y., Xue S.J., 2008. Separating tocotrienols from palm oil by molecular distillation. Food Reviews International, nr 24, 376–391.

Moreau R.A., Wayns K.E., Flores R.A., Hicks K.B., 2007. Tocopherols and tocotrienols in barley oil prepared from germ and other fractions from scarification and sieving of hulless barley. Cereal Chemistry, nr 84, 587–592.

Panfili G., Fratianni A., Di Criscio T., Marconi E., 2008. Tocol and beta-glucan levels in barley varieties and in pearling by-products. Food Chemistry, nr 107, 84–91.

Piironen V., Varo P., Koivistoinen P., 1988. Stability of tocopherols and tocotrienols during storage of foods. Journal of Food Composition and Analysis, nr 1, 124–129.

Pirisi F.M., Angioni A., Bandino G., Cabras P., Guillou C., Maccioni E., Reniero F., 1998. Photolysis of alpha-tocopherol in olive oils and model systems. Journal of Agricultural and Food Chemistry, nr 46, 4529–4533.

Posada L.R., Shi J., Kakuda Y., Xue S.J., 2007. Extraction of tocotrienols from palm fatty acid distillates using molecular distillation. Separation and Purification Technology, nr 57, 220–229.

Rennick K.A., Warner K., 2006. Effect of elevated temperature on development of tocopherolquinones in oils. Journal of Agricultural and Food Chemistry, nr 54, 2188–2192.

Sen C.K., Khanna S., Roy S., 2006. Tocotrienols: Vitamin E beyond tocopherols. Life Sciences, nr 78, 2088–2098.

Seppanen C.M., Song Q.H., Csallany A.S., 2010. The Antioxidant Functions of Tocopherol and Tocotrienol Homologues in Oils, Fats, and Food Systems. Journal of the American Oil Chemists Society, nr 87, 469–481.

Serbinova E., Kagan V., Han D., Packer L., 1991. Free-Radical Recycling and Intramembrane Mobility in the Antioxidant Properties of Alpha-Tocopherol and Alpha-Tocotrienol. Free Radical Biology and Medicine, nr 10, 263–275.

Siegel D., Bolton E.M., Burr J.A., Liebler D.C., Ross D., 1997. The reduction of alpha-tocopherolquinone by human NAD(P)H: Quinone oxidoreductase: The role of alpha-tocopherolhydroquinone as a cellular antioxidant. Molecular Pharmacology, nr 52, 300–305.

Ternes W., Drotleff A.M., Bohnsack C., Schneider I., Hahn A., 2011. Tocotrienol extracts from barley by-products: unique health benefits of the better vitamin E, [in:] Podbielska H., Trziszka T., (Eds.), Biomedical Engineering Acta. Indygo Zahir Media, Wroclaw, 43–51.

Tiwari U., Cummins E., 2009. Nutritional importance and effect of processing on tocols in cereals. Trends in Food Science & Technology, nr 20, 511–520.

Wagner K.H., Wotruba F., Elmadfa I., 2001. Antioxidative potential of tocotrienols and tocopherols in coconut fat at different oxidation temperatures. European Journal of Lipid Science and Technology, nr 103, 746–751.

Yang S.G., Wang W.Y., Ling T.J., Feng Y., Du X.T., Zhang X., Sun X.X., Zhao M., Xue D., Yang Y., Liu R.T., 2010. Alpha-tocopherol quinone inhibits beta-amyloid aggregation and cytotoxicity, disaggregates preformed fibrils and decreases the production of reactive oxygen species, NO and inflammatory cytokines. Neurochemistry International, nr 57, 914–922.

Yap S.P., Yuen K.H., Lim A.B., 2003. Influence of route of administration on the absortion and disposition of alpha-, gamma- and delta-tocotrienols in rats. Journal of Pharmacy and Pharmacology, nr 55, 53–58.

Yoshida Y., Niki E., Noguchi N., 2003. Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chemistry and Physics of Lipids, nr 123, 63–75.

4

ANTI-LISTERIA ACTIVITY OF ESSENTIAL OILS OBTAINED FROM EGYPTIAN AROMATIC PLANTS IN SKIMMED MILK AND FULL CREAM

Introduction

Many of the organoleptic and sensorial properties of food products diminish with time. This may be attributed to a large number of factors, including the action of oxygen, light and temperature, although the most important effect is that of microorganisms, whether they are molds, bacteria or yeasts [Viuda-Martos et al. 2008]. In addition, illnesses caused due to the consumption of foods contaminated with pathogens such as *Listeria monocytogenes*, *Clostridium botulinum* or *Sthaphylococcus aureus* have a wide economic and public health impact worldwide [Gandhi and Chikindas 2007]. For safety reasons, *Listeria innocua* is commonly used as a model species for predicting *Listeria monocytogenes* performance. *Listeria* genus is able to grow at refrigerated temperatures and is a facultative anaerobe that grows on a variety of complex media as foods. They are Gram-positive rods [Batt 2000]. *Listeria* genus can adapt to survive and grow in a wide range of environmental conditions as well as in a large variety of raw and processed foods, including milk and dairy products.

For many years, a range of different chemical and synthetic compounds have been used as antibacterial and antifungal agents to inhibit microbial food spoilage. Antimicrobial substances used in the food industry include chemical substances (added or already present naturally in foods), which are used in the food industry for two main reasons: (i) to control natural spoilage processes (food preservation), and (ii) to prevent/control growth of micro-organisms, including pathogenic micro-organisms (food safety) [Tajkarimi and Ibrahim 2010]. Although concerns about the economic and safety of these chemicals have increased consumer demand for naturally processed food.

Natural compounds from microbial (bacteriocins), animal (chitosan) and vegetal (plant products) origin have been and are under study. Among the plant derived compounds, recently, there has been considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and toxin producing microorganisms in foods [Soliman and Badeea 2002, Tepe et al. 2005]. In this context, plant essential oils (EOs) are gaining interest for their potential as preservative ingredients or decontaminating treatments, as they have GRAS (Generally Recognized As Safe) status and a wide acceptance from consumers [Burt 2004].

Essential oils extracted from spices and herbs have been extensively studied for antimicrobial activity even though their poor solubility, sedimentation and volatility make it difficult to use them in foods or to quantify their activity by diffusion or dilution assays [Kalemba and Kunicka 2003]. Thus, numerous *in vitro* studies have demonstrated

the effectiveness of spices essential oils against food-borne pathogens [Oroojalian et al. 2010, Alexopoulos et al. 2011, Viuda-Martos et al. 2011]. So far, little information is known about the inhibitory effect of spices EOs against specific food-borne pathogens in food and especially in milk and dairy products [Govaris et al. 2011]. These properties are due to many bioactive compounds. These bioactive compounds are defined as non-nutritive constituents of spices or aromatic herbs, which usually occur in very small quantities. A vast diversity of bioactive components in plants has been found: terpenes, lignans, sulfides, carotenoids, cumarins, saponins, phytosterols, and polyphenols, including flavonoids, anthocyanins and phenolic acids [Capecka et al. 2005]. The antimicrobial activity of EOs depends, mainly, on their chemical composition. EOs in general terms, are composed of more than seventy components [Russo et al. 1998], some of which may represent more than 85% of the total content, while others may only be present in trace amounts [Bakkali et al. 2008]. However, the role played by these minor compounds is very important since evidence suggests that they may contribute significantly to the functional properties of EOs, in which they sometimes act synergically [Viuda-Martos et al. 2011b]. However, the application of plant EOs for control of food-borne pathogens and food spoilage bacteria requires the evaluation of its efficacy within food products or in model systems that closely simulate food composition [Gutierrez et al. 2009]. Due to the complex mechanism of activity of EOs against pathogens and food spoilage microorganisms, their effect in complex matrices such as food/spoiled surfaces is only achieved with higher concentration of EOs as compared to the minimum inhibitory concentrations reported in conventional nutrient media [Burt 2004]. In general, the efficacy of many added and naturally occurring antimicrobials may be reduced by certain food components [Glass et al. 2004].

The aim of this study was investigated the effectiveness *in vitro* of the EOs from five aromatic herbs such as oregano (*Origanum syriacum*), marjoram (*Majorna hortensis*), rosemary (*Rosmarinus officinalis*), black cumin (*Nigella sativa*) and thyme (*Thymus vulgaris*) on growth of a food-borne indicators as *Listeria innocua* in skimmed milk and full cream.

Material and methods

Plant materials

Oregano (*Origanum syriacum*), marjoram (*Majorna hortensis*), rosemary (*Rosmarinus officinalis*), black cumin (*Nigella sativa*) and thyme (*Thymus vulgaris*) were collected from the Sekem company plantation in the city of Bilbeis in Sharkea region (NE, Cairo) during the flowering period. The plantation is certified for organic biodynamic agriculture by COAE (Center of Organic Agriculture in Egypt). The identification of the plant material was made by Prof Dr. Kamal Zayed, Plant Botany Faculty of Science, Cairo University (Egypt).

Extraction of essential oil

The EOs of oregano, marjoram, rosemary, black cumin and thyme were extracted from entire plant (stems, leaves and flowers) by hydro-distillation using a Clevenger-type apparatus for 3 h. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulphate (0.5 g). The extracted EOs was kept in sealed air-tight glass vials and covered with aluminium foil at 4ºC until further analysis.

Microbial strains

The essentials oils were individually tested against *L. innocua* CECT 910. This specie was supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia.

Agar disc diffusion method

The agar disc diffusion method described by Tepe et al. [2005] with some modifications was used to determine the antibacterial capacity of the EOs. Briefly, a suspension (0.1 mL of 10^6 CFU/mL) of *Listeria innocua* was spread on the solid medium plates made with skimmed milk or full cream. Milk model media and full cream media were made mixing skimmed milk powder or full cream with agar solution (Scharlau Chemie), both autoclaved separately, in order to obtain a final solid media solution with 1.5% agar. Full cream (35% fat) was directly blended with agar and skimmmed milk was made from powder and reconstituted with distilled water according to the manufacturer's instructions (10% milk solids). Sterile filter paper discs, 9 mm in diameter (Schlinder & Schuell, Dassel, Germany) were impregnated with 40 μL of the oil and placed on the inoculated plates; these plates were incubated at 37ºC for 24 h. The diameters of the inhibition zones were measured in millimetres. All tests were performed in triplicate.

Determination of concentration effect

The concentration effect (CE) was studied for to ascertain which doses of EO had an inhibitory effect on bacterial growth in the disc diffusion assay. The culture techniques used were those described in the previous paragraph (Agar disc diffusion method), but adding 40, 20, 10, 5 and 2 μL of EO which meant doses of 100, 50, 25, 12.5 and 5% of the initial volume. All tests were performed in triplicate.

Statistical analysis

Conventional statistical methods were used to calculate means and standard deviations of three simultaneous assays carried out with the different methods. Statistical analysis (ANOVA) was applied to the data to determine differences ($P < 0.05$). To discover where there were significant differences between the levels of the main factor, contrasts (Tukey test) between means were made [Afifi and Azen 1979]. ANOVA was made with the following factors: doses (five levels; 40, 20, 10, 5 and 2 μL) for each EO. The Statistical analyses were made using Statgraphics 5.1 for Windows.

Results and discussion

The chemical composition of the EOs used in this work was previously determined by Viuda-Martos et al. [2010] and Viuda-Martos et al. [2011a]. In the essential oil of *T. vulgaris* the major constituents being thymol (48.64%), *p*-cymene (22.94%) and α-terpinolene (6.49%). In *O. syriacum* essential oil the major constituent being thymol (21.04%) γ-terpinene (18.96%) and terpinen-4-ol (17.20%). In *M. hortensis* essential oil the main components being terpinen-4-ol (41.43%), γ-terpinene (9.26%) and α-terpineol (6.51%). In *N. sativa* essential oil the predominant compounds were *p*-cymene (33.03%), thymoquinone (32.18%) and α-thujene (13.01%). In the essential oil of *R. officinalis* the major constituents being 1,8-cineole (23.59%). Other important compounds were camphor (20.70%) and α-pinene (18.21%).

Antibacterial activity

Table 1 shows the antibacterial activity of *Origanum syriacum, Majorna hortensis*, *Rosmarinus officinalis, Nigella sativa* and *Thymus vulgaris* against *Listeria innocua* in skimmed milk and cream assessed for the presence or absence of inhibition zones.

In skimmed milk the essential oils of thyme, marjoram, rosemary, black cumin, and oregano, showed inhibitory effects ($P < 0.05$) on the tested bacteria (Fig. 1A). The agar disc diffusion method indicated that thyme EO showed the highest antibacterial activity against *L. innocua* with an inhibition zone of 29.00 mm, the second most effective essential oil in this respect was rosemary, which showed an inhibition zone of 28.50 mm. The other oils showed similar antibacterial activities. As regards full cream any of the five EOs assayed showed antibacterial activity (Fig. 1B).

Table 1

The antibacterial activity effect (CE) of *Origanum syriacum, Majorna hortensis*, *Rosmarinus officinalis, Nigella sativa* and *Thymus vulgaris* against *Listeria innocua* in skimmed milk and cream. Diameter (mean and SD) of inhibition zone (mm) including disc diameter of 9 mm

		Listeria innocua	
	Concentration [µL]	Skimmed milk [mm]	Cream [mm]
Thymus vulgaris	40	29.00±1.41	N.A.
Origanum syriacum	40	26.50±0.71	N.A.
Nigella sativa	40	26.00±0.00	N.A.
Rosmarinus officinalis	40	28.50±0.71	N.A.
Majorna hortensis	40	25.50±0.71	N.A.

N.A.: non active.

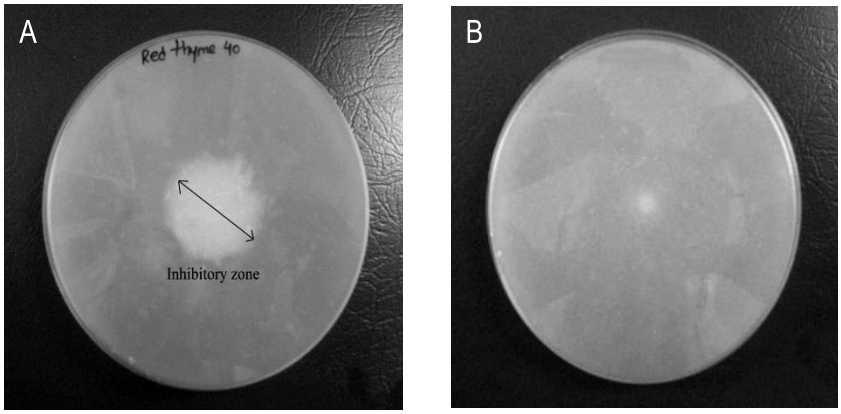

Fig. 1. (A) Inhibitory zone (antimicrobial effect) of *L. innocua* growth on a bacterial plate with skimmed milk induced by thyme essential. (B) Non active effect on *L. innocua* growth on a bacterial plate with full cream induced by thyme essential oil

Gutierrez et al. [2009] reported that when *Listeria monocytogenes* NCTC11994 and *Pseudomonas fluorescens* were exposed to oregano or thyme essential oil on milk model media, it was observed that the minimum inhibitory concentrations (MICs) of these EOs were approximately 10 fold higher than those obtained on the control media. MICs of oregano and thyme against the *Listeria* strain on the milk model media were 1000 and 3000 ppm respectively. Yoon et al. [2011] evaluated the effect of cone essential oil of *Metasequoia glyptostroboides* against *Listeria monocytogenes* inoculated in whole (8%), low (1%) and skim milks. Essential oil at the concentrations of 2% and 5% revealed strong antilisterial effect against *L. monocytogenes* in all categories of milks. High water activity positively affects the application of EOs in milk by speeding the transfer and movement of EOs toward the targeted micro-organisms [Cava et al. 2007].

Although EOs antimicrobial action is established, their mechanism of action has not been completely explained in detail [Lambert et al. 2001]. In fact, considering the large number of different chemical compounds present in EOs, it is most likely that their antibacterial activity is not ascribable to one specific mechanism, but that several targets exist in the cell [Carson et al. 2002]. For Cristani et al. [2007] the antibacterial effect of some components of EOs may be due, partially at least, to a perturbation of the lipidic fraction of microorganism plasmic membrane, resulting in alterations of membrane properties. In addition to being related to physicochemical characteristics of the compounds (such as lipophilicity and water solubility), this effect appears to be dependent also on net surface charge of microbe membranes. In this way, Goni et al. [2009] demonstrated that the antimicrobial effects of the EOs acts by causing structural and functional damages to the bacterial cell membrane. It is also indicated that the optimum range of hydrophobicity is involved in the toxicity of the EOs [Goñi et al. 2009]. For other authors [Skocibusic et al. 2006, Burt et al. 2007, Arques et al. 2008, Proestos et al. 2008] the essential oils bioactive compounds affect microbial cells by various antimicrobial mechanisms, including attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids. As regards full cream samples, many factors in food could be responsible for the reduction of antimicrobial activity of plant extracts when applied on different types of food. A protective effect of high fat levels was observed by Ismael and Pierson [1990] and Shelef el al. [1984]. However they reported that antimicrobial activity of spices and oils diminished in foods as a result of the solubilization of the antimicrobial agents into the food's lipid fraction. On the other hand EOs of clove, cinnamon, bay and thyme were tested against *L. monocytogenes* and *Salmonella enteritidis* in soft cheese; clove oil was found more effective against *Salmonella enteritidis* in full-fat cheese than in cheese slurry [Burt 2004]. The use of EOs as antimicrobials in food may be hampered by effective dosages, interference by food constituents or other food-grade compounds, unsuitable water activity, incompatible pH or processing regiment [Gutierrez et al. 2008].

Determination of Concentration effect (CE)

The CE values against *Listeria innocua* can be seen in Table 2. As can be seen, the inhibitory effect increased with increasing concentrations. In other words, their inhibitory effect was related to the concentration used (Fig. 2). Only thyme essential oil showed inhibitory effects ($P < 0.05$) on *L. innocua* in all concentrations tested in skimmed milk with inhibition zones ranging from 29.00 to 20.00 mm for the highest and the lowest concentration respectively. The disk impregnated with 2 µL of essential oils of oregano and black cumin and the disks impregnated with 5 and 2 µL of marjoram and rosemary essential oil did not have inhibitory

effects ($P > 0.05$) on *L. innocua* in skimmed milk. When oregano essential oil was analyzed, statistically significant differences ($P < 0.05$) existed between 40, 20, 10 and 5 µL concentrations used on *L. innocua* in skimmed milk. The same was true for black cumin essential oil. As regards rosemary, significant differences ($P < 0.05$) were observed between the 10, 20 and 40 concentrations on *L. innocua* in skimmed milk. The same was true for marjoram essential oil. As regards full cream none of the five EOs assayed showed antibacterial activity at the tested concentrations.

Table 2

The concentration effect (CE) of *Origanum syriacum, Majorna hortensis*, *Rosmarinus officinalis, Nigella sativa* and *Thymus vulgaris* against *Listeria innocua* in skimmed milk and cream. Diameter (mean and SD) of inhibition zone (mm) including disc diameter of 9 mm

		Listeria innocua	
	Concentration [µL]	Skimmed milk [mm]	Full cream [mm]
Thymus vulgaris	40	29.00±1.41[a]	N.A.
	20	27.00±0.00[b]	N.A.
	10	25.00±0.00[c]	N.A.
	5	22.50±0.71[d]	N.A.
	2	20.00±0.00[e]	N.A.
Origanum syriacum	40	26.50±0.71[a]	N.A.
	20	24.00±0.00[b]	N.A.
	10	20.50±0.71[c]	N.A.
	5	16.00±0.00[d]	N.A.
	2	N.A.	N.A.
Nigella sativa	40	26.00±0.00[a]	N.A.
	20	22.00±0.00[b]	N.A.
	10	15.50±0.71[c]	N.A.
	5	14.00±0.00[d]	N.A.
	2	N.A.	N.A.
Rosmarinus officinalis	40	28.50±0.71[a]	N.A.
	20	16.00±0.00[b]	N.A.
	10	12.00±0.00[c]	N.A.
	5	N.A.	N.A.
	2	N.A.	N.A.
Majorna hortensis	40	25.50±0.71[a]	N.A.
	20	21.00±0.00[b]	N.A.
	10	13.50±0.71[c]	N.A.
	5	N.A.	N.A.
	2	N.A.	N.A.

For the same essential oil, values followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple-range test. N.A.: non active.

