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THE ROLE OF THE EUROPEAN FEED INDUSTRY ON A SAFE AND SUSTAINABLE FEED AND FOOD PRODUCTION

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Abstract. The European feed industry produces about 140 million ton compound feed each year. Most of the feed is composed by feed ingredients imported from countries all over the world. The safety of this feed ingredients is a serious threat for the safety of food of animal origine.

Key words: feed legislation, feed production, feed safety

INTRODUCTION

The European feed industry produces about 140 million ton compound feed each year. Most of the feed is composed by feed ingredients imported from countries all over the world. The safety of this feed ingredients is a serious threat for the safety of food of animal origine. Some incidents in the past, like BSE and contamination of feed ingredients with dioxine, mycotoxins, nitrofen, heavy metals, pharmaceutical waste (mpa), a.s.o., had a clear political impact on the EU-regulations and ended up in the new General Food Law of the European Union who is in place since 1 January 2004. The European Parlament adapted in the framework of this GFL the Feed and Food Hygiene Regulation coming in place from 1 January 2006 onwards. Responsibility for food safety for all partners in the feed and food chain is the bases of this regulation. All operations should apply the HACCP principles to define there production process. Transparency and traceability are required to inform the competent authorities about the product streams.

Above all, traceability in the feed chain is FEFAC's aim to ensure the safety of feeds for the benefit of consumers (of products of animal origin), animals and the environment.

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The key to success: a feed legislation embracing the overall feed chain

The General Food Law Regulation (EC) No 178/200 together with the regulations on Feed Hygiene and the regulation on Feed and Food official controls are the main pillars for developing a feed legislation embracing the overall feed (and food) chain and ensuring adequate traceability systems to ensure feed safety. The General Food Law Regulation mirrors the farm to table approach („feed is for food") and manifests that the responsibility for the safety of food & feed products rests with feed and/or food business operators. Furthermore, it establishes traceability systems to be in place from 1 January 2006 on, recall procedures and notification to public authorities.

In the General Food law, traceability „means the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution" (Art. 3).

In the daily feed industry quality management, traceability plays an essential role and is realised via record keeping i.e. documentation and registration. For more than 15 years, EU feed compound feed producers developed individual traceability systems in accordance with the requirements of international quality assurance standards such as ISO 9000.

The EU Directive on the approval and registration of feed establishment (Directive 95/69/EC) already includes provisions regarding traceability. The new legal framework mirroring the „stable to table" approach intends to further develop the traceability systems in the feed chain with a view to embracing all operators in the chain. From a professional angle, traceability is the backbone of codes of good practice and also subject to FEFAC harmonising efforts to ensure the most efficient and transparent quality assurance system.

The Implementation of traceability is defined as follows: „2. (Food and) feed business operators shall be able to identify any person from whom they have been supplied with […] a feed or any substance intended to be, or expected to be, incorporated into a […] feed. 3. (Food and) feed business operators shall have in place systems and procedures to identify the other businesses to which their products have been supplied" (Art. 18). In other words, the scope of traceability is meant to cover one step back, one step forward.

The new proposal on Feed hygiene, adopted in April 2003, now represents the „missing link" for a feed legislation covering the whole feed chain, replacing Directive 95/69/EC on the approval of feed establishments and laying down, a.o. the general legal framework on traceability in the feed chain. The proposal mainly mirrors the food hygiene proposal and applies to the production of feed at all stages, including primary production.

Furthermore, mandatory registration of all feed businesses (primary producers, feed material suppliers (incl. food businesses), traders, compound feed manufacturers, livestock holders) is requested by competent authorities. In contrast to the food hygiene proposal, also a financial guarantee is foreseen to cover the costs of risks linked to feed business operations. The development of voluntary codes of practices both nationally and at EU level is encouraged, which may be subject to standardisation.

Once adopted, the new feed hygiene Regulation will then become the future legal reference of the FEFAC Action plan for feed safety.

HACCP – based safety assurance system

Feed business operators – other than primary producers – have to implement an HACCP system to ensure feed safety and operators must respect the hygiene requirements defined in the Annexes to the Regulation.

The HACCP – system includes a description of the process diagram of the production process. This does mean, that each individual company in the feed production chain has to describe the different steps of production from start to finish. In the compound feed industry this process diagram has to be described in the handbook of the company from the moment the recipe is draw up until the moment of distribution of the feed to the stable. That does mean, that for each badge the recipe, the purchasing and transport of feed ingredients and auxiliaries, the reception and storage after reception, the dosage, grinding and mixing, the pressing, conditioning, pelletting and cooling, the storage and delivery has to be described. The badge has to be identified and the registration of the delivery to the individual farms is mandatory. Based on a risk analyses critical control points in the process diagram for individual badges should be defined and monitored. The selection of this critical control points should be based on risk, probability of occurrence and severity of the risk. Depending on the category of the critical control point analytic results of the monitoring should be available before the start, before finalising the production or as control monitoring backwards.

The HACCP module in the feed mills forces the individual feed companies to discovery and register the critical control points in there production process and to monitor the process on critical risk factors.

For this reason f.i. in the Dutch Good Manufacturing Practice System (GMP+) regular monitoring does take place on Salmonella incidence. The results of this monitoring in animal feed is presented in table 1.

Table 1. Monitoring of Salmonella incidence in animal feed in 2003, 2004 and 2006 (internal PDV-report, June 2007)
Tabela 1. Monitoring występowania Salmonelli w paszach zwierzęcych w latach 2003, 2004 i 2006 (wewnętrzny PDV raport, czerwiec 2007)

	2003		2004		2006	
Feed	N	% pos.	N	% pos.	N	% pos.
Cattle	1375	0.7	2188	0.4	2438	0.3
Pigs	2857	0.8	3048	0.6	2917	0.3
Breeding	413	0.0	298	0.0	486	0.0
Multipliers	916	0.5	789	0.5	952	0.1
Broilers	1818	0.4	2753	0.3	2158	0.1
Laying hens	2262	0.4	3037	1.0	3001	0.7
Turkeys	324	0.0	145	0.7	258	0.0
Feed Ingredient	14903	3.6	19361	4.7	16359	1.7

Salmonella typing from the positive samples in 2006 resulted in the following findings:
Poultry: *3x Senftenberg; 2x Mbandaka; 5x Havana; 1x Heidelberg; 2x Parath.B.Java*; 1x Lexington; 1x Anatum 1x Enterica spp; 1x Bredeney;* 5x unknown
Cattle: *2x Infantis*; 3x Enterica spp; 1x Lexington*; 2x unknown
Pigs: *2x Senftenberg; 1x Angona*;1x Tenessee; 2x Livingstone; 1x Rissen ; 1x Anatum; 1x Corvallis;* 1x unknown

*The * marked Salmonella types are considered by the EU as risk factor for human health*

Compound Feed Producers response: The development of an European Feed Manufacturers Code (EFMC)

Many years ago, FEFAC decided to establish an action plan to develop a European quality assurance programme for animal feed. The aim was and is to evaluate and harmonise existing National Quality Assurance Systems and to support the development of this systems in other EU-countries.

In 1998, FEFAC drew up the first FEFAC guidelines for the implementation of national codes of practices for manufacturing of compound feed. They contained a set of principles covering the sourcing of quality feed materials, production, storage, transport and delivery of quality feed as well as the use of additives and veterinary medicinal substances in feed. One main requirement is also the record keeping ensuring an adequate traceability system.

In April 2001, the FEFAC guidelines were reviewed and the undertaking of a risk analysis based on HACCP principles, an authorisation procedure for the purchase of feed materials and a contingency procedure in case of contamination were introduced as new requirements to ensure feed safety.

Stepwise development of the European Feed Manufacturers Code

In 2002, the FEFAC guidelines have been further developed towards a «FEFAC benchmark standard». This document has been used to perform an independent benchmarking of each existing national Code of Good Practices for feed manufacturing with the aim to get an overview of the status of European Quality Assurance in the

compound feed sector, with a view to facilitate mutual recognition and convergence of national schemes. One main pillar of the benchmarking study was the demand for a full traceability system including a detailed record keeping procedure as a Quality management requirement. The benchmarking study was carried out by the independent certification body SGS at the end of 2002 and its results presented in February 2003. The outcome of the benchmarking study showed that there were too many codes and too many certification systems, little consistency and limited mutual recognition between schemes. Therefore, in order to reach more convergence of national codes of practice for compound feed manufacturing and to reach out to the whole supply chain, FEFAC has started the second stage of its Benchmarking project.

The second step of the FEFAC Benchmarking project embraces two objectives.

First priority was to establish a European Feed Manufacturers Code(EFMC), which covers all feed safety issues and also aims at harmonisation of independent (accredited) certification processes inside the feed production plants. However, national Codes will continue to exist to address specific national quality requirements or contractual provisions. The finalisation of the EFMC including an International Feed Material Standard (see B) did take place in September 2004 and was officially approved by the FEFAC Council in October 2004. The next step is to implement the EFMC in the national codes. This was finalised by the end of 2005 by independent approval of the national code. FEFAC did present the EFMC to the European Commission for endorsement in the scope of art. 22a of the Feed Hygiene Regulations. Finally in February 2007 the Standing Committee of the EU approved the EFMC as a guideline for the feed industry meeting all the requirements included in the EU-regulations.

Second part of the benchmarking project is the parallel development of an International Feed Ingredient Standard together with International Rules of Certification. This development does take place in the International Feed Safety Alliance (IFSA) where FEFAC cooperates with 4 standard owners for feed ingredients. The aim is to develop a basic harmonised core standard for feed materials and feed additives, which then could be completed step by step through sector notes with specific criteria for products and product categories. The final draft for the IFSA- Feed Ingredient Standard and the IFSA- Rules of Certification have been finished on 1 September 2005 and presented to the national, European and International supplier organisations and companies by putting them on the FEFAC-website.(www.fefac.org).

Sustainability: An increasing consumers concern

Sustainability is widely defined as meeting the needs of today without jeopardising the ability to future generations to meet their needs.

In the Millenium Development Goals (MDG's) agreed at the UN-Millenium Summit in 2000 concrete targets are set to avoid to continue to tax the earth ecological systems in favour of food, feed and energy demands of the global population. The MDG's remains vague about what sustainability really means.

Sustainability is very often linked to the 3 P's: People, Planet and Profit, expressing, that in the social corporate responsibility we have to take care for a balance between the interest of the environment, the social aspects and the economy.

Since 2000 Governmental and Non Governmental Organisations, like Greenpeace, Solidaridad, WWF etc., are more and more focusing on the sustainability aspects in the production of feed and food. The feed industry became very intensively involved in the

sustainability issue in relation to the soybean production in the Amazone region in Latin America.

Soybeans are used in the production of, foods, cosmetics and feed for cattle, pigs, poultry fish and bio-fuels. One of the important protein sources for the feed industry is the soybean meal, what is produced as by-product in the soy oil production. In Europe, soy traders and retailers have recently been under attack for buying soy that is fuelling deforestation in places such as the Amazon and the Cerrado (wooded savannahs) in South America. Some landuse-changes are occurring in Argentina, Uruguay, Southern Brazil and Southern Paraguay. Likewise, in some cases, the soy sector there has been criticized for illegal appropriation of public lands and displacement of small-farmers and indigenous peoples, while at the same time not keeping the capital investments made, in the regions where soybean is grown.

Round Table on Responsible Soy

The Roundtable on Responsible Soy (RTRS) was set up in 2004 by a group of organizations and companies committed to the responsible production, processing, trading, and use of soy. These institutions established an Organizing Committee (OC), with a view to leading the RTRS through its initial stages until a formal institutional framework had be put in place.

The main objective of Roundtable is to build a global participatory process that promotes economically viable, socially equitable and environmentally sustainable production of soy. FEFAC fully shares these objectives and will actively contribute to develop a common charter containing criteria and recommendations to the soy chain partners for responsible soy production, processing and trading.

The RTRS provides all stakeholders and interested parties with the opportunity to jointly develop global solutions leading to more responsible soy production worldwide. At the current time, the main deliverable of the RTRS is to develop a document that sets out principles, criteria, and indicators for the production. and sourcing of responsible soy. The principles and criteria will provide a market mechanism to address key negative environmental and social impacts of soy production and its expansion.

The objectives of the RTRS are to facilitate a global dialogue on responsible soy that:

* is a forum for reaching consensus on the key social and environmental impacts of soy among the various stakeholders;
* communicates issues regarding responsible soy production, processing and trading to a wide range of global stakeholders;
* develops and promotes criteria for responsible soy production, processing and trading. Mobilizes participants to the multi-stakeholder process;
* organizes Roundtable conferences and technical workshops;
* and acts as a recognized international body monitoring the status of responsible soy production, processing and trading.

The Board of the RTRS is composed by 5 representatives of the producers, 5 representatives of the trade, industry and finances and 5 representatives from the NGO's.

The RTRS recognises 9 principles as the starting point for the development of criteria, indicators and certification:

- Impact of infrastructure
- Compliance with Labor laws and requirements

- Respect of land rights
- Respect for small scale and traditional land use
- Rural communities and migration
- Water as a key resource
- Soil as a key resource
- Protection of biodiversity
- Responsible use of agrochemicals

Opinions on the benefits and risks of biotechnology and GMO's vary greatly. Therefore RTRS will not promote the production, processing or trading of either genetically modified or non-genetically modified soy.

Genetically Modified Organisms (GMO's) in Animal Feed

The feed industry recognises the potential benefits of the GM-biotechnology. GM-biotechnology can realise increased production, lesser use of agrochemicals and development of functional feed- and food products. Until now only by-products of genetically modified products are used to produce animal feed. At the other side risks can occur also. Therefore a extended scientific risk analyses by independent authorities of each new product is necessary before approval. An intensive communication program is needed to improve public information and acceptance.

In spite of a careful authorisation procedure consumers in Europe are very hesitating about GM-products. This has cultivated by some NGO's who suggest clear negative impact of the use of GM-products in the feed and food.

