SAFETY IN FOOD PRODUCTION CHAIN

edited by
Grażyna Krasnowska
and **Anna M. Salejda**

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Wrocław 2011

Authors:
Árvay Július, Bajzík Pavol, Bakker Evert J., Belej Lubomir, van Boekel M.A.J.S., Bojňanská Tatiana, Bulla Jozef, Bystrická Judita, Čičová Iveta, Czarniecka-Skubina Ewa, Godlewska Katarzyna, Goldsztejn Magdalena, Golian Jozef, Górecka Justyna, Górecki Jerzy, Górna Justyna, Grenda Tomasz, Grodzicki Tomasz, Harangozo Ľuboš, Jakubczak Antoni, Jałosińska Małgorzata, Ježo Peter, Jinap Selamat, Jónášová Diana, Kęsy Zbigniew, Kołożyn-Krajewska Danuta, Korzeniowska Małgorzata, Król Jolanta, Kukier Elżbieta, Kukułowicz Anita, Kwiatek Krzysztof, Lewandowski Włodzimierz, Litwińczuk Anna, Luning Pieternel A., Macherzyński Mariusz, Malicki Adam, Marczewska Sylwia, Mazur Robert, Musilová Janette, Nowicki Paweł, Ostrowska Monika, Revák Ondrej, Rola Jolanta G., Rzeźnik Radosław, Sanny Maimunah, Sikora Tadeusz, Stachelska Milena A., Steinka Izabela, Szmańko Tadeusz, Świsłocka Renata, Świątkowski Grzegorz, Tomáš Jan, Tóth Tomas, Trafiałek Joanna, Urminská Dana, Vollmannová Alena, Židek Radoslav

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PREFACE

Food safety is one of the most important factors necessary to ensure the desired quality of food products. Maintaining a high level of food and feed safety is one of the most important objectives in terms of health protection for consumers. Ensuring food safety requires taking up activities in a coordinated and integrated method across the entire food production chain. These requirements are enable to fulfill by legal systems included in the food law regulations, internal control system and in system of official food control. But even precisely developed procedures and a well-functioning system of food control not always protects consumers against the risks. May appear different, unknown so far, risk factors, or are not met all the conditions of good practices at various stages of the food chain. Problems of food products safety may be the result of the emergence of risks throughout the entire food chain.

The main hazards which threatened food and feed, whose use can cause damage to health or life, include threats microbiological, chemical, physical and adulteration of food. Microbiological pollution may naturally occur in the raw materials, may also appear as a secondary pollutant from the external environment. In developed countries, the major problem is the microorganism psychrophiles, yeasts and moulds. While in poor countries the problem main concerns of food contamination through pollution transmitted by rodents and other animals. Despite the use of modern technology and storage of food microbiological pollution still pose a significant problem difficult to take full control.

The main cause of chemical hazards in food is far from substantial contamination of the environment (including air, water and soil). Chemical impurities are characterized by the fact that for a long time are able to accumulate in the human body without any symptoms. Emerging symptoms are difficult to identify from the action of individual chemical substance. These substances include: heavy metals, dioxins and furans, residues of veterinary drugs, pesticides, polychlorinated biphenyls. Potentially hazardous compounds are also migrating from materials and articles intended to come into contact with food.

The rest of mentioned hazards are substantially more dependent on the proper handling of food and materials used in the process of its production.

Presented monograph included issues associated with the systems of designing consumers and producers awareness in the field of food safety. Introducing also selected items related to analysis of chemical, toxicological and microbiological hazards occurring in the production of food.

Grażyna Krasnowska

Anna M. Salejda

Wrocław, 2011

CHAPTER 1

REQUIREMENTS AND STANDARTS IN FOOD SAFETY ASSURANCE

1

THE IMPORTANCE OF LEGAL REUIREMENTS AND STANDARDS OF FOOD SAFETY MANAGEMENT FOR THE EFFECTIVENES OF THE TRACEABILITY SYSTEM

Introduction

Legal requirements on food safety oblige the companies to implement the rules of the HACCP system, whose main aim is to identify and analyse risks as well as to prevent their occurrence or minimise their influence. When building a food safety management system, companies must above all meet the requirements of the food law, but the system can also be based on more specific requirements described in such standards as ISO 22000:2005, IFS or BRC. A number of incidents which took place in the 1990s and in the last decade of this century only confirmed the significance of problems related to ensuring food safety e.g. the crisis related to BSE (Bovine Spongiform Encephalopathy), meat contaminated with dioxins, food contaminated with the pathogens of *Salmonella* and *Listeria monocytogenes*, or milk contaminated with melamine [Jonge et al. 2008, Kijowski and Cegielska-Radziejewska 2008, Shackell 2008, Górna 2009]. Requirements related to food safety in the light of the above mentioned and still occurring incidents impose a need to implement a traceability system and restore customer confidence in the safety of foods, its ingredients and in its quality. This obligation is imposed upon food operators [Kher et al. 2010]. Food safety and its quality are affected by growing consumer demands, strategies in the industry and any initiatives taken by the authorities. Diversification of the food processing industry together with the social policy which is carried out contribute to the fact that, in different countries, traceability has various criteria of requirements concerning the amount of information which is vital to ensure food safety [Hobbs et al. 2005]. Food safety can be ensured only when at all stages in the food chain, full traceability of raw materials, semi-finished products and processes is guaranteed. When one thinks about the notion of traceability at the level of an organisation, internal and external traceability have to be taken into consideration and their effectiveness needs to be assessed.

Target and methodology of the research

The aim of the following paper is to present the influence of legal requirements and food safety management standard requirements on the effectiveness of the traceability system and to demonstrate that food processing companies which take into account food safety management standards requirements in their operations are able to design a more effective traceability system than companies in which the operation of the food safety management system is based merely on legal requirements. For this purpose, the requirements of food law, as well as the requirements of food safety management's standards such as ISO 22000, BRC and IFS in the area of traceability were analysed. In addition to that, the paper presents the results of

a survey conducted in companies in the meat industry as far as identifying the factors affecting the effectiveness of the traceability system is concerned.

Legal requirements on traceability

The obligation to trace the movement and origin of products arises directly from the Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002, which came into force on 1 January 2005, laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Pursuant to requirements of Article 18 of this Regulations the traceability of food, feed and any other substance incorporated into a food or feed shall be established and business entities operating on the market of feed and food have to identify their suppliers. In case of such business entities, information regarding the suppliers of food, feed, food-producing animals or any substance incorporated into a food or feed has to be made available to the competent authorities on demand. Business entities operating within the food chain are also obliged to monitor the other businesses to which their products have been supplied. Under Article 100 of the Act of 25 August 2006 on food and nutrition safety (as amended) any person/entity failing to perform actions with regard to the traceability of food suppliers or consumers which is in defiance of the obligation stipulated in Article 18 of the Regulation (EC) No 178/2002 is subject to the penalty of fine. Neither the Regulation (EC) No 178/2002 nor the Act on the food and nutrition safety together with executive regulations specify what kind of data should be supplied to meet the requirement of identifying the supplier or end user [Taczanowski, 2009]. Moreover, Articles 19 and 20 of the Regulation (EC) No 178/2002 specify the responsibility of business entities operating on the food and feed market for withdrawing the products which are not in compliance with the food and feed safety requirements. Under Article 50 of this Regulation a rapid alert system (RASFF- Rapid Alert System for Food and Feed) was established to inform about direct or indirect risk to human health deriving from food or feed [Górna 2010]. All Member States in the European Union together with the countries belonging to the European Economic Area participate in the RASFF system. The system is also available to third countries and international organisations after they have signed an reciprocal non-disclosure agreement. Within the RASFF system the following kinds of notifications can be distinguished [Commission Regulation (EU) no 16/2011]:

1. Alert notification – means a notification of a risk that requires or may require rapid action in another member country.
2. Information notification – means a notification of a risk that does not require rapid action in another member country.
   2.1. Information notification for follow-up – means an information notification related to a product that is or may be placed on the market in another member country.
   2.2. Information notification for attention – means an information notification related to a product that:
      – is present only in the notifying member country; or
      – has not been placed on the market; or
      – is no longer on the market.
3. Border rejection notification – means a notification of a rejection of a batch, container or cargo of food or feed as referred to in Article 50(3)(c) of Regulation (EC)

No 178/200 Original notification – means an alert notification, an information notification or a border rejection notification.

4. Follow-up notification – means a notification that contains additional information in relation to an original notification.

Pursuant to Article 103 of the Act of 8 January 2010 amending the Act on food and nutrition safety and some other acts, whoever does not withdraw from the market a foodstuff posing a risk to human health or life, spoiled foodstuff and adulterated foodstuff is subject to financial penalty which can be imposed up to the five-fold gross value of the questioned quantity of the foodstuff or product which is not food but has been introduced into the market as food. As it can be seen above, food operators are obliged to notify any relevant parties and withdraw from the market any food which does not ensure food safety. However, these requirements do not include any guidelines stimulating the companies to take any actions as far as designing an effective traceability system is concerned.

Requirements of food safety management standards regarding traceability

Food safety management standards, such as ISO 22000 standard, BRC **(**British Retail Consortium, *BRC Global Standard for Food Safety)* **[**The BRC Global Standard for Food Safety, 2008] or IFS [International Food Standard, 2007] define some specific requirements concerning the traceability system.

Clause 7.9 "Traceability system" of the ISO 22000 standard [PN-EN ISO 22000:2006] defines the requirements according to which an organisation should implement a traceability system which enables the company to identify product lots and to match them with the batches of raw materials as well as with records related to product and material delivery and processing. In addition to that, a traceability system should help to identify and trace materials from immediate suppliers and trace the beginning of the distribution route for the final product. In such a system, records concerning traceability shall be maintained in order to assess the systems, as well as to handle potentially hazardous products. Such records shall be also maintained in the case of withdrawing a product from the market. The standard also imposes an obligation to initiate actions related to withdrawing a lot of the final product from the market once it has been identified as hazardous (see clause 7.10.4). Such actions must be recorded and in addition to that the organisation should verify the effectiveness of the withdrawal procedure by using an appropriate technique. Conducting such actions is not possible without an effectively planned and implemented system of external and internal traceability. Moreover, rules regarding the creation of a traceability system are specified in the PN-EN ISO 22005:2007 standard. The guidelines of this standard may serve as a tool for improving the traceability system operating in the organisation. These rules specify that such a system should be:

- verifiable,
- result-oriented,
- economical,
- used in a coherent and unbiased way,
- practical in use,
- in compliance with requirements or policy,
- in compliance with specified required level of accuracy.