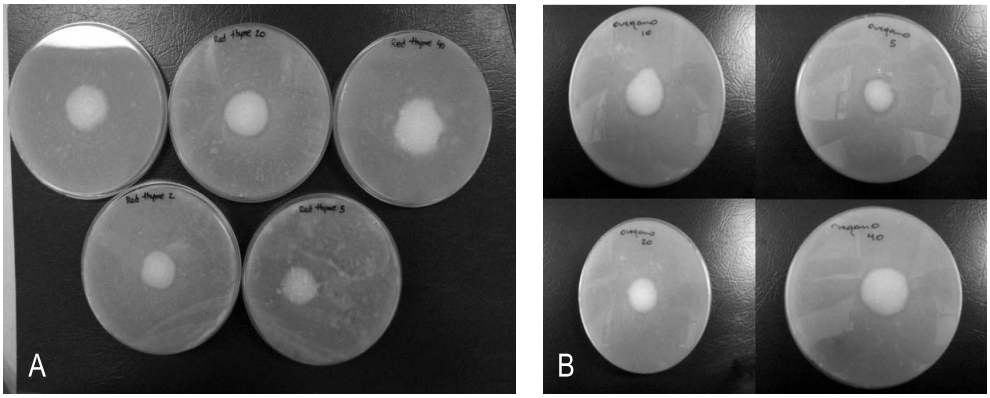

Fig. 2. (A) Concentration effect of thyme essential oil agaisnt *L. innocua* growth on a bacterial plate with skimmed milk. (B) Concentration effect of oregano essential oil against *L. innocua* growth on a bacterial plate with skimmed milk

Conclusions

Spices essential oils can be used as natural antimicrobials and represent a useful alternative for the food industry to reduce the quantity of synthetic additives used in their attempt to satisfy the demands of consumers, as long as the same consumers accept their effect on the organoleptic properties of the foods in question. However, their application for microbial control might be affected by food composition, therefore, careful selection of essential oils appropriate to the sensory and compositional status of the food system is required.

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References

Afifi A.A., Azen, S.P., 1979. Statistical analysis. A computer oriented approach. (p. 18). London: Academic.

Alexopoulos A., Kimbaris A.C., Plessas S., Mantzourani I., Theodoridou I., Stavropoulou E., Polissiou M.G., Bezirtzoglou E., 2011. Antibacterial activities of essential oils from eight Greek aromatic plants against clinical isolates of *Staphylococcus aureus*. (In Press).

Arques J.L., Rodriguez E., Nunez M., Medina M., 2008. Inactivation of Gramnegative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. Eur. Food Res. Technol. 227(1), 77–82.

Batt C.A., 2000. Listeria: Introduction, [in:] R.K. Robinson (Ed), Encyclopedia of Food Microbiology, London, Academic Press, 1195–1198.

Bakkali F., Averbeck S., Averbeck D., Idaomar M., 2008. Biological effects of essential oils-A review. Food Chem. Toxicol., 46, 446–475.

Burt S.A., 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. Int. J. Food Microbiol, 94 (3), 223–253.

Burt S.A., Der Zee R.V., Koets A.P., De Graaff A.M., Van Knapen F., Gaastra W., 2007. Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in Escherichia coli O157:H7. Appl. Environm. Microbiol., 73, 4484–4490.

Capecka E., Mareczek A., Leja M., 2005. Antioxidant activity of fresh and dry herbs of some *Lamiaceae*species. Food Chem., 93, 223–226.

Carson C.F., Mee B.J., Riley T.V., 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother., 46, 1914–1920.

Cava R., Nowak E., Taboada A., Marin-Iniesta F., 2007. Antimicrobial activity of clove and cinnamon essential oils against *Listeria monocytogenes* in pasteurized milk. J.Food Prot., 70(12), 2757–2763.

Cristani M., Manuela D'arrigo M., Mandalari, G., Castelli F., Maria Grazia Sarpietro M.G., Dorotea Micieli D., Vincenza Venuti, V., Bisignano G., Saija A., Trombetta D., 2007. Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. J. Agric. Food Chem., 55(15), 6300–6308.

Gandhi M., Chikindas M.L., 2007. Listeria: a foodborne pathogen that knows how to survive. Int. J. Food Microbiol., 113 (1), 1–15.

Glass K.A., Johnson E.A., 2004. Antagonistic effect of fat on the antibotulinal activity of food preservatives and fatty acids. Food Microbiol., 21, 675–682.

Goñi P., López P., Sánchez C., Gómez-Lus R., Becerril R., Nerin C., 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem., 116, 982–989.

Govaris A., Botsoglou E., Sergelidis D., Chatzopoulou P.S., 2011. Antibacterial activity of oregano and thyme essential oils against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in feta cheese packaged under modified atmosphere. LWT-Food Sci. Technol., 44, 1240–1244.

Gutierrez J., Barry-Ryan C., Bourke P., 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol., 124 (1), 91–97.

Gutierrez J., Barry-Ryan C., Bourke P., 2009. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. Food Microbiol., 26, 142–150.

Ismael A., Pierson M.D., 1990. Inhibition of growth and germination of *Clostridium botulinum* 33A, 40B and 1623E by essential oil of spices. J. Food Sci., 55, 1676–1678.

Kalemba D., Kunicka A., 2003. Antibacterial and antifungal properties of essential oils. Current Med. Chem., 10(10), 813–829.

Lambert R.J.W., Skandamis P.N., Coote P., Nychas G.J.E., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol., 91, 453–462.

Oroojalian F., Kasra-Kermanshahi R., Azizi M., Bassami M.R., 2010. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. Food Chem., 120(3), 765–770.

Proestos C., Boziaris I., Kapsokefalou S.M., Komaitis, M., 2008. Natural antioxidant constituents from selected aromatic plants and their antimicrobial activity against selected pathogenic microorganisms. Food Technol. Biotechnol., 46, 151–156.

Russo M., Gallet G.C., Bocchini P., Carnacini A., 1998. Essential oil chemical composition of wild populations of italian oregano spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): a preli-

minary evaluation of their use in chemotaxonomy by cluster analysis. 1. inflorescences. J. Agric. Food Chem., 46, 3741–3746.
Shelef L., Jyothi E., Bulgarelli M., 1984. Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. J. Food Sci., 49, 737–740.
Skocibusic M., Bezic N., Dunkic V., 2006. Phytochemical composition and antimicrobial activities of the essential oils from Satureja subspicata Vis. growing in Croatia. Food Chem., 96(1), 20–28.
Soliman K.M. and Badeea R.I., 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi, Food Chem. Toxicol., 40, 1669–1675.
Tajkarimi M.M., Ibrahim S.A., Cliver D.O., 2010. Antimicrobial herb and spice compounds in food. Food Control., 21, 1199–1218.
Tepe B., Daferera D., Sokmen A., Sokmen M., Polissiou M., 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem., 90, 333–340.
Viuda-Martos M., Ruiz-Navajas Y., Fernández-López J., Pérez-Alvarez J.A., 2008. Antibacterial activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. J. Food Safety., 28, 567–576.
Viuda-Martos M., Abd El-Nasser G.S. El Gendy, Sendra E., Fernández-López J., Abd ElRazik K.A., Omer E.A., Pérez-Alvarez J.A., 2010. Chemical Composition and Antioxidant and Anti-Listeria Activities of Essential Oils Obtained from Some Egyptian Plants. J. Agric. Food Chem., 58, 9063–9070.
Viuda-Martos M., Mohamady M.A., Fernández-López J., Abd ElRazik K.A., Omer E.A., Pérez-Alvarez J.A., Sendra E., 2011a. In vitro antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. Food Control., 22, 1715–1722.
Viuda-Martos M., Ruiz-Navajas Y., Fernández-López J., Pérez-Alvarez J.A., 2011b. Spices as functional food. Crit. Rev. Food Sci. Nutr., 51(1), 13–28.
Yoon J.I., Bajpai V.K., Kang S.C., 2011. Synergistic effect of nisin and cone essential oil of *Metasequoia glyptostroboides* Miki ex Hu against *Listeria monocytogenes* in milk samples. Food Chem. Toxicol., 49(1), 109–114.

5

ANTIOXIDANT ACTIVITY OF SPICES IN MEAT PRODUCTS

Introduction

Interest in plant-derived food additives has grown recently because of negative consumer perception of synthetic antioxidants (BHT, BHA). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are both effective syntethic antioxidants, but they are believed to lead to health risk [Martinez-Tome et al. 2001]. Therefore, some natural ingredients including spices has been studied as potential antioxidants in meat and meat products. Many spices, usually used to flavour dishes, are excellent source of natural antioxidants including phenolic diterpenes, flavonoids, tannins and phenolic acids [Dawidowicz et al. 2006]. These compounds have antioxidant, anti-inflammatory and anticancer activities [Ramarathnam et al. 1995]. Spices, like ginger, fenugreek, mustard, etc. may offer many health benefits and have been proven to counteract oxidative stress [Modak et al. 2007, Vasi and Austin 2009]. The study of Manda et al. [2010] showed that spices (fenugreek, mustard, ginger, turmeric, cardamon, coriander) contained different levels of biothiols. Biothiols are important antioxidants which reduce the oxidative damage in cells [Wlodek 2002]. According to Manda et al. [2010] spices can be a significant source of thiols and, along with the phenols present, can enhance the antioxidant status of the biological system. The high level of thiols observed in some spices may reinforce the idea that incorporation of spices into meals could be beneficial for health by protecting against oxidative damage related problems. Vasi and Austin [2009] determined the effect of herbal hypoglycemics on oxidative stress and antioxidant status in diabetic rats. Results of their study indicated that *G. sylvestre*, *S. reticulata, C. auriculata* and *E. jambolanum* not only have hypoglycemic activity but they also significantly reduce the plasma lipid peroxide levels in diabetic rats. Moreover, the activity of the anti-oxidant enzyme superoxide dismutase and serum albumin content were also increased.

The addition of natural antioxidants is a major way to inhibit lipid and myoglobin oxidation in meat products. Lipid oxidation, like most free radical reactions, is very sensitive to catalysts and inhibitors; nonlipid materials such as metals rapidly catalyze the reaction.

In food system, spices can inhibit lipid oxidation-induced food deteriorations. Natural antioxidants extracted from spices exhibit various degrees of efficacy in different food system. Several studies showed relationship between total phenolic content and antioxidant activity of selected plant extract. The data showed by Kong et al. [2010] indicated that the extracts of 13 culinary spices contained antioxidant activity. Six of the extracts (clove, rosemary, cassia bark, liquorice, nutmeg and round cardamom) were identified to be strong inhibitors of TBARS formation as well as to have the greatest total phenolic contents. They also suggested mechanism of antioxidant activities in the inhibition of lipid oxidation: stabilizing oxidizing radicals, donating hydrogen or electrons, and sequestering prooxidative metal ions.

Among spices, rosemary is known to have great antioxidant activity due to its high levels of phenolic content [Zhang et al. 2010]. Phenolic compounds are important constituents of

spices and their radical scavenging ability is due to their hydroxyl groups and conjugated double bonds. Hinneburg et al. [2006] indicated good correlation the content of total phenolics assays of extracts from selected culinary spices with most of antioxidants assays, such as iron reduction, inhibition of lipid peroxidation. They did not found a correlation between phenolic and DPPH· reduction and iron chelation.

Muscle has also a large number of endogenous catalysts (myoglobin and ionic irons) [Allen and Conforth 2006]. Ferrous ion is known to lipid oxidation prooxidant. Spice extracts could chelate iron ions as well as suppress their reactivity by occupying all the coordination sites of the specific metal ions. Results obtained by Kong et al. [2010] indicated that some spice extracts (clove, rosemary, cassia bark, liquorice, nutmeg and round cardamom) characterized by substantial capability to chelate ferrous ion. Of the six spice extracts, cardamom extract showed the highest ferrous ion chelating activity. They suggested that the extracts were able to donate electrons to reactive radicals, converting them into unreactive species.

In addition, spices have antimicrobial ability. Natural antimicrobial agents derived from spices have been used in food preservation. Tajkarimi et al. [2010] indicated that spices containing essential oils in the range of 0.05–0.1% have demonstrated activity against pathogens in food systems (*Salmonella typimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*). The main mechanisms of antimicrobial effect of plant substances include attacking of phospholipids bilayer of the microbial cells membrane, disrupting enzyme activity, compromising the genetic material of bacteria, forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids. Turgis et al. [2009] suggested that allyl isothiocyanate derived from mustard seed may have multi-targeted mechanisms of action in metabolic pathways, membrane integrity, cellular structure and statistically significant higher release of the cell compounds of *Escherichia coli* O157:H7.

Bajpai et al. [2008] suggested that antimicrobial ability of spices might be related to the phenolic compounds present. According to them, the possible mechanisms for antimicrobial effect of phenolic compounds include: altering microbial cell permeability; interfering with membrane function (electron transport, nutrient uptake, protein and nucleic acid sysnthesis and enzyme activity).

Among the spices, mustard seed is reported to have antioxiadant capacity followed by tocopherol content [Amarowicz et al. 1996, Schuster-Gajzágó et al. 2006]. According to Manda et al. [2010] mustard seed include biothiols, which protect cells from oxidative damage. They determined biothiols content in several species. The results of their study showed that mustard seed contained the highest level of γ-glutamylcysteine out of analysed spices (fenugreek, mustard, ginger, turmeric, cardamon, coriander). Moreover, mustard extract showed the highest phenolic content and the radical scavenging power.

White mustard seed has been used effectively for food (mustard production) and medical applications; however, its advantageous chemical composition and relatively low price offer wide possibilities for utilisation of mustard seed in human foods as additive. The limiting factor of use of mustard seed in human food applications has been its typical spicy flavor and the presence of a significant amount of eruic acid. Mustard seed contains glucosinolates which are responsible for the characteristic, pungent flavor of mustard and mustard products [Alireza Sadeghi et al. 2006]. The main glucosinolate in white mustard is sinalbin, sinigrin and glucobrassicin [Schuster-Gajzágó et al. 2006]. The pungent flavor develops by myrosinase enzyme activities which releases isothiocyanates from glucosinolates [Shahidi and Naczk 1990]. Deactivation of myrosinase prevents glucosinolate decomposition. According to the data published in literature the use of radio frequency energy was effective to myrosinase

enzyme inactivation. Schuster-Gajzágó et al. [2006] suggested that the use of radio frequency energy as an emerging low-cost environmentally friendly thermal process for enzyme inactivation proved effective to produce a product with acceptable sensory properties. Saleemi et al. [1993] used low-pungency ground mustard seed to the production of comminuted pork samples. They indicated that the addition of mustard in the amount of 0–2% improves the cooking and slicing characteristics of products, because of its good colloid-chemical properties such as water and fat binding capacity, as well as emulsifying characteristics. Moreover, they suggested that low-pungency ground mustard seed was capable of extending the shelf life of meat products and enhancing their cook yields.

The literature contains limited information regarding antioxidant activity of mustard seed in meat products. Therefore, the objective of this study was to asses the influence of ground mustard seed on pH, water activity, color parameters and lipid oxidation in cooked meat products made of pork during storage.

Materials and methods

Investigations were carried out on cooked meat products produced from organic pork meat (*m. biceps femoris*). Three variants of cured stuffed meat products samples (in collagen casings) were obtained: 1 – control (no antioxidants), 2 – experimental containing 0.2% of ground mustard seed and 3 – experimental containing 0.5% of ground mustard seed. Fresh pork was obtained from the local meat plant in Lublin region and stored at 4°C prior to use (48 hours). The meat was cured using 2% curing mixture (99.5% NaCl, 0.05% sodium nitrite) and was stored at 4°C for 24 hours. Then, the meat was ground through a 10 mm plate. Mustard seed (low erucic acid content mustard variety) purchased from local plant was ground in a coffee grinder. All ingredients were then blend in hand. After the meat is ground and mixed it has been stuffed into synthetic collagen casings. The cooking of the stuffed product was carried out to an internal temperature of 72°C. Chilled meat product samples were packed into the HDPE bags and stored at 4°C until assessed. The measurements of the meat products quality were carried out 1, 5, 10 day after production and included: pH values, water activity, CIE L*a*b* color parameters and lipid oxidation.

pH measurements

For pH measurements 10 g of minced meat product was homogenized with 100 ml of distilled water for 1 min using a homogenizator (IKA ULTRA-TURRAX T25 Basic, Germany). The pH of resulting homogenate was measured with a digital pH-meter CPC-501(Elmetron, Poland) equipped with a pH electrode (ERH-111, Elmetron, Poland). Measurements were carried out on four replicates.

Water activity

The water activity (a_w) measurements were carried out at 20°C using a water activity analyzer (Novasina LabMaster). Measurements were carried out on four replicates.

Lipid oxidation

Lipid oxidation was determined as thiobarbituric acid reactive substances (TBARS) method according to Pikul et al. [1989]. The rose-pink color obtained by the reaction between malondialdehyde (MDA) and 2-thiobarbituric acid was measured at 532 nm (Nicole Evolution

300, Thermo Electron Corporation). Results were expressed as mg of MDA per kg of the sample (TBA units). Measurements were carried out in four replications for each sample.

Color measurements

Color (CIE L*a*b*) was assessed on the freshly cut surface of a meat products. Visible reflectance spectra (from 360 to 760 nm) were determined with an X-Rite Color® Premiere 8200 colorimeter (X-Rite Incorporated, Michigan, USA) with a D65 illuminant and a 10° standard observer [AMSA 2005]. The spectrocolorimeter was calibrated daily against a standard white plate. Samples for color measurements were 5 cm thick and excided at the depth of 20 mm. Oxygenation index (ΔR) was determined by reflectance difference between 630 nm (maximum of the oxidized myoglobin) and 580 nm (maximum of the oxygenated myoglobin) [Hunt et al. 1991]. Six measurements were taken on each sample and averaged for statistical analysis.

Statistical analysis

Three series of experiments were conducted. Obtained results were statistically analysed using the Microsoft Office Excel 2007. One-way analysis of variance was carried out. Significance of differences between samples at the same storage time and the same sample at different storage times was determined (at the significance level $p \leq 0.05$) using T-Tukey's test.

Results and discussion

The use of spices may offer meat processors the opportunity to develop novel products with enhanced nutritional and health benefits as well as improved shelf-life. The possible mechanism of antioxidant activity of spice extracts include reducing activity, free radical termination. Moreover, some plant extract can act as metal ion chelators and singlet oxygen quenchers. The data presented by Manda et al. [2010] suggested that antioxidant abilities of spices may not only be a result of phenolic content, but may also be connected with other antioxidant components, like thiols and flavonoids. They indicated that despite the highest phenolic content of mustard extract, none of antioxidant studies showed its strong effect due to its phenolic content.

The pH of meat and meat product is important to meat science since it affect many quality factors, including color, texture [Dutson 1983]. Incorporation of mustard seed to the composition of experimental meat products had no significant ($p \leq 0.05$) effect on pH values at 1, 5 and 10 day of storage (Fig. 1). Results of pH measurements also indicated that storage time had no significant effect on its property in case of control and experimental samples (MS 0,2%, MS 0,5%). The pH values tended to increase for meat products sample with storage time.