Due to the BSE-crisis the use of animal proteins for farm animal is fully banned in Europe since January 2000. Alternative sources for protein-rich animal feedstuffs are available through soybean meal, corn gluten feed (CGF) and distillers dried grains (DDG). CGF and DDG are excellent protein –rich sources for cattle feeding. Soybean and soybean meal are important protein sources for the rest of the livestock. The EU is heavily depended on imports of these feeding stuffs. Due to the EU-climatic and agronomic conditions the selfsufficient rate of this protein-rich products is only 23%. The import of CGF in 2005 was 2.5 million tons, of DDG 0.72 mln tons and of soybean meal 33,6 mln tons (including imported soybeans). The EU-import of this soybean meals origins to nearly 100% from the USA (2,6 ml. ton), Brazil (17.2 mln ton) and Argentina (12.0 mln ton). Maize and maize products consist of 20% of GM-origin and the soybean products consist of 90% of GM origin.

The EU doesn't accept authorisation of GM-products by non-EU countries. Every new GM event has to be analysed by the European Food Safety Authority (EFSA) on safety for health and environment. Finally the European government has to approve the event for entrance on the European market.

There are 3 different thresholds in the control on GMO's in the EU:

- Non-EU authorised products: 0% (zero-tolerance)
- Products under EU-approval procedure: 0.5%
- EU authorised products: 0.9%

Based on the Regulations (EC) No. 1829/2003 and 1830/2003 feed operators are obliged to label all feed produced from GMO's as soon as they pass the threshold. Transparency and traceability is required in all stages of placing in the market.

Although the industry was already faced in the past with the negative trade impact on feed material supply as a result of the growing gap between worldwide approvals

of GM plants and EU approvals, the EU has so far been unable to come forward with a practical proposal to allow trade to continue. As a result we have almost lost the entire supply of Corn gluten feed. Unless a „miracle“ happens over the next two years we may risk losing the vital supply of soybean meal from South America, should the second generation of GM soybean events be authorised and grown in key export markets before their approval in the EU.

CONCLUSIONS

The European Feed Industry produces 142 million tons of compound feed yearly to produce food of animal origin. Some feed incidents in the past like the MPA – crisis focused the activities of the feed industry towards the assurance of feed safety. Implementation of HACCP in the feed mills and monitoring the production process created clear information about the risks and the opportunities to minimize the feed safety risks. Salmonella monitoring for instance demonstrates this opportunity very clear.

Feed safety is an essential part of food safety. Feed and food business operators have to recognise their shared responsibility and overcome mistrust among partners in the chain. In the feed sector, there is an ongoing implementation of codes of practice but still a lack of harmonisation of traceability systems also. Therefore, FEFAC has developed the European Feed Manufacturers Code linked with an International Feed Ingredient Standard to ensure feed safety, for which an effective traceability system tracing back the Quality Assurance systems all along the feed chain is crucial.

In the last years more attention is given on sustainability. The feed industry is heavily involved in the sustainability of soy production. The objective of the RTRS initiative is to achieve a certification system for the global soy production to assure responsible soy production.

As soon as Genetically Modified (GM)-products are authorised by European governmental body's safety is not longer an issue in the use of GM-products for compound feed production. Labelling of GM from feed to food should assure a traceable and transparent tool to inform the consumer about the GM status of the food.

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Once adopted, Feed Hygiene Regulation became the legal reference of the FEFAC Action plan for feed safety.

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DZIAŁANIE EUROPEJSKIEGO PRZEMYSŁU PASZOWEGO NA ZAPEWNIENIE BEZPIECZEŃSTWA PRODUKCJI PASZ I ŻYWNOŚCI

Streszczenie. Europejski Przemysł Paszowy produkuje rocznie ok. 140 mln ton komponentów paszowych. Większość z mieszanek paszowych jest wytwarzana ze składników importowanych z całego świata. Właściwa jakość tych składników paszowych stanowi poważne zagrożenie dla bezpieczeństwa żywności pochodzenia zwierzęcego.

Słowa kluczowe: przepisy, produkcja pasz, bezpieczeństwo żywności

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SAFETY AND QUALITY OF MILK PRODUCTS FROM GOAT'S FARM IN CZECH REPUBLIC*

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Abstract. The composition and selected physical and technological properties of raw White-Shorthaired Goat milk and fresh goat cheeses were monitored. Milk and cheeses samples coming from a goat farm which situated in the Czech Republic were collected in the course of the lactation period. Milk composition and properties were monitored in close relationship to the lactation stage, season, nutrition and method of breeding. The following average values have been found: protein content 27.80 ± 2.30 $g.l^{-1}$, fat content 30.60 ± 3.1 $g.l^{-1}$, lactose content 45.20 ± 0.40 $g.l^{-1}$, non-fat solids content 78.40 ± 2.20 $g.l^{-1}$, titrable acidity 5.54 ± 0.86 °SH, rennetability 93.33 ± 14.76 s, freezing point – 0.550 ± 0.004°C and somatic cell count 1875 ± 476.$10.10^3.ml^{-1}$. The following average values of fresh goat cheeses were obtained: pH 4.87 ± 0.14, titratable acidity 98.09 ± 4.93 °SH, solid 46.83 ± 1.57%, fat in solid 52.74 ± 5.24%, NaCl 2.08 ± 0.54%, a_w 0.979 ± 0.007. All samples were evaluated as excellent from the senzoric point of viev and their composition corresponded with the composition declared by producer. Results were use for calibration of the spektrometer FT NIR Nicolet Antaris (Thermo electron Corporation, Madison, USA).

Key words: goat milk, goat cheese, physical and chemical parameters, milk composition, milk properties

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INTRODUCTION

Goats are bred worldwide and therefore there is a great variance in its composition. The White Shorthaired breed is the most widely spread breed in the Czech Republic. Goat milk is a nutritious, healthy and easily digestible foodstuff. Raw goat milk tastes almost identically as cow milk. The nutritious value and the dry matter content are also virtually identical with cow milk (Späth and Thume 1996) although Grieger and Holec (1990) present it to be higher in fat, protein and non-protein nitrogenous compound contents as opposed to cow milk. Dostálová (2004) presents goat milk to be slightly richer in lipids. The most frequent use for goat milk is its processing for cheese production. Goat cheese brands, classified as fresh non-ripening cheese (Decree No. 77/2003), belong to the most commonly produced goat milk cheese brands (Fantová 2000).

MATERIALS AND METHODS

Milk and cheeses samples were collected at farm in the South Moravian Region of the Czech Republic where White Shorthaired goats are reared. 75 goats were kept at their 1st and 8th lactations, the average milk yield was 2–3 litres of milk, and average yearly yield was 600–800 litres of milk. In the period between mid May and mid November, goats had access to pasture, 0.5 kg of hay was added and a maximum of 1 kg of grain cereals, vitamin and mineral mixture and a salt block for licking. In the winter, the feed ration contained 3 kg of grass haylage, 1 kg of beet silage, 1 kg of hay and a maximum of grain cereals, vitamin and mineral mixture and salt for licking. Milking was machine operated twice a day. Milk sampling was carried out after the weaning of the kids in the period between the end of April and the beginning of November 2006 in regular time intervals. Altogether, a total of 48 tank raw milk samples were collected. Milk composition was determined using the method detailed in the Czech National Standard No. ČSN 570536/1999 – Determination of milk composition using an infrared absorption spectrophotometer. Our machine was Bentley 2500 (Bentley Instruments, Minnesota, USA). The somatic cell count was determined by the flurooptoelectronic method as detailed in the ČSN EN ISO 133366-3/1998. Freezing point was determined as detailed in the ČSN 57 0538 (1998) on a Milk cryoscope 4D2 machine, by Advanced Instruments, Inc., USA.

Soxhlet-Henkel method of titrable acidity was used and non-fat solids were determined as detailed in the ČSN 570530 (1972). Rennetability was determined using the Černá and Cvak method (1986). Statistical assessment was carried out with the Unistat software (1998).

Selected parameters were monitored in 22 samples of natural non-ripening goat cheese brands (Fresh Goat Cheese: average dry matter content 48%, average fat content in dry matter 40%, vacuum packed, shelf life of 8 weeks).

Samples were taken twice a month over a period of four months in 2006. Sensory evaluation has been done and selected physical and chemical parameters have been established – active acidity, titration acidity, fat content, dry matter contents, fat in dry matter content, sodium chloride and water activity. Cheese analyses were done at the Department of Milk Hygiene and Technology of the UVPS in Brno was evaluated based on the applicable Czech Standard ISO norms. pH was evaluated based on ČSN 57 0107: 1966 entitled entitled Methods of evaluating cheese, curdle, mousse and spread brands.

Titration acidity was evaluated based on ČSN 0107: 1966 entitled Methods of evaluating cheese, curdle, mousse and spread brands. Dry matter was evaluated based on ČSN 0107 Section 3: 1982 entitled Methods of evaluating natural cheese and cheese spreads. Fat was evaluated using the butyric acid method based on ČSN 0107: 1966 entitled Methods of evaluating cheese, curdle, mousse and spread brands. Sodium chloride was evaluated based on ČSN 0107 Section 12: 1982 entitled Methods of evaluating natural cheese and cheese spreads. Water activity was evaluated based on ČSN ISO 21807 (56 0627) Microbiology of food and feedstuffs – Water activity determination. The results were processed in a statistics application Stat plus (Matoušková et al. 1992).

The aim of this study was determinate the composition and chosen physical parametres of 22. symplex fresh goat cheeses (Goat fresh cheese: solids content on the average 48%, fat in soil on the average 40%, vacual packaged, durability 8 for weeks).

Exhibits was withdawing 2 x monthly during the lactation period, was classification sensorial and was fixed term choice physical and chemical characteristics – active sourness, titracni sourness, content oil, dry matter, oil in dry matter, sodium chloride and activity waters. Investigation cheese was fulfilment on constitution hygiene and technology milks VFU Brno according to valid CSN ISO specification. pH according to CSN 57 0107: 1966 method checking cheese, curd, cream and spread. Titracni sourness according to CSN 0107: 1966 method checking cheese, curd, cream and spread. Susinu according to CSN 0107 part 3: 1982 method checking natural cheese and molten cheese. Oil acidobutyrometrickou method according to CSN 0107: 1966 method checking cheese, curd, cream and spread. Sodium chloride according to CSN 0107 part 12: 1982 method checking natural cheese and molten cheese. Water activities according to CSN ISO 21807 (56 0627) Mikrobiologie groceries and pasturage – assesment water activities. Record was evaluated in statistical programme Stat plus (Matouskova et al. 1992). Was following choice characteristics in 22 figure natural nezrajicich goaty cheese (goaty cheese green).

RESULTS AND DISCUSSION

An overview of the average values in the milk composition at the Farm A is presented in the Table 1. Average protein content was 27.80 ± 2.30 $g.l^{-1}$ %. The lowest protein content of 23.80 $g.l^{-1}$ was detected in Month 4; the following months brought an increase on the protein content with a slight fluctuation in the values in the summer months. The highest protein content of 31.50 $g.l^{-1}$ was determined at the end of the lactation. Protein content levels were influenced by the lactation stage and nutrition but especially by the transfer from the summer to the winter feed and vice versa. Kuchtík and Sedláčková (2003) published that protein content was in course of monitoring between Day 35 to Day 163 of the lactation relatively balanced but in the following period, a gradual increase in the content set in, lasting until the end of monitoring. Similar protein content results are reported also by Boroš et al. (1985).

The average value of fat content was 30.60 ± 3.10, $g.l^{-1}$ which is a value considerably lower that the value of 34.00 $g.l^{-1}$ which Späth and Thume report (1996). Fat content was at the beginning of the lactation at 31.60 $g.l^{-1}$ and in Month 5 after the transfer to the summer feed, a decrease to the value of 28.40 $g.l^{-1}$ was detected. In the following months, with the exception of extremely warm Month 7, there was a gradual fat content increase onto the value of 35.70 $g.l^{-1}$ in Month 10. After the shift to the winter feed, the

value of fat content in milk was determined to be at 34.70 g.l^{-1}. Growing fat content in the course of the lactation is reported by Prasad and Sengar as well (2002). On the contrary, Antunac (2001b) witnessed a decrease in fat content from Day 50 to Day 100 of the lactation but after Day 150 of the lactation, its increase set in again.

Table 1. Average values for goat milk composition (g·l^{-1})
Tabela 1. Średnie wartości składu mleka kóz (g·l^{-1})

Month	Protein	Fat	Lactose	NFS
4	23.80 ± 0.50	31.60 ± 1.80	45.20 ± 0.10	73.80 ± 1.20
5	26.40 ± 1.20	28.40 ±2.20	45.70 ± 0.50	77.40 ± 1.10
6	27.70 ± 0.60	28.70 ± 1.70	45.70 ± 0.20	78.50 ± 0.50
7	28.10 ± 1.50	27.50 ± 0.40	45.30 ± 0.20	79.00 ± 0.20
8	27.20 ± 0.40	29.00 ± 1.30	45.30 ± 0.20	78.50 ± 0.60
9	27.80 ± 1.10	29.20 ± 3.50	45.20 ± 0.60	79.00 ± 1.40
10	29.60 ± 0.70	35.70 ± 1.50	45.20 ± 0.50	80.00 ± 2.30
11	31.50 ± 1.60	34.70 ± 0.90	44.30 ± 0.70	81.00 ± 0.40
$\overline{x}$	27.80 ±2.30	30.60 ± 3.10	45.20 ± 0.40	78.40 ± 2.20

Lactose content from Month 4 to Month 9 fluctuated mildly, values of 4.52–4.57% were found, more significant decline was experience in Months 10 and 11 (44.20 and 44.30 g·l^{-1}). The average value of lactose content was 45.20 ± 0.40 g·l^{-1} which corresponds to the data of a series of authors: Zadražil (2002), Gajdůšek (2003), Späth and Thume (1996).

The average level of non fat solids (NFS) was 78.40 ± 2.20 g·l^{-1}. The lowest level of 73.80 g·l^{-1} was determined in Month 4 and the highest level of 81.00 g·l^{-1} in Month 11. On the whole, the content of non fat solids is low. Kuchtík and Sedláčková (2003) report a gradual increase in NFS to Day 100 of the lactation. In Day 135, they found an inconclusive decline and from then on till the end of monitoring, there was a gradual growth. The level that we found does not meet the requirements of the Slovak Technical Standard STN 57 0520 (1995) entitled „Rules on Goat Milk" that requires the composition of milk to have a minimum fat content of 30.0 g·l^{-1}, protein at 30.0 g·l^{-1} and a minimum fat-free dry matter at 8.3%.