In the BRC standard, clause 3.9 "Traceability" was classified as a ‘fundamental’ one. If the requirements of this clause are not met when the system is audited for compliance with

the said standard, such case is identified as critical non-compliance. The organisation shall have a system to identify and trace product lots and follow them through all raw materials, all stages of processing and the distribution of the finished product to the customer. The traceability system shall ensure traceability from the stage of raw material to the final product and the other way round. What is more, quantity test should be taken into account here and a mass balance should be conducted. Apart from this, pursuant to the requirements of this standard, primary materials and any other packaging materials as well as auxiliary materials used in processing need to be identified and traced. Traceability must be also ensured if the product is modified or any modification action is carried out. It is crucial that the organisation can show that the performed actions do not affect the safety or legal status of the final product, e.g. its declared composition, information regarding allergies nor change the identity. In addition to that clause 3.11 "Management of incidents, product withdrawal and product recalls" determines specific rules for effective management of incidents and potential emergency situations and imposes the obligation to implement a withdrawal and recall procedure upon the organisation. According to the standard, the procedure for product withdrawal and recall shall be tested regularly (at least once a year).

The IFS standard in section 4.16 "Traceability" determines the obligation of an organisation to have a traceability system in place which shall enable it to identify product lots, determine the relation with batches of raw materials and packaging materials. The traceability system shall also entail records of the realisation of production processes (including modification) and distribution. Section 4.16.2 of the IFS standard imposes on the organisation an obligation to test the effectiveness of the traceability system used in two directions – from the final product to the raw material and the other way round, together with conducting a mass balance. Section 5.9 of this standard specifies the rules for management of incidents, product withdrawal and product recall. In this standard the organisation should establish a procedure for product withdrawal, which, as it was the case with the BRC and ISO 22000 standard has to undergo regular internal tests.

The influence of legal requirements and on the effectiveness of traceability

When legal requirements and the requirements of standards with regard to traceability are analysed, an essential difference between them can be seen. Taking into consideration the effectiveness of traceability systems, the requirements of standards are more rigorous in this respect than legal requirements. In the said standards apart from external traceability (first supplier and first end user) also stress the need to use an internal traceability system. They specify the requirement to identify the descriptors related to traceability at different stages of the production process (as well as in case of modifications/corrections). A very important requirement is the need to test the effectiveness of the traceability system taking into consideration the mass balance (BRC standard). As a rule the effectiveness of the traceability system is verified when the procedure of withdrawing a product from the market is tested. The obligation to perform this kind of test is specified in all food safety standards discussed in this paper. On the other hand, legal requirements oblige companies to identify the first supplier and first customer, whereas the standards oblige the companies to keep record helping to identify the processes/production activities and post-production activities which the raw material and later, the product, was subject to. In the standards, there is an obligation to establish and maintain an internal traceability system, which is not the case with legal requirements.

Summing up, legal requirements oblige food operators to trace the supplier of raw materials and the end user of products. In other words, food operators are obliged to maintain an external traceability system. However, when the effectiveness of a traceability system in general is concerned, the internal traceability system should not be disregarded. In other words, legal requirements are not a significant factor motivating the companies to design an effective internal traceability system. An effective traceability system itself is not sufficient to achieve food safety. However, it is a very significant tool which helps the organisation to realise the food safety aims specified by it. Legal regulations do not outline any specific methodology which could be accepted by all operators in the food chain to build a traceability system. Instead of this, companies are free to choose the mechanisms they use and thanks to which they can ensure an effective traceability system [Folinas et al. 2006]. Such mechanisms can be found in food safety management standards, such as, among others, the ISO 22000 standard, BRC or IFS in which specific requirements regarding the traceability system were defined.

Factors influencing the effectiveness of the internal traceability system

The survey which was conducted on the sample of 180 companies representing the meat industry in Poland allowed to distinguish factors influencing the effectiveness of the internal traceability system. The respondents were given the task of ranking a number of factors according to their significance from 1 to 5, where 1 stands for the least significant factor and 5 for the most significant one. The following factors were indicated by the respondents as definitely significant (Tab. 1):

- correct identification of production batch (70.7%),
- clearly specified rules for the traceability of raw materials/products/processes (65.9%),
- top management awareness (65%),
- employee awareness (59.5%),
- clearly specified rules of the traceability system (59.5%).

The average grades for the above mentioned factors amounted ranged from 4.40 to 4.61 and the value of standard deviation (from 0.71 to 0.85) shows that the diversity in the opinions of the respondents is not high.

The respondents consider correct identification of production batch as one of the most significant factors influencing the effectiveness of the traceability system. When identifying the production batch the company should take into consideration the fact that a product batch/lot is a set of product units which was produced and/or processed or packaged in similar conditions. Whereas, the lot should be specified by means of parameters previously determined by the organisation and the set of units can be limited to a single product unit [PN-EN ISO 22005:2007]. The rules for raw material/product/process traceability, as well as the rules for the traceability system itself which are clearly specified are also significant according to the respondents. However, clearly specified criteria for traceability and traceability system shall not be enough if there is no top management and employee awareness on the significance of their actions as far as ensuring the effectiveness of the traceability system is concerned.

Table 1

Factors influencing the effectiveness of the internal traceability system

Factors	Definitely insignificant	Rather insignificant	Of average significance	Rather significant	Definitely significant	In total
employee awareness	0.0	0.0	11.9	28.6	59.5	100
clearly specified rules for the traceability of raw materials/products/processes	0.0	2.4	17.1	14.6	65.9	100
clearly specified rules of the traceability system	0.0	0.0	19.0	21.4	59.5	100
frequent training of employees on traceability	2.5	0.0	30.0	42.5	25.0	100
financial resources of the company	13.2	15.8	36.8	26.3	7.9	100
frequent testing of the traceability system	2.4	19.5	29.3	31.7	17.1	100
evaluating the effectiveness of performed corrective actions in order to manage non-compliance related to the implemented traceability system	0.0	7.5	27.5	50.0	15.0	100
evaluating the effectiveness of implemented preventive actions	0.0	5.0	25.0	55.0	15.0	100
records of process/parameter monitoring	2.5	5.0	12.5	32.5	47.5	100
traceability system audits	4.9	9.8	17.1	39.0	29.3	100
the time for which the records of process/parameter monitoring are stored	7.3	9.8	26.8	26.8	29.3	100
correct identification of production batch	0.0	2.4	4.9	22.0	70.7	100
implementing an IT system in the area from obtaining the raw material, through the production process to distribution	5.1	5.1	15.4	28.2	46.2	100
the ability to determine how different batches of the same raw material are combined in a lot of the final product	2.4	12.2	19.5	24.4	41.5	100
top management awareness	0.0	5.0	7.5	22.5	65.0	100
technical way of identifying the raw materials/products	2.6	2.6	12.8	48.7	33.3	100

Source: Own research

When the obtained results are analysed according to the level of significance at the value from 4 to 5 (rather significant to definitely significant), the ranking of factors significant for the effectiveness of the traceability system as assessed by the meat industry companies is the following:

(1) correct identification of production batch (92.7%),

(2) employee awareness (88.1%),

(3) top management awareness (87.5%),

(4) technical way of identifying the raw materials/products (82%),
(5) clearly specified rules of the traceability system (80.9%).
(6) clearly specified rules for the traceability of raw materials/products/processes (80.5%),
(7) records of process/parameter monitoring (80%).

For the rest of factors the percentage of answers was at the level from 34.2 to 74.4%. The smallest percentage of answers ranked from 4 to 5 scale bracket was given to such factors, as: frequent testing of the traceability system (48.8%) and financial resources of the company (34.2%).

Summary

Requirements of the food law oblige food operators to implement external traceability systems, whereas standard requirements define specific requirements both for external and internal traceability. On the other hand, companies in which the function of the system responsible for food safety is only based on legal requirements, are not stimulated to apply a wider spectrum of actions aimed at improving safety in the area of traceability. Such stimulating mechanisms can be found in food safety management standards, such as, the ISO 22 000 and 22 005 standards, BRC or IFS in which specific requirements regarding the traceability system were defined.

The survey conducted among meat industry companies helped to distinguish a group of factors significantly influencing the effectiveness of an internal traceability system, which were for example correct identification of the production lot, clearly specified rules of the identification and traceability system and awareness of the management and employees. Only full awareness of the management and of the employees of the need to use a system for internal and external traceability can guarantee that the system will be effective.

To recap, the effectiveness of the traceability system is more significantly affected by the requirements of food safety standards than by legal requirements. The most significant factors influencing the effectiveness of such system are correct identification of the product lot and management and employee awareness.

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References

Folinas D., Manikas, I. Manos B., 2006, Traceability data management for food chains, British Food Journal, 108, 622–633.

Górna J., 2009, Konkurencyjność przedsiębiorstw przemysłu mięsnego i mleczarskiego na terenie Wielkopolski w aspekcie spełnienia wymagań prawa żywnościowego Unii Europejskiej [Competitiveness of the meat and dairy industry companies in the Wielkopolska region as far as meeting the requirements of EU food law is concerned], [in:] Problemy rolnictwa światowego [Problems of world agriculture], Zeszyty Naukowe SGGW, 7 (XXII), Wyd. SGGW, Warszawa.

Górna J., 2010. Realizacja wymagań prawnych w zakresie identyfikowalności a wymagania normy ISO 22000 oraz ISO 22005 – studium przypadku [Realisation of legal requirements on traceability and ISO 22000 and 22005 standards requirements – case study], [in:] Jakość i bezpieczeństwo produktu oraz ochrona środowiska w sektorze rolno-spożywczym [Quality, product safety and environmental protection in the farm and food industry], Prace i Materiały Wydziału Zarządzania Uniwersytetu Gdańskiego 2/1, Sopot.