Water activity is an important criterion for the evaluation of food quality since it predicts safety and stability with respect to microbial growth, chemical and biochemical reaction rates, and physical properties [Vulkov 2006]. Water activity helps reduce some undesirable reactions, such as lipid oxidation, vitamin degradation or enzymatic reactions [Wolf et al. 1984]. Typically, as the water activity level is lowered, the rate of chemical degradative reactions decreases. Since water activity indicates the amount of water in the total water content which is available to microorganisms, it is important to control water activity to predict which microorganisms will be potential sources of spoilage [Vulkov 2006]. Results obtained in current study did not show the influence of mustard seed addition on water activity

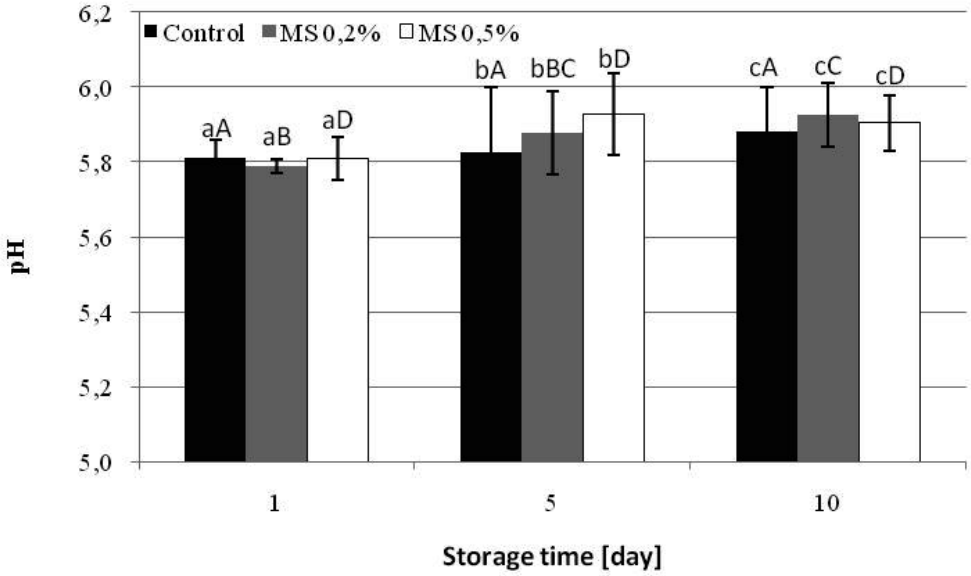

Fig. 1. pH of meat products during storage. Means followed by the same lower case letters between the samples at the same storage time and capital letters between the same sample at different storage times are not significantly different at $p \leq 0.05$

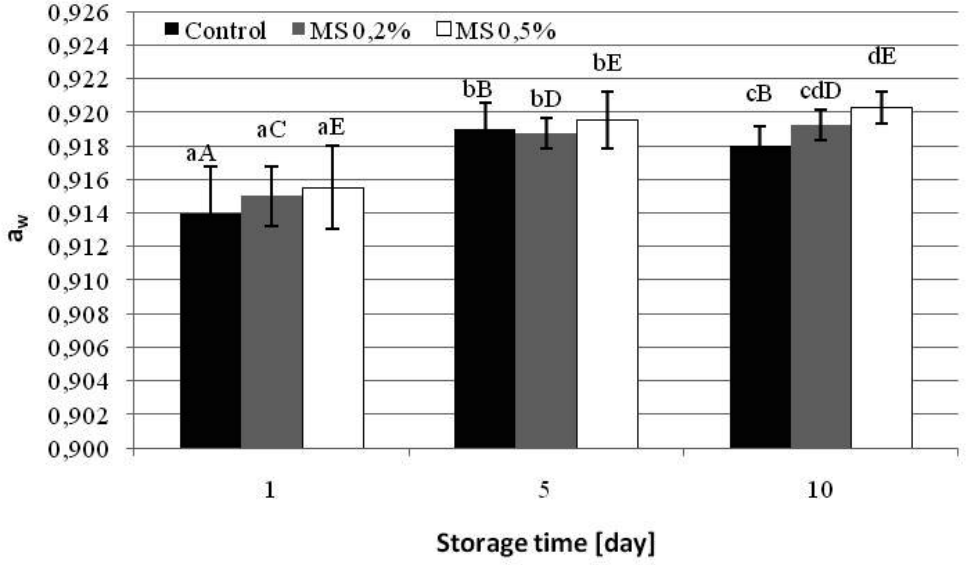

Fig. 2. Water activity (aw) of meat products during storage. Means followed by the same lower case letters between the samples at the same storage time and capital letters between the same sample at different storage times are not significantly different at $p \leq 0.05$

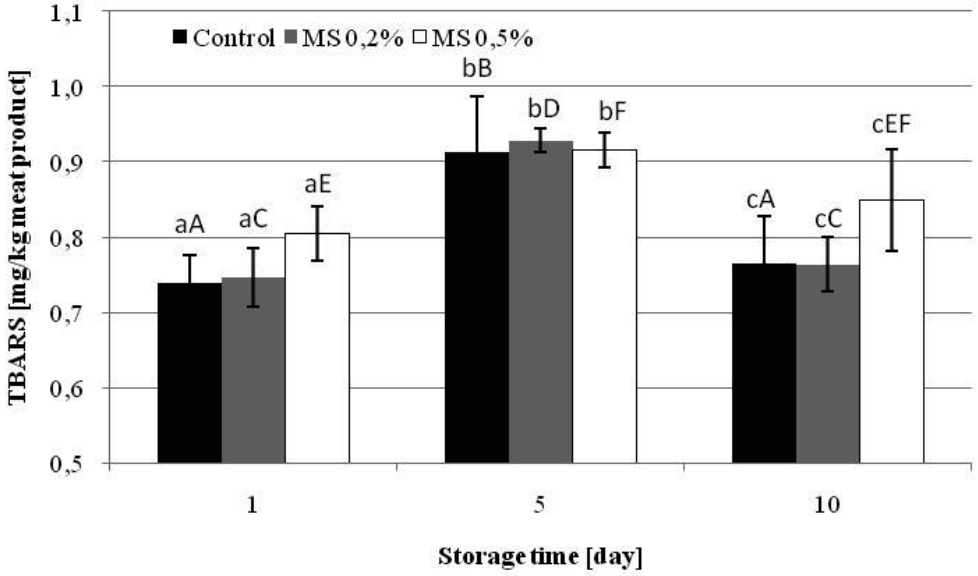

Fig. 3. Lipid oxidation (mg MDA/kg meat product) of meat products during storage. Means followed by the same lower case letters between the samples at the same storage time and capital letters between the same sample at different storage times are not significantly different at $p \leq 0.05$

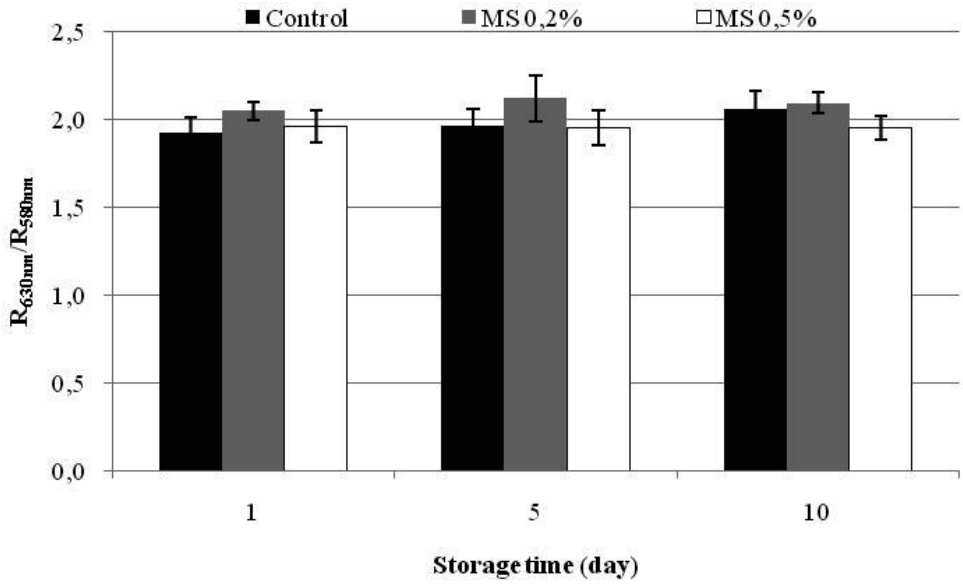

Fig. 4. R630/R580 reflectance ratio of meat products during storage. A statistically significant difference between samples was not detected ($p \leq 0.05$)

values of cooked meat products at 1 and 5 day of storage. The statistically significant effect of storage time on water activity values of meat products was noted at 5 day of storage in case of control and MS 0.2% sample. Leistner et al. [1975] reported that the main hurdles for microbiologically stability of meat products are water activity, pH and temperature. According to them meat products with water activity between 0.95±0.91 are perishable and should be stored at temperatures below 10°C.

Moreover, spices contain essential oils which show antioxidant and antimicrobial activity. The antimicrobial activity of mustard essential oils may be due to the ability of its components to disrupt the membranes of bacterial cells. According to Burt [2004] essential oils comprise a large number of components that has been effective antibacterials, e.g. carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid. Their mode of action involves several targets in the bacterial cell. Turgis et al. [2009] demonstrated that mustard essential oil affected the cell membrane integrity, resulting in a loss of cell homeostasis of bacterial. They suggested, that mustard essential oil can be used as an effective antibacterial agent.

Oxidation processes are one of the major problems encountered in meat processing and storage. Meat components oxidation affects the sensory attributes; it is responsible for the loss in flavor and attractive color [Gray and Pearson 1987, Fautsman et al. 1989]. Moreover, lipid oxidation causes loss of nutritional values and generates compounds that may pose continual risks to human health, e.g. cholesterol oxides [Monahan et al. 1992, Pearson et al. 1983]. It was indicated that cholesterol oxidation products characterized by the mutagenic, cancerogenic, antitoxic, cytotoxic activities [Chan et al. 1997].

Much research has been carried out to better understand the main processes of oxidation of polyunsaturated fatty acids, antioxidant action and the effects of products of lipid oxidation. To learn about the effects of antioxidants, it is important to obtain specific chemical information about what is a mechanism of lipid or protein oxidation. McDonald and Hultin [1987] reported that lipid oxidation is affected by pH, lipid composition, ionic strength, temperature, redox potential, light exposure and iron content. Lipid peroxidation mediated by free radicals is considered to be the major problems encountered in meat processing and storage. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxynonenal (HNE) upon decomposition. 4-Hydroxy-2-nonenal (HNE) is a highly reactive product of lipid peroxidation of unsaturated lipids, particularly arachidonic acid. It was indicated that 4-HNE is highly reactive and destabilizes myoglobin redox state, affecting meat color [Naveena et al. 2010]. According to Faustman et al. [2010] HNE accelerates oxymyoglobin oxidation by binding covalently to specific histidine residue in the protein's primary sequence. In the current study, lipid peroxidation was determined by measurement of the thiobarbituric acid value (TBARS).

The results of the present study show that application of ground mustard seed did not significantly ($p \leq 0.05$) improve the lipid stability of meat products, as measured by TBARS values (Fig. 3). It was also noticed that TBARS values significantly increased on 5 day of storage. TBARS measurements at 10 day of storage indicated that samples of meat products were characterized by the same TBARS values than at 1 day of storage.

Less is known about effectiveness of mustard seed in retarding oxidation processess of meat and meat product. According to our previous results the addition of ground mustard seed to roasted meat products inhibited lipid oxidation during storage as reflected in the 2-thiobarbituric acid (TBA) values [Karwowska and Dolatowski 2010].

It has been established that lipid oxidation can cause meat discoloration due to coupled reactions between lipid and pigment oxidation. Lipid oxidation and myoglobin oxidation

appear to be linked and the oxidation of one of these leads to the formation of chemical species that can exacerbate oxidation of the other [Faustman et al. 2010]. It was indicated that free radicals derived from lipid oxidation can initiate the reaction of oxidizing oxymyoglobin to metmyoglobin. Similarly, hydrogen peroxide activates metmyoglobin to form ferrylmyglobin radicals which area catalysts for lipid oxidation in muscle foods [Hultin 1980]. Not all studies that have accessed the effect of spices on lipid and myoglobin oxidation in meat and meat products have demonstrated results that the two processes are linked. Hayes et al. [2009] indicated that sesamol addition to porcine and bovine meat led to decreased lipid oxidation but enhanced oxymyoglobin oxidation. In contrast, addition of ellagic acid and olive leaf extracted to decreased the oxidation of both meat constituent. Haak et al. [2009] reported that extract of rosemary minimized lipid oxidation but were without effect to inhibit myoglobin oxidation in pork patties.

Color of meat is the most important quality attribute for consumers. The surface color of meat depends on the quantity of myoglobin and its chemical state. It is also dependent on the chemical and physical state of other components in the meat [Renerre 2000]. Meat showing a bright red color is assumed to be fresh, while oxidation of heme iron to form metmyoglobin (MetMb) produces the brown color which consumers find undesirable.

Analysis of color parameters showed that the use of mustard seed to the composition of meat products did not cause significant changes on L*a*b* values of cooked meat products (Tab. 1). It was also noticed that color parameters slightly changed during storage.

Table 1

Color parameters of meat products during storage (means ± standard error)

1		Storage time [day]		
		5	10	
Control	L*	62.8±2.6	59.5±1.2	59.8±0.9
MS 0,2%		61.8±2.0	57.8±2.6	59.4±1.5
MS 0,5%		59.8±1.7	58.6±0.9	60.3±1.2
Control	a*	10.4±0.5	11.1±0.4	10.6±0.8
MS 0,2%		10.6±0.6	11.6±0.8	10.2±0.8
MS 0,5%		11.3±0.7	11.5±0.5	10.6±0.5
Control	b*	8.8±0.7	8.5±0.5	8.2±0.6
MS 0,2%		8.4±0.6	8.7±0.5	8.3±0.4
MS 0,5%		9.4±0.3	9.0±0.7	8.2±0.5

Since color of meat is closely related to the myoglobin content [Reneree et al. 1996] and meat discoloration is due to the conversion of oxymyoglobin to metmyoglobin [Faustman et al. 2010], oxygenation index (ΔR) was determined by reflectance difference between 630 nm determined maximum of the oxidized myoglobin and 580 nm (maximum of the oxygenated myoglobin). Figure 4 shows the reflectance ratio R_{630}/R_{580} of cooked meat products throughout the 10-day period. Results of present study indicated that the addition of mustard seed to cooked meat products did not cause significant changes ($p \leq 0.05$) in oxymyoglobin content during storage.

Conclusions

due to the increased demand and commercialization of botanicals because of their health-related benefits, many spices have been evaluated to determine their benefits in muscle food. In addition, incorporation of functional ingredients, such as spices into meat products can improve their functional value for consumers. Mustard seed contains several antioxidant compounds (tocopherols, glucosinolates and phenolics); these compounds may play role in human and animal health protection and on the other side they could extend the self-life of the products prepared with mustard seed.

Obtained results pointed out that the addition of mustard seed at the level of 0.2 and 0.5% to the composition of cooked sausage had little effect on TBARS values and water activity in examined samples. Incorporation of mustard seed to the composition of experimental meat products had no significant effect on L*a*b* parameters and oxymyoglobin content during storage.

These data did not suggest that the application of mustard seed at the level of 0.2 and 0.5% to the composition of cooked sausages improve their antioxidant potential during storage. Considering that mustard seed has an advantageous chemical composition, further research is needed to identify the effect of higher level of mustard seed on oxidation stability of meat products.

Acknowledegments

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References

Alireza Sadeghi M., Appu Rao A.G., Bhagya S., 2006. Evaluation of mustard (Brassica juncea) protein isolate prepared by steam injection heating for reduction of antinutritional factors. Lebensm.--Wiss. Technol., 39, 911–917.

Allen K.E., Cornforth D.P., 2006. Myoglobin oxidation in a model system as affected by nonheme iron and iron chelating agents. J. Agric. Food Chem., 54, 10134–10140.

Amarowicz A., Wanasundra U.N., Karamac M., Shahidi F., 1996. Antioxidant activity of ethanolic extract of mustard seed. Nahrung, 5, 261–263.

American Meat Science Association (AMSA), 2005. Guidelines for Meat Colour Evaluation – AMSA, Savoy.

Bajpai V.K., Rahman A., Dung N.T., Huh M.K., Kang S.C., 2008. In vitro inhibition of food spoilage and food borne pathogenic bacteria by essential oil and leaf extract of Magnolia liliflora Desr. J. Food Sci., 73, 314–320.

Burt S., 2004. Essential oils: Their antibacterial properties and potential applications in foods – A review. Int. J. Food Microb., 94(3), 223–253.

Chang Y.H., Abdalla D.S.P., Sevanian A., 1997. Characterization of cholesterol oxidation products formed by oxidative modification of low-density lipoprotein. Free Radic. Biol. Med., 23, 202–214.

Dawidowicz A.L., Wianowska D., Baraniak B., 2006. The antioxidant properties of alcoholic extracts from Sumbucus nigra L. (antioxitants properties of extracts). Lebensm.-Wiss. Technol., 39, 308–315.

Dutson T.R., 1983. The measurement of pH in muscle and its importance to meat quality. In Proceedings of the 36th Annual Reciprocal Meat Conference, 92–97.

Fautsman C., Cassens R.G., Schaefer D.M., Buege D.R., Williams S.N., Scheller K.K., 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E.J. Food Sci., 54, 858–862.

Faustman C., Sun Q., Mancini R., Suman S.P., 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci., 86, 86–94.

Gray J.I., Pearson A.M., 1987. Rancidity and warmed-over flavour, [in:] Advances in Meat Research, vol. 3: Restructured Meat and Poultry Products (eds. Pearson AM, Dutson TR). Van Nostrand Reinhold Co. NY, 221–269.

Haak L., Raes K., De Smet S., 2009. Effect of plant phenolics, tocopherol and ascorbic acid on the oxidative stability of pork patties. J. Sci. Food Agric., 89, 1360–1365.

Hayes J., O'Brien N., Kerry J., O'Grady M., Stepanyan V., Allen P., 2009. The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. Meat Sci., 83, 201–208.

Hinneburg I., Dorman H.J.D., Hiltunen R., 2006. Anioxidant activities of extracts from selected culinary herbs and spices. Food Chem., 97, 122–129.

Hultin H.O., 1980. Enzyme-catalyzed lipid oxidation in muscle microsomes, [in:] Autooxidation in Food and Biological Systems. Simic M.G., Karel M., New York.

Hunt M.C., Acton J.C., Benedict R.C., Calkins C.R., Cornforth D.P., Jeremiach L.E., Olson D.G., Salm C.P., Savell J.W., Shivas S.D., 1991. AMSA guidelines for meat color evaluation. In Proceedings 44th annual reciprocal meat conference. Manhattan, KS, USA: Kansas State University, 1–17.

Karwowska M., Dolatowski Z.J., 2010. The effect of mustard seed on oxidative stability of meat products [in:] Meat technology and functional additives. Modern trends in meat production. Red. Migdał W., Cilew G., Zikoviċ B., Jukna V. Kraków 2010.

Kong B., Hang H., Xiong Y.L., 2010. Antioxidant activity of spice extracts in a liposome system and in cooked pork patties and the possible mode of action. Meat Sci., 85, 772–778.

Leistner W., Rodel W., Krispien K., 1981. Microbiology of meat and meat products in high- and intermediate- moisture ranges. In L.B. Rockland, & G.F. Stewart (Eds.), Water activity: influences on food quality, New York/London/Toronto/Sydney/San Francisco: Academic Press, 855–916.

Manda K.R., Adams C., Ercal N., 2010. Biologically important thiols in aqueous extracts of spices and evaluation of their in vitro antioxidant properties. Food Chem., 118, 589–593.

Marcone M.F., Yada R.Y., Aroonkamonsri W., Kakuda Y., 1997. Physico-chemical properties of purified isoforms of the 12S seed globulin from mustard seed (Brassica alba). Biosci., Biotechnol. Biochem., 61(1), 65–74.

Martinez-Tome M., Jimenez A., Ruggieri S., Frega n., Strabbioli R., Murcia M., 2001. Antioxidant properties of Mediterranean spices compared with common food additives. J. Food Prot., 64, 1412–1419.

McDonald R.E., Hultin H.O., 1987. Some characteristics of the enzymatic peroxidation system in the microsomal fraction of flounder skeletal muscle. J. Food Sci., 52, 15–21.

Modak M., Dixit P., Londhe J., Ghaskadbi S., Paul A., Devasagayam T., 2007. Indian herbs and herbal drugs used for the treatment of diabetes. J. Clin. Bioch. Nutr., 40, 163–173.

Monahan F.J., Gray J.I., Booren A.M., Miller E.R., Buckley D.J., Morrissey P.A., Gomma E.A., 1992. Influence of dietary treatment on lipid and cholesterol oxidation in pork. J. Agric. Food Chem., 40, 1310–1315.

Naveena B.M., Faustman C., Tatiyaborworntham N., Yin S., Ramanathan R., Mancini R.A., 2010. Detection of 4-hydroxy-2-nonenal adducts of turkey and chicken myoglobins using mass spectrometry. Food Chem., 122, 836–840.

Pikul J., Leszczyński D.E., Kummerow F.A., 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agric. Food Chem., 37*,* 1309.

Pearson A.M., Gray J.I., Wolzak A.M., Horenstein N.A., 1983. Safety implications of oxidised lipids in muscle foods. Food Tech., 37, 121–129.

Ramarathnam N., Osawa T., Ochi H., Kawakishi S., 1995. The contribution of plant food antioxidants to human health. Trends Food Sci. Technol., 6, 75–82.

Reneree M., 2000. Oxidative processes and myoglobin. Antioxidants in muscle foods. Nutritional strategies to improve quality. New York: John Wiley & Sons, Inc.

Rhee K.S., Anderson L.M., Sams A.R., 1996. Lipid oxidation potential of beef, chicken, and pork. J. Food Sci., 61, 8–12.