The values of physical and chemical parameters are reported in the Table 2. Titrable acidity was at an average of 5.54 ± 0.683 °SH. Kuchtík and Sedláčková (2003) report that the highest value was measured at the beginning at 5.14 °SH and at the end of the lactation at 6.24 °SH, the lowest value was at 5.14 °SH in Day 100 of the lactation. Similar values are reported by Antunac (2001a), too. The lowest values of our monitoring were found to be in Months 4 and 5 (4.78 and 5.00), the highest acidity was detected at the end of the lactation (6.65).

Rennetability is a technological property influenced by the lactation period. The average value of 93.33 ± 14.76 s was found, the highest value of 104.8 and 123.00 s in Months 8 and 9, the lowest value of 76.83 s in Month 6. Kuchtík and Sedláčková (2003) present the values of 50–134 s.

Table 2. Average values for goat milk – physical and chemical properties
Tabela 2. Średnie wartości mleka kóz – fizyczne i chemiczne właściwości

Month	Titrable acidity SH	BM (°C)	Rennetability (s)	SCC $.10^3.ml^{-1}$
4	4.78 ± 0.15	-0.548 ± 0.004	87.80 ± 14.46	2219 ± 373.80
5	5.00 ± 0.44	-0.547 ± 0.004	89.22 ± 25.53	1396 ± 120.70
6	5.47 ± 0.80	-0.551 ± 0.006	76.83 ± 8.50	1726 ± 1132.00
7	5.07 ± 0.12	-0.557 ± 0.002	95.33 ± 4.16	1395 ± 387.30
8	5.27 ± 0.21	-0.555 ± 0.003	104.80 ± 17.84	1540 ± 834.00
9	5.57 ± 0.45	-0.551 ± 0.004	123.00 ± 33.48	1948 ± 238.80
10	6.47 ± 0.21	-0.551 ± 0.006	80.11 ± 4.45	1971 ± 261.70
11	6.65 ± 0.53	-0.523 ± 0.002	89.50 ± 5.20	2802 ± 606.20
$\overline{x}$	5.54 ± 0.68	-0.551 ± 0.004	83.33 ± 14.76	1875 ± 476.10

The average value of the freezing point was -0.550 ± 0.004°C and it matches the values of –0.551 to –0.548°C that were detected, for instance, by Petrova et al. (2001). It is apparent from the results that there is a fluctuation of the freezing point of goat milk which due to the lactation stage, the weather conditions and it mirrors seasonal feeding practices. Lower values were detected at the beginning of the lactation, being –0.548 and -0.547°C. A rise to the value of – 0.523°C occurred in Month 11.

Somatic cell count (SCC) was 1875 ± $10^3 \cdot ml^{-1}$. As opposed to dairy cow milk, for which the Directive of the European Parliament and Council of the European Community No. 853/2004 established the limit to be at $400 \cdot 10^3 \cdot ml^{-1}$, goat milk limit is not established. The values of goat milk are higher which is caused by a higher number of cytoplasmatic bodies as a result of apocrine type secretion in the goat mammary glands (Olechnowicz and Jaskowski 2004). Contreras et al. (2003) report that the cause of the increased somatic cell count can be the *Staphylococcus aureus* which is considered in goats to be the most frequently found pathogenic microorganism. Somatic cell count that we detected was much higher than, for instance, Zadražil (2002) reports 800 000 ml^{-1}.

An overview of mean, minimum and peak values of the selected physical and chemical parameters is presented in the following Table No. 3. A total of 22 fresh goat cheese varieties was assessed.

Sensory evaluation by an evaluating team ranked all products as very good to excellent. The mean value of pH was 4.87 ± 0.14, mean titration acidity was 98.09 ± 4.93 °SH. As becomes clear from the value of SD, titration acidity was balance in the course of monitoring. The mean dry matter content declared by the producer on the label was 48%. In 4 samples, it was established in excess of 48%. The mean value of dry matter content was 46.83 ± 1.57%, minimum being at 44.08, maximum at 50.05. Raw goat milk is not standardized before processing. With respect to the fact that the production takes place under strict observance of technological parameters, the variation is caused by the differences in the composition of the original foodstuff depending on the lactation stage and the season. Because the producer does not present minimal values, the determined content is suitable.

Table 3. Average values for goat cheeses – physical and chemical properties
Tabela 3. Średnie wartości serów kozich – fizyczne i chemiczne właściwości

Physical parametres						
	pH	Titrable acidity (°SH)	solid %	Fat in solid %	NaCl %	a_w
$\overline{x}$	4,87 ± 0,14	98,09 ± 4,93	46,83 ± 1,57	52,74 ± 5,24	2,08 ± 0,54	0,979 ± 0,014
Min.	4,75	84,50	44,08	43,66	1,08	0,969
Max.	5,12	106,50	50,05	64,31	2,75	0,990

Fat in dry matter content in soft cheese varieties ranges between 40 and 65%. Cheese producers declare fat in dry matter content at an average of 40%. All samples displayed the value of over 40%. The mean value was 52.74 ± 5.24%, with the highest fat content in dry matter at 64.31%, lowest being at 43.66%. The results collected were used to create calibration models for a FT NIR spectrometer Nicolet Antaris (Thermo Electron Corporation, Madison, USA) which allows for fast food analysis (Lee 2004).

Sodium chloride in cheese can range from 0.7 to 4.5% depending on the cheese brand. The mean value was at 2.08% ± 0.54, the highest established per cent content of sodium chloride was 2.75%, the lowest at 1.08%. Water activity ranged in the interval of values that are optimal for the growth of all microorganisms including pathogenic ones, being between 0.969 and 0.990. The mean value was 0.979 ± 0.007, which is a risk factor (due to giving ground to *L. monocytogenes* growth) when combined with the detected acidity (pH >4.4). The presence of *L. monocytogenes*, however, was not confirmed with the microbiological analysis.

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BEZPIECZEŃSTWO I JAKOŚĆ PRODUKTÓW MLECZARSKICH POCHODZĄCYCH Z FARM KÓZ W REPUBLICE CZECH

Streszczenie. Przedmiotem badań był skład wybranych fizycznych i technologicznych właściwości surowego mleka koziego (rasy białej-krótkowłosej) oraz świeżego sera koziego. Skład mleka i jego właściwości badano w zależności od okresu laktacji, pory roku, żywienia i metody hodowli. Stwierdzono następujące przeciętne zawartości w mleku: białko 27.80 ± 2.30 $g.l^{-1}$, tłuszcz 30.60 ± 3.1 $g.l^{-1}$, laktoza 45.20 ± 0.40 $g.l^{-1}$, składniki stałei niekwasowe 78.40 ± 2.20 $g.l^{-1}$, kwasowość 5.54 ± 0.86 °SH, punkt zamarzania-0.550 ± 0.004°C i liczba komórek somatycznych 1875 ± 476.10.10^3.ml^{-1}; w serach: pH 4.87 ± 0.14, kwasowość 98.09 ± 4.93 °SH, składniki stałe 46.83 ± 1.57%, tłuszcz w składnikach stałych 52.74 ± 5.24%, NaCl 2.08 ± 0.54%, a_w 0.979 ± 0.007.

Wszystkie próbki oceniono jako doskonałe pod względem sensorycznym, a ich skład odpowiadał deklarowanemu przez producenta. Wyniki użyto do kalibracji spektrometru FT NIR Nicolet Antaris (Thermo electron Corporation, Madison, USA).

Słowa kluczowe: mleko kozie, ser kozi, fizyczne i chemiczne właściwości, skład mleka

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QUALITY AND SAFETY OF CZECH HONEY*

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Abstract. In this project, parameters are presented that prove a high quality of honey produced by Czech beekeepers. Physical and chemical parameters whose limit (of tolerable) levels for honey are stipulated for by the Czech legislation, which is compatible with the Council Directive No. 2001/110/EC relating to honey. All these parameters were established based on methods described in the Harmonised methods of the European Honey Commission. Moreover, selected contaminant contents were monitored, too. Analyzing an extensive number of samples proved the already traditional high health safety and quality of Czech honey.

Key words: honey, quality, physicochemical parameters, contaminants

INTRODUCTION

Honey is an easily digestible, energy-rich foodstuff that contains a lot of nutritionally valuable ingredients. Composition of the honey and quality varies and is reflected by physicochemical and chemical parameters. They vary according to the honey types (blossom and honeydew) and many of them provide information on how the honey was handled and on possible procedures that can significantly reduce its dietary value (adulteration). Several of the physicochemical parameters are covered by legislative limits on the national level (Decree No.76/2003 Coll. as amended in later regulations) that match international legislation (Council Directive 2001/110/EC). Many honeys produced by the Czech honey beekeepers are of a high quality, which is evidenced by physicochemical and chemical parameters (Bartáková et al. 2007).

The aim of this study was to monitor these parameters and evaluate the quality of honeys of Czech origin.

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MATERIAL AND METHODS

Samples of honey were obtained directly from Czech beekeepers, the majority of them originated in the South Moravia region over period 1999–2007. Physicochemical parameters were established based on the methods described in Harmonised methods of the European Honey Commission (Bogdanov et al. 1997).

Water content in honey was determined using the refractometric method on the Abbe refractometer (AR 2, A-Kruss, Optronic, Germany). Electrical conductivity was determined by the conductometric method on a inoLab Cond Level 2 (WTW, Germany). Diastase activity in honey was determined by the Schade method on a UV/VIS spectrophotometer Lambda 11 (Perkin Elmer, USA). Invertase activity was determined on the same instrument. The pH value was measured on an Orion Ph meter (Orion Research, USA). Water activity was determined using an a_w-meter (Thermoconstanter, Switzerland).

Hydroxymethylfurfural (HMF) concentration was determined by HPLC method on a liquid chromatograph Alliance 2695 with a PDA detector 2996 (Waters, USA) and Zorbax Eclipse XDB C18 chromatographic column, 4.6 x 150 mm, 5 um (Agilent, USA). Linear gradient elution was applied, water and methanol as a mobile phase were used, flow rate 1.0 ml·min^{-1} Detection was performed at 285 nm. An external standard method for evaluation was used.

Polycyclic aromatic hydrocarbons were determined by HPLC method on a liquid chromatograph Alliance 2695 with a FLD 2475 detector (Waters, USA), PAHs chromatographic column 250 x 4.6 mm, 5um, detection at programmable changed wavelengths, linear gradient water/acetonitrile, flow rate 1.4 ml·min^{-1}.

Three modes of sample preparation were used. The first procedure: 10 g sample of honey was dissolved in 100 ml of deionised water, 1g NaCl and 10 ml hexane were added and thoroughly shaken for 30 min. By means of separatory adapter an aliquot of organic layer was taken and evaporated to dryness. The residue was dissolved in 1 ml of acetonitrile, filtered through nylone membrane filter (0.45 um) and analysed by HPLC.

The second procedure: 10 g of honey sample was mixed with anhydrous sodium sulphate, added 40 ml of dichloromethane and extracted by means of ultrasonic bath and Ultrathorax. After filtration the solvent was evaporated and next process was the same as in the first procedure.

The third procedure: 10 g of sample was dissolved in 100 ml of deionised water and extracted by means of solid phase of SPE cartridges. The next process was as in the first procedure.

RESULTS AND DISCUSSION

Diastase activity

Diastase (α-, β-, γ-amylase) is a member of an enzyme group which breaks up starch contained in honey. Its activity in honey depends on the plant source. It is a thermally instable enzyme whose low activity can indicate that honey was warmed up. Its activity decreases also with storing time. Diastase activity should range at least 8 degrees of the Schade scale with the exception of honey with natural low enzyme content which at the

HMF content up to 15 mg·kg^{-1} can display diastase activity at least 3 degrees according to Schade (Horn and Lüllmann, 2002; Vorlová et al., 2002; Decree No. 76/2003 Coll.).

In our 2003 study, we established diastase activity in 37 honey samples, coming directly from beekeepers. Values in the range of 9.8 – 40.9 degrees on the Schade scale (Bartáková et al. 2007) were detected. In this study, our results on diastase activity were in interrelation with the botanical origin of the honey.

Water content

Water content ranks among important parameters of honey quality, too. It depends on the degree of honey ripeness or the method of storing. Higher levels of water content present honey fermentation hazard. According to the legislation, water content should not exceed 20%. Honey samples, taken in 2003, met the current legislation requirements. We detected water content which ranged in the interval between 15.00 and 20.00% (Bartáková et al. 2007).

Electrical conductivity

Based on this parameter, honey is divided onto blossom and honeydew varieties while the legislation sets the conductivity for blossom honey up to 80 mS.m^{-1} and for honeydew above 80 mS·m^{-1}.

In our project by the authors Bartáková et al. (2007), we focused on monitoring the physical and chemical parameters and botanical origin of Czech honeys. The electrical conductivity levels detected were in the range of 9.8 – 127.3 mS·m^{-1}. Dependence of the electrical conductivity on the botanical origin was observed, with rape and acacia honeys displaying the lowest electrical conductivity. The highest electrical conductivity of all blossom honeys was found in lime tree honeys.

Contaminants

Primary introduction of contaminants into honey is much smaller than in other foodstuffs because bees detoxify them selectively. Honey contaminants can be divided based on their origin onto endogenous and exogenous.

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (5-hydroxymethyl-2-furancarboxaldehyde) is a significant representative of endogenous contaminants in honey. We focused on its determination in our project.

Hydroxymethylfurfural is one of the most important indicators of honey quality. This cyclic aldehyde appears in honey both by hexose dehydration (glucose and fructose) in acidic environment and as a result of Maillard reaction. HMF content in fresh honey is very low, almost null, its concentration increases in the course of storing (depending on pH, temperature, storage times) and also in the course of honey heating. Therefore, judging by HMF content, we can establish not only honey freshness but also potential inappropriate handling when processing and storing this dietetically important food item. High levels of HMF can also be caused by adultering honey by adding invert sugar or starch syrup (Kubiš and Ingr 1998, Nozal et al. 2001, Vorlová et al. 2002, Wunderlin et al. 1998).

In our study (Kalábová et al. 2003), we monitored the HMF levels in 56 honey samples collected in years 1999–2002. The range of the recorded values of HMF were: 24.8–66.1 mg·kg^{-1} in samples from the year 1999, 15.2–38.7 mg·kg^{-1} in the samples

from the year 2000. Samples from the year 2001 contained 5.7 – 38.4 mg·kg^{-1} of HMF. HMF in the honey samples collected in 2002 ranged between the values lower than the limit of detection up to the concentrations of 24.2 mg·kg^{-1}. There were significant differences ($p<0.01$) in the concentrations of HMF in the individual years, with insignificant differences between the years 2001 and 2002. The limit of 40 mg·kg^{-1} specified by the Czech legislation for HMF content in honey was exceeded in 55.6% of the samples collected in 1999. The obtained results demonstrate that the putting the limit of the minimal shelf life at three years is legitimate. In another study from 2003, we monitored HMF contents in 37 samples. We detected the concentrations in the range of 0.00–15.51 mg·kg^{-1} (Bartáková et al. 2007).