Hobbs J.E., Bailey D.V., Dickinson, D.L., Haghiri M., 2005. Traceability in the Canadian Red Meat Sector: Do Consumers Care?, Canadian J. Agric. Econ., 53, 47–65,

International Food Standard, 2007. Issue 5, HDE Trade Services, Berlin.

Jonge J., Trijp H., Goddard E., Frewer L., 2008. Consumer confidence in the safety of food in Canada and the Netherlands: The validation of a generic framework, Food Quality and Preference, 19, 439–451.

Kher S.V., Frezer L.J., De Jonge J., Wentholt M., Howell Davies O., Luijckx N.B.L., Cnossen H.J., 2010. Experts' perspectives on the implementation of traceability in Europe, British Food Journal, 112, 3.

Kijowski J., Cegielska-Radziejewska R., 2008. Kontrola zagrożeń żywności auditowanym i certyfikowanym systemem ISO 22000/ HACCP [Food hazards control by an audited and certified ISO 22000/HACCP system], Wyd. UP w Poznaniu, Poznań.

PN-EN ISO 22000, 2006. Systemy zarządzania bezpieczeństwem żywności. Wymagania dla każdej organizacji należącej do łańcucha żywnościowego [Food safety management systems. Requirements for all organisations in the food chain], PKN, Warszawa.

PN-EN ISO 22005, 2007. Identyfikowalność w łańcuchu pasz i żywności. Ogólne zasady i podstawowe wymagania przy projektowaniu i wdrażaniu system [Traceability in the food and feed chain. General rules and basic requirements for designing and implementing the system], PKN, Warszawa.

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

Commission Regulation (EU) No 16/2011 of 10 January 2011 laying down implementing measures for the Rapid alert system for food and feed.

Shackell G.,H., 2008. Traceability in the meat industry – the farm to plate continuum, J. of Food Sci. and Tech., 43, 2134–2142.

Taczanowski M., 2009. Prawo żywnościowe w warunkach członkostwa Polski w Unii Europejskiej [Food law in the conditions of Poland's membership in the EU], Wydawnictwo Wolters Kluwer Polska, Warszawa.

The BRC Global Standard for Food Safety, 2008, Issue 5, British Retail Consortium, London.

Act of 25 August 2006 on food and nutrition safety, Dz.U. (Polish Journal of Laws) No 171(1225) (as amended).

Act of 8 January 2010 amending the Act on foody and nutrition safety and some other acts Dz.U. (Polish Journal of Laws) No 21(105).

2

BENEFITS OF SAFETY SYSTEMS IMPLEMENTATION IN PRODUCTION AND DISTRIBUTION OF FOOD

Introduction

Food safety is of paramount importance not only for consumers and food industry, but also for economics. Disclosure of food scandals automatically causes a drop in consumption of the product, loss of consumer confidence and financial losses, which in recent years and even months, took place in Europe. The health safety of food corresponds to the producer in manufacturing plants or the person who places the product on sale in stores. Food safety is ensured, if the processing plants and grocery stores have implemented and applied the principles of Good Manufacturing Practice, Good Hygiene Practice and HACCP system. Implementation of these systems is a legal obligation arising from EC Regulation 852/2004. Food business operators must ensure health safety of food.

The question is whether compliance with legal requirements actually affect the safety of foods in the food production and distribution, and that allows users to reach other benefits. In the literature there are few findings concerning the benefits after the implementation of the HACCP system. The application of the HACCP system in Poland is the new issue and still controversial. Therefore, knowledge of the benefits achieved by the food enterprises and shops after the implementation of the safety system, can be proof of the correctness of system implementation by the national food industry and distribution.

Furthermore, the implementation and application of HACCP and GMP/GHP is a prerequisite to adapt Polish food businesses to the requirements of the European Union. It is important that the system was used both in food plants and grocery stores. The literature data shows that lack of good practices and HACCP system in all stages of food chain, e.g. in shops, was in the past, the most important factor for many diseases from food sources in the United States [Ehiri and Morris 1996, Howes et al. 1996]. Regulation (EC) No 178/2002 is the framework constitution of the European Union food law. The Regulation applies to all stages of food production and distribution, thus covering the full chain "from farm to fork", and is therefore relevant to the entire area of the food economy and affects all operators in the production and marketing of food and services in the food chain in the European Union.

Purpose and methods of research and the characteristics of survey respondents

The aim of the research conducted by the authors was to analyze and assess the degree of safety system implementation and benefits of implementing mandatory methods and systems to ensure the health safety of food in grocery stores and processing plants. The study was conducted using two different questionnaires. Research survey was conducted in food

processing plants in 2005, while research in the stores in 2009. Questionnaire sent to food plants included 17 open and closed questions concerning the degree of implementation of the HACCP system, the difficulties in his introduction to the plant and the benefits after implementation. The questionnaire sent to grocery stores included 56 open and closed questions about the degree of implementation of the safety system, system documentation, difficulties and benefits after the implementation of the system, issues relating to Good Hygiene Practice and questions to verify their workers knowledge of GMP/GHP. Five hundred factories and five hundred grocery stores were tested. Processing plants were represented by 250 medium and 250 small enterprises from 13 different branches according to the directory "Agribusiness in Poland" [Agrobazar-Multipress, 2001] – Table 1. Grocery stores were represented by shops of all sizes, as shown in Table 2.

Table 1

Characteristics of the study food processing enterprises

Total number			
		N	[%]
Together		304	100.0
Small		131	43.1
Medium		173	56.9
The number of enterprises			
		N	[%]
Bakery and farinaceous products		25	8.2
Meat products		30	9.9
Poultry products		29	9.5
Fruit and vegetable products		24	7.9
Dairy products		30	9.9
Fish products		24	7.9
Drinks and beverages		24	7.9
Ready-to-eat product		22	7.2
Cereal products		29	9.5
Food components		29	9.5
Oils and fats		14	4.6
Potatoe products		12	3.9
Sugar industry		12	3.9
Location	Country	122	40.1
	Sity	182	59.9

Source: the authors' own studies

Table 2

The percentage of groceries depending on the surface of the shop [m^2]

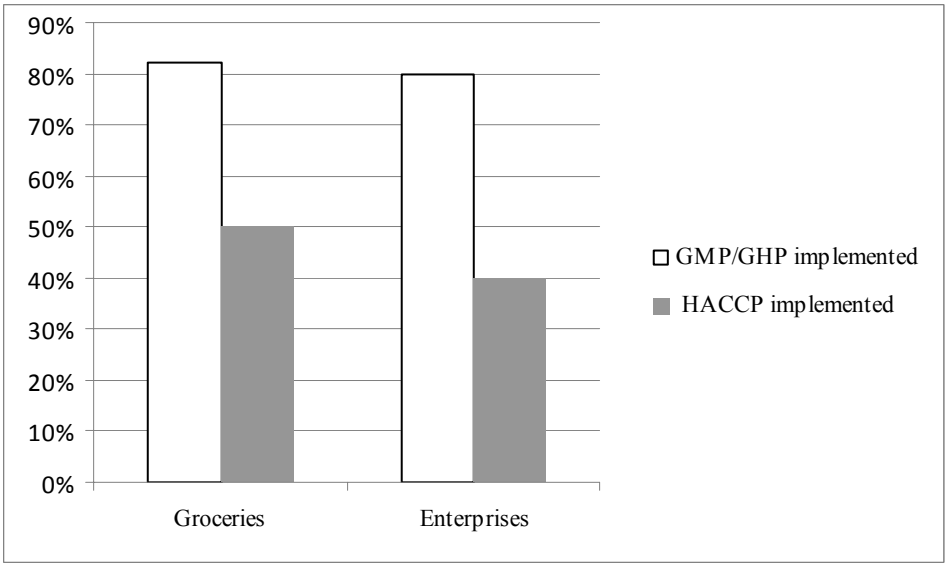
Grocery surface	Percentage of evaluated
< 50	41%
50–100	31.6%
101–200	12%
201–500	9.8%
> 500	5.6%

Source: the authors' own studies

The collected results were statistically analyzed using Statistica package, procedure 6.0 [Hill and Lewicki 2006]. The method consisting in verifying the significance of differences between the two structure indexes (structure index = percentage share/100), the χ^2 test of independence , the φ-Yule factor and cluster analysis.

Selected results

Implementation of the safety system in evaluated food plants and grocery stores is a legal obligation. Although not in all evaluated enterprises and shops food safety system have been implemented (Fig. 1). Research shows that the implementation of HACCP in food shops and processing plants were at a similar level, i.e. about 40–50%, although studies were conducted in plants 4 years earlier than in the shops.



Source: the authors' own studies

Fig. 1. The degree to which Polish food processing plants (in 2005) and Polish groceries (in 2009) have implemented GMP/GHP and HACCP system