Saleemi Z.O., Janitha P.K., Wanasundra P.D., Shahidi F., 1993. Effect of low-pungency ground mustard seed on oxidative stability, cooking yield, and colour characteristics of comminuted pork. J. Agric. Food Chem., 41, 641–643.

Schuster-Gajzágó I., Kiszter A.K. Tóth-Márkus M., Baráth A., Márkus-Bednarik Z., Czukor B., 2006. The effect of radio frequency heat treatment on nutritional and colloid-chemical properties of different white mustard (Sinapis alba L.) varieties. Innov. Food Sci. Emerg. Technol., 7, 74–79.

Shahidi F., Wanasundara U., Amarowicz R., 1994. Natural antioxidants from low-pungency mustard flour. Food Res. Intern., 27, 489–493.

Shahidi F., Yang Z., Saleemi Z.O., Omar S., 2007. Stabilization of mechanically deboned chicken meat lipids with ground mustard seed. J. Food Lipids, 1, 89–96.

Shahidi F., Naczk M., 1990. Removal of glycosinolates and other antinutrients from canola and rapeseed by methanol/ammonia processing. In: F. Shahidi, Editor, *Canola/Rapeseed*, Van Nostrand Reinhold, New York, 291–306.

Vasi S., Austin A., 2009. Effect of herbal hypoglycemics on oxidative stress and antioxidant status in diabetic rats. The Open Diab. J., 2, 48–52.

Vulkov P., 2006. Water activity concept for safety food storage. Proceedings of the 3rd Central European Congress on Food, Sofia (Bulgaria).

Wlodek L., 2002. Beneficial and harmful effects of thiols. Pol. J. Pharm., 54, 215–223.

Wolf W., Spiess W.E. L., Jung G., Weisser H., Bizot H., Duckworth R.B., 1984. The water sorption isotherms of bacteria growth. Results of collaborative study. J. Food Eng., 3, 51–73.

Zhang W., Ciao S., Samaraweera H., Lee E.J., Ahn D.U., 2010. Improving functional value of meat products. Meat Sci., 86, 15–31.

6

QUALITY CHARACTERISTIC OF NEW HULL-LESS BARLEY GENOTYPES WITH HIGH LEVEL BETA-GLUCAN

Introduction

Barley is the fourth cereal after wheat, rice and corn. In ancient times barley was grown mostly to provide food staples, but today only small part of barley is used for human nutrition, except for barley used in beer production. Nevertheless, recently some increase of interest in introducing barley into a food production can be seen. Hull-less barley does not require dehulling and has some advantages for food uses. Nutrients, which are present in outer layers, are not lost due to pearling. Moreover, with compare to the hulled forms, the nutrients concentration in caryopsis is higher due to removal of the hull. Hull-less barley contains the same amount of crude fiber as wheat and corn, while hulled barley contains only 5–6% crude fiber, which limits its use in foods [Kinner et al. 2011, Izydorczyk and Dexter 2008, Izydorczyk et al. 2008, Knutsen and Holtekjølen 2007, Yalçin et al. 2007].

The main components of barley grain are starch, dietary fiber and protein, and grain chemical composition depends on both genotypic and environmental factors. Total dietary fiber consists of insoluble and soluble fractions. The insoluble fraction contains cellulose, which id beneficial in the gastrointestinal tract. The soluble fraction consists of pectin, arabinoxylan and β-glucan. Unique for barley is high concentration of β-glucans, from 2.5% to 11.3%, which is said to have a cholesterol-lowering effect, improving lipid metabolism, reducing glycaemic index and lowering coronary heart disease. The positive effect of β-glucans is related to its high viscosity in aqueous solutions, which increases the viscosity of the contents within the intestinal trial. Crucial in β-glucans properties is ratio of trisaccharide unit (DP3) to tetrasaccharide unit (DP4), which can be attributed to genotypic and environmental factors. However, the effect of environmental factor on DP3/DP4 molar ratios has not been established. β-glucans solubility seems to decrease with increasing molar ratio DP3/DP4 in the β-glucans chain. The nutritional values of other fiber components, mostly arabinoxylans, have not been well investigated. It has been already known that arabinoxylans properties depend on the ratio of arabinose to xylose residues (Ara/Xyl), which is higher in hull-less varieties, than in hulled types. However, there are some studies concerning positive effect of arabinoxylans on cecal fermentation, production of short-chain fatty acids, reduction of serum cholesterol and improved adsorption of calcium and magnesium [Kinner et al. 2011, Izydorczyk and Dexter 2008, Izydorczyk et al. 2008, Knutsen and Holtekjølen 2007, Yalçin et al. 2007, Storsley et al. 2003].

Nevertheless, barley flour still is hardly used for food production for some reasons. Gluten network is hardly formed and, additionally, high β-glucans concentration decreases the water availability, which reduces baking properties. Moreover, naked barley flour deteriorates bread sensory properties – lowers bread volume and worsens crumb structure. Thus, to keep

the appropriate properties of bread the maximum level of barley flour in wheat bread should not exceed 10%. But as a compromise between sensory attractiveness and health benefits, share of the barley flour may be increased to 20% [Kinner et al. 2011]. On the other hand, introducing of barley β-glucans and arabinoxylans into foods may also improve quality parameters such as processing behaviour and stability or shelf-life [Storsley et al. 2003].

Barley dietary fiber constituents solubility seems to be an important determinant of their technological functionality and physiological efficacy. Water solubility and extractability of β-glucans in oats is greater than in barley, which is, in turn, greater than in wheat. And water solubility and extractability of arabinoxylans, compared to β-glucans, is low [Izydorczyk and Dexter 2008].

The interaction of β-glucans and arabinoxylans with other constituents and their localization in grain are important from the technological point of view. Other constituents influence on isolation and purification of fractions enriched in these polysaccharides, which affects the utilization of barley grain for human and animal nutrition. β-glucans and arabinoxylans are main structural constituents of cell walls in various tissues of barley grain. They may represent up to 85% of total polysaccharides building cell walls in the starchy endosperm of mature grain. Those cell walls are mainly built of β-glucans and contain smaller quantities of arabinoxylans. Meanwhile, aleurone cell walls are built of arabinoxylans (67–71%) with smaller amounts of β-glucans (26%) [Izydorczyk and Dexter 2008, Knutsen and Holtekjølen 2007]. From this it follows that white barley flour contains a sufficiently high amount of soluble dietary fiber and there is no need for using whole meal flour to gain cereal based products with high dietary fiber content when naked barley is used for food production [Kinner et al. 2011].

In 2005 the US Food and Drug Administration (FDA) concluded that there is a cause and effect relationship between the consumption of β-glucans and lowering coronary heart disease. Similarly, the European Food Safety Authority (EFSA) has indicated that regular consumption of β-glucans maintains normal blood cholesterol concentration and has assumed that barley β-glucans have the same effects as oat β-glucans. Thus EFSA demands daily intake of β-glucans in a quantity of at least 3 g from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed β-glucans in one or more servings. Therefore, it is anticipated that interest in using naked barley for food purposes will increase [Kinner et al. 2011].

Material and methods

The material was grain of 6 lines of hull-less barley acquired from Plant Breeding Strzelce Ltd., Co. Grain physical properties and chemical composition were estimated. Among physical properties thousand grain weight, hectoliter weight, grain filling, grain vitreousity and falling number were evaluated according to Polish Standards. Grain chemical composition evaluation concerned total protein, starch, beta-glucans, fiber (total, soluble and insoluble) and pentosans (total, soluble and insoluble) contents. Total protein content was evaluated according to Polish Standards, starch content according to Lintner method, described by Jakubczyk and Haber [1983], beta-glucans content according to McCleary and Codd [1991], fiber content according to SIGMA standards, and pentosans (arabinoxylans) content according to Subda [1984].

Lintner method of starch analysis is based on measuring the rotation of polarized light. Flour was homogenized with water, and then concentrated hydrochloric acid was added.

After 30 minuts the mixture was transferred into the flask, phosphoric-tungsten acid was added in order to precipitate protein. The flask was filled with hydrochloric acid and then the solution was filtered. The filtrate was used to measure the rotation of polarized light.

McCleary and Codd β-glucans analysis method is based on β-glucans decomposition by lichenase to β-gluco-oligosaccharides, and then by β-glucosidase to glucose. Glucose is determined with a reagent GOPOD – a mixture of glucose oxidase and peroxidase.

In fiber content analysis method according to SIGMA standards, samples were gelatinized with thermo-resistant α-amylase, and then they were digested with protease and amyloglucosidase in order to remove protein and starch from samples. To precipitate the soluble fiber fraction, ethanol was added. The precipitate was filtered and rinsed with ethanol and acetone. The precipitate was weighed after drying. A half of samples were analized for protein content, and the rest was mineralized. Total fiber content was the difference between the masses of the precipitate and protein with ash.

In total pentosans content assay according to Subda [1984], flour samples were boiled for 6 hours with sodium chloride, hydrochloric acid and xylene. After cooling, the solutions were separated and the upper fractions were taken for further examination. They were mixed with ethyl aniline and ethanol and then their extinctions were measured at a wavelength of 540 nm. The soluble pentosans assay differed in the way of sample preparation. At first, aqueous flour suspension were shaken for 3 hours. After centrifugation the liquid was boiled for 6 hours with sodium chloride, hydrochloric acid and xylene. Further treatment was the same as in the case of total pentosans. Insoluble pentosans fraction was the difference between total pentosans and their soluble fraction.

Statistical analysis based on estimation of mean value, minimum, median, maximum, standard deviation, coefficient of variability, range and correlation coefficient.

Results

Physical properties of all barley lines were rather equal, which was indicated by quite low variability coefficient, lower than 10%, however grain vitreousity and falling number were varied by genotype factor (Tab. 1). Although thousand grain weight ranged from 43.1 g to 51.1 g, mean value was 46.4 g and median was 45.25 g, which indicates that thousand grain weight mostly scored around 43–45 g. Coefficient of variability equal to 7.3% shows some differentiation of results. Hectoliter weight was very close. It ranged from 76.7 $kg \cdot hl^{-1}$ to 79.3 $kg \cdot hl^{-1}$, and both mean value and median were the same and reached 78.0 $kg \cdot hl^{-1}$. Coefficient of variability was only 1.1%, which hardly indicates differentiation of results. Although coefficient of variability of grain filling was quite low – 4.3%, the results were slightly different. Grain filling ranged from 82.7 to 92.7%, so it differed by as much as 10%. Besides median was only 84.9%, thus half of the results were very similar to each other. The mean value was higher then median by 1.7%, which must have been caused by quite high maximum – 92.7%. Grain vitreousity was very low and very varied – it ranged from 0 to 25%. The mean value, median and standard deviation were 11, 12.5 and 9%, respectively, which led to a very high coefficient of variability – 81.8%. The falling number ranged from 62 s to 108 s, however median was very close to minimum – it was only 68.5 s, and consequently the mean value was 77 s. The coefficient of variability was 23.4%, but it was rather caused by very high maximum than by general scattering of the results.

Table 1

Physical features of hull-less barley grain

Line \ Feature		Thousand grain weight [g]	Hectoliter weight [kg·hl^{-1}]	Grain filling [%]	Vitreousity [%]	Falling number [s]
STH 7007		43.3	76.7	82.8	1	69
STH 7809		50.7	78.3	84.5	25	68
STH 1192		45.2	79.3	88.5	14	62
STH 1292		45.3	78.6	92.7	11	62
STH 1246		43.1	77.7	82.7	0	92
STH 4933		51.1	77.5	85.3	16	108
Statistical analysis	Mean	46.4	78.0	86.1	11	77
	Minimum	43.1	76.7	82.7	0	62
	Median	45.3	78.0	84.9	12.5	68.5
	Maximum	51.1	79.3	92.7	25	108
	Range	8.0	2.6	10.0	25	46
	Standard deviation	3.4	0.9	3.7	9	18
	Coefficient of variability [%]	7.3	1.1	4.3	81.8	23.4

Table 2

Chemical composition of hull-less barley grain

Line \ Feature		Total protein content [%]	Starch content [%]	Beta-glucans content [%]
Sth 7007		12.5	60.0	4.89
Sth 7809		16.4	55.8	5.85
Sth 1192		16.3	56.0	5.48
Sth 1292		14.5	58.4	5.26
Sth 1246		15.0	59.7	4.96
Sth 4933		14.9	58.3	5.43
Statistical analysis	Mean	14.9	58.0	5.31
	Minimum	12.5	55.8	4.89
	Median	15.0	58.4	5.35
	Maximum	16.4	60.0	5.85
	Range	3.9	4.2	1.0
	Standard deviation	1.4	1.7	0.39
	Coefficient of variability [%]	9.4	2.9	7.3

Grain chemical composition was not as varied by genotype factor as its physical properties (Tab. 2). Among examined components, total protein content varied the most. It ranged from 12.5 to 16.4%, with median at 14.95% and mean value at 14.9%. It resulted with the coefficient of variability outcome of 9.4%. Starch content was a little varied by genotype factor. It ranged from 55.8 to 60.0%. Median and mean value reached 58.35 and 58.0%, respectively.

Low coefficient of variability (2.9%) confirms the small diversity of results. beta-glucans content ranged from 4.89 to 5.85% and was quite diversified, which was supported by coefficient of variability – 7.34%. Median and mean value were very close to each other, and they were 5.345 and 5.31%, respectively.

Fiber content was investigated only in lines with the highest content of beta-glucans (Tab. 3). It can be seen, that the higher content of beta-glucans, the higher content of total fiber. However, in the line STH 7809, in which beta-glucans content was the highest, total fiber content was much higher than in other 2 lines. STH 7809 contained 12.23% of total fiber, while the other lines contained only 9.99% and 9.83% of that component. Share of soluble and insoluble fractions in grain was various depending on barley line and no trend can be observed.

Table 3

Fiber content in hull-less barley grain

Feature / Line		Fiber content		
		Total [%]	Soluble [%]	Insoluble [%]
STH 7007		–	–	–
STH 7809		12.23	5.04	7.19
STH 1192		9.99	4.64	5.35
STH 1292		–	–	–
STH 1246		–	–	–
STH 4933		9.83	6.35	3.48
Statistical analysis	Mean	10.72	5.34	5.34
	Minimum	9.83	4.64	3.48
	Median	9.99	5.04	5.35
	Maximum	12.23	6.35	7.19
	Range	2.40	1.71	3.71
	Standard deviation	1.34	0.89	1.86
	Coefficient of variability [%]	12.5	16.8	34.8

Pentosans content in grain was quite varied by genotype factor, which is confirmed by the coefficient of variability greater than 5% (Tab. 4). Total pentosans were the least varied – coefficient of variability was 5.97%. Their content ranged from 3.71 to 4.36% with median at 4.00% and mean value at 4.02%. Although soluble pentosans ranged only from 0.48 to 0.64%, their diversity was the biggest; coefficient of variability was 8.8%. It can be also seen that in all lines soluble fraction of pentosans was around 15% of total pentosans. Insoluble fraction was less diversified; coefficient of variability was 7.8%. That fraction content ranged from 3.15 to 3.88%. Median and mean value were close and they were 3.405 and 3.46%, respectively.

Correlations between grain properties were determined (Tab. 5). Fiber content were not regarded due to the less number of results. It can be seen that the higher grain vitreousity, the higher thousand grain weight and lower starch content. Starch content was negatively correlated with total protein content, either. Moreover, the higher insoluble pentosans content, the higher total pentosans content.

Table 4

Pentosans content in hull-less barley grain

Feature / Line		Pentosans content		
		Total [%]	Soluble [%]	Insoluble [%]
STH 7007		4.36	0.48	3.88
STH 7809		3.93	0.64	3.29
STH 1192		4.07	0.56	3.51
STH 1292		4.21	0.60	3.61
STH 1246		3.71	0.56	3.15
STH 4933		3.86	0.56	3.30
Statistical analysis	Mean	4.02	0.57	3.46
	Minimum	3.71	0.48	3.15
	Median	4.00	0.56	3.41
	Maximum	4.26	0.64	3.88
	Range	0.65	0.16	0.73
	Standard deviation	0.24	0.05	0.27
	Coefficient of variability [%]	6.0	8.8	7.8

Discussion

The results of present work do not confirm the results of other researchers. In the Yalçin et al. [2007] research 1000 Kernel weight of 16 hull-less barley lines was lower by around 10–15% depending on location than in present studies, while standard deviation was 3.55–5.79 g in cited work and 3.4 g in own studies. It suggests that Turkish lines were more diverse in terms of this characteristic. In studies of Wiewióra [2006] also thousand grain weight was lower than in present studies, and it was 40.4 g.

Yalçin et al. [2007] studied hectoliter weight. Depending on location, the results were close to own results or lower by around 8 $kg \cdot hl^{-1}$. Moreover, dispersion of the results was the same as in own studies or higher. Thus barley grown in one of three locations had very similar hectoliter weight to barley evaluated in present work, while in other locations grain density was lower and more distracted.

According to Izydorczyk et al. [2008], hull-less barley grain contains 53.8–61.2% starch, depending on its structure. When starch is normal, its content is the highest, and when is high amylose – the lowest. Waxy starch content is average. Storsley et al. [2003] came to similar conclusions. In own studies starch structure had not been evaluated, therefore reference to these observations can not be done. Meanwhile, Spychaj et al. [2002] inform that hull-less barley grain contains 54%, which is slightly lower than in present work.

The results of available researches are not compatible in terms of protein content in hull-less barley grain. In studies of Yalçin et al. [2007], total protein content was little lower (less than 2%, depending on location), than in own studies. Meanwhile, in Izydorczyk et al. [2008] work total protein content was 13.0–14.5%, depending on starch structure (normal, waxy or

Table 5

Correlation of physical properties and chemical composition of hull-less barley grain

	TGW	HW	GF	GV	FN	TPC	SC	B-G	TP	SP	IP
TGW	1.00	0.13	0.01	0.87 *	0.35	0.48	-0.56	0.79	-0.34	0.58	-0.42
HW	0.13	1.00	0.69	0.52	-0.46	0.80	-0.79	-0.10	-0.11	0.62	-0.23
GF	0.01	0.69	1.00	0.28	-0.47	0.22	-0.33	-0.06	0.34	0.39	0.23
GV	0.87 *	0.52	0.28	1.00	-0.11	0.71	-0.87 *	0.66	-0.17	0.76	-0.30
FN	0.35	-0.46	-0.47	-0.11	1.00	-0.06	0.37	0.20	-0.67	-0.13	-0.58
TPC	0.48	0.80	0.22	0.71	-0.06	1.00	-0.86 *	0.25	-0.58	0.78	-0.68
SC	-0.56	-0.79	-0.33	-0.87 *	0.37	-0.86 *	1.00	-0.28	0.13	-0.68	0.25
B-G	0.79	-0.10	-0.06	0.66	0.20	0.25	-0.28	1.00	-0.27	0.68	-0.38
TP	-0.34	-0.11	0.34	-0.17	-0.67	-0.58	0.13	-0.27	1.00	-0.43	0.98 *
SP	0.58	0.62	0.39	0.76	-0.13	0.78	-0.68	0.68	-0.43	1.00	-0.58
IP	-0.42	-0.23	0.23	-0.30	-0.58	-0.68	0.25	-0.38	0.98 *	-0.58	1.00

Abbreviations: TGW – thousand grain weight, HW – hectoliter weight, GF – grain filling, GV – grain vitreousity, FN – falling number, TPC – total protein content, SC – starch content, B-G – beta-glucans content, TP – total pentosans content, SP – soluble pentosans content, IP – insoluble pentosans content

* – correlation coefficient significant at $p < 0.05$

high amylose). Spychaj et al. [2002] reported that protein content in hull-less barley grain was 15.5%, which was even higher than in present studies.

Barley grain is regarded for a high content of β-glucans, from 2.5 to 11.3%, which is much higher than in other cereals. It depends on genetic and environmental factors, as well as on interaction between them two. However, no significant differences in contents of β-glucans between two-row and six-row barley or hulled and hull-less barley with normal starch had been observed. Nevertheless, the largest effect on the content of β-glucans in barley is associated with a locus on chromosome 2(2H). And if look at the impact of weather, β-glucan content in grain is higher when barley is grown in dry and hot conditions, however it can be reduced by short periods of very high temperature or drought stress [Izydorczyk and Dexter 2008]. The own studies results do not confirm the observations of other workers concerning β-glucans contents. Regardless of location, β-glucan content in studies of Yalçin et al. [2007] was much lower (4.3–4.6%) than in own results (5.31%) and less dispersed, as standard deviations in both studies were very close to each other. However, dietary fiber content was a little higher than in own work (10.8–12.3% and 10.72%, respectively), but with similar distraction of the results. In work of Spychaj et al. [2002] it was 4.43%, which was slightly lower than in present studies. Izydorczyk and Dexter [2008] suggest that β-glucans content, both total and water soluble fraction, depends on starch structure. When starch is normal, the total β-glucans content is the lowest, around 4%, and when the starch is high amylose, total β-glucans content is the highest (around 9%). In hull-less barley grain with waxy starch the total β-glucans content is average, around 7%. Also Izydorczyk et al. [2008] and Storsley et al. [2003] claim that β-glucans contents depends on starch structure and is higher, when starch is abnormal (waxy or high amylose). In own studies starch structure and weather conditions during barley grown had not been analyzed, so the impact of those factors cannot be confirmed. Moreover, Storsley et al. [2003] found some disproportions in β-glucans and arabinoxylans in different barley types, which was observed in present studies, either.