Our projects focused also on monitoring other parameters: water activity 0.485–0.607, pH value 3.64 – 4.95 and invertase activity 6.8–238.1 U·kg^{-1} (Bartáková et al. 2007). We confirmed good quality of honey collected from beekeepers also in studies of samples collected in years 2004 (n = 45), 2006 (n = 10) and 2007 (n = 6). In these honeys, we determined only water content, HMF, electrical conductivity and diastase activity. With the exception of 3 samples, in which higher water content was detected, all the samples met the above mentioned requirements.

Exogenous contaminants
Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) belong to the family of persistent organic contaminants having many interconnections with and negative impact on human organism (carcinogenicity, mutagenicity).

The data on PAHs content in honey is rare (Albero et al. 2003). In the study dealing with the PAHs content in honey, considerably high concentrations are reported (Dobrinas et al. 2008), especially for the honeydew honey, not only for those PAHs with 3–4 aromatic rings (acenaphten 187 ug·kg^{-1}, fluoren 163 ug·kg^{-1}, phenanthren 625 ug·kg^{-1}, anthracen 635 ug·kg^{-1}) predominantly occurring in foodstuffs and materials of plant origin (European Commission 2002) but also for the toxic PAHs (benzo-k-fluoranthen 58 ug·kg^{-1}, benzo-a-pyren 126 ug·kg^{-1}).

The origin of PAHs in honey is ascribed to beekeeping in contaminated areas, controlled burn of meadows, beekeeping activities (smoking) or endogenous PAHs precursor change in plants, e.g. the Mustard Family (*Brassicaceae).*

We estimated the contents of 15 priority contaminants according to the US EPA, namely naphthalene (NAPT), acenaphthen (ACENAPT), fluorene (FLU), anthracene (ANT), fluoranthene (FLT), pyrene (PY), benzo-a-anthracene (BaA), chrysene (CHRY) benzo-b-fluoranthene (BbF), benzo-k-fluoranthene (BkF), benzo-a-pyrene (BaP), dibenzo-a,h-anthracene (DBahA), benzo-g,h,i-perylene (BghiPE) and indeno-1,2,3-cd--pyrene (IPY).

The concentrations of polycyclic aromatic hydrocarbons in analysed samples were very low, they ranged between 0.02 and 2.22 ug·kg^{-1} for individual PAHs. These values are usually found in uncontaminated foodstuffs.

Additional exogenous contaminants

Exogenous contaminants in honey can be divided, from the chemical point of view, to inorganic and organic.

Inorganic contaminants are contamination hazard elements, they are especially as follows: Pb, Hg, Cd and As. The results of our study in the previous years (2000–

2001) showed that contamination hazard elements in honey from beekeepers (30 samples) statistically differed significantly in blossom and honeydew honeys but values lay deeply under the specified hygienic limits (Čelechovská and Vorlová 2001, Vorlová and Čelechovská 2002).

Organic contaminants involve compounds with pharmacological effect for which no maximum limits can be set (e.g. chloramfenicol and nitrofurans), drug residua (e.g. sulphmethoxazol, sulphathiazol, tetracyclines; ectoparasitics) and contaminants (e.g. chlorinated hydrocarbons – DDT, PCB, pesticides and organophosphates).

Incidence of external compounds (residua and contaminants) in honey is monitored by the State Veterinary Administration of the Czech Republic (SVS ČR) as part of the monitoring under the Decree No. 291/2003 Coll. as amended by the Decree No. 232/2005 Coll. For PCB concentrations (congener sum) and also for DDT sums, there are hygienic limits set in the legislation. In none of the samples were they exceeded. These compounds levels were always under 50% of the given hygienic limit. The other monitored agents haven't got a limit set for them but when they were detected in Czech honeys, their levels never ranged detectable levels.

CONCLUSIONS

With respect to the fact that honey as one of the few animal origin food products reaches the consumer in its natural state free of additives and major processing modifications, the issue of its quality and health safety is very important. If the proper hygienic and production practice is observed, the parameters should meet the requirements specified in the legislation. Analyzing an extensive number of samples, which came directly from beekeepers, proved their already traditional high health safety and quality.

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JAKOŚĆ I BEZPIECZEŃSTWO CZESKIEGO MIODU

Streszczenie. W badaniach przedstawiono parametry świadczące o wysokiej jakości miodu produkowanego w Czechach. Fizykochemiczne parametry jakości miodu obowiązujące w czeskich przepisach są zgodne z zawierającą wymagania dla miodu Dyrektywą Rady Nr 2001/110/EC. Wszystkie te parametry badane były przy zastosowaniu metod opisanych w Harmonised Methods of the European Honey Commission. Badaniami objęto ponadto zawartości wybranych zanieczyszczeń. Wyniki na szeroką skalę prowadzonych badań potwierdziły tradycyjnie już wysoką jakość i bezpieczeństwo dla zdrowia czeskiego miodu.

Słowa kluczowe: jakość miodu, fizykochemiczne parametry, zanieczyszczenia

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POTENTIAL THREAT OF TYRAMINE PRODUCTION AMONG ENTEROCOCCI ISOLATED FROM DAIRY PRODUCTS*

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Abstract. The decarboxylation of tyrosine by certain enterococci strains leads to the undesirable presence of tyramine in fermented foods. Tyramine is the most frequent biogenic amine found in cheese and is also commonly found in other fermented foods and beverages. In total 75 different strains of enterococci from various dairy products were studied. A multiplex PCR was designed for the genotypic differentiation of various enterococci strains and to determine the presence to tyramine producing (*tyrdc*) gene. *E. faecalis* followed by *E. faecium* was found to be the most prominent strains present in dairy products. Presence of *E. mundtii, E. malodoratus, E. durans, E. casseliflavus, E. raffinosus* was also found but to a lesser extent. 82% of the total strains were found to be carrying *tyrdc* gene responsible for tyramine production. *E. faecalis* was found to be the most active and *E. casseliflavus* was found to be the least active in the production of tyramine.

KEY WORDS: Enterococci, dairy products, multiplex PCR, biogenic amines, tyramine, tyrdc gene.

INTRODUCTION

Enterococci are ubiquitous lactic acid bacteria that frequently occur in dairy products and other fermented foods, and are also present in the gastrointestinal tract of humans and animals (Morandi et al. 2006). Enterococci are found in large numbers in traditional cheeses made with both pasteurized and raw milk (Andrighetto et al. 2001, Giraffa 2003, Suzzi et al. 2000). The reason for the prevalence of enterococci in dairy products has long been considered as a result of unhygienic conditions during the production and processing of milk (Giraffa 2003). Due to their psychotrophic nature, their

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heat resistance and their adaptability to different substrates and growth conditions, enterococci can increase during milk cooling and in low numbers can even be present after pasteurization. Therefore, Enterococci are part of both raw and pasteurized milk. Different species of enterococci are found in dairy products, but *E. faecalis and E. faecium* are the species most importance. (Franz et al. 1999, Gelsomino et al. 2001). High levels of contaminating enterococci in some cheeses, especially fresh or soft industrial cheeses made with pasteurized milk and selected lactic starter cultures, usually result from poor hygienic practices during cheese manufacture and lead to deterioration of sensory properties of these products (Giraffa et al. 1997, Franz et al. 1999). Therefore, the presence of enterococci in these cheeses is undesirable. On the other hand, they also, play an important role in determining cheese taste and texture and help in cheese ripening and flavor development (Morandi et al. 2006). Thus they have been proposed as a part of defined starter culture combinations for different European cheeses. But, these enterococci present in various dairy products are found to be highly active in the production of biogenic amines (BAs) mainly tyramine.

Tyramine and histamine are able to cause various symptoms in sensitive population such as alteration in blood pressure, headache, urticaria, nausea and vomiting (Shalaby 1996). Many authors have discussed the toxicological effects of biogenic amines and their occurrence and formation in foods, with a special emphasis on cheeses and fermented foods. The accumulation of BAs in various dairy products requires the presence of microorganisms that produce specific amino acid decarboxylases (Fernandez et al. 2007). The amount and type of BA formed depends on the nature of food and particularly on the kind of microorganisms present (M. Carmen et. al. 2001). Biogenic amines and polyamines occur in various tissues and play an important role in cell regeneration and differentiation (Pircher et al. 2007). If in case the intake is higher or if an individual's natural detoxification mechanisms are inhibited or deficient than even a low BA concentration can be problematic. (Fernandez et al. 2007, De Las Rivas et al. 2006).

The aim of the study was to check the presence of *tyrdc* gene responsible for the production of tyramine in enterococci isolated from various dairy products using multiplex PCR method.

MATERIALS AND METHOD

75 different enterococci strains originating from the collection of Department of Hygiene and Milk Technology (University of Veterinary and Pharmaceutical Sciences, Brno) isolated from various dairy products and stored at -75°C were used in this study. The cultures were grown at 37°C on Slanetz-Bartley Medium (Oxiod, UK).

DNA was extracted from bacterial cultures by boiling procedure.

A multiplex PCR was designed for the genetic identification of bacteria and check for the presence of tyramine producing *tyrdc* gene.

Species specific identification of *E. faecalis, E. faecium, E. mundtii, E. malodoratus, E. durans, E. casseliflavus,* and *E. raffinosus* was performed; the primers used are presented in table 1.

The amplification program for the multiplex PCR is as follows: 95°C 5 min, 32 cycles of 95°C 45 sec, 52°C 45 sec, 72°C 1 min 15sec with a final extension at 72°C

for 5 min. All multiplex experiments were done in a Peltier Thermal Cycler PTC-200 (MJ Research, U.S.A.) using Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) in the total volume of 25 ul. PCR products were detected by electrophoresis in 2% (wt/vol) agarose gels in 0.5 X TBE buffer at 120 V for 45 min and visualized with ethidium bromide staining.

Table 1. List of primers used in this study
Tabela 1. Lista primerów użytych w badaniach

Designation	Primer Sequence	Gene	Product Size	Species	Reference
EFS 1 EFS 2	*F: 5'-TAA TGG TGA ATC TTG GTT TGG-3' *R: 5'-TAA TGG TGA ATC TTG GTT TGG-3'	*sod* A	360 bp*	*E. faecalis*	Jackson *et al.*, 2004
EFM 1 EFM2	F: 5'-GAA AAA ACA ATA GAA GAA TTA T-3' R: 5'- TGC TTT TTT GAA TTC TTC TTT A-3'	*sod* A	215 bp	*E. faecium*	Jackson *et al.*, 2004
EDU 1 EDU 2	F: 5'-CCT ACT GAT ATT AAG ACA GCG-3' R: 5'-TAA TCC TAA GAT AGG TGT TTG-3'	*sod* A	295 bp	*E. durans*	Jackson *et al.*, 2004
EMAL 1 EMAL 2	F: 5'- GTA ACG AAC TTG AAT GAA GTG-3' R: 5'-TTG ATC GCA CCT GTT GGT TTT-3'	*sod* A	134 bp	*E. malodoratus*	Jackson *et al.*, 2004
ECAS 1 ECAS 2	F: 5'-TCC TGA ATT AGG TGA AAA AAC-3' R: 5'-TCC TGA ATT AGG TGA AAA AAC-3'	*sod* A	288 bp	*E. casseliflavus*	Jackson *et al.*, 2004
Ent 1 Ent 2	F: 5'-TAC TGA CAA ACC ATT CAT GAT G-3' R: 5'-AAC TTC GTC ACC AAC GCG AAC-3'	*tuf*	112bp	*E. mundtii*, *E. raffinosus*	Ke *et al.*,1999
TD 2 TD 5	F: 5'-AGA GTT TGA TCC TGG CTC AG-3' R: 5'-AAG GAG GTG ATC CAG CCG CA-3'	*tyrdc*	>970 bp		Coton *et al.*, 2004
BSF 8 BSR1541	F: 5'-AGA GTT TGA TCC TGG CTC AG-3' R: 5'-AAG GAG GTG ATC CAG CCG CA-3'		>970 bp		Wilmotte *et l.*,1993

*F – forward primer, *R – reverse primer, bp* – base pair

RESULTS AND DISCUSSION

BA in foods is considered potentially dangerous to human health. Thus, the development of methods for the detection BA-producing bacterial strains is of top priority (De Las Rivas et al. 2006).

By species specific PCR method 7 different species were detected out of the raw milk and dairy products, 40 strains out of 75 were found to be *E. faecalis*, 20 were

reported to be *E. faecium*, 6 were *E. casseliflavus*, 3 of each *E. durans*, and *E. mundtii*, 2 were *E. raffinosus*, and 1 was found to be *E. malodoratus.* Detailed information regarding the multiplex PCR results is shown in table 2.

Table 2 Detailed Information Regarding Multiplex PCR Result
Tabela 2. Szczegółowe informacje dotyczące wyników multiplex PCR

Enterococcus species	Number of strains identified	Strain origin	Positive *tyrdc* gene (%)
E. faecalis	40	Raw and pasteurized milk, hard and soft cheeses	37 (92.5)
E. faecium	20	Raw and pasteurized milk, hard eidam cheese	17(85)
E. casseliflavus	6	Cheese from raw milk	2(33)
E. durans	3	Raw milk	2(66.6)
E. mundtii	3	Soft cheese	2(66.6)
E. raffinosus	2	Raw milk	1(50)
E. malodoratus	1	Pasteurized milk	1 (100.0)
Total	75		62(82)

The result from table 2 shows that almost 82% of the strains were able to produce tyramine. Recently carried out some experiments by Trivedi Krina (Trivedi et al. 2008) showed 90% of the strains of *E. faecalis* and *E. faecium* from various dairy products were able to produce tyramine. *E. faecalis* was found to be highly active in the production of this biogenic amine. Out of 40 strains of *E. faecalis* 37 (92.5%) were found to be carrying *tyrdc* gene responsible for tyramine production. *E. casseliflavus* was found to be least active in the production of tyramine. Out of 6 identified strains of *E. casseliflavus* only 2 (33%) were found to be carrying *tyrdc* gene. Also, from the results it was noticed that *E. faecium* was also much active in the production of tyramine. It was determined that out of 20 strains of *E. faecium* 17 (85%) were found to be carrying *tyrdc* gene. There was only 1 strain of *E. malodoratus* found in our study and it was found to be positive for tyramine production.