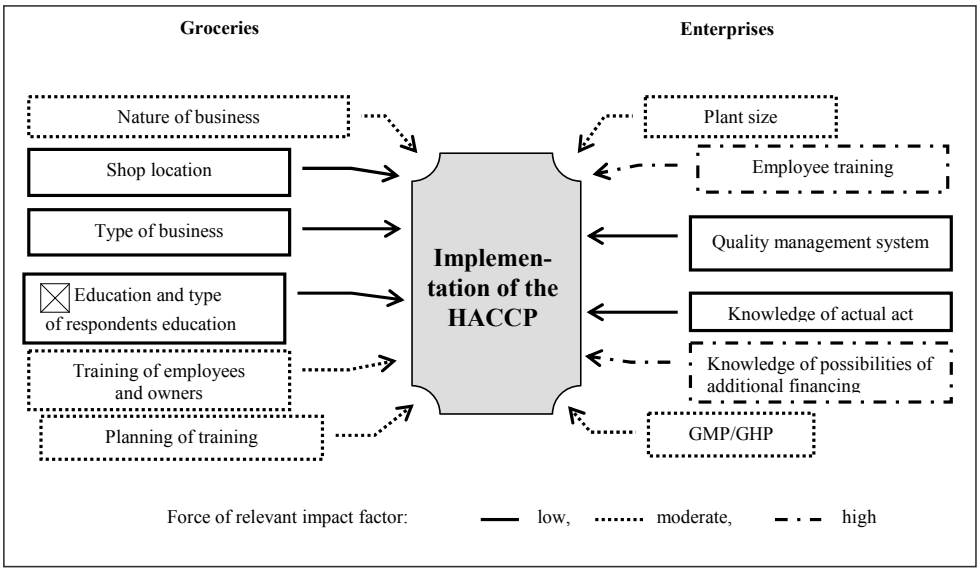
From the literature data shows that in different EU countries, the degree of implementation of mandatory systems is different. Panisello et al. [1999] based on surveys conducted in 1998 in the UK in Yorkshire and Humberside, found that 72.6% of plants had implemented the HACCP system, 15% of enterprises were in the process, and 13% of enterprises did not have a system in place. In Ireland, on the basis of Teagasc (The National Food Centre) [Przegalińska 2003] HACCP system was implemented in 71% of the plants, while in the course of implementation was 15% of enterprises. Based on research conducted at the University of Bonn in Germany, in April 2003, among 300 food industry it was found that the HACCP system was implemented to about 83% of surveyed companies [Beyer and Krieger, 2004]. Surveys conducted in 2004 in Turkey show that the majority of respondents were not even trained in food safety [Bass et al. 2006], hence other studies realized in this country show that only about 16% of enterprises has implemented HACCP system [Bass et al. 2007]. It is worth noting that the food safety systems have been developed in Western countries for 40 years, while in Poland, this process began in the early 90s, hence the system implementation process is not yet ended. To a lesser extent than in Poland, the system implemented food enterprises in China. According to data from 2005, only 23% of food companies have implemented the HACCP system, while the situation is different in the case of grocery stores in China: the system has implemented 71% of foreign shops and only 29% of the national [Jin et al. 2008]. There is no current data on the degree of international implementation of the HACCP system. Most likely it was that, since the system is mandatory in EU and other countries, all companies have implemented it and apply, so there is no need to verify degree of implementation.

In the studied plants and stores the degree of implementation of the HACCP system depend on many factors. One of the factors in the case of processing plant was size of enterprise calculated in terms of employment size. A similar dependence showed Mortlock et al. [1999] and Panisello et al. [1999] in the establishments in England, Azanza and Zamora-Luna [2005] in plants in the Philippines, and in national studies Bernat and Majka [2004], Konecka-Matyjek et al. [2005] and Morkis [2005].

Obtained results showed that in the case of plants, the degree of implementation of the HACCP system was not affected by industry branch or location, but these factors determined the degree of implementation of the system in grocery stores. Both in plants, and stores the important role played training of employees/owners. It is often concluded in the literature that the success of the HACCP system depends on the education and training of production personnel and officers [Vela and Fernández 2003], and that without a high level of training, the desired awareness and commitment of the crew cannot be achieved [Ziajka et al. 2001].

Principles of Good Hygiene and Manufacturing Practices are the prerequisites and should be developed prior to implementation of the HACCP system. Hence the lack of GMP /GHP developed for the specific site is the barrier of implementation of the HACCP system. The effective HACCP system cannot be introduced into the plant, which has serious hygiene neglect [Steinhauser 2004], hence the implementation of GMP/GHP was requirement of the HACCP system implementation in the studied plants.

Helpful in implementing safety system in food production were and still are the various forms of financial support. Knowledge about the possibilities of financing the implementation activities have a significant impact on the degree of implementation of HACCP system in processing plants. Factors influencing the implementation of HACCP in enterprises and stores, having regard to their impact, are shown in Figure 2.



Source: the authors' own studies

Fig. 2. Factors affecting the degree of implementation of HACCP in Polish processing plants and grocery shops

The study shows that both processing plants and shops, saw a lot of benefits after the implementation of the safety system. The benefits of processing plants were analyzed in much broader terms than in grocery stores. They were divided into three categories: benefits for improving health safety (questionnaire question No. 10), nonmaterial benefits (questionnaire question No. 11) and economic benefits (questionnaire question No. 12). In the case of grocery stores, only one question (No. 25) was designed to test the benefits after the implementation of safety system. Summary of questions about the benefits and response options in questionnaires for factories and shops are presented in Table 3.

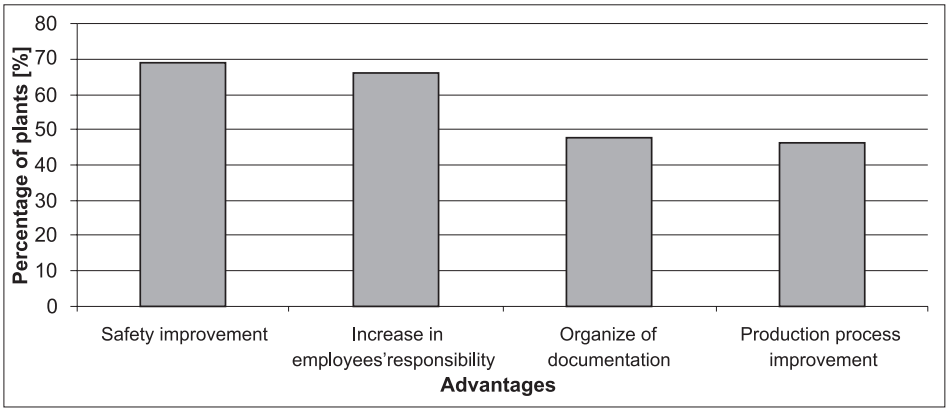
In processing plants among the benefits of health safety of the most importance was to improve the safety of products (70%) and increase of accountability of employees (66%). Among the nonmaterial benefits the most important was to increase the prestige of the company (78%), while the stay in business (60%) and gaining new customers in the country (58%) were pointed as economic benefits. Other benefits indicated in processing plants are shown in Figures 3–5. In the questionnaire survey plants could make multiple selections and it is must be noted that most of the benefits were indicated by about 50% of the evaluated enterprises.

Table 3

Analyzed benefits after implementation of the safety system in food processing enterprises and groceries

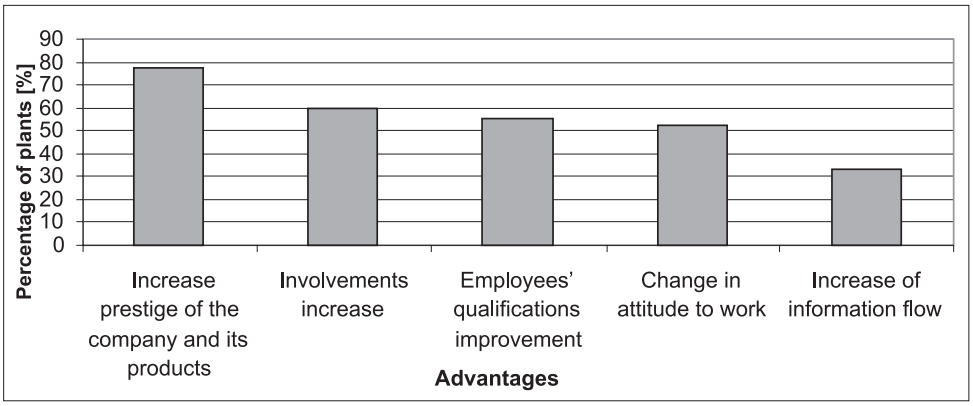
Questions No	Questions	Response options
Enterprises		
10	What advantages concerning improvement in food safety you expect after HACCP implementation?	– Safety improvement – Production process improvement – Organize of documentation – Increase in employees'responsibility for production hygiene
11	What non-material advantages you expect after HACCP implementation?	– Increase prestige of the company and its products – Change in attitude to work – Involvements increase – Employees' qualifications improvement – Increase of information flow
12	What economic and material advantages you expect or observe after HACCP implementation?	– Demand increase – Product competitiveness growth – Maintenance of market position – Acquisitions of clients at home – Sales expansion abroad – Fewer complaints – Eemployment rightsizing
Groceries		
25	What benefits you can see after the implementation of safety systems (GMP/GHP, system HACCP)?	– Increase competitiveness – Demand increase – Organize of documentation – Fulfillment of legal requirements – Safety improvement – Improving conditions and quality of work – No benefits

Source: the authors' own studies



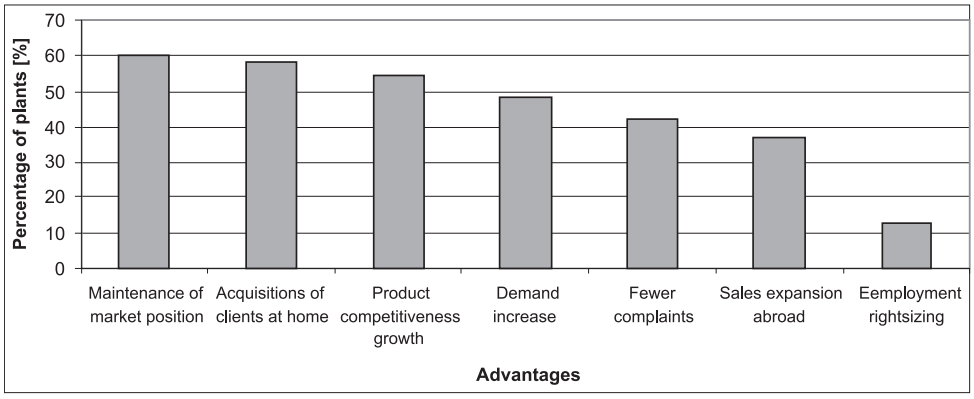
Source: the authors' own studies

Fig. 3. Advantages concerning health safety improvement following the implementation of the HACCP system in Polish enterprises



Source: the authors' own studies

Fig. 4. Declared non-material advantages following the implementation of the HACCP system in Polish enterprises



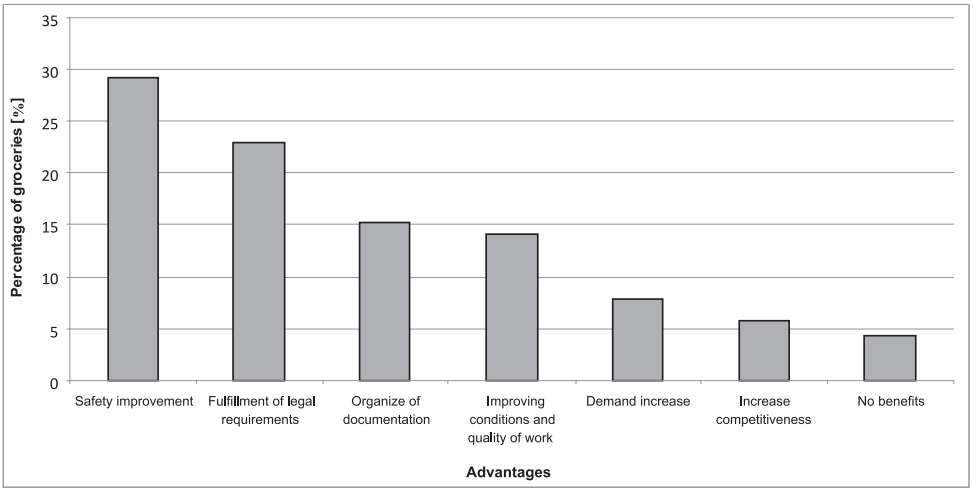
Source: the authors' own studies

Fig. 5. Declared economic advantages following the implementation of the HACCP system in Polish enterprises

Based on studies carried out in the shops, it was found that the most important benefit resulting from the safety system implementation, claimed by over 29% of grocery stores, was to improve food safety. No benefits were declared only in about 4% of stores (Fig. 6).