The content of arabinoxylans in barley is similar to that in wheat, lower than in rye, but higher than in other cereals (oat, sorghum, rice). It depends on genetic and environmental factors, but it seems to be less variable than that of β-glucans. Generally, six-rowed barley types contain slightly higher levels of arabinoxylans than two-rowed cultivars. And the presence of the waxy gene in barley does not affect the content of arabinoxylans as much as the content of β-glucans [Izydorczyk and Dexter 2008]. According to Storsley et al. [2003] and Knutsen and Holtekjølen [2007], only a part of arabinoxylans is soluble in water, while they are more soluble in alkali. Knutsen and Holtekjølen [2007] also suggest that the water-soluble arabinoxylans of the barley, both hulled and hull-less, have a relative uniform structure, which is expressed by the very similar Ara/Xyl ratios (around 0.6), found in water soluble, thermo-stable amylase and pancreatine treated, alcohol insoluble fractions. However, in present studies this ratio had not been determined, so this observation cannot be confirmed. Spychaj et al. [2002] were evaluating content of total, soluble and insoluble pentosans in hull-less barley grain. Results they obtained, especially those concerning soluble pentosans, were slightly lower than results of present studies. Izydorczyk et al. [2008] found out that contents of arabinoxylans in hull-less barley were 4.1–4.3%, depending on starch structure (normal, waxy or high amylose). Storsley et al. [2003] found out that if starch was high amylose, the total arabinoxylans concentration was the highest. Knutsen and Holtekjølen [2007] also reported that arabinoxylans concentration (total and water soluble fraction) in hull-less barley varies depending on starch structure. Nevertheless, average concentrations were 4.40% of total arabinoxylans and 0.53% of water soluble fraction. Comparing to own

results, there were more total arabinoxylans and less soluble fraction than in barley evaluated in this work. Moreover, in the work of Norwegian scientist standard deviations were higher than in present studies, which suggest that barley grain examined in this work was more equal in terms of these features.

Storsley et al. [2003] found out that molecular features of non-starch polysaccharides, like length of cellulosic regions in β-glucans, Ara/Xyl ratio in arabinoxylans and weight of water- and alkaliextractable fractions vary significantly depending on barley genotype. The impact of such molecular differences on grain physiological properties is not certain. However, the varying viscoelastic properties of the water-soluble fractions indicate the possibilities for two barley varieties containing similar amounts of soluble non-starch polysaccharides to have different nutritional characteristics. In present studies molecular features of non-starch polysaccharides had not been evaluated, so comparison to presented observations cannot be done. Nevertheless, the extent of this issue indicates the need for further research.

Conclusions

1. New lines of hull-less barley are diverse in terms of quality features and chemical composition. Line STH 7809 had the highest content of total protein, β-glucans and total fiber and the lowest content of starch. Meanwhile, line STH 7007 had the lowest content of total protein and β-glucans and the highest content of starch and total pentosans.
2. Grain physical properties were less diverse than its chemical composition. The exceptions were grain vitreousity and falling number.
3. There was a strong negative correlation between total protein content or grain vitreousity and starch content.

References

Izydorczyk M.S., Dexter J.E., 2008. Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products – a Review. Food Res. Int., 41, 850–868.

Izydorczyk M.S., Chornick T.L., Paulley F.G., Edwards N.M., Dexter J.E., 2008. Physicochemical properties of hull-less barley fibre-rich fractions varying in particle size and their potential as functional ingredients in two-layer flat bread. Food Chem., 108, 561–570.

Jakubczyk T., Haber T. (red.), 1983. Analiza zbóż i przetworów zbożowych. SGGW-AR, Warszawa, 339.

Kinner M., Nitschko S., Sommeregger J., Petrasch A., Linsberger-Martin G., Grausgruber H., Berghofer E., Siebenhandl-Ehn S., 2011. Naked barley – Optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. J. Cereal Sci., 53, 225–230.

Knutsen S.H., Holtekjølen A.K., 2007. Preparation and analysis of dietary fiber constituents in whole grain from hulled and hull-less barley. Food Chem., 102, 707–715.

McCleary B.V., Codd R., 1991. Measurement of (1→3), (1→4)-β-D-glucan in barley and oats: A streamlined enzymic procedure. J. Sci. Food Agri., 55, 303–312.

PN-68-R-74017. Cereals and pulses. Determination of the mass of 1000 grains.

PN-R-74007:1973. Cereals. Determination of density.

PN-R-74008:1970. Cereals. Determination of glittering grains.

PN-ISO 3093:1996/Az1:2000. Cereals – Determination of falling number.

PN-75/A-04018. Agricultural food products. Determination of nitrogen by the Kjeldahl method and expressing as protein.

Spychaj R., Sowa M., Gil Z., Liszewski M., 2002. Wpływ technologii uprawy i terminu zbioru na wybrane wyróżniki wartości żywieniowej ziarna jęczmienia jarego nieoplewionego i oplewionego. Nauka. Żywność. Technologia. Jakość., 3 (32), Supl., 96–102.

Storsley J.M., Izydorczyk M.S., You S., Biliaderis C.G., Rossnagel B., 2003. Structure and physicochemical properties of β-glucans and arabinoxylans isolated from hull-less barley. Food Hydrocolloids, 17, 831–844.

Subda H., 1984. Metoda oznaczania zawartości pentozanów rozpuszczalnych w wodzie (Instrukcja). Biul. IHAR., 155, 325–326.

Wiewióra B., 2006. Porównanie wybranych cech nieoplewionego i oplewionego ziarna jęczmienia jarego. Pam. Puł., 146, 547–560.

Yalçin E., Çelik S., Akar T., Sayim I., Köksel H., 2007. Effects of genotype and environment on β-glucan and dietary fiber contents of hull-less barleys grown in Turkey. Food Chem., 101, 171–176.

www.sigmaaldrich.com: Total dietary fiber assay

7

ISOFLAVONS COMPOSITION VARIABILITY OF SOYBEAN IN RELATION TO THE GROWN LOCALITY AND STORAGE DURATION UNDER NATURAL CONDITIONS

Introduction

Soybean seeds contain a variety of biologically active compounds. They are known to contain high amounts of protein composed of all the essential aminoacids. Soybean oils is also known to be rich in ω-3 fatty acids and fat-soluble vitamins. The carbohydrates of soybean are largely polysaccharides and indigestible fiber, which reduces the diseases of the lower gastrointestinal tract [Wang et al. 1990]. However, soybean is a unique source of components called isoflavons. They are classified as phytoestrogens (plant-derived compounds that have estrogenic properties). Phytoestrogens include a wide variety of plant products with weak estogenic activity discovered after isoflavons were found to be responsible for the infertility problems of livestock feeding on forage plants such as clover [Franke et al. 1994, Benešová et al. 1996]. Nowadays, they are being studied for their potential role in the prevention and treatment of a number of chronic diseases including certain forms of cancer, osteoporosis, and heart disease, and also for their ability to relieve woman menopausal symptoms.

The highest amounts of isoflavons are generally found in Fabacae family plant (including soybean) that are widely used to human and animal feeding. The major isoflavons genistein and daidzein have been intensively studied in the past 10 years. Isoflavons are biologically active metabolites that accumulate in soybean during development. Amounts of isoflavones fluctuate in different plant species [Klejdus et al. 2005]. The amount of isoflavons present in soybean is variable, depending on genetic and environmental factors (the type of cultivar, and growth location can influence the content of isoflavones). Therefore the comparing the contents of selected isoflavones in the soybean seeds grown in the different localities in Slovakia was the object of our study.

Material

Plant material. Soybean (*Glycine* max. *L.)* seeds were purchased in cooperation with the farms so that they represented a significant area of that crop in Slovakia. Company PRIVA-TEX-Agro Company Ltd. New Locks supplied soybean varieties Bolyi-45, Erin, Korada, Supra and Quito, which were grown in the area Marcelová. Varieties Crystal and Belmont delivered by Jalšové and varieties Korada and Supra were from Oponice. Korada variety was also grown in the area of Belá over Cirochou and Malacky. The seeds were manual cleaned a stored in paper sacks in normal storage conditions for further analysis.

Chemicals. Methanol (99%, HPLC grade), hydrochloric acid, ammonium acetate were obtained from Merck (Germany). All chemicals and reagents were of analytical grade. Isoflavons standards daidzein, genistein were purchased from Sigma Aldrich (USA). The standards were dissolved in methanol to a concentration of 1mg/mL and kept protected from light at – 18°C. Calibration standards in methanol were prepared from these stock solutions.

Soils samples. Collection of soil samples was performed from the same plots, which were collected for the tested pulses. Sampling and adjustment of soil samples followed the „Binding methodologies of soils analysis" [Fiala et al. 1999]. Soil samples were taken from the soil science probe depths of 0 to 0.1 m. By way of illustration, the location of delivery points of soybean and soil samples is indicated on the map of the SR (Fig. 1).

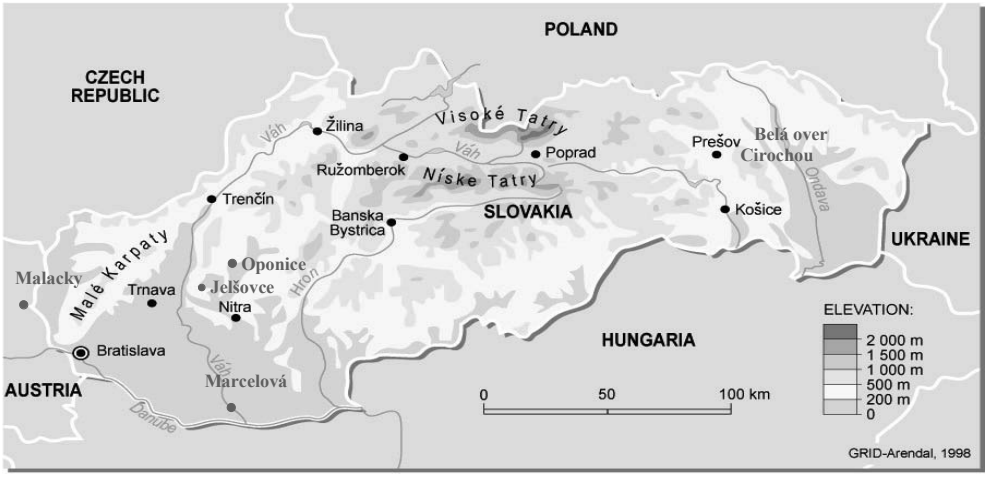

Fig. 1. Location of delivery points of soybean and soil samples

Types of soils in the individual localities of Slovakia: Belá over Cirochou – EutricCambisols (East Slovakia), Marcelová – Haplic Chernozems (South Slovakia), Oponice – Haplic Luvisols (West Slovakia), Jelšovce – Haplic Luvisols (West Slovakia), Malacky – Eutric Regosols (West Slovakia)

Methods

Isoflavons. The contents of observed isoflavons – daidzein, genistein in $mg.kg^{-1}$ DM – were determined according to the modified method by Wang et al. [1990]. Acid hydrolysis was chosen to convert the selected isoflavons conjugates into their aglycons. The samples were prepared as follows: Ten grams finely ground sample were placed in a 250 mL stoppered Erlenmayer flask, mixed with 50 mL of 1M HCl. The flavonoids were hydrolysed inside a thermostat at 100°C. After 2 hours, the extract was cooled to room temperature and methanol (25 mL) was added. The mixture was filtered under vacuum and an aliquot of leachate was evaporated to approximately 10 mL at less than 50°C for purifying by SPE (C18). The cartridge was conditioned with 2 mL of methanol and 2 mL of water. A sample was passed

through the cartridge. Impurities were washed out twice more of water. Retained isoflavons were eluted with 99% methanol. The eluate was reduced with the evaporator to dryness and dissolved in a small volume of a mobile phase. The final sample was filtered through a 0,22 μm (Chromservis) filter and injected into the HPLC.

Isocratic HPLC analysis of soybean samples with UV DAD detection was performed- UV for a HPLC-DAD. The HPLC system consisted of a Waters system HPLC (Agilent Technologies, USA) with autosampler. Compounds were separated on a RP C_{18} column (NovaPack, 150 x 3,9 mm, 4 μm, fy Waters) with methanol : 1mM acetate ammonium (6:4), as a mobile phase at a flow rate of 1 $mL.min^{-1}$. Identification of chromatographic peaks was achieved by comparing retention times of standards and, if necessary, by comparison of spectra. The results were obtained by peak area measurement at 254 nm, using calibration standards at the same wavelenght.

Soil. Nutrients contents (K, Ca, Mg) were determined by Mehlich II. procedure and the total (HF+HNO_3 solution) and releasable (in the solution of 2 M HNO_3) contents of risk elements were determined using a Varian AA240FS atomic absorption spectrometer equipped with a D2 lamp background correction system, using an air–acetylene flame. Between 0.9 and 1.1 g of dried sample was weighed into digestion tubes and HNO_3 were added. The samples were incinerated in a Nabertherm muffle furnace. Ashes were dissolved in nitric acid and passed through an ash-free, acidwashed filter paper and diluted to a certain volume with water. Phosphorus was measured by a colorimetric method, which used tartate antimonylo–potassium and molybdate ammmonium reagent. Nitrogen was determined by the Kjeldhal method.

Results of the determination of soil reaction (was determined as pH/ KCl), content of available nutrients, total heavy metal content and risky compounds concentrations in the extract of 2 $mol.dm^{-3}$ HNO_3 in soil samples from various sampling sites are listed in the Appendix (Tab. 4–6). The results were compared according to the Decision of Ministry of Agriculture in Slovak republic about highest acceptable limits of toxic compounds in soil No. 531/1994–540 (Tab. 7, Appendix).

Results and discussion

Two varieties (Korada, Supra) were grown in the four different parts od Slovak Republik (localities Marcelová, Belá over Cirochou, Oponice, Malacky), some varieties of soybean samples were grown in the same environmental conditions (localities Marcelová, Jelšovce).

The isoflavons amounts ($mg.kg^{-1}$) in soybean seeds of different varieties in full maturity, which came from five growing areas of Slovakia (Tab. 1), were evaluated.

Results in Table 1 shows that the interval of values was quite broad. In all varieties of soybean were detected high levels of isoflavons (daidzein and genistein). According Klejdus et al. [2003] these high concentrations are likely related to the protection of seeds against stress conditions (pathogen attack). Kempherol a apigenine content (flavonoids of pea or bean) can not be detected even in trace amounts because of low selective method for the determination of those flavonoids, or likely absence of these substances in soybeans (their presence will not appear in literary sources).

Table 1

Isoflavons content (mg.kg^{-1}) in seeds of different soybean varieties grown in full maturity in different localities in Slovakia

Locality	Variety	Daidzein content	Genistein content
Marcelová	Bolyi-45	721.0 c	142.9 b
	Erin	370.8 b	278.2 c
	Korada	477.1 b	243.9 b
	Quito	382.2 c	244.6 b
	Supra	358.9 c	223.3 b
Jelšovce	Crystal	527.5 c	307.2 b
	Belmont	538.0 c	232.9 b
Oponice	Supra	470.7 a	425.8 c
	Korada	617.0 b	304.2 b
Belá over Cirochou	Korada	668.9 c	258.9 a
Malacky	Korada	402.3 b	254.2 b

Values in the same column with different letters present significant differences $p < 0.01$

According to literature data content of isoflavons in soybean seeds varies considerably. According to Genovese et al. [2005], the levels of isoflavons in Brazilian navy breeding varieties vary in the range from 570 to 1 888 mg.kg^{-1}, while Sakai et al. [2005], by analysis of japanese varieties, found greater variability in the isoflavons amount (from 235 to 8 485 mg. kg^{-1}). In the Italian varieties, Heimler et al. [2004] determined the content of isoflavons range from 1 830 to 11 880 mg per kg of dry matter. Those results are consistent with the view of Nakamura et al. [2000] on the impact of country of origin for soybean composition and content of these substances in variety. Our results of the isoflavons contents are by the values in these published intervals.

In the variety Korada exerting a statistically significant effect of locality (P <0.01) for daidzein content. Results of genistein determination in this variety didn't showed differences in relation to the locality. The order of sites for the isoflavones content is Malacky <Marcelová <Oponice <Belá over Cirochou. Effect of site diversity was significantly (P <0.01) affected the amount of genistein variety Supra. Variety Supra grown in the area Oponice is characterized by a surprisingly approximately the same daidzein and genistein content (1:1), while the same variety from locality Marcelová differed significantly in representation of both isoflavons and also reached the lowest content of daidzein in the whole samples group. Soil of land cadastre Oponice has neutral soil reaction (pHKCl = 7,10) with very high Mg content. Higher content genistein was also observed in varieties Korada (Oponice) and Crystal (Jelšovce) also grown in soils with neutral soil reaction. The relation between the interaction of pH soil and isoflavons levels in the crop not indicate a lower genistein content found in the variety Belmont (Jelšovce), which is comparable with values in a variety of localities with alkali, resp. acidic soil. Significant differences (P <0.01) were also found in daidzein content in varieties Korada (Malacky, Belá over Cirochou) and the Supra (Marcelová) obtained from plots with acid soil reaction.

A comparison of results from all localities, Table 1. shows that the highest daidzein content end concurrently the lowest genistein content was determined in a variety Bolyi-45. The site that has been grown variety Bolyi-45 (Marcelová) is characterized by the lowest nutrient content, but also the risk of heavy metals, which may be closely associated with alkaline soil

reaction. In this area it is necessary to monitor the heavy metals content in harvested agricultural production, because of changes in soil properties is an increased risk of metal mobility and bioaccessibility for cultivated crops. Even in the case of a variety Korada from locality Belá over Cirochou (pH_{KCl} = 5.51) confirms the impact of low levels of nutrients and heavy metals to increased production of daidzein as a determining factor content of total polyphenols. In contrast to these findings are, higher levels of daidzein and genistein of this variety harvested in adequate environmental conditions (Oponice).

Within 5 rated soybean varieties in the locality Marcelová did not determine extremely different values between the contents of daidzein and genistein. Given similar soil quality of site from which they were collected with reference varieties, it is not possible to consider the causation of available nutrients, resp. soil acidity and isoflavons content. Furthermore, it can be concluded that the varieties grown in this area (soil type chernozem) show the lowest content of isoflavons in the entire samples set.

Examination of the phenolic composition of soybean, we arrive at a favorable view of Mebrahtu et al. [2004] that the contents of daidzein, genistein in soybeans are equivalently involved in environmental conditions, as well as genetic disposition of crops. In terms of content studied isoflavons as optimal variety show variety Korada and Crystal grown in adequate environmental conditions – locality Nitra (Oponice, Jelšovce).

Sakai et al. [2005] at studying of japanese soybean varieties found tend to find lower content of isoflavons in early varieties. The result of their work is also finding a lower difference in isoflavone content in early varieties in comparison with the differences found between the middle and late ripening varieties of soybean seeds, independently of seed color. Some consistent indications of this trend can be observed when we monitored early varieties Korada (Malacky, 656.5 mg.kg^{-1}) and Erin (649.0 mg. kg^{-1}), which is characterized by both a lower amount of isoflavones (the sum) compared with late varieties Bolyi-45, Crystal and Belmont. Relatively greater difference in the amount of isoflavons was also observed between the late varieties, and variety Belmont (771.1 mg. kg^{-1}) matures a little more than the variety Crystal (834.6 mg.kg^{-1}). In contrast with these facts is to determine the highest isoflavons content in early Korada variety from sites Belá over Cirochou and Oponice. These facts are important, but in terms of experiment implementation (few varieties) will not allow us to declare the conclusion presented in Sakai et al. [2005].