The results of our study also show that in the dairy and raw as well as pasteurized milk the prevailing strain was *E. faecalis* followed by *E. faecium*. Here, after *E. faecalis* and *E. faecium* the strain dominating was found to be *E. casseliflavus*. These results are contradictory to the results shown by Morandi et al. (2006) who showed that along with *E. faecalis* and *E. faecium* strains of *E. durans* are frequently found in dairy products. Also, various other strains of enterococci have been isolated in our study and they should be taken into consideration because in total 82% of the strains are able to produce tyramine.

CONCLUSION

Enterococci because of their ability to help in ripening and flavor development in cheese production have been used as a starter culture. Thus, the technological aspects of enterococci should be taken into consideration. Yet, obstacles like production

of biogenic amine such as tyramine should also be taken into consideration. One should always remember that, excess consumption of tyramine can lead to food poisoning and imply to various kind of food exportation problems. This can prove to be a potential threat to the society. Therefore, it becomes highly necessary to monitor various dairy and related products for the presence of tyramine at industrial and farm level.

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TYRAMINA WYTWARZANA PRZEZ ENTEROKOKI WYOSOBNIONE Z PRODUKTÓW MLECZNYCH POTENCJALNYM ZAGROŻENIEM ZDROWIA

Streszczenie. Dekarboksylacja tyrozyny przez niektóre szczepy enterokoków skutkuje niepożądaną obecnością tyraminy w produktach fermentowanej żywności. Tyramina jest najczęściej wykrywaną biogenną aminą w serach, a także w innych produktach fermentowanych i napojach. Zdolność do jej wytwarzania badano u 75 szczepów, różnych gatunków enterokoków, wyosobnionych z różnych przetworów mlecznych. W genotypowym rozpoznaniu badanych szczepów enterokoków oraz wykrywaniu u nich genu *tyrdc* posługiwano się metodą multiplet-PCR. Stwierdzono że *E. faecalis*, a następnie *E. faecium* dominowały wśród enterokoków obecnych w produktach mlecznych. *E. mundtii, E. malodoratus, E. durans, E. casseliflavus* oraz *E. raffinosus* były stwierdzane także, ale znacznie mniej często. 85% badanych szczepów posiadało gen tyrcd, determinujący produkcję tyraminy. *E. faecalis* w przeciwieństwie do *E. casseliflavus* okazał się najaktywniejszym producentem histaminy.

Słowa kluczowe: enterokoki, produkty mleczne, multiplet PCR, aminy biogenne, tyramina, gen tyrdc

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THE OCCURRENCE OF BIOGENIC AMINES IN DAIRY PRODUCTS ON THE CZECH MARKET*

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Abstract. Recent trends in food safety promote an increasing search for compounds that can affect human health. Biogenic amines belong to this group of substances. Under normal conditions in humans, exogenous amines absorbed from food are rapidly detoxified by the action of amine oxidases but in the case of allergic individuals and if MAO inhibitors are applied, the detoxification process is disturbed. Cheese is a foodstuff most frequently connected with biogenic amine production.

The levels of biogenic amines in different cheese types on the Czech market were investigated. 215 samples of 10 cheese brands were purchased in retail stores. Amines were extracted using hydrochloric acid, derivatized with dansylchloride and separated using RP-HPLC method with fluorometric detection. Tyramine, putrescine and cadaverine were the prevalent amines, followed by histamine. Spermine, spermidine, tryptamine and 2-phenylethylamine were detected at low levels (max. 51 $mg \cdot kg^{-1}$). Tyramine, putrescine, cadaverine and histamine were present at levels up to 1123, 591, 1110 and 283 $mg \cdot kg^{-1}$, respectively. There was variability in the types and levels of amines in every cheese brand. The highest levels of biogenic amines were determined in swiss-type cheese or surface ripened cheese and the sum of biogenic amines was in excess of 3000 $mg \cdot kg^{-1}$. However, cheeses with low degree of cultivation are noted for low contents of amines. Susceptible individuals should be advised to consume cheese brands with low biogenic amine contents.

Key words: biogenic amines, cheese brands, RP-HPLC, Czech market

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INTRODUCTION

Biogenic amines (BA histamine, tyramine, tryptamine, 2-phenylethylamine, cadaverine, putrescine) are low molecular organic bases mainly produced by the decarboxylation of certain amino acids with microbial action (Silla-Santos 1996). Polyamines (putrescine, spermidine and spermine, introduced as members of the BA family) are nowadays considered as a distinct group because they can also be produced in another metabolic pathway and for their specific physiological action (putrescine for its dual role is presented as BA as polyamine) (Kalač and Krausová 2005).

Some of them play a major role in many human and animal physiological functions, such as regulation of body temperature, stomach pH and brain activity. However, when these compounds are consumed in high quantities they could give rise to different changes in the organism.

Histamine and tyramine are the most frequently studied BA due to their toxicological effect. Histamine may lead to the dilatation of blood vessels, capillaries and arteries, causing headaches, hypotension, gastrointestinal distress and oedemas (Stratton et al. 1991). Tyramine is a potent vasoconstrictor, provoking headache, hypertension and migraine, it can induce brain haemorrhage and heart failure (Křížek and Kalač 1998). Histamine and tyramine are broken down in the organism by the oxidative deamination catalyzed by monoamine and/or diamine oxidase (MAO and DAO) (Bardócz et al. 1993). The presence of MAO and DAO inhibitors or the presence of other BA such as diamines (putrescine and cadaverine) can potentiate their toxicity. Attention has been paid to diamines such as putrescine and cadaverine and polyamines such as spermidine and spermine due to the fact that they are precursors of carcinogenic nitrosamines and they enhance the growth of chemically induced aberrant crypt foci in the intestine (Paulsen et al. 1997). As polyamines are required for cell proliferation, it is very important to control polyamine content in the diet of cancer patients (Kalač and Krausová 2005).

There are cases when human detoxification mechanisms fail: high individual susceptibility (e.g. allergic individuals, patients consuming drugs with MAO inhibitors), too high dietary intake of BA, presence of other catalyst compounds. Due to these reasons, it is difficult to establish toxic BA doses (Halász et al. 1994, Komprda 2005). However, the value suggested by Spanier et al. (1991), is used for cheese conventionally. It says that the aggregate of histamine + tyramine + putrescine + cadaverine should not exceed 900 $mg \cdot kg^{-1}$. Cheese is the next (after fish) most frequently presented food item associated with BA poisoning (Stratton et al. 1991). The presence of microorganisms with decarboxylase activity is one of the main factors affecting BA content in cheese: it can be the lactic acid bacteria (LAB) used as starter culture (Fernández-García et al. 2000) and the action of non-starter lactic acid bacteria or some other spontaneous microflora (Roig –Sagués et al. 2002). Strains of a wide range of genera, such as enterobacteria, *Pseudomonas* and LAB, are capable of producing BA. The capability to form BA seems to be strain dependent rather than related to species specificity. It is thus difficult to find precise correlations between BA contents and microorganism counts (Halász et al. 1994, Valsamaki et al. 2000). There are factors which influence BA content in cheese: milk pasteurization (Novella-Rodríguez et al. 2003), conditions for cheese production (Křížek and Kalač 1998), time of ripening (Valsamaki et al. 2000) and starter culture (Roig-Sagués et al. 2002).

Cheese usually contains one to three orders of magnitude mg·kg^{-1}of histamine, tyramine, putrescine and cadaverine; one to two orders of magnitude of 2-phenylethylamine and very low levels of tryptamine (Silla-Santos 1996). BA contents, however, can exceptionally reach levels of grams per a kilogram of cheese, which depends on the method of processing of the primary raw material and technological factors (Křížek and Kalač 1998). Significantly higher content of BA was found in cheese made of unpasteurized milk (Pinho et al. 2001).

The objectives of the present study were threefold. Firstly, to determine the contents and BA profiles of cheese brands from the Czech market depending on the cheese type including special cheese products. Secondly, to compare the obtained results with the literature data. Finally, to try and evaluate the health risk of consuming different cheese types by various consumer groups.

MATERIAL AND METHODS

Analysed samples

Samples under analysis (n = 215) were brands available and consumed in the Czech Republic and selected specific milk products (soft maturing cheese types – brynza, goat cheese) that can be bought in retail market. Among them, there were hard cheese varieties of the Emmenthaler type (Ementál etc.), Edammer type (Eidam etc.), cheese types with mould on top (Hermelín etc.), cheese types with mould inside (Niva), soft maturing cheese types (Romadur etc.), also cream and thermally treated cheese types (Lučina etc.), goat cheese and processed cheese types.

Biogenic amines determination

Biogenic amines (tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) were extracted from the food matrix using hydrochloric acid. Subsequently, they were established as dansyl-derivates. Quantitative determination was then carried out using RP-HPLC with gradient elution and fluorescence detection.

HPLC determination parameters

Analyses were carried out on a liquid chromatographer Alliance 2695 (Waters, USA) with PDA 2996 detectors and a 2475 fluorescence detector. A chromatographic column Polaris C 18 (Varian, USA), 150 mm × 4.6 mm, particle size of 3 um with a Meta Guard Polaris C 18 precolumn, 30 mm × 4.6 mm, 3 um and Zorbax Eclipse XDB C18 (Agilent, USA), 150 x 4,6 mm, 5um with a Zorbax precolumn 30 mm x 4,6 mm, 5 um were used. Determination was carried out using gradient elution. Mobile phase A was a mixture of 0.1 M of acetic acid, acetonitrile and methanol (90:5:5), mobile phase B was a mixture of 0.1 M acetic acid, acetonitrile and methanol (10:45:45), flow rate was 1 ml·min^{-1}, inject volume of 10 ul. Detection was carried out at lambda$_{ex}$/lambda$_{em}$ = 330/500 nm, detection in the UV region at 254 nm. Every sample was analyzed at least in two parallel sessions; every series had a involved. Determination was done using the outer standard method.

RESULTS AND DISCUSSION

Biogenic amine levels and profile in cheese brands varied significantly by cheese types, with every cheese having a characteristic range. In the monitored cheese sample collection, tyramine and cadaverine were most frequent, with putrescine and histamine following while tryptamine, 2-phenylethylamine, spermidine and spermine levels were low (Fig. No. 1). Levels of the individual biogenic amines in cheese types ranged in the interval of 0.1 – 1123 mg·kg^{-1}. Biogenic amine levels assessed based on the method by Spanier et al. (1991) in 6% of samples exceeded the level of 900 mg·kg^{-1}. The cause may be the sample contamination during inadequate storage, use of inadequate technological procedures or inferior raw materials for production. The highest biogenic amine concentrations were found in Emmenthaler type cheese and in soft maturing cheese while cream and thermally treated cheese types did not display biogenic amines presence or they were present at a low level. In thermally treated cheese types, biogenic amine levels depend on the technological process: products with low degree of processing display low levels of biogenic amines (Rak 2005).

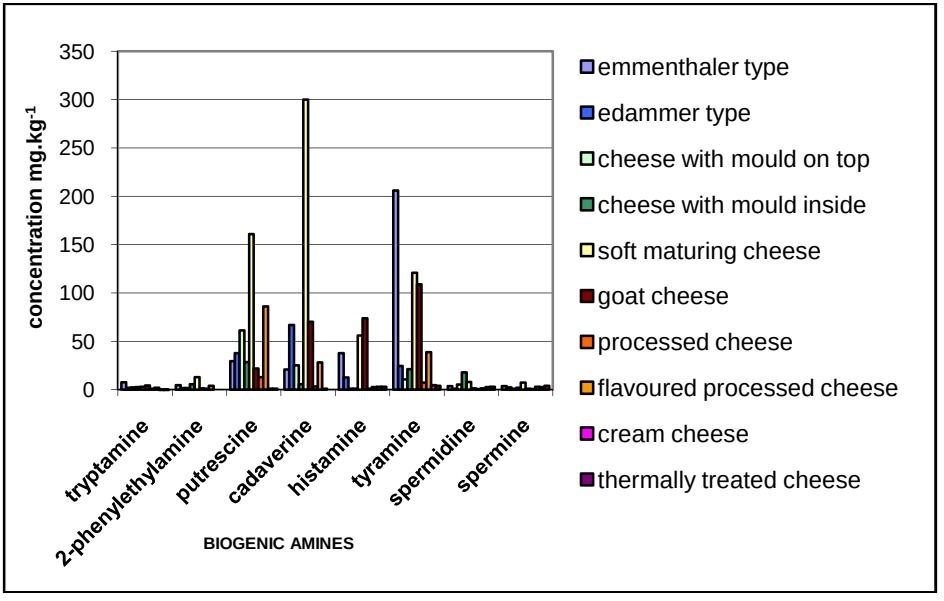

Fig. 1. Biogenic amine content in cheeses on the Czech market
Rys. 1. Zawartość amin biogennych na rynku czeskim

Emmenthaler type cheese brands

The determined tyramine levels match the results of earlier studies (Askar and Treptow 1986). The highest tyramine concentrations were found in the Ementál cheese brand at 1123 mg·kg^{-1} while foreign cheese brands usually have higher concentrations, e.g. parmesan is at 2460 mg·kg^{-1} (Sieber and Lavanchy 1990). In Czech Emmenthaler type cheese brands, histamine levels were lower by one order of magnitude (in Ementál at 151 mg·kg^{-1}) as compared to foreign cheese types of the same type (Emmenthaler

types up to 1500 mg·kg^{-1}) (Polo et al. 1985). Cadaverine and putrescine concentrations exceeded 100 mg·kg^{-1} only rarely (cadaverine and putrescine reached concentrations 233 and 223 mg·kg^{-1}, respectively).

Edammer type cheese brands

Hard Edammer cheese types contain mostly tyramine (70.2 mg·kg^{-1} on average), cadaverine (66.9 mg·kg^{-1} on average), putrescine (37.7 mg·kg^{-1} on average) and histamine (12.6 mg·kg^{-1} on average). The other biogenic amines display low levels only. In the above mentioned biogenic amines, samples exceeded the concentration of 100 mg·kg^{-1} very rarely. Low and medium BA levels of this cheese type are presented by Křížek and Kalač (1998). In these most frequently sold cheese brands on the Czech market, high levels of BA were shown only rarely (Standara 1998).