Comparing the benefits declared after the implementation of HACCP system in shops and plants should be noted that manufacturing plants and grocery stores differently perceive the positive aspects of system implementation. The main difference relates to food safety – the most important from the point of view of HACCP idea. Safety is usually declared as the benefit of safety system implementation, both in grocery stores and processing plants. However, in the case of shops much less of the respondents, i.e. about 30%, showed this advantage. However until about 70% of processing plants cited the improvement of food safety as the main benefit after the implementation of the system. Differences in perception of the benefits can be due to different characteristics of the enterprises and the various determinants

of achievement. In the case of plants, important factor was to train staff and implement the principles of GMP/GHP. Conducting training was important for grocery stores too, but other factors as the type and nature of the business, shop location, education, and training schedules of the respondents, were also important.

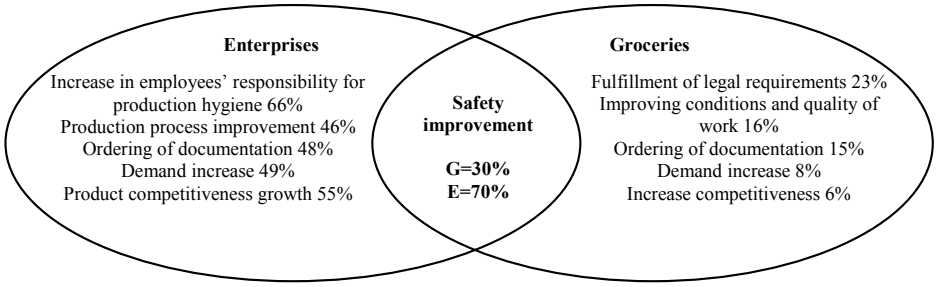


Source: the authors' own studies

Fig. 6. Declared advantages following the implementation of the HACCP system in Polish groceries

In the case of shops, the opinion of employees and managers on the impact of HACCP on the health safety of marketed products, was examined. Research shows that more workers reported that the implementation of the HACCP system helps to ensure food safety and organization of work in the shops (about 68%) than managers (ok.49%), and far fewer workers believed that the implementation affects the deterioration of food safety and organization of work in the stores (approximately 3.5% of staff, management staff – about 13%).

Comparison of selected benefits in shops and factories are shown in Figure 7.



Source: the authors' own studies

Fig. 7. Comparison of selected benefits in enterprises (E) and groceries (G) after the implementation of mandatory health safety assurance systems (GMP/GHP & HACCP)

Calculations carried out by cluster analysis showed which groups of analyzed enterprises and shops have noticed the most improvement of health safety by implementing a system. Only hypermarkets located in the city declared the achievement of such benefit in the most degree (approx. 64%). However, all medium-sized enterprises and small animal origin plants declared achievement of the benefit in about 50–70%. Only small plant origin enterprises and food branches such as oil, sugar, potato and ready-to-eat food, noticed safety improvement by implementing a system to a much lesser extent (30–50%).

Safety improvement as a benefit after the implementation of the system has also been shown in other national studies [Borusiewicz and Sikora, 2007, Mokrosińskia and Malenta 2008] and in foreign studies: English [Panisello et al. 1999], Finnish [Hielm et al. 2006], American [Kvenberg et al. 2000] and was even considered in terms of international trade [Motarjemi et al. 1996]. Improving of health safety is associated with a reduction of number of food-related illnesses – which is difficult to estimate [Unnevehr and Jansen 1999] and to guarantee food production without the health risks [Bernat and Krupa 2004].

It should be pointed that, in grocery stores, another declared benefit is to meet the requirements of food law, which clearly indicates that HACCP system is not perceived as a necessary tool to guarantee the quality of food in this sector. In addition, by about 40% of the surveyed stores, system does not change anything in the sphere of food safety and organization of work in the shop.

Research shows that the case of system documentation, which according to the seventh HACCP principle must be conducted and maintained, is viewed very differently in shops and processing plants. In grocery stores, only about 15% of respondents expressed the view that the implementation of the system affected the arrangement of the documentation. Contrast to approximately 50% of SMEs have noted the positive impact of the system implementation for plant documentation.

The available literature data also demonstrated many other benefits of implementing HACCP system as: providing evidence of product safety and ensure the safety and reproducibility of customer trust [Panisello et al. 1999], reducing the number of microbiological hazards in the product and extend shelf life [Henson et al. 1999], improving consumer confidence and preventing food poisoning [Bas et al. 2007], customer satisfaction [Panisello et al. 1999, Beyer and Krieger 2004], confidence in their products [Panisello et al. 1999] and increase of employee satisfaction [Beyer and Krieger 2004].

Application of the HACCP system reduces the costs of all food chain participants: less research, fewer accidents, fewer complaints, satisfied customers, less of the losses at each stage [Bernat and Krupa 2004], which initiates a series of economic benefits. HACCP prevents the loss of reputation and provides an opportunity to remain on the market [Suwanrangsi 2000], which is confirmed by the results of its own production facilities. Over 60% of the surveyed companies acknowledged that maintaining the market is the most important economic advantage.

Another declared benefit of an economic nature was increasing of the competitiveness of products, which was declared by 55% of manufacturing plants and only 6% of grocery stores. Differences in perception of the position of this benefit may be explained by the fact that processing plants compete to get customers (shops and wholesalers). In the case of competition it is an important task to provide a safe product with consistent quality This will allow plants effectively winning products of EU member states in international markets. But for shops competitiveness through the implementation of the system does not play a significant role.

Respondents from groceries were asked in questionnaire whether possession of the HACCP system is important for their consumers. According to over 60% of shops respondents customers do not pay attention to the ownership of the implemented HACCP system. In another question (question No 31) they were asked about the impact of the implementation of the system on the course of official controls. Approximately 30% of surveyed shops expressed the view that the system will simplify the official control of food and 20% thought that will affect the efficiency of control.

Conclusions

1. Implementation of the safety system in food processing plants and stores, in addition to meeting mandatory legal requirements is resulting in positive consequences for the current operation of the enterprises, such as: the guarantee of food safety, increase accountability of employees, improving the production process, increase competitiveness, organize documentation and fulfilment of the requirements of food law.
2. Food processing plants and other grocery stores see the benefits after the implementation of the safety system. Major differences in the perception of the benefits relate to food safety, competitiveness, and system documentation.

References

Agrobazar-Multipress, 2001. Katalog Agrobiznes in Poland. Published by Agrobazar-Multipress, Warszawa.

Azanza Ma. P.V., Zamora-Luna M.B.V., 2005. Barriers of HACCP team members to guideline adherence. Food Control, 16, 1, 15–22.

Bas M., Ersun A.S., Kivanç G., 2006. The evaluation of food hygiene knowledge, attitudes, and practices of food handlers in food businesses in Turkey, Food Control, 17, 317–320.

Bas M., Yüksel M., Cavusoglu T., 2007. Difficulties and barriers for the implementing of HACCP and food safety systems in food businesses in Turkey. Food Control, 18, 2, 124–130.

Bernat E., Krupa K., 2004. The owner's surveillance and production quality in the meat industry. Sikora T. [edd.] Quality tools in quality improvement and management. Published by AE Kraków, 142–150.

Bernat E., Majka A., 2004. The HACCP system in meat industry enterprises. Sikora T. [edd.]. Quality tools in quality improvement and management. Published by AE Kraków, 151–155.

Beyer J., Krieger S., 2004. Kunden und Mitarbeiter profitieren. Fleischwirtschaft, 84, 6, 59–60.

Borusiewicz A., Sikora T., 2007. Motives and barriers to implementation of HACCP in the opinion of staff of the selected company. Przem. Ferment. i Owoc.-Warzyw, 9, 28–30.

Ehiri J.E., Morris G.P., 1996. Hygiene training and education of food handlers: Does it work? Ecology of Food and Nutrition, 35, 243–251.

Henson S., Holt G., Northen J., 1999. Costs and benefits of implementing HACCP in the UK dairy processing sector. Food Control, 10, 2, 99–106.

Hielm S., Tuominen P., Aarnisalo K., Raaska L., Maijala R., 2006. Attitudes torwards own-checking and HACCP plans among Finnish food industry employees. Food Control, 17, 5, 402–407.

Hill T., Lewicki P., 2006. Statistics Methods and Applications. StatSoft, Tulsa.