Relationships between storage time and changes in the isoflavons level in soybean seeds present results in Tables 2–3. Due to high daidzein and genistein amounts of soybean samples were collected after 3 months of storage, then in two months and after the interim evaluation at the end of storage (April-June) in monthly intervals.

The obtained results suggest that in soybean varieties within the stipulated seven months of the monitored isoflavons concentrations declined, but in any case not reached the threshold limit of detection. At the same throughout the field of storage was maintained higher daidzein level compared with genistein level. Testing the daidzein levels by method variance in varieties Erin, Belmont and Korada (Bela over Cirochou) showed significant decrease in daidzein in relation to initial value to after 5 months. In all other varieties were found significant decrease in daidzein already after the quarterly storage (November – February). On the basis of continuous evaluation of genistein we may noted that the process of this isoflavon degradation is significantly expressed only in the beginning of storage. With the exception of the varieties Erin and Supra (Oponice) in the next period is no longer statistically significant loss of genistein ($P > 0.01$) in soybean seeds between the period and between samplings.

Table 2

Changes in isoflavone content (mg.kg^{-1}) in soybean seeds grown in the Marcelová area in dependence on the storage period

Variety	Measurement	Daidzein content	Genistein content
Bolyi-45	November	721.0c	142.9b
	February	269.5b	47.5a
	April	172.9a	40.8a
	May	126.3a	31.2a
	June	111.6a	25.1a
Erin	November	370.8b	278.2c
	February	314.2b	81.0b
	April	145.6a	51.7a.b
	May	126.3a	35.4a.b
	June	107.9a	28.4a
Korada	November	477.1b	243.9b
	February	225.1a.b	46.9a
	April	211.0a.b	40.9a
	May	110.6a	38.3a
	June	83.4a	24.0a
Quito	November	382.2c	244.6b
	February	236.4b	63.6a
	April	95.7a	28.4a
	May	90.5a	27.4a
	June	73.5a	21.3a
Supra	November	358.9c	223.3b
	February	208.9b	53.7a
	April	109.1a	33.2a
	May	78.5a	26.7a
	June	57.1a	25.9a

Values in the same column with different letters present significant differences $p < 0.01$

In comparing the values obtained in 5 varieties of locality Marcelová was in the variety Bolyi-45 detected the lowest decline in genistein and at the same time the largest decline of daidzein level (despite a high concentration at the beginning of storage).

V 2nd collection (February) in this variety was found 62.6% decrease of daidzein, which during the remaining five months gradually declined. The variety Erin with lowest values at the beginning of storage, retained relatively highest levels of daidzein and genistein

On the basis of percentage of final values of isoflavons against the initial values can be created the following order of varieties in locality Marcelová:

- *daidzein*: Erin (29,1%) > Quito (19,2%) > Korada (17,5%) > Supra (16,0%) > Bolyi-45 (15,5%)
- *genistein*: Bolyi-45 (17,6%) > Supra (11,6%) > Erin (10,2%) > Korada (9,8%) > Quito (8,7%)

Table 3

Changes in isoflavone content (mg.kg^{-1}) in soybean seeds grown in different localities of Slovakia dependence on the storage period

Locality	Variety	Measurement	Daidzein content	Genistein content
Jelšovce	Crystal	November	527.5c	307.2b
		February	394.4b	71.1a
		April	260.0a	63.1a
		May	199.1a	40.8a
		June	167.3a	38.5a
	Belmont	November	538.0c	232.9b
		February	386.1bc	50.5a
		April	235.6ab	44.1a
		May	188.2a	30.3a
		June	145.2a	30.3a
Oponice	Supra	November	470.7d	425.8c
		February	365.1c	106.6b
		April	213.5b	49.5a
		May	117.6ab	49.0a
		June	95.3a	33.4a
	Korada	November	617.0b	304.2b
		February	226.1a	64.3a
		April	213.2a	49.2a
		May	168.1a	48.8a
		June	157.4a	46.0a
Belá over Cirochou	Korada	November	668.9c	258.9a
		February	511.3bc	122.5a
		April	427.5b	105.2a
		May	206.7a	70.8a
		June	120.3a	44.9a
Malacky	Korada	November	402.3b	252.4b
		February	173.8a	54.7a
		April	162.7a	50.4a
		May	148.9a	49.6a
		June	146.9a	39.6a

Values in the same column with different letters present significant differences $p < 0.01$

Among all the tested varieties grown in different localities in Slovakia the highest daidzein content have preserved the variety Crystal (Jelšovce, 167.250 mg.kg^{-1}) and the highest value of genistein was found in the variety Korada (Oponice, 46.048 mg.kg^{-1}). It is interesting that although at the beginning of storage isoflavons contents in different varieties varied, genistein values determined at the end of storage in all varieties were in a relatively narrow range

of values (from 21.269 to 46.048 mg.kg^{-1}). The range of values was wider at daidzein from 57.079 to 167.250 mg.kg^{-1}.

Interaction of influence of the storage and locality on the isoflavons content has been studied in the varieties Korada and Supra. In the variety Korada during three months of storage has rapidly decreased the genistein value (an average of five times compared to the value obtained after sample collection), with the exception of the variety obtained from the locality Belá over Cirochou. Reducing levels of daidzein at the same time period was variable. Differences found in isoflavons content over the next period were no longer statistically significant and showed a slight downward trend in the values of both isoflavons.

On the basis of the results of the isoflavons determination in the 1st measurements in soybean samples cv. Korada can set the following order of localities:

daidzein: Belá nad Cirochou > Oponice > Marcelová > Malacky

genistein: Oponice > Belá over Cirochou > Malacky > Marcelová

From the results of the isoflavons determination in the 5th measurement in soybean samples cv.Korada can set the following order of localities:

daidzein: Oponice > Malacky > Belá over Cirochou > Marcelová

genistein: Oponice > Belá over Cirochou > Malacky > Marcelová

From a comparison of locality order it is clear that between the samples of Korada variety has shown an impact on the storage on the daidzein content . In terms of the dynamics of change sharpest decline occurred in the variety Korada grown in the locality Marcelová (82,5%), which is comparable to the loss of daidzein in this variety from locality Belá over Cirochou (82%). Despite the lowest baseline value, least significant change is passed a variety grown in Malacky (63,6%). The variety Korada grown in Oponice area retained 25,5% of daidzein.

In the case of genistein has been shown to consensus sequence sites from the initial measurement and final measurement, on what basis can say that a storage effect of genistein in the individual sites not shown. However, differences were found in the dynamic process of degradation of genistein. Korada variety grown in the Oponice content while maintaining the level of genistein compared with the same varieties grown in Malacky, but the total loss of genistein on the whole section of storage was comparable (85%). The most significant decrease was observed in genistein Korada variety grown in the area Marcelová (90,2%) and the smallest decrease was observed in the same variety of locations Belá over Cirochou (82%) (low levels of nutrients and hazardous elements). With the percentage of final values to the initial values of genistein may say that the above sequence of sites is not identical with the percentage decline in genistein.

Impact assessment of the storage and locality is not reflected significantly in the case of a variety Supra. The ongoing monitoring of changes can be observed a tendency to maintain a higher content of isoflavones in this variety grown in Oponice compared with values obtained from the locality Marcelová.

Conclusions

Analysis of changes to the isoflavons content in dry soybean seeds during long-term storage, it was found that differences in the degradation of these substances are due to the different structure of compounds. Long-term storage of soybean seeds for animal feed in terms of the decline in the isoflavons levels (mainly daidzein, that the action of bacteria in the colon

changes to equol, thus causing infertility in animals) appears to be a suitable alternative for reducing the risk of animal sterility. On the other hand, relatively high concentrations of daidzein and genistein in soybeans down after a long storage period shows the appropriateness of the use of soybean in our menu for the declared beneficial effects of isoflavons on human health throughout the year. In terms of studied isoflavons content, their degradation dynamics in our soybean varieties evaluated as optimal for consumption appear varieties grown in Nitra (Oponice, Jelšovce).

References

Benešová L. et al., 1996. Potravinářství 94. ÚZPI, Praha, 157.

Decision of the Ministry of Agriculture SR No. 531/1994–540 o najvyšších prípustných hodnotách rizikových látok v pôde. MP SR: Vestník MP SR, roč. XXVI, čiastka I., rozhodnutie 3, číslo 531/1994.

Fiala K. et al., 1999. Záväzné metódy rozborov pôd. Čiastkový monitorovací systém – pôda. Edičné stredisko Výskumného ústavu pôdoznalectva a ochrany pôdy, Bratislava, 142.

Franke A.A., Custer L.J., Cerna C.M., Narala K.K., 1994. Quantitation of phytoestrogens in legumes by HPLC.J. Agric. Food. Chem., 42, 1905–1913.

Genovese M.I., Hassimotto N.M.A., Lajolo F.M., 2005. Food Science and Technology International, 11, nr 3, 205–211.

Heimler D., Vignolini P., Galardi C., 2004. Simple extraction and rapide quantitative analysis of isoflavones in soybean seeds. Chromatographia, 59, nr 5–6, 361–365.

Klejdus B., Štěrbová D., Stratil P., Kubáň V., 2003. Identifikace a charakterizace isoflavonú v rostlinných extraktech za použití kombinace HPLC s hmotnostným detektorem a detektorem s diodovým polem (HPLC-DAD-MS). Chemické listy, 97, 1–10.

Mebrahtu T., Mohamed A., Wang C.Y., 2004. Analysis of isoflavone contents in vegetable soybeans, [in:] Plant Foods for Human Nutrition, 59, nr 2, 55–61.

Nakamura Y., Tsui S., Tonogai Y., 2000. Determination of the levels of isoflavonoids in soybean and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake. Journal of AOAC International, 83, nr 3, 635–650.

Sakai T., Kikuchi A., Shimada H. et al., 2005. Evaluation of isoflavone contents and compositions of soybean seeds and its relation with seeding time. Japanese Journal of Crop Science, 74, nr 2, 156–164.

Wang G., Kuan S. et al., 1990. A simplified HPLC methods for the determination of phytoestogens in soybean and its processed products. J. Agric. Food Chem., 38, 185–190.

Appendix

Table 4

Available nutrients content in mg.kg^{-1} (Mehlich II), soil reaction and humus content [%] in soils of different sites of soybean cultivation

Locality	Site (Variety)	Ca	Mg	K	P	N	pH_{KCl}	% humus
Marce-lová	MČ-3 (Supra)	851.0	153.0	269.5	74.3	1050.0	5.01	2.79
	Markacová (Quito)	4440.0	148.0	225.5	62.6	1487.5	7.30	2.44
	Pri Virte (Erin)	6280.0	173.5	148.0	44.4	1312.5	7.46	2.35
	Dolnožitavská (Korada)	1909.0	287.0	207.0	144.4	1400.0	6.88	2.25
	Ármaiho (Bolyi45)	4155.0	99.5	97.0	33.4	875.0	7.55	1.52
Jelšovce		2930.0	265.5	266.5	82.1	1575.0	7.11	2.51
Oponice		3950.0	314.0	296.0	45.7	962.0	7.10	2.32
Belá over Cirochou	Štaň	978.5	86.0	107.5	55.1	1575.0	5.51	2.64
Malacky	Prostredná	2455.0	251.0	135.0	76.9	1750.0	5.12	3.27

Table 5

Total content of heavy elements [mg.kg^{-1}] in soils of different sites of soybean cultivation

Locality	Site (Variety)	Zn	Cu	Cr	Cd	Pb	Ni	Co
Marce-lová	MČ-3 (Supra)	55.2	28.4	39.6	0.72	22.4	26.4	13.2
	Markacová (Quito)	53.2	22.4	45.2	0.92	24.0	28.0	14.0
	Pri Virte (Erin)	47.2	20.0	42.0	0.84	23.6	28.4	14.4
	Dolnožitavská (Korada)	61.6	24.8	50.8	0.88	26.0	30.0	13.6
	Ármaiho (Bolyi45)	41.2	16.8	34.8	0.84	23.2	23.2	12.0
Jelšovce		78.8	36.8	57.6	1.00	28.8	34.4	17.2
Oponice		95.2	37.2	60.0	1.08	31.6	36.0	17.2
Belá over Cirochou	Štaň	55.6	21.6	51.2	0.72	25.2	20.4	11.6
Malacky	Prostredná	63.2	30.0	64.8	0.92	28.4	31.2	12.0

Table 6

Risky elements content (mg.kg^{-1}) in HNO_3 extract (c=2 mol.dm^{-3}) in soils of different sites of soybean cultivation

Locality	Site (Variety)	Zn	Cu	Cr	Cd	Pb	Ni	Co
Marcelová	MČ-3 (Supra)	5.8	8.3	1.2	0.16	6.8	5.1	1.7
	Markacová (Quito)	6.2	7.6	1.9	0.29	8.3	5.9	3.8
	Pri Virte (Erin)	5.0	6.9	1.9	0.38	7.7	5.7	3.9
	Dolnožitavská (Korada)	9.5	8.3	2.0	0.21	7.9	5.5	2.9
	Ármaiho (Bolyi45)	4.3	5.7	1.4	0.26	6.1	3.7	2.7
Jelšovce		10.1	11.3	2.1	0.29	11.1	6.5	4.4
Oponice		17.3	9.8	2.4	0.34	14.9	4.6	4.2
Belá over Cirochou	Štaň	7.9	4.4	2.1	0.23	10.9	1.7	2.2
Malacky	Prostredná	10.5	8.6	3.4	0.21	8.8	6.4	2.0

Table 7

The Decision of Ministry of Agriculture of Slovak Republic about highest acceptable limits of toxic compounds in soil No. 531/1994–540

Riziková látka	A	A_1	B	C
As	(29)	5.0	30	50
Ba	500	x	1 000	2 000
Be	3	x	20	30
Cd	(0.8)	0.3	5	20
Co	20	x	50	300
Cr	(130)	10	250	800
Cu	(36)	20	100	500
Hg	(0.3)	x	2	10
Mo	1	x	40	200
Ni	(35)	10	100	500
Pb	(35)	30	150	600
Se	0.8	x	5	20
Sn	20	x	50	300
V	120	x	200	500
Zn	(140)	40	500	3 000

A – background value, A_1 – background value in HNO_s extract (c=2 M), B – indicative value for the contamination, C – indicative value for soil remediation

8

FRUIT QUALITY, POLYPHENOLIC CONTENT, AND ANTIOXIDANT CAPACITY OF ORGANICALLY AND CONVENTIONALLY GROWN STRAWBERRIES

Introduction

Polyphenols are an important class of phytochemicals in fruits. They have a number of functions in the plant including resistance to disease, phytoprotection, are colorants and attractants to fruit-eating animals and aid in identifying when fruits are edible. Plant polyphenols may also have positive effects on human health although mechanisms by which they might exert such effects are unclear. High human intake of foods rich in polyphenol compounds, such as fruits and vegetables, have been inversely associated with risks of coronary heart disease and mortality [Hertog et al. 1994, Dauchet et al. 2006, He et al. 2007] and stroke [He et al. 2006]. Polyphenols have strong antioxidant activities, and the onset of these diseases is associated with oxidation of low density lipoproteins in the vasculature. However, *in vivo* action of dietary antioxidants may also include induction of protective enzymes and processes [Scalbert et al. 2005].

Strawberries (*Fragaria x ananassa* Duch.) are an important crop in temperate regions such as Poland in Central Europe. They are widely consumed fresh and in processed forms especially as frozen fruits. These attractive fruits are favored for their excellent taste and can be considered a very potent source of bioactive phenolic compounds including hydroxycinnamic acids, ellagic acid, ellagitannins, flavan-3-ols, flavonols, and anthocyanins [Määttä-Riihinen, Kamal-Eldin, Törrönen 2004]. Compared with other fruits, strawberries possess high antioxidant activity [Sun, Chu, Wu, Liu 2002). Guo, Cao, Sofic and Prior [1997] found that strawberry had 1.3 times the antioxidant activity of oranges, twice that of red grapes, five times that of apples and bananas, and thirteen times that of honeydew melon [Guo, Cao, Sofic, Prior 1997]. Antioxidant activity of strawberry phenolics is important in the prevention of cancer, cardiovascular and other chronic diseases [Hannum 2004].

Organic food is an expanding sector of the agricultural industry in many parts of the world, and it is possible to find organically produced food in most supermarkets in Europe and North America.

The overall goal of organic farming is to use agricultural methods that have the smallest impact on the environment and provide the greatest benefit to people [Becharrell and MacFie 1991, Bourn and Prescott 2002].

One main difference between organic and conventional farming is in the use of fertilizers. The latter uses chemical fertilizers as well as manure, compost, sewage sludge, and other soil amendments, whereas most certified organic farming allows only the use of manure, compost, and natural soil additives. A second difference between the 2 systems is the use of pes-

ticides and herbicides. Conventional farming, once again, uses any product available on the market (not forbidden by law), whereas organic farming allows only a few pesticides that are believed to leave no residue on the products, for example, copper ammonium carbonate, copper sulfate, copper oxychloride, sulfur, rotenone, pyrethrum, and soft soap [IFOAM 1998].

The application of pesticides and fertilizers has previously been reported to modulate the biosynthesis and nature of phenolics in plants and other chemical composition [Lea and Beech 1978, Nicolas and others 1994, Daniel and others 1999, Carbonaro and Mattera 2001].

The aim of the study was to estimate whether the effect of cultivation technique on the chemical composition (dry matter, soluble solids, total acidity, sugars and vitamin C) and phenolic profiles of strawberry fruits.

Materials and method

Strawberry fruit came from the Certified Organic Experimental Field of the Fruit Experiment Station in Brzezna of the Research Institute of Pomology and Floriculture in Skierniewice. In the experiment, there were also used their conventional equivalents from the same Institute. The experiment was conducted in 2010, whereas strawberry fruit were picked in the period from 5 to 20 June. There were selected average samples of four strawberry varieties for studies: Elkat, Kent, Honeoye, Alioth.

In fresh, completely mature fruit, there was determined a content of following components: dry matter by the weight method (PN-90/A-75101/03), soluble solids (PN-90/A-75101/02), total and directly reducing sugars by the Lane–Eynona method (PN-90/A-75101/07), total acidity (PN-90/A- 75101/04), the content of vitamin C by the Tillman's method (PN-90/A-75101/11). The content of phenolic compounds, including phenolic acids, flavonols and anthocyanins, was determined by the HPLC method, according to Oszmiański et al. [2009] and proanthocyanidins by Kenedy et al. [2001]. Antioxidant activity of the studied varieties was determined by the ABTS radical cation method, according to Re et al. [1999]. The results obtained were submitted to statistical analysis with the use of one-way analysis of variance, by Duncan's test, at a significance level of $p<0.05$, using a computer programme Statistica 8.0 (StatSoft Inc.)

Results and discussion

The obtained results of the chemical composition analysis, i.e. the content of dry matter, soluble solids, total acidity, total and reducing sugars and vitamin C for strawberry fruit from organic and conventional production were presented in Table 1.

The results of fruit chemical analysis, presented in the table, show that the applied cultivation method did not have a significant influence on the dry matter content. Both in organic and conventional production, there were obtained very similar contents of dry matter. However, the examined variety considerably determined a capacity of dry matter accumulation in fruit. Among organic and conventional strawberry, the highest level of dry matter was characteristic of the variety Honeoye.

Table 1

The content of dry matter, soluble solids, total acidity, total and reducing sugars and vitamin C in strawberry fruit from organic and conventional production

Production	Variety	Dry matter [%]	Soluble solids [%]	Total acidity [g/100g of citric acid]	Reducing sugars [%]	Total sugars [%]	Vitamin C [mg/100g]
Organically	KENT	10.20±0.32c	9.2±0.02c	0.62±0.09d	6.86±2.12bc	7.46±2.15c	15.29±1.55e
	ELKAT	11.33±0.96b	10.4±0.11b	0.60±0.13d	7.41±1.24b	8.04±1.04b	26.12±1.33d
	HONEOYE	11.94±0.11b	9.8±0.16b	0.85±0.06b	7.15±0.10b	7.90±0.29c	17.28±0.96e
	ALIOTH	9,78±0.32d	8.3±028d	0.74±0.19c	5.65±1.08d	6.48±1.07d	39.31±1.05b
	mean	10.81	9.43	0.70	6.77	7.47	24.55
Conventionally	KENT	8.96±0.13d	7.3±0.33e	0.72±0.13c	5.47±0.10d	6.15±2.05d	13.67±1.08f
	ELKAT	9.26±1.09d	8.0±0.26d	0.72±0.11c	5.55±2.11d	6.23±1.11d	34.08±1.02c
	HONEOYE	15.36±0.14a	13.2±0.40a	1.02±0.31a	10.21±1.03a	11.47±0.6a	39.31±2.03b
	ALIOTH	9.69±1.02d	8.4±0.51d	0.71±0.15c	6.27±1.15c	6.91±1.03cd	47.18±1.08a
	mean	10.82	9.23	0.79	6.88	7.69	32.65

Values are mean ± SD. $n = 3$ mean values within a verses with different letters are significantly different at $p < 0.05$

With respect to the measurement of soluble solids, also conventional fruit were characterised by their higher content, comparing to organic ones, respectively at the average content of 9.43 and 9.23%. The lowest content of soluble solids included an conventional variety of Kent (7.3%), whereas the soluble solids content of this variety – in case of organic production – amounted to 9.2%.