Cheese brands with mould on top

Earlier studies reported that cheese with mould on top can display a high cadaverine concentration. For instance, cadaverine concentration exceeded 1000 mg·kg^{-1} (Brink et al. 1990) in Camembert cheese types. In the sample collection monitored by our team, cadaverine concentration reached the levels of 388 mg·kg^{-1}. Putrescine concentration in Czech cheese brands was relatively low (17,9–166 mg·kg^{-1}). From among the individual mould cheese brands, the highest tyramine concentration was found in Hermelín at 187 mg·kg^{-1}.

Cheese brands with mould inside

In Czech cheese brands with mould inside, tyramine levels were found to be high (63 mg·kg^{-1} on average). In the Niva brand, cadaverine concentration reached 699 mg·kg^{-1}. The established putrescine (28.5 mg·kg^{-1} on average), histamine (8.3 mg·kg^{-1} on average) and 2-fenylethylamine (5.6 mg·kg^{-1} on average) levels were low in other biogenic amines just as well. In tyramine and putrescine, samples exceeded the concentration of 100 mg·kg^{-1} only rarely (tyramine and putrescine concentration reached 444 and 123 mg·kg^{-1} , respectively). The results match those in literature on BA levels in blue vein cheese brands (Standara et al. 2007) but also such levels of BA were found which would exceed the suggested maximum tolerable amine levels in cheese according to Spanier et al. (1991).

Soft maturing cheese brands

Soft maturing cheese brands contained a high concentration of cadaverine (brynda at 1110 mg·kg^{-1}, quargels at 739 mg·kg^{-1}), putrescine (brynza at 591 mg·kg^{-1} and quargels at 514 mg·kg^{-1}) and histamine (Beer cheese at 283 mg·kg^{-1}). The detected tyramine levels in soft maturing cheese brands were the highest also in brynza (417 mg·kg^{-1}). High BA levels in brynza were detected by Greif et al. (1997) in the past. Polyamines (spermine and spermidine) and 2-phenylethylamine were found in the monitored cheese brands in concentrations up to the orders of tens of mg·kg^{-1}. Antila et al. (1984) report similar putrescine levels in soft maturing cheese brands but histamine levels are by one order of magnitude higher.

Goat cheese brands

Biogenic amine levels in goat cheese are similar to cow cheese (Křížek and Kalač 1998). The cheese contained mostly tyramine (109 mg·kg^{-1} on average) and histamine

(74 mg·kg^{-1} on average), with cadaverine (70.2 mg·kg^{-1} on average) and putrescine (22 mg·kg^{-1} on average).

Processed cheese brands

Biogenic amines profile was monitored also in processed cheese brands. In the monitored cheese brands, putrescine, tyramine and cadaverine were detected in concentrations in the order of tens of mg·kg^{-1} in the most. The levels of biogenic amines in Czech cheese brands correspond to those in foreign products. The study confirmed that the concentration of biogenic amines in processed cheese brands is lower than in hard cheese ones (Křížek and Kalač 2002) although samples were found displaying increased levels of biogenic amines, which was apparently connected to the use of the initial raw material of inferior quality (Standara 1998).

Flavoured processed cheese brands

Flavoured processed cheese brands contained putrescine (86.2 mg·kg^{-1} on average), tyramine (38.3 mg·kg^{-1} on average) and cadaverine (28.2 mg·kg^{-1} on average). Other biogenic amines levels in the analyzed samples were low. In a some of the samples, putrescine, tyramine and cadaverine concentrations were found to be higher than 100 mg·kg^{-1} (422, 161 and 242 mg·kg^{-1}, respectively).

CONCLUSIONS

A comparison of Czech and foreign cheese samples was done. Biogenic amine concentrations in Czech cheese are lower or they correspond to the biogenic amine concentrations in cheese of foreign origin with the exception of brynza and quargels, which matches earlier findings (Křížek and Kaláč 2002). Biogenic amine levels and profile depended mostly on the cheese type. Based on the results that we acquired as regards the BA profile and with respect to the toxicological aspects bearing on the susceptible consumer groups, the individual cheese types were classified as to the health risk with biogenic amines as follows: cheese types presenting great risk (soft maturing cheese, Emmethaler type cheese), cheese types with increased health risk (Edammer type cheese, mould containing cheese and goat cheese) and cheese types with low health risk (cream and thermally treated cheese).

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WYSTĘPOWANIE AMIN BIOGENNYCH W PRODUKTACH MLECZNYCH OBECNYCH W SPRZEDAŻY DETALICZNEJ W CZECHACH

Streszczenie. Aktualne tendencje w działaniach na rzecz zapewnienia bezpieczeństwa żywności promują badania wszelkich czynników mogących zagrażać zdrowiu konsumentów. Do tej grupy czynników należą aminy biogenne. U zdrowych ludzi egzogenne aminy absorbowane z żywności są szybko detoksyfikowane przez oksydazy aminowe. Jednakże w przypadku alergików oraz występowania inhibitorów MAO proces detoksyfikacji ulega zakłóceniu. W serach najczęściej wśród produktów żywności stwierdzane są aminy biogenne.

Badano zawartość amin biogennych w 215 próbkach pobranych z 10 różnych gatunków serów dostępnych w sprzedaży detalicznej w Czechach. Ekstrakty amin uzyskiwano przy użyciu kwasu solnego (derivatized with dansylchloride) i oddzielane (separatek) przez RP-HPLC z fluorometryczną detekcją. Dominującymi aminami były: tyramina, putrescyna i kadaweryna, a następnie histamina. Wykryto również niewielkie koncentracje sperminy, spermidyny, tryptaminy i 2-fenyl-oetyloaminy. Zawartość tyraminy, putrescyny, kadaweryny i histaminy wynosiła odpowiednio aż do 1123, 591, 1110 i 283 $mg \cdot kg^{-1}$. Obserwowano różnorodność rodzajów jak i zawartości amin w każdym asortymencie serów. Najwyższe koncentracje amin biogennych stwierdzono w serach szwajcarskich lub na powierzchni serów dojrzewających, gdzie ich suma przekraczała 30000 $mg \cdot kg^{-1}$. Natomiast sery w mniejszym stopniu przetworzone zawierają mniej amin. Dla wrażliwych ludzi zalecana jest konsumpcja gatunków sera o niskich zawartościach amin biogennych.

Słowa kluczowe: aminy biogenne, gatunki sera, RP-HCLP, czeski rynek

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JAKOŚĆ MIKROBIOLOGICZNA MIĘSA DROBIOWEGO ODDZIELONEGO MECHANICZNIE

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Streszczenie. Mięso drobiowe oddzielone mechanicznie (MDOM) jest rozdrobnioną masą mięsno-tłuszczową otrzymywaną z fragmentów lub całych tuszek, przeznaczoną jako surowiec do produkcji przetworów mięsnych poddawanych obróbce cieplnej. Mięso to stwarza możliwość optymalnego wykorzystania wszystkich elementów i części zwierząt uzyskiwanych podczas rozbioru. Jako surowiec technologicznie i sanitarnie wrażliwy oraz podatny na zanieczyszczenia mikrobiologiczne podlega specjalnym wymaganiom mikrobiologicznym. Celem pracy jest określenie stopnia zanieczyszczenia mikrobiologicznego mięsa drobiowego oddzielonego mechanicznie oraz właściwa ocena sanitarno-weterynaryjna MDOM-u.

Słowa kluczowe: mięso drobiowe oddzielone mechanicznie, higiena mięsa, zanieczyszczenia mikrobiologiczne

WSTĘP

Mięso drobiowe oddzielone mechanicznie (MDOM) jest to rozdrobniona masa mięsno-tłuszczowa otrzymana z fragmentów lub całych tuszek patroszonych, przeznaczona jako surowiec do produkcji przetworów drobiowych poddawanych obróbce cieplnej [Anon 1992]. Surowcem do otrzymywania mięsa drobiowego oddzielonego mechanicznie są schłodzone lub zamrożone elementy tuszek patroszonych kurcząt, kur, indyków, kaczek, gęsi lub całe tuszki, uznane przez Weterynaryjną Inspekcję Sanitarną za zdatne do spożycia bez zastrzeżeń. Surowiec, zgodnie z wymaganiami, może być przechowywany w temperaturze od 0°C do -2°C przez 48 h od momentu rozbioru. Do produkcji mrożonego mięsa drobiowego oddzielonego mechanicznie należy stosować wyłącznie surowiec schłodzony (świeży) [Anon 1992, Anon 1999]. Surowiec pozyskiwany jest przez wyciskanie kości pozostałych po wykrawaniu tuszek drobiowych lub ich części na specjalnych urządzeniach (separatorach) [Michalski 2006].

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Szczegółowe wymagania dla mięsa drobiowego oddzielonego mechanicznie, w tym mikrobiologiczne, określone są w normie PN-A-86522:1992 „Mięso drobiowe oddzielone mechanicznie", a także w obowiązującym od 1 stycznia 2008 r. Rozporządzeniu Komisji (WE) Nr 1441/2007 z dnia 5 grudnia 2007 r. zmieniającym rozporządzenie (WE) nr 2073/2005 w sprawie kryteriów mikrobiologicznych dotyczących środków spożywczych. Warunki produkcji mięsa drobiowego oddzielonego mechanicznie określone są w obowiązującym od 1 stycznia 2006 r. Rozporządzeniu (WE) nr 853/2004 Parlamentu Europejskiego i Rady z dnia 29 kwietnia 2004 r. ustanawiającym szczególne przepisy dotyczące higieny w odniesieniu do żywności pochodzenia zwierzęcego. Sposób produkcji MDOM-u powoduje, że uzyskuje się surowiec technologicznie wrażliwy oraz podatny na zanieczyszczenia mikrobiologiczne. Obecność różnorodnych bakterii, w tym patogennych, szczególnie z rodziny *Enterobacteriaceae*, w surowcu drobiowym podczas uboju i obróbki jest dobrze udokumentowana [Gill i in. 2006, Jimenez i in. 2003]. Na kościach i surowcu mogą być obecne chorobotwórcze bakterie *Salmonella* spp., *Campylobacter* spp., enterokrwotoczne *Escherichia coli* (EHEC), *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus* i inne, a także bakterie saprofityczne, głównie z rodzaju *Pseudomonas*, odpowiadające za procesy psucia [Anon 1999, Gill 2004]. Mięso drobiowe oddzielone mechanicznie jest powszechnie używane jako surowiec do produkcji wyrobów mięsnych i często jest przyczyną obniżenia jakości zdrowotnej produktów, zatruć pokarmowych, a także podwyższonej zawartości fosforu, co stanowi potencjalne zagrożenie zdrowotne [Nurmi i Ring 1999, Michalski 2006].

Celem pracy było określenie jakości mikrobiologicznej mięsa drobiowego oddzielonego mechanicznie, pochodzącego z różnych zakładów na terenie kraju w odniesieniu do wymagań stawianych w normie PN-A-86522:1992. Wymagania mikrobiologiczne dla mięsa drobiowego oddzielonego mechanicznie są następujące:

- ogólna liczba drobnoustrojów tlenowych w 1 g : nie więcej niż 1 x 10^6 jtk,
- pałeczki z grupy *coli*: nieobecne w 0,001 g,
- gronkowce chorobotwórcze (koagulazododatnie): nieobecne w 0,1 g,
- pałeczki z rodzaju *Salmonella*: nieobecne w 25 g,
- beztlenowe laseczki przetrwalnikujące: nieobecne w 0,01 g.

MATERIAŁY I METODY

Przedmiotem badań były próbki mięsa drobiowego oddzielonego mechanicznie pochodzące z różnych zakładów na terenie kraju. Próby do badań pobierane były w latach 2005–2006 w zakładach drobiarskich, zamrażane a następnie dostarczane w warunkach chłodniczych (2–6°C, termotorba z wkładami chłodzącymi) do Państwowego Instytutu Weterynaryjnego – Państwowego Instytutu Badawczego w Puławach (PIWet-PIB). Świeży MDOM, dostarczany także w warunkach chłodniczych, poddawano badaniu najpóźniej dnia następnego po dostarczeniu do laboratorium lub zamrażano. Surowcem do produkcji MDOM-u były głównie korpusy kur, kurcząt i indyków.

Badania mikrobiologiczne określone w wymaganiach dla mięsa drobiowego oddzielonego mechanicznie wykonywano w Zakładzie Higieny Żywności Pochodzenia Zwierzęcego PIWet-PIB, zgodnie z polskimi normami:

- ogólna liczba drobnoustrojów tlenowych wg PN-EN ISO 4833:2004 „Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby drobnoustrojów. Metoda płytkowa w temperaturze 30°C";
- obecność pałeczek z grupy *coli* wg PN-ISO 4831:1998 „Mikrobiologia. Ogólne zasady oznaczania bakterii z grupy *coli*. Metoda najbardziej prawdopodobnej liczby";
- obecność gronkowców chorobotwórczych (koagulazo-dodatnich) wg PN-EN ISO 6888-3:2004 „Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby gronkowców koagulazododatnich (*Staphylococcus aureus* i innych gatunków). Część 3: Wykrywanie obecności i oznaczanie małych liczb metodą NPL";
- obecność pałeczek z rodzaju Salmonella wg PN-EN ISO 6579:2003 „Mikrobiologia żywności i pasz. Horyzontalna metoda wykrywania *Salmonella* spp.";
- obecność beztlenowych laseczek przetrwalnikujących wg PN-A-82055-12:1997 „Mięso i przetwory mięsne. Badania mikrobiologiczne. Mięso i przetwory mięsne. Wykrywanie obecności beztlenowych bakterii przetrwalnikujących i beztlenowych bakterii przetrwalnikujących redukujących siarczany (IV)".

WYNIKI I OMÓWIENIE

Analizie i ocenie jakości mikrobiologicznej poddano 46 próbek mięsa drobiowego oddzielonego mechanicznie. Szczegółowe wyniki badań mikrobiologicznych przedstawiono w tabeli 1.