Howes M., McEwen S., Griffiths M., Harris L., 1996. Food handler certification by home study: Measuring changes in knowledge and behaviour. Dairy, Food and Environmental Sanitation, 16, 737–744.
Jin S., Zhou J., Ye J., 2008. Adoption of HACCP system in the Chinese food industry: A comparative analysis. Food Control, 19, 8, 823–828.
Konecka-Matyjek, Turlejska H., Pelzner U., Szponar L., 2005. Actual situation in the area of implementing quality assurance systems GMP, GHP and HACCP in Polish food production and processing plants. Food Control, 16, 1, 1–9.
Kvenberg J., Stolfa P., Stringfellow D., Garrett E.S., 2000. HACCP development and regulatory assessment in the United States of America. Food Control, 11, 5, 387–401.
Mokrosińska K., Malenta B., 2008. Checking system – part. III. Patisserie and Baking, 109, 11, 96–98.
Morkis G., 2005. Quality management systems in food industry enterprises. A multiple-year program 2005–2006 – Economic and social conditions of development of Polish food economy following Poland's accession to the European Union, Published by IERiGŻ.
Mortlock M.P., Peters A.C., Griffith C., 1999. Food hygiene and hazard analysis critical control point in the United Kingdom food industry: practices, perceptions and attitudes. Journal of Food Protection, 62, 7, 786–792.
Motarjemi Y., Käferstein F., Moy G., Miyagawa S., Miyagishima K., 1996. Importance of HACCP for public health and development. The role of the Word Health Organization. Food Control, 7, 2, 77–85.
Panisello P.J., Quantick P.Ch., Knowles M.J., 1999. Torwards the implementation of HACCP: results of a UK regional survey. Food Control, 10, 2, 87–98.
Przegalińska M.J., 2003. HACCP w sejmie. Przem. Ferment. i Owocowo-Warzywny, 47, 9, 30–31.
Steinhauser L., 2004. Implementation of HACCP in the meat industry in new EU member states. International Conference in Athens 12–14.05.2004 Farm to Fork Food Safe.
Suwanrangsi S., 2000. HACCP implementation in the Tai fisheries industry. Food Control, 11, 5, 377–382.
Unnevehr L.J., Jensen H.H., 1999. The economic implications of using HACCP as a food safety regulatory standard. Food Policy, 24, 6, 625–635.
Vela A.R., Fernández J.M., 2003. Barriers for the developing and implementation of HACCP plans: results from a Spanish regional survey. Food Control, 14, 5, 333–337.
Ziajka S., Dzwolak W., Cybulski A., Tarczyńska A., 2001. Conditions of implementation and operation of the HACCP system. Przem. Spoż., 55, 4, 12–16, 26.

3

FOOD SAFETY AND FOOD CONTROL SYSTEM IN THE SLOVAK REPUBLIC IN 2010

Introduction

Food safety in the Slovak Republic is guaranteed by legislation, official food control systems and is included in the National Plan of official control of foodstuffs. In 2010, were in line with the national control plan provided for the number of inspections (frequency and intensity of controls) and number of samples on the basis of risk assessment.

In 2010 were carried out 107.190 inspections, most inspections were carried out in the retail sector and producers and packers. Shortcomings were found in 11.590 from 38.005 objects controlled, which is 30.50% of objects. Totally was carried out 107.190 inspections. Most deficiencies were detected in overall hygiene – in 8.128 objects, which is 51.01%. Weaknesses were also identified in the application of HACCP system in 3.761 objects, which is 32.45% in food labeling in 2.853 objects, which is 24.62% and sales of end-consumption / minimum durability was established in 3.566 objects, which is 30.78%.

In terms of individual sectors was most deficiencies identified in the retail trade up to 5.873 objects, which is 50.67% in the services sector for 3.727 objects, which is 32.16%.

Overall, in 2010, official control authorities collected 36.677 samples, of which 1.722 samples were unsatisfactory, which is 4.7%, with most samples did not comply with the microbiological point of view, labeling and physicochemical properties. In 2010 it was examined at 38.005 objects, which in 3.761 the facility was disagreement in the application of good manufacturing practice. Most breaches were identified in the service sector in 1.321 buildings and retail premises in 1.243. The highest number of deficiencies were found in the retail sector, where the 15.932 examined at objects to the 8.128 objects were found in the application of non-compliance with hygiene establishments.

Official inspection of foodstuff

The Official controls of foodstuffs have been made by the authorities of the State Veterinary and Food Administration (SVFA) of the Slovak Republic (SR) in 2010 according to official controls on multi-annual plan drawn up on the years 2007–2011 (updated for the year 2010) in accordance with Commission Decision 2007/363/EC, and to ensure a high level of human health and consumer interests throughout the food chain from primary production to retail. Official food control is carried out according to Law of the National Council No.152/1995 and according to Law of the National Council No. 39/2007 in accordance with Regulation (EC) No 882/2004 and Regulation (EC) No. 854/2004.

The main objective of official controls on products of animal origin was verification of compliance with legislation in this area. Priority control activity SVFA in food in 2010

followed the guidelines defined in the multi-annual official food control in SR 2007–2011 to ensure a high level of human health and consumer interests throughout the food chain, from primary producers to retail. The main task of official controls has been to ensure the elimination of defects in foods produced in our country and also efficient enough to prevent entry into hazardous food imported from third countries so as to ensure the health of EU consumers. Great emphasis was placed to detect the introduction and consumer deception and adulteration of food.

When carrying out official controls were based on annual plans, inspections, audits and sampling of raw materials and foods of plant origin and setting priorities on the principles of risk assessment of an individual commodity or analytical indicator. Other criteria when deciding on control activities were knowledge of previous audits, the findings of other official control authorities, consumers and media initiatives, the Commission's recommendations and information from the Rapid Alert System for Food and Feed (RAFF). All audit activity based on the fact that responsibility for food safety under the Food Act, food business operators have, therefore, the authorities of the SVFA in addition to the planned official controls were carried out and targeted checks according to established priorities and also to respond flexibly and effectively on actual cases that occurred during the year on the market.

Total conformity of operators and products

Under the Food Act No.152/1995 are in the Slovak Republic 25.614 registered food business operators. From this number were 21.012 controlled operators, which is 82.03% and in 2010 was carried out by these operators 55.835 controls.

From the total number of inspected food business operators were in 7.338 operators (34.92%) weaknesses. Frequently when official controls have been identified weaknesses in the overall hygiene (11.262), also has been detected weaknesses like the time of consumption after its goods and shelf-life (3.566), followed by weaknesses in labeling (2387) and deficiencies in good manufacturing practice (2.238).

The most weaknesses were in retail trade, where the checks were discovered 5.681 objects with defects, which is 38.36% of the number of controlled retail buildings. Deficiencies were mainly related to the overall hygiene of the findings of the goods after the time of consumption, durability and minimal labeling.

For producers and packagers of products of plant origin have been found weaknesses in 494 objects, which is 35.14% of the total number of controlled objects, while weaknesses were mainly detected in the overall hygiene and good manufacturing practice. In 2010 the authorities of the SVFA implemented in the food business operators and plant commodities audits focusing on compliance verification system established to ensure control of food hygiene and good manufacturing practice with current legislation.

From the total number of audits (435) which were performed were 221 audits with disagreements, in which was identified 490 weaknesses.

Frequency and types of discord, the classification of system which was used:

- disagreement in the use of good manufacturing practice – in 2010 were examined 21.012 objects, which in 2.238 objects were found non-compliance in the application of good manufacturing practice. Most breaches were identified in retail trade 1.243 objects. The most common weaknesses were inadequate by the documentation prepared by the application of good manufacturing practice.

The various operations related to production, handling and placing the food on the market based on the general hygiene requirements for food production under current legislation were not sufficiently implemented in practice, inadequate record keeping the implementation of the plan to a system of control of food hygiene and lack of monitoring of CCP and also in non-compliance plan for pest control,

- weaknesses in implementation with hygiene establishments – the highest number of deficiencies were found in the retail sector, where from the 14.810 examined objects were 8086 objects found in the application of non-compliance with hygiene establishments. Weaknesses mainly related to the lack of operational hygiene, dirty walls, ceilings, dirty and damaged floors, worn and dirty processing equipment, the occurrence of mold, unsecured windows against insects and rodents, door intrusion, lack of evidence carried out sanitation facilities construction and technically not always relevant legislative requirements,
- weaknesses in implementing the requirements for personal hygiene – cover missing scalp, neglect wearing work clothes, inappropriate clothing, soiled work clothes, failure to meet the sanitation needs in sink (liquid soap, disposable towels, etc..),
- weaknesses in the verification of origin (traceability) – inadequate record keeping of all suppliers and consumers of food and food ingredients at all stages of production, processing and distribution – lack of delivery notes, incomplete data on the origin of food, keeping food in containers without any labels or insufficient sign and also insufficient designation of food in the state language,
- weaknesses in the application requirements for storage of food – food consumption after the date or date of minimum durability, lack of storage facilities measuring devices, common storage incompatible foods, cold chain breach of stored materials and food, store food directly on the floor,
- weaknesses in the composition and labeling of products in each commodity, application of non-harmonized legislation – weaknesses in the composition of products implementation of harmonized legislation – weaknesses in labeling and in terms of microbiological contamination and contaminants – exceeding the limits for microbiological criteria, food additives and contaminants.

Analysis of discord, occurrence of discord

The highest frequency of disagreements occurred in retail trade and also the producer and packer, mostly in non-compliance on the overall hygiene. In retail trade, excluding non-hygienic requirements has been an increased number of food products after the date of consumption or date of minimum durability, also the number of non-compliance with requirements for labeling food products and weaknesses in good manufacturing practice. Found disagreements were mainly operational, but also occurred in the finished products. The food products were stored action on deficiencies found.

A. Christmas controls fruit, vegetables and dried fruit shell

During targeted control was carried out with 656 controls by manufacturers, the wholesale stores and retail establishments were examined at 9.802 varieties of dried and candied fruit and dried fruit shell. Most common weaknesses were:

- non-labeled product in the official language,
- missing % in the composition of components listed in the product name,

– lacks of any sign of unpacked goods,
– lack of warning that the presence of dyes can cause hyperactivity in children,
– illegible date of minimum durability,
– composed of the product is not mentioned additive identified in the analysis, 188 samples were collected, 8 samples has not complied.

B. Control of food of plant origin imported from third countries

It was carried out 4032 in import controls, which were examined at 52 452 951.44 kg of food non-animal origin. Released into free circulation was 3.891 total supply of 50.991 weight 365.38 kg. Into free circulation has been released about 146 deliveries a total weight of 1 461 586.16 kilograms. Most items were unreleased in Turkey (figs, hazelnuts, pickles, pasta, jams, raisins, etc.), Ukraine (soft drinks, cereals, legumes, fruits and vegetables, oils), Serbia (red gourd, spices, confectionery, compotes, fruit in alcohol, beer, oil), Argentina (peanuts, prunes) and Vietnam (dehydrated foods, beverages, processed fruit and vegetables).