Organic acids content in organic strawberry fruit was lower than in conventional ones. Kent, Elkat and Alioth varieties were characteristic of lower total acidity, comparing to Honeoye variety. The conventional variety Honeoye included a slightly higher content of total acidity in fruit, comparing to organic ones (Tab. 1), and the differences were statistically significant ($p<0.05$). For organic production, it amounted to 0.70 g/100 g fw., and for conventional – 0.79 g/100 g fw.

The content of total and reducing sugars is closely connected with the soluble solids content. In this case as well, such regularity was maintained that the varieties of the highest soluble solids content also included the highest content of total and reducing sugars. But differences between production methods of the same varieties were slight and statistically insignificant (Tab. 1).

The obtained values of the basic chemical composition in case of most of examined determinants, i.e. the content of dry matter, soluble solids, total acidity or total and reducing sugars, were similar or lower for organic fruit, comparing to conventional ones. Cayuela et al. [1997] showed a significantly higher content of dry matter, total acidity and sugars in the examined organic strawberry fruit, comparing to conventional ones. However, Anttonen and Karjalainen [2006], analysing the values of soluble solids and total acidity for black currant fruit, ascertained that organic fruit varieties were characterised by very equalised values of the examined determinants, but lower values of these parameters than conventional fruit. Also in case of studies on other raw materials, i.e. apple, carrot, beet, tomato, onion, a higher content of dry matter, soluble solids or total sugars was found in organic crops [Kolbe et al. 1995, Rembiałkowska et al. 2005, Hallmann and Rembiałkowska 2007a].

The average content of ascorbic acid in conventional raspberry fruit (32.65 mg/100 g fw) was higher than in organic one (24.55 mg/100g fw), but separate organic and conventional varieties included different contents of this substance. Only the organic Kent variety was characteristic of a higher content of vitamin C than their conventional equivalents, contrary to the rest varieties. However, among conventional strawberry, the content of vitamin C increased as follows: Alioth > Honeoye > Elkat > Kent, and in case of organic production – Alioth > Elkat > Honeoye > Kent. Conventional fruit included a higher content of ascorbic acid by 1.4 times, comparing to organic production.

According to literature data, strawberry include approx. 30–50 mg/100 g of vitamin C, whereas organic fruit do not differ in the vitamin content from strawberry traditionally grown. Some article show that organic fruit poses higher value than conventional production, i.e. for black currant – Anttonen and Karjalainen [2006], strawberries [Cayuela et al. 1997] or peaches [Carbonaro et al. 2002]. Contrary studies results are presented by Lombardi-Boccia et al. [2004]. The other studies, conducted by Kazimierczak et al. [2008] and also concerning black currant fruit indicate that organic fruit – irrespective of a variety – were typical of a higher content of dry matter and vitamin C. The differences in the ascorbic acid content of organic and conventional fruit can be explained by a natural attitude of plants towards external factors, among others, an access of light or over-fertilising. An application of the excessive nitrogen fertilisation, which takes place in conventional farming, results in extreme growth of a green plant part and a lush growth of leaves. It causes the fruit shading effect,

which leads to a decrease in the intensity of the ascorbic acid synthesis in fruit [Premuzic et al. 1998]. It is confirmed by the experiment by Liptay et al. [1986] on tomatoes grown at poorer light access, in autumn season, which included a significantly lower content of ascorbic acid, comparing to the same varieties grown at full sun light access in spring.

Besides vitamin C and mineral components, strawberry fruit represent a great source of natural bioactive compounds [Wang et al. 1996, Wang and Lin 2000]. Obtain result show that organic fruit – comparing to conventional ones – were characteristic of a higher content of bioactive compounds, including phenolic compounds. The average content of phenolic compounds was higher for organic varieties (except for Kent variety) than for conventional varieties (Fig. 1). The highest content of the compounds was found in organic cultivars: Alioth – 2 126.60 mg/kg fw, Elkat 2 070.40 mg/kg fresh weight (fw) and Honeoye 1 933.28 mg/kg fw, contrary to conventional production, respectively 1 764.92 mg, 1 870.70 mg and 1 604.48 g/kg fw.

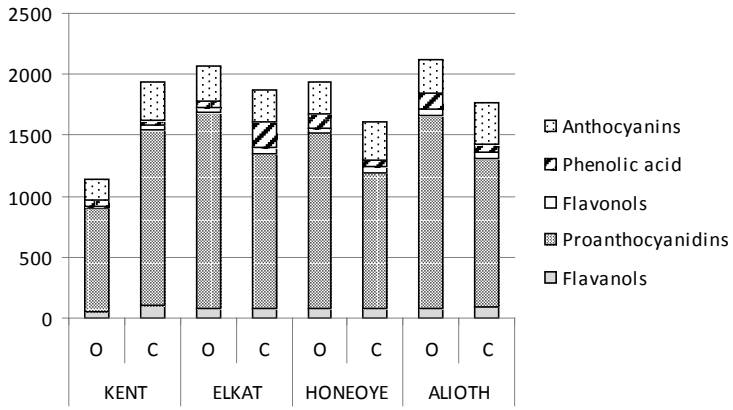

Fig. 1. The content of phenolic compounds [mg/kg fw] in strawberry fruit from organic [O] and conventional [C] production

The conducted HPLC analysis showed that proanthocyanins belonging to flavanols compounds represented a predominant compounds group in strawberry fruit, in particular for organic varieties, where they amounted to 79–83% of total polyphenols content. In organic variety, the highest content of proanthocyanidins included Alioth variety (1 584.99 mg/kg fw) and Elkat variety (1 612.16 mg/kg fw). Other groups of phenolic compounds are represented by anthocyanins > phenolic acids > quercetin derivatives and rest of monomer and dimeric flavanols. Analysing the results obtained, it was ascertained that the content of particular groups of phenolic compounds in strawberry fruit was significantly influenced by a production method as well as the examined variety. Organic strawberry included a higher level of proantocyanidins and anthocyanins but a lower content of phenolic acids and flavonols, comparing to conventional fruits (Fig. 1).

The higher content of phenolic compounds – according to this paper were obtained in the strawberry studies conducted by Olsson et al. [2006] and the American blueberry studies by Wang et al. [2008]. Present studies and literature data confirm the regularity concerning a higher content of the compounds in organic raw materials. It is related to Bryant et al. [1983] theory of the balance of nitrogen to carbon, which says that organic plants – having

at their disposal a lower level of easily-available carbon – produce a higher amount of precious bioactive compounds, including phenolic compounds, than conventional plants [Caris-Veynard et al. 2004].

Also the studies conducted by Asami et al. [2003] showed that strawberry and blackberry fruit included a higher content of phenolic compounds in organic fruit than conventional ones. However, Häkkinen and Torronen [2000] showed that irrespective of production system the content of flavonols and phenolic acids was similar in case of Polka and Honeoye varieties. Only the examined organic variety Jonsok included a higher content of total polyphenols by 12%, comparing to conventional variety.

An analysis of antioxidant activity was conducted by ABTS method. The results obtained are presented in Figure 2. The mean values of antioxidant activity for the examined strawberry fruit amounted to 27.38 and 69.88 μM Trolox/1g fw for organic and conventional production. A determined antioxidant activity of strawberry fruit was comparable to the content of bioactive compounds, especially for phenolic compounds. The lowest activity was characteristic of Alioth variety, in case of which – regardless of a growing method – the measured antioxidant activity amounted to 69.88 μM Trolox/1g fw. In both strawberry production systems, the highest antioxidant activity was typical of Alioth variety.

These results suggest that the antioxidant capacity of blueberry fruit could have been derived from the contribution of phenolic and anthocyanin compounds.

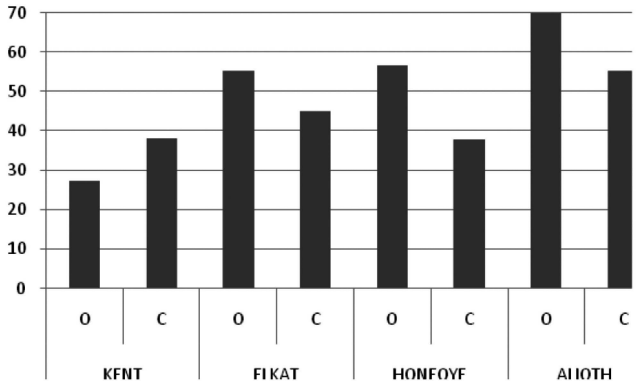

Fig. 2. The antioxidant activity measured by ABTS method [μM Trolox/1g] in strawberry fruit from organic [O] and conventional [C] production

Previous studies showed that strawberries have strong antioxidant characteristics Wang et al. [2000] and Oszmiański et al. [2009]. Therefore, the consumption of organic fruit, including strawberries, may contribute to increasing the antioxidant protection of human organism through prophylactic and pro-healthy effects. Studies indicate a close connection between the content of bioactive compounds and antioxidant activity. A high correlation ratio (r=0.97) for strwberry fruit confirms that the activity mainly depended on the polyphenols content, but not the content of vitamin C (r=0.35). Similar result was obtain by Wang et al. [2008] for blueberry fruits and Wojdylo et al. [2010] for raspberry fruits.

Conclusions

The obtained results of the studies showed that conventional fruit included a similar content of dry matter, but higher content of soluble solids, total acidity, total and reducing sugars and vitamin C compared to organic production. However, some organic variety of strawberry fruits were specific of a higher content of bioactive compounds, including phenolic compounds. Such contents resulted in a higher antioxidant activity of organic soluble solids. Therefore, at this stage of the studies, it is difficult to present unequivocal conclusions allowing to state which production system provides fruit of better pro-healthy quality, or which varieties are more advantageous for organic production. Concerning the above mentioned, the studies should be continued to next years to confirm the regularities observed.

Acknowledgements

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References

Anttonen M.J., Karjalainen R.O., 2006. High-Performance liquid chromatography analysis of black currant (*Ribes nigrum* L.) fruit phenolics grown either conventionally or organically. Journal of the Agricultural and Food Chemistry, 54, 7530–7538.

Asami, D.K., Hong, Y., Barrett D.M., Mitchell A.E., 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. Journal of the Agricultural and Food Chemistry, 51, 1237–1241.

Becharrell B., MacFie J.H., 1991. Consumer attitudes to organic foods. Br. Food J., 93, 25–30.

Bourn D., Prescott J., 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Crit. Rev. Food. Sci. Nutr., 42, 1–34.

Bryant J.P., Chapin III F.S., Klein D.R., 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos., 40, 357–368.

Carbonaro M, Mattera M., 2001. Polyphenoloxidase activity and polyphenol levels in organically and conventionally grown peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv. Williams). Food Chemistry, 72, 419–24.

Carbonaro M., Mattera M., Stefano N., Bergamo P., Cappelloni M., 2002. Modulation of antioxidant compounds in organic vs conventional fruit (peach, *Prunus perlica* L., and pear, *Prunus communis* L.). Journal of the Agricultural and Food Chemistry, 50, 5458–5462.

Caris-Veyrat C., Amiot M.J., Tyssandier V., Grasselly D., Buret M., Mikolajczak M., Guilland J.-C., Bouteloup-Demange C., Borel P., 2004. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. Journal of the Agricultural and Food Chemistry, 52, 6503–6509.

Cayuela J.A., Vidueira J.M., Albi M.A., Gutiérrez F., 1997. Influence of the ecological cultivation of strawberries (*Fragaria* × *ananassa* cv. Chandler) on the quality of the fruit and on their capacity for conservation. Journal of the Agricultural and Food Chemistry, 45, 1736–1740.

Daniel O., Meier M.S., Schlatter J., Frischknecht P., 1999. Selected phenolic compounds in cultivated plants: ecologic functions, heath implications, and modulation by pesticides. Environ. Health Perspect., 107, 109–14.

Dauchet L., Amouyel P., Hercberg S., Dallongeville J., 2006. Fruit and vegetable consumption and risk of coronary heart disease: A meta-analysis of cohort studies. J. Nutr., 136, 2588–2593.

Guo C., Cao G., Sofic E., Prior R.L., 1997. High-performance liquid chromatography coupled with coulmetric array detection of electroactive components in fruits and vegetables. Relationship to oxygen radical absorbance capacity. Journal of Agricultural and Food Chemistry, 45, 1787–1796.

Häkkinen S., Törrönen A.R., 2000. Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. Food Research International, 33, 517–524.

Hallmann E., Rembiałkowska E., 2007a. Zawartość związków bioaktywnych w owocach papryki z uprawy ekologicznej i konwencjonalnej. Żywienie Człowieka i Metabolizm, XXXIV, 1/2, 538–543.

Hannum S.M., 2004. Potential impact of strawberries on human health: A Review of the Science. Critical Review Food Science, 44, 1–17.

He F.J., Nowson C.A., Lucas M., MacGregor G.A., 2007. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J. Human Hypertens., 21, 717–728.

He F.J., Nowson C.A., MacGregor G.A., 2006. Fruit and vegetable consumptionand stroke: meta-analysis of cohort studies. Lancet, 367, 320–326.

Hertog M.G.L., Kromhout D., Aravanis C., Blackburn H., Buzina R., Fidanza F., Giampaoli S., Jansen A., Menotti A., Nedeljkovic S., Pekkarinen M., Smic B.S., Toshima H., Feskens E.J.M., Hollman P.C.H., Katan M.B., 1994. Flavanoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch. Intern. Med., 155, 381–386.

IFOAM – Intl. Federation of Organic Agricultural Movements. 1998. Basic standards for organic production and processing. IFOAM General Assembly; Buenos Aires, Argentina; 9–21 Nov 1998. Tholey-Theley, Germany: IFOAM.

Kazimierczak R., Hallmann E., Rusaczonek A., Rembiałkowska E., 2008. Antioxidant content in black currants from organic and conventional cultivation. Electronic Journal of Polish Agricultural Universities: Food Science and Technology, 11/ 2.

Kennedy J.A., Jones G.P., 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. Journal of Agricultural and Food Chemistry, 49, 1740–1746.

Kolbe H., Meineke S., Zhang W.-L., 1995. Differences in organic and mineral fertilization on potato tuber field and chemical composition compared to model calculations. Agribiology Research, 48, 63–7311.

Lea A.G., Beech F.W., 1978. The phenolic of ciders: effect of culture conditions. J. Sci. Food Agric., 29, 493–6.

Liptay A., Papadopoulos P., Bryan H.H., Gull D., 1986. Ascorbic acid levels in tomato (Lycopersicon esculentum Mill) at low temperatures. Agricultural, Biological, Chemistry, 50, 3185–3187.

Lombardi-Boccia G., Lucarini M., Lanzi S., Aguzzi A., Cappelloni M., 2004. Nutrients and antioxidant molecules in yellow plums (*Prunus domestica* L.) from conventional and organic productions: a comparative study. Journal of the Agricultural and Food Chemistry, 52, 90–94.

Määttä-Riihinen K.R., Kamal-Eldin A., Törrönen A.R., 2004. Identification and quantification of phenolic compounds in berries of Fragaria and Rubus species (family Rosaceae). Journal of Agricultural and Food Chemistry, 52, 6178–6187.

Nicolas J.J., Richard-Forget F.C., Goupy P.M., Amiot M.J., Aubert S., 1994. Enzymatic browning reactions in apple and apple products. Crit. Rev. Food Sci. Nutr., 34, 109–157.

Olsson M.E., Gustavsson K., Andersson S., Nilsson Å., Duan R., 2006. Inhibition of Cancer Cell Proliferation in Vitro by Fruit and Berry Extracts and Correlations with Antioxidant Levels. Journal of the Agricultural and Food Chemistry, 52, 7264–7271.

Oszmiański J., Wojdyło A., Kolniak J., 2009. Effect of L-ascorbic acid, sugar, pectin and freeze-thaw treatment on polyphenol content of frozen strawberries. LWT-Food Science and Technology, 42, 581–586.

Premuzic Z., Bargiela M., Garcia A., Rendina A., Iorio A., 1998. Calcium, iron, potassium, phosphorus, and vitamin C content of organic and hydroponic tomatoes. Horticulture Science, 33, 255–257.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical & Biology and Medicine, 26, 1231–1237.

Rembiałkowska E., Hallmann E., Szafirowska A., 2005. Nutritive quality of tomato fruits from organic and conventional cultivation. Culinary Arts and Sciences V. Global and National Perspectives. (ed. J. S. A. Edwards, B. Kowrygo, K. Rejman), 193–202.

Scalbert A., Johnson I.T., Saltmarsh M., 2005. Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr., 81 S, 215–217.

Sun J., Chu Y-F., Wu X., Liu R.H., 2002. Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry, 50, 7449–7454.

Wang S.Y., Chen C., Sciarappa W., Wang C.Y., Camp M.J., 2008. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. Journal of the Agricultural and Food Chemistry, 56, 5788–5794.

Wang H., Cao G., Prior R.L., 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry, 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. Journal of the Agricultural and Food Chemistry, 48, 140–146.

Wojdyło A., Oszmiański J., Rozpara E., 2010. Biological composition, phenolic compounds, and antioxidant capacity of organic and conventional grown raspberry (*Rubus ideaus*). Monografia: The Impact of Organic Production Methods on The Vegetable Product Quality. Edited by E. Rembiałkowska, Warsaw, 245–258.

9

BIOLOGICALLY ACTIVE HISTIDINE DIPEPTIDES – FUNCTIONAL COMPONENT OF POULTRY MEAT

Introduction

Lee and Ho [2002] defined a bioactive component of a diet as any food or part of food which provides medical or health benefits, including treatment of a disease and prevention. Numerous compound of meat are not recognized as nutrients, but carnosine and related compounds have been reported to have bioactive properties under certain conditions [Purchas et al. 2004].

Carnosine (β-alanyl L-histidine) and its related dipeptide anserine are a group of histidine – containing molecules Figure 1. The former presents in the non-protein fraction of skeletal muscle and certain others issue of vertebrates whereas anserine has been reported to be present at millimolar concentration in several mammalian tissues including brain and skeletal muscle [Guiotto et al. 2007]. The carnosine present in the human body come from endogenous and exogenouse sources. The endogenous part is synthesized from the essential amino acids β-alanine and histidine through the action of a specific synthetase carnosinase. Histidine is essential amino acids, whereas β-alanine (a constituent of coenzyme A) is an unusual amino acid absent from proteins [Boldyrev 2007]. The highest activity of carnosine synthetase in mice was observed in olfactory epithelium (495 nmol per g tissue per h), olfactory bulb (90 nmol per g tissue per h), cerebral cortex (11 nmol per g tissue) and cerebellum (7 nmol per g tissue per h). The rate of carnosinese biosynthesis was found to be three to five times higher than that of homocarnosine biosynthesis. Exogenouse carnosine is introduced with the diet , which can introduce an amount of approximately capable of hydrolizyng 50–250 mg/day (whith at least one portion of beef, pork or chicken per day). Intake of red meat cause increased level of carnosine in human blood.

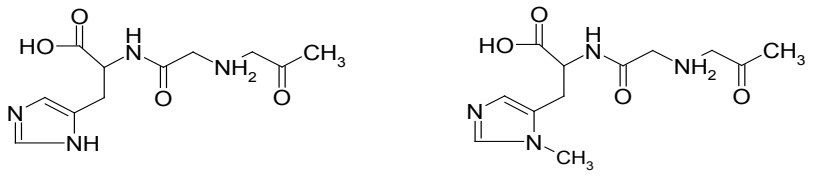

Fig. 1. Structure of carnosine and its natural derivative- anserine

Carnosine concentration in tissues depends on specific features of diet. Histidine deficiency in food causes a decrease in the carnosine concentration in skeletal muscles of rats, whereas a dietary supplements containing only 5% excess of histidine increases carnosine concentration in muscle tissue [Boldyrev 2007]. Normal human muscle contain carnosine

about 4.4 mM per kg raw weight at the age of 14–16 years. Moreover carnosie content in a man is significantly higher than woman. Carnosine level also depends on age and stress. Stuernburg and Kunze [1999] reported that in patients with neuromuscular disease, muscle concentration of carnosine decrease 63% from age 10 to 70.