Porównując stan mikrobiologiczny zbadanych próbek z wymaganiami normatywnymi przekroczenie ogólnej liczby drobnoustrojów w 1 g ponad kryterium 1 x 10^6 jtk stwierdzono w 3 próbkach na 46 badanych, co stanowi 6,5%. Pałeczki *Salmonella* spp. w 25 g stwierdzono we wszystkich próbkach badanych, a więc żadna z próbek nie spełniała wymagania w tym zakresie. Obecność beztlenowych bakterii przetrwalnikujących w 0,01 g odnotowano w 27 próbkach, co stanowi 58,7%. W 40 próbkach stwierdzono obecność bakterii z grupy *coli* w 0,001 g, czyli 87%. Gronkowce koagulazododatnie w 0,1 g zarejestrowano w 35 próbkach, co stanowi 76%.

Analiza wyników przeprowadzonych badań mikrobiologicznych w odniesieniu do wymagań normatywnych dla mięsa drobiowego oddzielonego mechanicznie wskazuje, że żadna z próbek badanych nie spełniała przedmiotowych wymagań. Bezwarunkowo dyskwalifikującym surowiec jest stwierdzenie obecności pałeczek z rodzaju *Salmonella*. Na jakość mikrobiologiczną MDOM-u wpływ mają takie czynniki, jak traktowanie surowca do jego produkcji jako gorszego, prawdopodobnie nieodpowiedni stan higieniczny urządzeń i pomieszczeń produkcyjnych, niewłaściwie przeprowadzane procesy mycia i dezynfekcji a także wadliwy stan techniczny urządzeń wykorzystywanych w procesie mechanicznego oddzielania. Ponadto na zanieczyszczenie mikrobiologiczne mięs odzyskiwanych mechanicznie przy użyciu technik naruszających strukturę kości wskazują liczne dane literaturowe.

Tabela 1. Wyniki badań mikrobiologicznych MDOM
Table 1. Results of microbiological examinations of MSPM

Nr próbki No of sample	Ogólna liczba drobnoustrojów w jtk/g Total colony count cfu/g	Obecność *Salmonella* spp. w 25 g Detection of *Salmonella* spp. in 25 g	Obecność beztlenowych bakterii przetrwalnikujących w 0,01 g Detection of spore forming anaerobe bacteria in 0,01 g	Obecność bakterii z grupy *coli* w 0,001 g Detection of coliforms in 0,001 g	Obecność gronkowców koagulazo-dodatnich w 0,1 g Detection of coagulase-positive staphylococci in 0,1 g
1	2	3	4	5	6
1	$1{,}3 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
2	$3{,}0 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
3	$1{,}1 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
4	$3{,}4 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
5	$5{,}2 \times 10^3$	Obecne Present	Obecne Present	Nieobecne Not present	Obecne Present
6	$9{,}1 \times 10^4$	Obecne Present	Nieobecne Not present	Nieobecne Not present	Obecne Present
7	$4{,}3 \times 10^3$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
8	$1{,}8 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
9	$1{,}1 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Nieobecne Not present
10	$6{,}1 \times 10^6$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
11	$9{,}4 \times 10^5$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
12	$8{,}4 \times 10^5$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
13	$3{,}4 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
14	$5{,}2 \times 10^3$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
15	$3{,}1 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
16	$1{,}7 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
17	$3{,}4 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
18	$5{,}2 \times 10^3$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
19	$9{,}5 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
20	$1{,}3 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
21	$3{,}6 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
22	$1{,}1 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
23	$1{,}1 \times 10^5$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present

Tabela 1 cd.
Table 1 cont.

1	2	3	4	5	6
24	$1{,}8 \times 10^4$	Obecne Present	Obecne Present	Nieobecne Not present	Obecne Present
25	$2{,}2 \times 10^6$	Obecne Present	Nieobecne Not present	Nieobecne Not present	Obecne Present
26	$3{,}1 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
27	$1{,}7 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
28	$1{,}1 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Nieobecne Not present
29	$1{,}3 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
30	$9{,}1 \times 10^3$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
31	$2{,}9 \times 10^5$	Obecne Present	Obecne Present	Obecne Present	Nieobecne Not present
32	$3{,}4 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
33	$5{,}2 \times 10^3$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
34	$9{,}5 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
35	$1{,}3 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
36	$3{,}0 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
37	$1{,}1 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
38	$3{,}4 \times 10^4$	Obecne Present	Nieobecne Not present	Obecne Present	Obecne Present
39	$5{,}2 \times 10^3$	Obecne Present	Obecne Present	Nieobecne Not present	Obecne Present
40	$9{,}1 \times 10^4$	Obecne Present	Nieobecne Not present	Nieobecne Not present	Obecne Present
41	$4{,}3 \times 10^3$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
42	$1{,}8 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
43	$1{,}1 \times 10^4$	Obecne Present	Obecne Present	Obecne Present	Nieobecne Not present
44	$6{,}1 \times 10^6$	Obecne Present	Nieobecne Not present	Obecne Present	Nieobecne Not present
45	$9{,}4 \times 10^5$	Obecne Present	Obecne Present	Obecne Present	Obecne Present
46	$8{,}4 \times 10^5$	Obecne Present	Obecne Present	Obecne Present	Obecne Present

Zły stan mikrobiologiczny mięsa drobiowego oddzielonego mechanicznie oraz jego szerokie zastosowanie w przetwórstwie mięsnym powoduje, że istnieje wysokie prawdopodobieństwo wyprodukowania produktu niebezpiecznego dla zdrowia konsumenta.

PODSUMOWANIE

Analiza mikrobiologiczna mięsa drobiowego oddzielonego mechanicznie wykazała brak zgodności z wymaganiami normatywnymi dla MDOM-u dla wszystkich próbek badanych. Można przypuszczać na tej podstawie, że nadzór technologiczny i weterynaryjny jest niewystarczający w zakresie produkcji mięsa drobiowego oddzielonego mechanicznie. Wyniki przeprowadzonych badań wskazują bowiem jednoznacznie, że surowiec ten nie powinien być dopuszczony do produkcji żywności przeznaczonej do spożycia przez ludzi i bezwarunkowo poddany procesowi utylizacji.

Zastosowanie mięsa drobiowego oddzielonego mechanicznie jest uzasadnione nie tylko ze względów technologicznych, ale również ekonomicznych. Jednak aby zapewnić pożądaną jakość, a szczególnie czystość mikrobiologiczną tego bardzo wrażliwego na obróbkę surowca, niezbędne jest rygorystyczne przestrzeganie wymogów jego pozyskiwania. Kryteria takie jak dobre właściwości technologiczne, niewielki koszt wytworzenia oraz nie budząca wątpliwości natury higienicznej jakość mogą być gwarantem, że mięsa oddzielane mechaniczne, w tym mięsa drobiowe, stanowią dobry, a co najważniejsze, zdrowotnie bezpieczny surowiec przetwórczy.

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MICROBIOLOGICAL QUALITY OF MECHANICALLY DEBONED POULTRY MEAT

Abstract. Mechanically separated poultry meat is a minced meat-fatty mass produced from elements of poultry carcasses or whole poultry carcasses, as a raw material component for production of heat-treated poultry products. Mechanically separated poultry meat (MSPM) is giving the possibility of optimal using of all elements and parts of animals gotten during the disassembly. As a technologically and sanitary very sensitive material, susceptible for microbiological contamination, mechanically deboned poultry meat is subjected to special requirements, among other things, microbiological. The aim of this study is to evaluate a level of microbiological contamination of MSPM an and appropriate sanitary-veterinary evaluation of MSPM.

Key words: mechanically separated poultry meat, meat hygiene, microbiological contamination

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ZAWARTOŚĆ DODANYCH POLIFOSFORANÓW W WYBRANYCH OWOCACH MORZA („FRUTTI DE MARE")

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Streszczenie. W pracy określono zawartości fosforu fizjologicznego dla tzw. „owoców morza". Przebadano 72 próbki. Średnia zawartość fosforu całkowitego (w procentach, jako P) dla badanych prób wynosi od 0,041±0,002 do 0,181±0,062. Maksymalna stwierdzona zawartość fosforu wyliczonego jako fosforan dodany (w procentach, jako P_2O_5) wynosi 0,322. W 30 próbach na 72 wykazano fosfor jako dodany przy stosowaniu współczynnika 0,01, co stanowi 41,7% badanych prób. Wymagane jest badanie metodą TLC. Ponad 2/5 dostępnych na rynku „owoców morza" zawiera wielofosforany.

Słowa kluczowe: fosforany dodane, owoce morza, fosfor

WSTĘP

Fosfor i jego pochodne należą do tzw. związków pokarmowych. Występują w każdej żywności, a dodatkowo różnorakie związki fosforu dodaje się również celowo jako dodatek funkcjonalny oraz technologiczny. Są one związkami chemicznymi szeroko stosowanymi w różnych działach przemysłu spożywczego. Wśród nich znajdują się wielofosforany mające duże zastosowanie w przetwórstwie żywności pochodzenia zwierzęcego. Jednakże poszczególne sole mają zastosowanie w ustalonych grupach żywności, co wiąże się najczęściej z osiągnięciem zamierzonego celu technologicznego (Michalski 2007, PN-A-04018:1975, Rozporządzenie Ministra Zdrowia 2004). I tak w przemyśle mięsnym, drobiowym i rybnym dzięki stosowaniu polifosforanów uzyskujemy obniżenie ilości wycieku mięsnego, zwiększenie zdolności utrzymywania się wody w mięsie, zmniejszenie strat przy gotowaniu produktów i zmniejszenie ilości galarety w konserwach, lepszą kruchość i soczystość. Jest to wynikiem związania dodatkowej wody przez włókna mięśniowe pod wpływem obecności polifosforanów. Również skurcz cieplny tkanki mięśniowej podczas obróbki cieplnej jest mniejszy. Pozwala to na lepsze uformowanie się produktu w osłonce czy też w puszce. Barwa produktu jest bardziej trwała, równomierna i intensywniejsza. Jednakże przy zastoso-

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waniu fosforanów pogarsza się smakowitość gotowego wyrobu mięsnego. Produkty z fosforanami nie wykazują tzw. oparzelin mrozowych podczas przechowywania mroźniczego, co jest istotne przy długotrwałym przechowywaniu w niskich temperaturach magazynów mroźniczych. Te same właściwości wykazują wielofosforany w odniesieniu do ryb i produktów rybnych (Ahmed 1995, Duda 2000).

W związku ze szkodliwością stosowanie polifosforanów podlega ścisłym ograniczeniom. Maksymalne ilości związków fosforu dopuszczone do stosowania dla poszczególnych grup żywności reguluje Rozporządzenie Ministra Zdrowia z dnia 23 kwietnia 2004 r., Dziennik Ustaw Nr 94, poz. 933 w sprawie dozwolonych substancji dodatkowych i substancji pomagających w przetwarzaniu. Problem wpływu fosforanów spożywanych przez nas jest przedmiotem stałych dyskusji. Nie są one obojętne dla naszego zdrowia, a spożyte w nadmiarze mogą zaburzyć w organizmie równowagę wapniowo-fosforanową. Mogą też być między innymi przyczyną zmniejszonej gęstości kości, prowadzić do osteoporozy czy też powodować alergie (Duda 1998, Duda 1998a, Duda 2000). W odniesieniu do ryb i przetworów rybnych zakazuje się stosowania polifosforanów w przemyśle rybnym przy produkcji i obrocie rybami świeżymi/mrożonymi. Dopuszczalne jest natomiast przy produkcji pasty rybnej oraz ze skorupiaków morskich. Można je stosować do glazury do produktów mięsnych/rybnych. Używa się wielofosforanów w filetach rybnych nieprzetworzonych świeżych, mrożonych czy też głęboko mrożonych, w nieprzetworzonych i przetworzonych mięczakach i skorupiakach mrożonych i głęboko mrożonych oraz puszkowanych przetworach ze skorupiaków w ilości do 5 g/kg, jako P_2O_5. Lód używany do przesypywania ryb może również zawierać wielofosforany w ilości do 10g/kg lodu (Duda 1998, Duda 1998a, Michalski 2006, Rozporządzenie Ministra Zdrowia 2004).

Coraz większa popularność tzw. „owoców morza" spowodowała zwiększony napływ tychże produktów na rynek polski. W związku z tym importem i częstym stosowaniem polifosforanów należałoby kontrolować obecność związków fosforu lub wymagać deklaracji producenta o niestosowaniu fosforanów w procesie przerobu czy też pozyskiwania.

W analizie żywności nie ma metody, która bezpośrednio umożliwiłaby oznaczenie fosforu dodanego do produktów żywnościowych, w tym pochodzenia zwierzęcego. Ortofosforany są również naturalnym składnikiem tkanki mięsnej. Stosowana dotychczas metoda określenia zawartości fosforu dodanego do produktów mięsnych, drobiowych czy też rybnych polega na wyliczeniu różnicy pomiędzy całkowitą zawartością fosforu całkowitego zawartego w próbie a szacunkową zawartością fosforu fizjologicznego występującego w określonej tkance, przy użyciu współczynnika przeliczeniowego skorelowanego z zawartością białka. Problemem jest tutaj określenie stosunku fosforu naturalnego do białka dla różnych tkanek zwierzęcych. Zawarty w polskiej normie współczynnik przeliczeniowy wynosi 0,01 i został on określony empirycznie, lecz tylko dla mięsa wołowego, drobiowego oraz wieprzowego i niektórych produktów z nich wyprodukowanych (Michalski 2006, PN-A-82060:1999).

W pracy określono zawartości fosforu fizjologicznego dla dostępnych na rynku „owoców morza" i produktów wyprodukowanych z ich udziałem, spotykanych w handlu (importowane).

MATERIAŁ I METODY

Przedmiotem badań były tzw. „owoce morza" dostępne w sprzedaży detalicznej. Próby do badań kupowano w sklepie i dostarczano w warunkach izotermicznych do laboratorium. Przebadano paluszki krabowe, małże „Abba" w sosie własnym, krewetki „Tiger", małże, ośmiornice, krewetki koktajlowe, raki, omułki i kalmary (tuby).

Oznaczenia zawartości fosforu, wykrywania wielofosforanów oraz białka w badanych próbach wykonywano w Zakładzie Higieny Żywności Pochodzenia Zwierzęcego zgodnie z polskimi normami, to jest PN-A-82060:1999 „Mięso i przetwory mięsne. Oznaczanie zawartości fosforu" oraz PN-A-04018:1975 „Produkty rolniczo-żywnościowe. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko". Oznaczenie zawartości białka jest niezbędne do określenia zawartości wielofosforanów dodanych.