Progress on strategic objectives described in the national monitoring plan

For the area of food were provided for two basic objectives:

1. Ensure a high level of human health and consumer protection consistent and integrated control of the entire food chain from production to sale of food.
2. Increase consumer confidence in processed foods and putting them on the EU's single market by communicating with the public concerned.

Both goals are being met and in 2010 was the fulfillment of their recorded progress has been achieved through intensive and good cooperation and official control bodies. In terms of goal. 1 was:

- official control of foodstuffs, in accordance with the annual plan, a total of 107 190 was carried out checks in 38 005 buildings inspected,
- official controls were carried out on the basis of reports from RASFF,
- conducted a risk assessment based on scientific opinions using national professional scientific groups, which are composed of experts from the scientific and research community as well as professional associations,
- in official laboratories to diversify the analysis carried out under official control of foodstuffs, introduced a new highly sensitive analytical methods,
- updated to documented procedures for the newly adopted food law,
- continuation of the continuous education of professional staff,
- month to evaluate the official control of foodstuffs and receive proposals for corrective action when deficiencies.

In improving consumer confidence in processed foods in the Single Market Union, which is the second strategic objective was the food industry kept informed about upcoming and newly adopted legislation and for the latest news in food hygiene and food safety through professional presentations at conferences and seminars at regional and national level, further training for the food industry.

In 2010, official control authorities Food also continue to develop the documented procedures and training to carry out official controls in a uniform manner, the implementation of internal audits in District Veterinary and Food Administrations (DVFA) and Regional Veterinary and Food Administrations (RVFA) under the program of internal audits for 2007–2011, drawn up and updated for the year and in conducting tests by trained people/hunters in

accordance with Regulation (EC) No. 853/2004 (DVPS in 2009 by passing the test issued by trained persons with 378 certificates of competency for initial examination of wild game on the ground after the catch.).

In 2010, health authorities performing official control of foodstuffs also continue to develop the documented procedures (a total of 18 of the Guidelines were created by Public Health Authority of the Slovak Republic (PHA SR) for (RPHA SR)) and training to carry out official controls in a uniform manner. PHA SR continues to carry out internal audits in RPHA SR by the internal audit program developed for 2007–2011 and updated for the actual year.

Effectiveness of official controls carried out by the national control plan

Official controls are carried out on the basis of documented procedures, they contain guidance for inspectors who carry out official controls. If deficiencies are identified in accordance with current of the national legislation imposed measures to eliminate the deficiencies and penalties laid down by law to correct the identified deficiencies. Official controls are carried out on the basis of risk assessment. In 2010 were prepared plans for controls and sampling. Implementations of these plans were reviewed when carrying out audits. The audits were monitored compliance with operating procedures, guidelines and instructions as well as established control plans and sampling. Where discrepancies are imposed corrective measures to eliminate discrepancies, and follow-up audit check implementation of corrective measures. For 2010, each control organization set goals that were complied during the year 2010. Confirms the effectiveness of official controls correspondent with the number of messages sent from the SR RASFF. In 2010 was sent total 58 notices. 2 Of this number were later withdrawn from the system. From the total of 56 notive the EC assessed follows:

- 27 notifications as warning (i.e. 48% of the total),
- 24 notices as information (i.e. 43% of the total),
- 5 were sent as a notice of returned goods from the border, i.e. 9% of the total.

The Table 1 shows the overview and evaluation of initiatives by various food processors and distributors with point of view of reasonably, undulty, abandoned and total number of notice.

Following three tables (Tab. 2, 3, 4) are highlights of countries from point of view of imports and representation of commodities in import.

The Table 5 shows the overview of the number of different samples analyzed in the Laboratory of State Veterinary and Food Institute.

The Table 6 shows the overview of samples analyzed for mycotoxins in cereals.

The Table 7 shows kinds of taken samples from materials and items designed for food contact and their main monitored risk indicators.

Table 1

Overview and evaluation of initiatives

	Total number of notice	Reasonably	Unduly	Could not be prove, abandoned
Packager	11	4	7	0
Carriers and distributors	11	1	2	8
Hypermarkets, Supermarkets	887	212	390	285
Small stores	441	104	193	144
Medium stores	462	131	186	145
VO storage	61	21	20	20
Manufactures	166	48	78	40
Other	28	8	15	5
Total	**2067**	**529**	**891**	**647**

Table 2

Overview of third countries with the largest number of imports

Rank	Country	Number of deliveries
1.	China	804
2.	Turkey	706
3.	Croatia	283
4.	Serbia	282
5.	Vietnam	198

Table 3

Overview of imports by commodity

Rank	Commodity	Number of deliveries
1.	Sterilized and pickled vegetables	159
2.	Compotes	153
3.	Other sugars	150
4.	Dietary supplements	138
5.	Oil	106

Table 4

Overview of third countries with the largest number of unreleased supply

Rank	Country	Number of unreleased supply
1.	Turkey	32
2.	Ukraine	14
3.	Serbia	12
4.	Argentina	10
5.	Vietnam	8

Table 5

Overview of the number of samples analyzed in the Laboratory of State Veterinary and Food Institute

Type of sample	Result of qualitative analysis	Laboratory SVFI		Total
		Dolný Kubín	Bratislava	
		Number of samples		
maize and maize products	negative	31	35	66
	pozitive	2	0	2
soya and soya products	negative	36	23	59
	pozitive	5	0	5
rice and rice products	negative	60	41	101
	pozitive	0	0	0
seed of flax	negative	2	0	2
	pozitive	0	0	0
other	negative	3	1	4
	pozitive	0	0	0
Total		139	100	239

Table 6

Overview of samples analyzed for mycotoxins in cereals

Commodity Overview – samples analyzed	Number of samples	Number of unsatisfactory samples
1	2	3
Conventional wheat	96	1
wheat BIO	11	0
Barley	11	0
Rye	8	0
Rye BIO	2	0

Table 6 cd.

1	2	3
Oats	1	0
Oats BIO	2	0
Corn	11	0
Buckwheat	1	0
Grain mill products	52	1
Other	4	0
Other BIO	1	0
Total	**200**	**2**

Table 7

Kinds of taken samples and monitored risk indicators

A: Materials and items designed for food contact	Monitored risk indicators
melamine kitchen utensils	formaldehyde, sensory evaluation
nylon kitchen utensils	primary aromatic amines, sensory evaluation
plastic baby bottles (preferably PC)	bisphenol A, sensory evaluation
plastic products for children (plates, bowls, cups, cutlery etc.)	formaldehyde, primary aromatic amines sensory evaluation
glass jars with color printing intervening with the oral edge	Cd, Pb
ceramic products	Cd, Pb
plastic packaging materials	microbiological examination, the total migration of substances, octene, formaldehyde, sensory evaluation
The special campaign in 2010	
B: Materials and items designed for food contact	Monitored risk indicators
ceramic products for children	Cd, Pb
plastic sports bottles for children	eg. total migration of substances, formaldehyde, primary aromatic amines, bisphenol A, Cd, Pb, sensory evaluation, migration of pigment
plastic snack boxes for children	eg. total migration of substances, formaldehyde, primary aromatic amines, bisphenol A, Cd, Pb, sensory evaluation, migration of pigment
plastic containers for food storage	eg. total migration of substances, formaldehyde, octene, sensory evaluation
thermos	eg. Cd, Pb, Cr, Ni, appearance, overall migration agents, migration of pigments, formaldehyde, primary aromatic amines
cups	eg. Cd, Pb, Cr, Ni, appearance, overall migration agents, migration of pigments, formaldehyde, primary aromatic amines

Conclusions

Food control system in Slovak Republic can be characterized as functional and satisfactory. The role of official food control authorities is to optimize the annual and five-year plans for food control. Based on these checks, we can conclude that the number of health deficiencies is reduced, decreasing the number alimentary diseases. Over the past 10 years, the decrease of salmonellossis by more than 50 %. In addition to routine inspections, an increase in the number of audits of food premises. The most health problems remains to the sale of food. In this area will in future need to increase the frequency of inspections with a focus on efficiency and lack of preventing in the hygiene of sale.

4

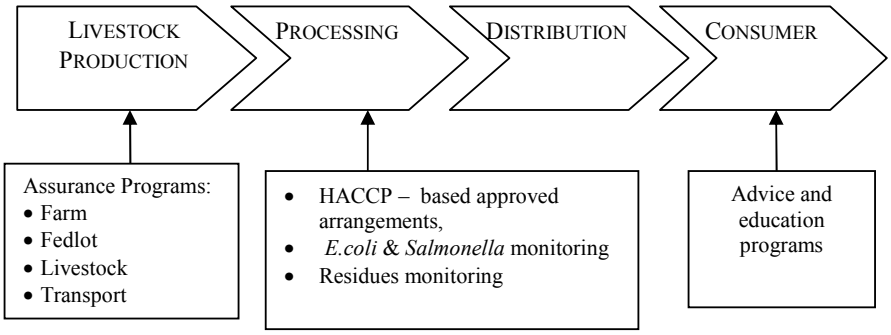
EVALUATION OF THE HYGIENIC LEVEL OF MEAT PROCESSING PLANT BASIS ON THE MICROBIOLOGICAL RESEARCH OF RAW MEAT PRODUCT

Introduction

Risk assessment is necessary along the whole food chain in order to ensure food safety to the final consumer. At all stages of the food chain is very important to prevent contamination, mainly microbiological, due to the possibility of microorganisms rapid growth and direct hazards to the consumer. Food production requires adequate hygiene and sanitary conditions which need to be fulfilled to prevent cross-contamination during processing and distribution, thus preventing food poisoning consumers.

Production cycle of animal origin food is longer and contains more cells than plant origin food. These products may include zoonotic pathogens that directly hazardous of human health. In addition, the characteristic attribute of this chain is that the manufactured product requires specific storage conditions ie. Maintaining the cold chain from the slaughter of animals until consumption of meat by the consumer.