Experimental data taken all together shows that carnosine undergoes intense metabolic conversion in biological tissues. There are several biological roles in with carnosine parcipitates, including decarboxylation (resulting in histamine formations, which interact with β- alanine leading to carcinine formation), methylation (giving anserine and ophidine) or hydrolysis (resulting in histidine and β-alanine). Human body tissue also contain two types of dipeptydases capable of hydrolazing carnosine. These enzymes differ from one another in activation by metal ions molecular weight, substrate specificity etc. One of these enzymes tissue carnosinse is similar to nonspecific cytosol dipeptidase animal tissue. Neither homocarnosine and anserine were hydrolyzed by this enzyme, also its activity in carnosine hydrolysis in vivo is rather low. The second enzyme is able to hydrolaze carnosine, anserine and homocarnosine. This enzyme is abundant in blood serum where it plays an important role in hydrolysis of dietary carnosine.

For the firs time, the role of histidine – containing dipeptides as a phisological pH buffer was suggested by Bata-Smith in1939 [Boldyrev 2007]. Indeed the amount of dipeptides in tissue with active glycolisys (mainly skeletal muscles was estimated to be as high as to provide up to 60% the total buffer capacity of cells). Because of small size and relatively high mobility dipeptides are superior to proteins, particulary under conditions of hinderd diffusion of protons, which is restricted by limited volume of cytosol. Presence of mobile proton buffering system is of particulary importance for normal function of mitochondrial reticulum an elaborate three- dimensional system of membranes resides extending throughout the entire myofibril and providing the possibility of propagate of transmembrane potential, that is generated by the respiratory chain along the whole length of myocyte [Boldyrev 2007].

Natural histidine-containing dipeptides are regulators of many biological systems. They regulated process of excitation and concentration of skeletal muscles, where the effect of dipeptides is implemented at the level of electrochemical coupling. These effects of carnosine related compounds are indeed observed in case of individual event of muscle concentration they reduce the duration of both the stage of concentration and the stage of relaxation of exhausted skeletal muscle of frog [Boldyrev 2007].

Bioactive constituents of food have been shown to regulate aspects of immune responses. Carnosine is thought to be neuromodulator in the olfactory system, has been shown to inhibit the growth of neoplastic cells and it also known to have effects on vascular smooth muscle tone [Tan and Candlish 1998]. The olfactory receptor neurons abundantly contain carnosine in their perikoria and cell process, inducting the exonal projections to the main and accessort olfactory bulb. These olfactory fibers reach the glomerular layer of the olfactory bulb and contain the dipeptide in their synaptic terminals [Bonfanti et al. 1999].

Carnosine and anserine are thought to have antioxidant effect in preventing membranes lipid peroxidation and stabilizing cell membranes and membrane-bound enzymes. The antioxidant mechanism has been postulated to be due to metal chelation and free radical scavenging. Moreover, recent studies have shown that carnosine can protect proteins against crosslinking mediated by aldehyde-containing sugars and glycolitic intermediates. Carnosine is protective against cellular toxicity and malonealdehyde (MDA)-induced protein damage. Result from Hipkiss A.R et al. [1997] studies showed that carnosine can protected cultured rat brain endothelial cells against MDA-induced toxicity protein modification. In addition carnosine can reduced certain proteolytic reactions associated with cell ageing [Hipkiss et al. 2002] and that way can be considered as anti ageing agent.

Materials and method

The aim of this research was to determine the content of carnosine and anserine in the tissue from different poultry animal species i.e. chicken and turkey.

Basic chemical compounds were analyzed according to following methods: dry matter -PN-ISO 1442:2000, the protein by Kjeldahl-method (984.13) using Kjeltec 2300 Foss Tecator apparatus (Sweden), by multiplying of N-content by 6.25, crude fat PN-ISO 1444:2000.

Samples for dipeptides (carnosine/anserine content) determination were prepared as described by Aristoy and Toldra [2004], except ratio of muscle to redistilled water, (1:1) and use of the Mixer B-400 homogeniser (Buchi Switzerland). The homogenate was centrifuged at 10,000 x g and the supernatant was deproteinized by adding 3 vol of methanol (HPLC grade). Then, the samples were centrifuged (12,000x g). Dipeptides were quantified by HPLC method after derivatization with O-phthalaldehyde (OPA pre-column derivation method) (Fig. 1).

The chromatographic separations were run on a HPLC 1100 Series (Agilent Technologies) coupled to a fluorescence detector. Samples were analysed using C18 column (Agilent 150x4,6, 3 μm particle size) at a 200 μl/min flow rate. Mobile phases consisted of solvent A, containing 40 mM Na_2HPO4, (pH 7.8), in water and solvent B, containing acetonitryle ACN: methanol MeOH: water (45:45:10). The injection volume was 40μl.

All obtained data were evaluated statistically by one- factorial ANOVA using StatSoft Statistica® Software [2009].

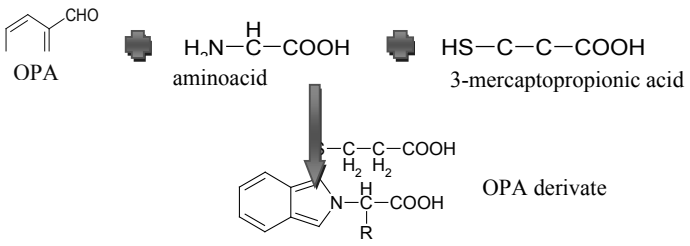

Fig. 2. The pre-column derivatiztion

Results and discussion

The Table 1 presents the results of basic chemical components analyses of different poultry sources. Type of raw material significantly influenced the content dry matter, protein and fat. In low quality sources (MDM-mechanically deboned meat) the highest dry matter and fat contents were observed. Fat content in all analyzed sources was from 0,64% in turkey breast to 27,30% in mechanically deboned meat from spent hen. The highest protein content was observed in turkey breast muscle tissue (24,38%) whereas the lowest value was noticed in mechanically deboned meat from spent hen (6,75%). The highest amount of dry matter was noticed mechanically deboned meat from spent hen.

Table 1

Chemical component in raw meat obtained from different kind of poultry sources

Meat sample	Dry matter [%]	Protein [%]	Fat [%]
Turkey breast	24.38 a	24.66 g	0.64 a
Turkey wing	25.92 ab	23.63 f	1.03 a
Chicken breast	22.84 ab	22.21 e	0.95 a
Breast from hen spent	28.04 b	23.26 f	1.10 a
Brest from meat type hen	24.03 c	22.51 g	0.99 a
MDM from turkey	34.64 e	15.39 d	17.81 b
MDM	40.81 f	6.75 a	27.30 d
MDM from meat type hen	30.32 d	10.8 c	11.75 b
Frame from spent hen	33.78 e	9.70 b	20.69 c

Carnosine and anserine concentrations in raw poultry meat are shown in Table 2. Both dipeptides were determined in the homogenates on the basis of the preliminary separation study. Muscle from turkey breast were characterized by higher anserine content then muscle from chicken breast. Also the sum of dipeptides was the highest in turkey meat (5.22 mg/g). The similar concentration of both dipeptides we can noticed in turkey wing. Whereas sum of carnosine and anserine in mechanically deboned turkey meat was twofold lower.

In chicken breast muscle we can observed the twofold lower level of carnosine carnosine whereas anserine concentration was similar.

The low quality sources such as breast muscle from spent hen and meat type hens were characterized by two times lower concentration of both dipeptides. Level of anserine was slightly lower.

Mechanically deboned poultry meat from spent hens and meat type hens was characterized by the lowest concentration of both dipeptides. Sum of anserine and carnosine was about 2 mg/g tissue.

The frame from spent hen was the worst sources of histidine dipeptides. The sum of both dipeptides amounted 1,5 mg/g.

Table 2

Carnosine and anserine contents in raw meat obtained from different kind of poultry sources

Meat sample	Carnosine [mg/g]	Anserine [mg/g]	Sum of carnosine and anserine [mg/g]
Turkey breast	1.62 d	3.60 h	5.22 g
Turkey wing	1.60 d	3.13 f	4.73 f
Chicken breast	0.94 ab	3.32 g	4.26 e
Breast from hen spent	1.36 c	2.04 e	3.40 d
Brest from meat type hen	1.28 c	2.13 e	3.41 d
MDM from turkey	1.67 d	1.78 d	3.46 d
MDM from spent hen	1.08 b	0.72 b	1.80 b
MDM from meat type hen	1.06 ab	0.91 c	1.97 c
Frame from spent hen	0.9 a	0.59 a	1.49 a

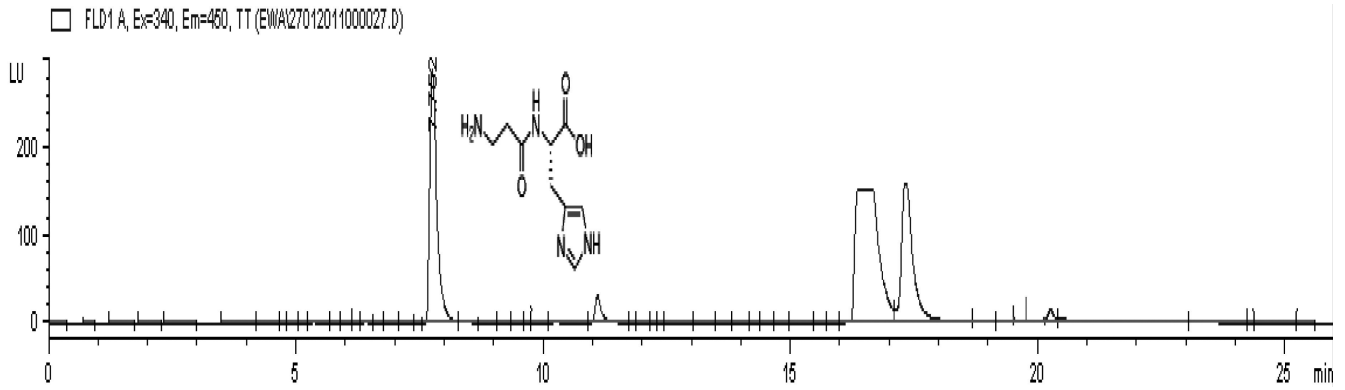

Fig. 3. Chromatogram of carnosine standard

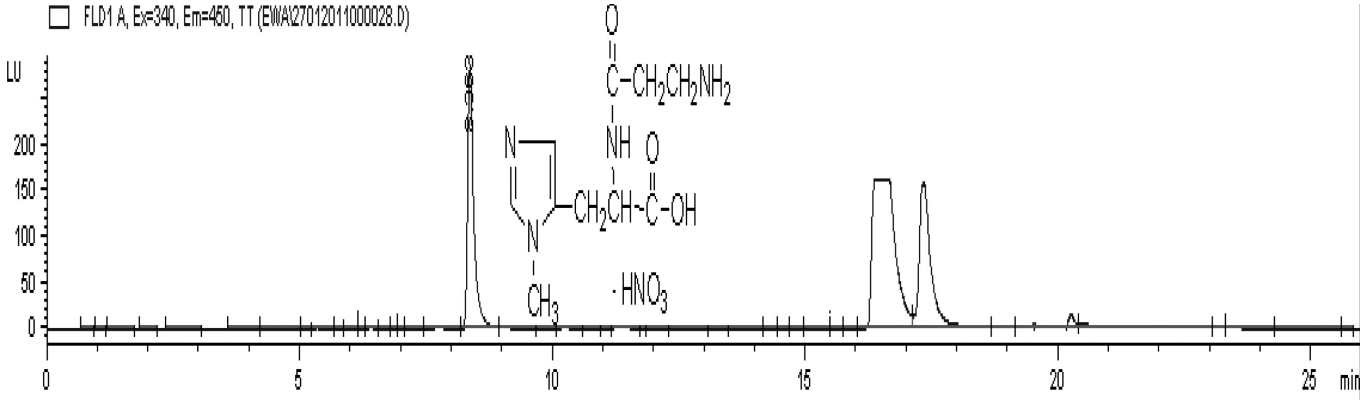

Fig. 4. Chromatogram of anserine standard

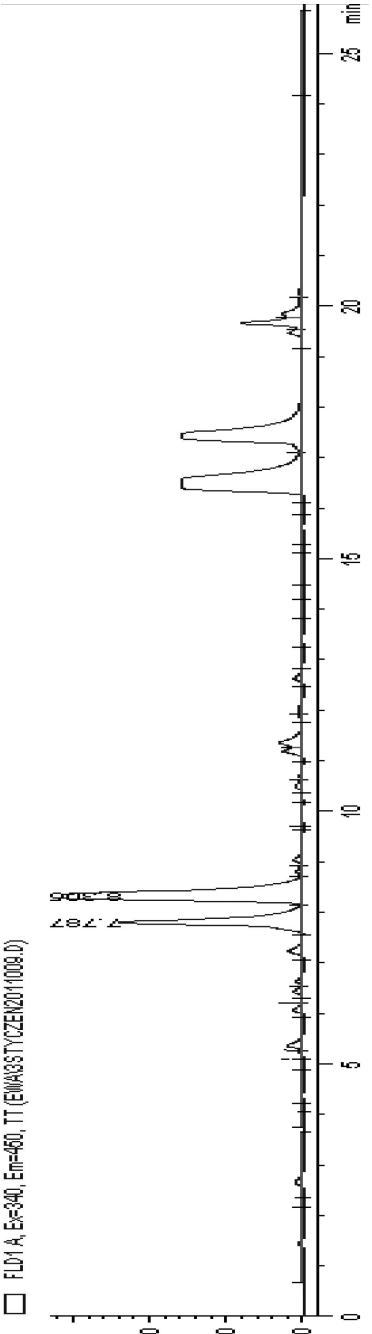

Fig. 5. Chromatogram of turkey meat sample

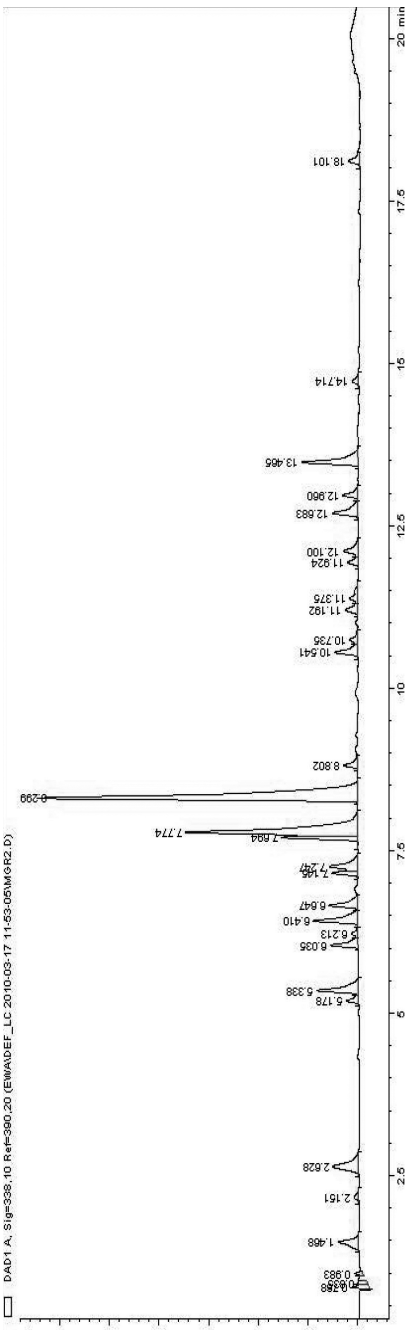

Fig. 6. Chromatogram of chicken meat sample

In own study we can observed the similar trend for the dipeptide content to the Aristoy and Toldra [2004] and Peiretii at al. [2011]. The content of carnosine is higher in beef and pork meat, while in turkey meat lower level was observed. In contrast higher anserine content levels were found in poultry meat.

Results from Peiretii et al. [2011] studies showed that anserine in the turkey breast was about 6 times more abounded than carnosine. In own study we can observed only 2.5 times higher level of carnosine in turkey breast muscle.

The carnosine contain in animals tissue differ according to sex and genotype [Intrapichet and Maikhutand 2005]. Moreover with increasing age the carnosine concentration may be reduced [Stuerburg and Kunze 1999], hence lower level of dipeptides in meat from spent hens.

In addition Hu et al. [2009] suggested that dietary carnosine improves chicken meat quantity and quality and it could be candidate for feed additive as a growth promoter.

Conclusions

1. Turkey meat is the richest source of bioactive histidine dipeptides occurring in tissue.
2. Low quality sources contain twofold lower content histidine dipeptides than its content in muscle
3. Anserine is the main dipeptide in muscle tissue of poultry meat whereas in trunk and mechanically deboned meat from hens carnosine dominates

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References

Aristoy M.C., Toldra F., 2004. Histidine dipeptides HPLC-based test for the detection of mammalian origin proteins in feeds for ruminants. Meat Science, 67, 211–217.

Boldyrev A.A., 2007. Carnosine and Oxidative Stress in Cells and Tissues. Nova Science Publishers.

Bonfati L., Paretto P., De Marchis S., Fasolo A., 1999. Carnosine –related dipeptides in the mammalian brain. Progress in Neurobiology, 59, 333–353.

D'Arrigo P., Kanerva L.T., Li X.G., Saraceno C., Servi S., Tessaro D., 2009. Enzymatic synthesis of carnosine derivatives catalysed by Burkholderia cepacia lipase. Tetrahedron:Asymetry, 20, 1641–1645.

Fu H., Katsumura Y., Lin M., Muroya Y., Hata K., Fujii K., Yokoya A., Hatano Y., (2009) Free radical scavenging and readioprocetive effes of carnosine and amserine. Radiation Physics and Chemistry 78, 1192–1197.

Guiotto A., Calderan A., Ruzza P., Borin G., 2005. Carnosine and carnosine-related antioxidants: a review, Curr. Med. Chem., 12, 2293—2315.

Hipkiss A.R., Preston J.E., Himswoth D.T.M., Worthington V.C., Abbot N.J., 1997. Procetive effect of carnosine against malondialdehyde- induced toxicity towards cultured rat brain endothelial cells. Neuroscience Letters 238, 135–138.

Hipkiss A.R., Brownson C., Bertani M.F., Ruiz E., Ferro A., 2002. Reaction of carnosine with aged proteins. Another protective process?", Annals of the New York Academy of Science, 959, 285–294.

Hollidy R., McFarland G.A., 1996. Inhibition of the growth of transformed and neoplastic cells by the dipeptide carnosie. British Journal of Cancer, 73, 966–971.

Hu X., Hongtrakul K., Ji C., Ma Q., Guan S., Song C., Zhang Y., Zhao L., 2009. Effect of carnosine on growth performance, carcass characteristics, meat quality and oxidative stability in broiler chickens. Journal of Poultry Science, 46, 4, 296–302.

Intraoichet K.O., Maikhuntand B., 2005. Genotype and gender differences in carnosine extracts and antioxidant activities of chicken breast and thigh meats. Meat Sciences, 71, 634–642.

Lee T.C., Ho C.T., 2002. Bioactive compounds in foods. Effects of processing and storage. American Chemical Society, Symposium Series, 816.

Nardiello D., Cataldi T.R.J., 2004. Determination of carnosine in feed and meat by high-performance anion-exchanged chromatography with integrated pulsed amperometric detection. Journal of Chromatography, 1035, 285–289.

Peiretti P.G., Medana C., Visentin S., Giancotti V., Zunino V., Meineri G., 2011. Determination of carnosine, anserine, homocarnosine, pentosidine and thiobarbituric acid reactive substances contents in meat from different animal species. Food Chemistry, 126, 1939–1947.

Purchas R.W., Rutherfurd S.M., Pearce P.D., Vather R., Wilkinson B.H.P., 2004. Concentrations in beef and lamb of taurine, carnosine, coenzyme Q10, and creatine. Meat Science, 66, 629–537.

Sanchez-Escalate A., Djenane D., Torrescanol G., Gimezez B., Beltran J.A, Roncales P., 2003. Evaluation of the antioxidane ability of hydrazine-purified and untreated commercial carnosine in beef patties. Meat Science, 64, 59–67.

Stuerenburg H.J., Kunze K., 1999. Concentrations of free carnosine (a putative membrane-protective antioxidant) in human muscle Biopsies and rat muscles. Archives of Gerontology and Geriatrics, 29, 107–113.

Tan K.M.L, Canslish J.K., 1998. Carnosine and anserine as modulators of neurophil function . Clin Lab Haem, 20, 239–244.

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