WYNIKI I OMÓWIENIE

Wyniki oznaczeń zawartości fosforu ogólnego, dodanego, zawartość białka przedstawiono jako wartości średnie z podaniem odchylenia standardowego dla poszczególnych produktów – w tabeli 1. Przebadano 72 próbki „owoców morza" (9 asortymentów). Tabela zawiera również wykaz ilości próbek w których stwierdzono wielofosforany wykazane jako dodane przy zastosowaniu współczynnika przeliczeniowego 0,01 oraz procent prób ze stwierdzonymi wielofosforanami. Średnia zawartość fosforu całkowitego (w procentach, jako P) dla badanych próbek wynosi od 0,041±0,002 do 0,181±0,062. Porównując 9 rodzajów „frutti de mare", zauważamy bardzo zróżnicowaną zawartość fosforu tzw. fizjologicznego. Maksymalna stwierdzona zawartość fosforu wyliczonego jako fosforan dodany (w procentach, jako P_2O_5) wynosi 0,322. W 30 próbach na 72 wykazano fosfor jako dodany przy stosowaniu współczynnika 0,01, co stanowi 41,7% badanych próbek.

Tabela 1. Zawartość fosforu ogólnego, fosforu wykazanego jako dodany oraz białka w „owocach morza”

Table 1. Content of total phosphorus, added phosphates and protein in „frutti de mare”

Asortyment Assotrment	Ilość prób Number of samples	Zawartość [%] Contents in [%]			Ilość prób, w których stwierdzono wielofosforany wg PN Number of samples with addend polyphosphates	Ilość prób, w których wykryto wielofosforany [%] Number of samples with addend polyphosphates [%]
		Białko protein ± std	Fosfor Pcałk. Total phosphor ± std	Fosfor wykazany jako dodany (P_2O_5), wg PN[1)] Added phosphorus as P_2O_5		
Paluszki krabowe Crab fingers	4	6.01±0.54	0.041±0.002	0	0	0
Małże „ABBA” w sosie własnym Moule in own sause	4	13.96±0.29	0.17±0.008	0.075±0.013	4	100
Krewetki „TIGER” Prawn	4	17.64±2.44	0.181±0.062	0.081±0.021	3	75,0
Małże Claus	7	13.48±2.59	0.154±0.052	0.08±0.041	5	71,4
Ośmiornica Octopus	14	11.23±1.91	0.072±0.014	0	0	0,0
Krewetka koktajlowa Cocktail prawn	15	11.39±1.85	0.163±0.088	0.322±0.083	8	53,3
Raki Cryfish	3	13.76±3.17	0.155±0.041	0.248	1	33,3
Omułki Moule	7	12.92±2.37	0.144±0.053	0.113±0.037	3	42,8
Kalmary Kalmar	13	12.22±0.97	0.122±0.060	0.156±0.03	6	46,2

[1)] w próbkach, w których wyliczono dodany P – (for samples with added phosphates)

PODSUMOWANIE

Decyzja o jakości zdrowotnej produktu w oparciu o wyniki analizy chemicznej w kierunku pozostałości fosforanów dodanych nie może być podejmowana, jak to się często zdarza, na podstawie ww. PN z zastosowaniem współczynnika 0,01. Wymagane jest badanie próbek metodą TLC w celu potwierdzenia obecności wielofosforanów oraz ich identyfikacji. Średnio około 2/5 dostępnych na naszym rynku „owoców” morza zawiera dodane wielofosforany.

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CONTENTS OF ADDED POLYPHOSPHATES IN SOME ASSORTMENTS OF „FRUTTI DE MARE"

Abstract. Total phosphorus (P) and added polyphosphates were determined in product. A total 72 samples of frutti de mare were tested. Medium content of total P (in %) were from 0,041±0,002 up to 0,181±0,062. Maximum level of added phosphates, expressed as P_2O_5, was found on level 0,322%. In 30 samples were detected added polyphosphates ie. in 41,7 percentage of samples. It was calculated with 0,01 calculation factor. It is necessary to use TLC for confirmation and identification added components as phosphorus salts.

Key words: phosphorus, added polyphosphates, frutti de mare

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WPŁYW WYSOKIEGO CIŚNIENIA HYDROSTATYCZNEGO NA WŁAŚCIWOSCI ORGANOLEPTYCZNE RYB WĘDZONYCH*

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Streszczenie. Wyniki naszych wcześniejszych badań oraz liczne prace innych autorów wskazują, że wysokie ciśnienie hydrostatyczne może skutecznie eliminować z żywności pochodzenia zwierzęcego bakterie chorobotwórcze oraz mikroflorę gnilną, zwiększając bezpieczeństwo mikrobiologiczne i trwałość produktów spożywczych. Celem pracy było określenie wpływu wysokiego ciśnienia hydrostatycznego na cechy organoleptyczne wybranych ryb wędzonych. Stwierdzono, że poddanie działaniu wysokiego ciśnienia hydrostatycznego (300 i 400 MPa przez 5, 10 i 15 minut) pakowanych próżniowo próbek łososia wędzonego na zimno oraz makreli wędzonej na gorąco nie wywiera statystycznie istotnego wpływu na barwę, zapach, smak i teksturę tych produktów.

Słowa kluczowe: wysokie ciśnienie hydrostatyczne (UHP), wyróżniki sensoryczne, łosoś wędzony, makrela wędzona

WSTĘP I CEL BADAŃ

Wyniki naszych wcześniejszych badań oraz liczne prace innych autorów wskazują, że wysokie ciśnienie hydrostatyczne może skutecznie eliminować z żywności pochodzenia zwierzęcego bakterie chorobotwórcze oraz mikroflorę gnilną, zwiększając bezpieczeństwo mikrobiologiczne i trwałość produktów spożywczych [Farkas i Hoover

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2000, Fonberg-Broczek i in. 2005, Szczawińska i Szczawiński 2000, Szczawiński i in. 1995, 1998, 2003], w tym również ryb wędzonych [Jackowska i in. 2008].

Celem niniejszej pracy było określenie wpływu wysokiego ciśnienia hydrostatycznego na cechy organoleptyczne łososia wędzonego na zimno oraz makreli wędzonej na gorąco.

MATERIAŁY I METODY

Zakupione w supermarkecie, zapakowane próżniowo w typowe opakowania jednostkowe wykorzystywane w handlu, próbki łososia wędzonego na zimno oraz makreli wędzonej na gorąco poddano działaniu wysokiego ciśnienia hydrostatycznego w Centrum Wysokich Ciśnień Polskiej Akademii Nauk. Zabieg ciśnieniowania przeprowadzono w urządzeniu specjalnie skonstruowanym do badania żywności. Do komory urządzenia wypełnionej wodą destylowaną wkładano zapakowane próżniowo próbki, a następnie po zamknięciu górnej pokrywy generowano wysokie ciśnienie hydrostatyczne za pomocą tłoka zagłębiającego się w komorze w ciągu 10–60 sekund do momentu uzyskania zaplanowanego ciśnienia. Pomiaru ciśnienia hydrostatycznego dokonywano pośrednio za pomocą manometru wskazującego ciśnienie pod tłokiem. Po zakończeniu zabiegu ciśnienie w komorze stopniowo obniżano w czasie ok. dwukrotnie krótszym od czasu jego generowania.

Próbki ryb wędzonych poddawano działaniu wysokiego ciśnienia hydrostatycznego w wysokości 300 i 400 MPa w czasie 5, 10 i 15 minut w temperaturze 4°C.

Cechy sensoryczne próbek (barwa, zapach, smak i tekstura) oceniane były przez 10 lub 11-osobowy zespół, używający zamieszczonej poniżej następującej 9-punktowej skali hedonicznej, przy której opracowaniu kierowano się zasadami podanymi w PN-ISO 4121:1998 .

Skala ocen od 1 do 9	Ocena jakości
1 – skrajnie zła (nie do zaakceptowania)	Zła
2 – bardzo zła	
3 – zła	
4 – poniżej przeciętnej	Średnia
5 – przeciętna	
6 – poniżej dobrej, powyżej przeciętnej	
7 – dobra	Dobra
8 – bardzo dobra	
9 – znakomita	

Do obliczeń statystycznych wykorzystano analizę wariancji (ANOVA) przeprowadzoną w oparciu o ogólny model liniowy programu matematyczno-statystycznego SPSS 14.0 for Windows. Wartości średnie porównano testem Tukey'a.

WYNIKI I OMÓWIENIE

Wyniki badań przedstawiono w tabelach 1 i 2.

Tabela 1. Wpływ wysokiego ciśnienia hydrostatycznego na wyróżniki sensoryczne łososia wędzonego na zimno
Table 1. Effect of high hydrostatic pressure on sensory parameters of cold-smoked salmon

Ciśnienie Pressure	Czas Time	Średnia liczba punktów (n=10; skala od 1 do 9 punktów) Mean score (n =10; scale from 1 to 9 points)			
		Barwa Colour	Zapach Smell	Smak Taste	Tekstura Texture
0 MPa	0 min	7,3[a] (1,5)*	7,6[a] (1,2)	7,2[a] (1,7)	7,3[a] (1,4)
300 MPa	5 min	6,7[a] (1,3)	7,1[a] (1,1)	6,3[a] (1,8)	5,4[a] (2,0)
300 MPa	10 min	6,7[a] (1,5)	7,4[a] (1,0)	7,1[a] (0,9)	6,7[a] (0,9)
300 MPa	15 min	6,7[a] (1,4)	7,1[a] (1,0)	7,1[a] (1,1)	6,5[a] (1,6)
400 MPa	5 min	6,4[a] (1,6)	7,1[a] (1,0)	7,2[a] (1,2)	6,7[a] (1,6)
400 MPa	10 min	5,9[a] (1,7)	6,7[a] (1,6)	7,1[a] (1,7)	5,8[a] (2,2)
400 MPa	15 min	6,9[a] (1,7)	6,9[a] (1,2)	6,8[a] (0,9)	6,2[a] (1,5)

[a] Średnie w tej samej kolumnie oznaczone różnymi literami różnią się przy P < 0,05
* Odchylenie standardowe

[a] Means within the same column bearing different superscripts are different at P < 0,05
* Standard deviation

Tabela 2. Wpływ wysokiego ciśnienia hydrostatycznego na wyróżniki sensoryczne makreli wędzonej na gorąco
Table 2. Effect of high hydrostatic pressure on sensory parameters of hot-smoked mackerel

Ciśnienie Pressure	Czas Time	Średnia liczba punktów (n=10; skala od 1 do 9 punktów) Mean score (n =11; scale from 1 to 9 points)			
		Barwa Colour	Zapach Smell	Smak Taste	Tekstura Texture
0 MPa	0 min	8,1[a] (0,8)*	8,2[a] (0,8)	8,1[a] (0,8)	7,8[a] (1,2)
300 MPa	5 min	7,4[a] (1,2)	7,7[a] (0,9)	7,2[a] (1,5)	6,8[a] (2,0)
300 MPa	10 min	7,5[a] (0,9)	8,2[a] (0,4)	7,6[a] (1,0)	7,5[a] (1,1)
300 MPa	15 min	7,8[a] (0,9)	7,9[a] (0,9)	7,1[a] (1,6)	6,8[a] (1,8)
400 MPa	5 min	7,6[a] (0,9)	7,8[a] (0,8)	7,2[a] (0,9)	7,5[a] (0,8)
400 MPa	10 min	7,5[a] (0,8)	7,9[a] (0,5)	7,3[a] (0,9)	7,4[a] (1,3)
400 MPa	15 min	7,9[a] (0,8)	8,0[a] (0,6)	7,0[a] (1,8)	6,9[a] (2,1)

[a] Średnie w tej samej kolumnie oznaczone różnymi literami różnią się przy P < 0,05
* Odchylenie standardowe

[a] Means within the same column bearing different superscripts are different at P < 0,05
* Standard deviation

Na podstawie uzyskanych wyników (tab. 1 i 2) można stwierdzić, że średnie arytmetyczne dla wszystkich wyróżników sensorycznych (barwy, zapachu, smaku i tekstury) ryb wędzonych są najwyższe w przypadku próbek kontrolnych, nie poddanych wysokiemu ciśnieniu hydrostatycznemu. Obserwowane różnice pomiędzy próbkami kontrolnymi i ciśnieniowanymi są jednak bardzo niewielkie i nieistotne statystycznie przy $P < 0,05$.

Na podstawie analizy wariancji również stwierdzono, że zarówno wysokość ciśnienia, jak i czas jego oddziaływania nie wywierają statystycznie istotnego wpływu na wyróżniki sensoryczne badanych próbek ryb wędzonych. Nieistotna statystycznie okazała się również interakcja obu wymienionych czynników.

W dostępnym piśmiennictwie niewiele jest szczegółowych informacji na temat wpływu wysokiego ciśnienia na cechy organoleptyczne ryb wędzonych [Jackowska i in. 2008]. Uzyskane wyniki generalnie potwierdzają opinie podawane w innych pracach, że zastosowanie wysokiego ciśnienia w dawkach wywołujących wyraźną redukcje bakterii w produktach pochodzenia zwierzęcego w minimalnym stopniu wpływa na ich cechy organoleptyczne [Fonberg-Broczek i in. 2005, Karłowski i in. 2002, Szczawiński i in. 2003].

PODSUMOWANIE

Poddanie działaniu wysokiego ciśnienia hydrostatycznego (300 i 400 MPa przez 5, 10 i 15 minut) pakowanych próżniowo próbek łososia wędzonego na zimno oraz makreli wędzonej na gorąco nie wywiera statystycznie istotnego wpływu na barwę, zapach, smak i teksturę tych produktów.

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EFFECT OF HIGH HYDROSTATIC PRESSURE ON SENSORY PROPERTIES OF SMOKED FISH

Abstract. Results of our previously conducted studies demonstrated that high hydrostatic pressure can be effective in elimination of pathogenic and spoilage microflora from food products and may extend their shelf-life. The aim of the present study was to determine the effect of ultra high pressure treatment on sensory properties of selected smoked fish. It was found that high hydrostatic pressure (300 i 400 MPa applied for 5, 10 i 15 min) did not exert statistically significant influence on the color, smell, taste and texture of vacuum-packaged, cold-smoked salmon and hot-smoked mackerel.

Key words: ultra high pressure (UHP), sensory parameters, smoked salmon, smoked mackerel

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