To successfully manage food safety and quality risk in meat production a fully integrated assurance system, containing effective control, has to be applied at all stages (Fig. 1).



Source: based on Desmarchelier et al. 2007

Fig. 1. Assurance system in meat production

The EFSA Report [2008a] indicated that 10.3% of pigs and 5.1% of pig carcasses slaughtered in the European Union are vectors of various strains of Salmonella spp. According to another EFSA report [2008b] Salmonella spp. was the reason for 55–95% of cases of food poisoning described in humans. According to Polish data [PZH 2008] in Poland, were recorded 11,701 cases of salmonellosis, which represent approximately 26% of all quoted food poisoning. More food poisoning in the EU is cause only viruses.

In order to effectively manage the problem of human salmonellosis attributable to pork and pork products, control measures should be taken simultaneously at all levels of production. Pork and pork products are recognized as one of the major sources for human salmonellosis [Wong et al. 2002].

The aim of this study was to assess the level of hygienic meat processing plant as an example for the microbiological analysis of raw meat product.

Materials and methods

The study was conducted in meat processing plant as an example of one group of products over the years 2005–2010. The plant engages with the cutting of pig carcasses, quarters of beef and poultry carcasses. It produces elements of culinary meat (food elements for warehouse of meat) and processing (meat and fats for processing), and produces raw meat products. The plant is permitted to trade which means that the produced items can be move freely throughout the European Union. In the plant were implemented GHP, GMP and HACCP systems.

The research material was delicatessen meat, part of which consisted of pork and/or beef or poultry meat, spice mixture and ice. The characteristics of analysed meat are presented in Table 1.

Research methods were: audit of plant according to the guidelines specified in Codex Alimentarius, inspection of the establishment and control documentation of plant, review complaints and documentation of veterinary, as well as microbiological analysis.

Table 1

Characteristics of delicatessen meat

<table>
<tr><td colspan="2">Product: Delicatessen meat (raw meat product)</td><td colspan="3">Destination: All consumer groups except infants under 6 months of age and people with contraindications (health, religious etc.).</td></tr>
<tr><td colspan="5">Raw material composition: Pork meat and/or beef meat or poultry meat, spice mixture, ice.</td></tr>
<tr><td colspan="2">Product susceptible to spoilage:
☐ no ☑ yes</td><td colspan="3">Physicochemical parameters – depending on a type of used meat</td></tr>
<tr><td colspan="5">Description of production: Elements for the production of delicatessen meat are taken from the cooler, weigh in accordance with recipe and then mixing and first grinding with ice and additives. Then meat is grinding a second time, after which the bites portioning (mass range: 0.5–5 kg), vacuum packed in foil and labeled. After producing the goods will be sent to the warehouse.</td></tr>
<tr><td>Consistency:
As minced meat</td><td colspan="2">Colour: Characteristic of used meat from cream to deep pink</td><td colspan="2">Contaminants:
Allowed only with established range of appropriate legislation</td></tr>
<tr><td colspan="5">Apperance: Transparent packaging with a clearly visible minced meat and fat.</td></tr>
<tr><td colspan="2">Taste: Characteristic of meat used, inadmissible strange flavours</td><td colspan="2">Odour: Characteristic of meat used, inadmissible other odours, e.g. rancid fat odour</td><td>Transport conditions:
Temperature 0–4°C</td></tr>
<tr><td colspan="2">Storage: Temperature 0–4°C</td><td colspan="3">Shelf life: determined on the basis of storage researches, for each recipe separately</td></tr>
<tr><td colspan="5">Customer information on the label: Name, address and veterinary number of plant, date of production, storage conditions, shelf life, information that date of production is also signature of production batch, information "to be eaten after heat treatment".</td></tr>
</table>

Source: materials of studied plant, 2011

Microbiological analysis concerned on determining the level of bacteria: *Salmonella* spp., *Escherichia coli* and aerobic mesophilic bacteria. Analyses of specific types of microorganisms were carried out according to the methodology contained in: PN-EN ISO 6579, 2003 (*Salmonella* spp.), PN-ISO 16649-2, 2004 (*Escherichia coli*), PN-EN ISO 4833-2004+Ap 1, 2005 (bacteria aerobic mesophiles).

The requirements were determined basis on the relevant legislation on microbiological contamination [Regulation (EC) 2005] – Salmonella absent, *Escherichia coli* up to 100 cfu/g, the total count of microorganisms 2 of 5 samples may be in the range m-M, where m=5 x 105, M=5 x 106.

To the study packaged products as for sale was used. Every time production batch i.e. 5 package units or their multiplicity was analysis. If the correct results were obtained for the next eight weeks, the plant could reduce the frequency of analysis from once a week to once every two weeks.

Results and discussion

Technology of meat delicatessen production

Delicatessen meat (SWM) is raw meat product made from minced meat with functional--spice mixture and ice. In the studied plant have been designated rooms to storage such as cold store of carcasses and quarters, cold store of poultry carcasses, cold store of parts culinary elements, cold store of industrial elements, freezers own elements (shock and cold store), packaging and storage of spices warehouse, and finished products cooler, in which delicatessen meats were kept before receiving. The second type of rooms are production areas e.g. cutting rooms and hall production SWM. The other rooms are wash containers with dirty and clean storage containers, as well as social rooms.

Cutting technology is a separate station for cutting beef (1 table), poultry (1 table) and a line for cutting pork (5 linked sliding tapes integrated with saws and tables).

For the production of delicatessen meat was used pork of class I, IIA (lean), IIB (fatty), III (with lots of connective tissue), IV (bloody), beef of class I and II and poultry meat with fat. Meat is generally directed to produce chilled, but the technology allowed the use of thawed meat. The plant produced several kinds of meat delicatessen according to specially developed recipes.

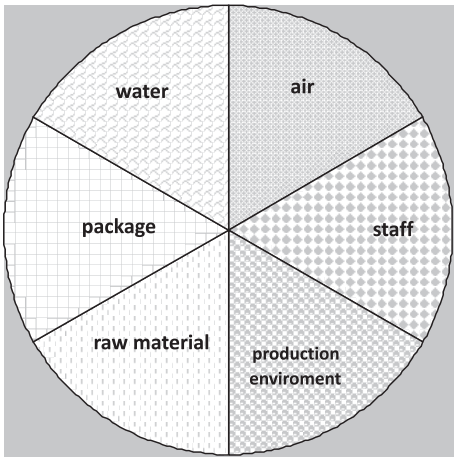
For the study only the results of one product marked with his product brand were used. For production of that meat only pork was used.

Delicatessen meat was produced in the production hall SWM equipped with mixer--grinder (hybrid), portioning-cut machine (portioner) and machine packing and labeling, as well as a small equipment such as scales. In the hall was also an ice maker. Meat obtained from cutting was weighed in accordance with recipe to the containers, and then given to mixer-grinder together with a mixture of spices and ice, and after mixed ground to a thick mesh sieve with a diameter of 8 mm. The meat was minced into containers and set of and maturation (one component of the spice mixture was salt curing). After the entire daily production grinding on the coarse sieve, sieve exchanged on the smaller sieve with φ 4 mm and re-milled. The minced meat was served on the portioning-cut machine separating the pieces

about 500 g, and then manually was placed in the pressed forms of packaging foil in packaging machine. Sliding machine was transfer pieces of delicatessen meat under develop top foil, which in vacuum formation hermetic closed of product manufactured. Closed package passed by the guillotine, and then by weight labeller. The resulting product after abandoned the line, packed into plastic containers and put into cold storage of finished product, where expected the issue to the customer.

Hygiene in plant (HACCP)

The level of hygiene in plant directly influences on the safety of produced articles. Figure 2 shows the components of hygiene in meat industry plant.



Source: own work

Fig. 2. The components of hygiene of studied plant

The source of potential contamination brought into the plant and may affect the safety of the finished product are raw materials. In described case, the microbial quality of meat delicatessen is affected by the initial contamination in the plant from the raw materials. Depending on the purity of the slaughter, post-mortem cooling and transport of carcasses, meat cutted may be more or less contaminated. The studied plant conducted audits of suppliers and qualified their selection to get to the best (the cleanest) raw materials for production. Critical control point of plant' HACCP system was acceptance of goods, where carefully estimated and analyzed delivered half-carcasses and quarters. In accordance with records of the CCP monitoring, corrective actions involving sending back the delivery were carried out, which actually affected the improvement of hygiene of the plant. For delicatessen meat production is also used seasoning-functional additive, because it is not in the composition of natural spices, and used amount is small (3.6 kg/100 kg of meat). Effect of contamination of the finished product can be regarded as irrelevant.

Another factor affecting the hygiene of the plant was water. Water in the plant was used to clean the rooms, production areas such as tables, tapes, cleaning containers, hygienic procedures of personnel and for the production of ice used as a raw material for the production of meat delicatessen.

Partition of pig carcasses were made on portable tapes and tables, using saws, knives and butcher's steels. A very important factor in preventing the transfer of the cross contamination was to maintain separation between different types of cutted meat. As stated on the basis of risks and complaints analysis, cutting meat on the tables dedicated for cutting other kinds of meat immediately followed changes in hygiene and sanitation of studied products. Condition of the production environment is also an appropriate technical condition of used machinery and equipment. The raw material for production of delicatessen meat were usually small meat obtained in the process of cleaning of large culinary elements such as: pork loin, chuck, shoulder, ham, or for removing the meat from the fatty elements such as back fat. Small meat due to the developed area had increased contact with the air which causes more easily increases temperature and the settling of pollutants in the air.

The air present in production rooms and coolers has a direct impact on the quantity of pollutants seated on its blowing surfaces. The level of impurities in the air is affected by the filters used in the establishment of ambient air and the frequency of their exchange, external environment facility (neighborhood) which is the amount of pollutants in the air outside the plant, the frequency and length of the door opening to the outside plant which favors the penetration of pollutants into the interior. Another factors are the type of ventilation used in the plant and cooling system. Both force the circulation of air and the formation of over- or under vacuum which may result in the aspirated of contamination from dirty zones of plant.

A very important factor in the importance of plant hygiene is the man, who can be first vector of pollution (from outside plant, inside of plant and inside of your body). Second, the operations performed on the site may increase the levels of contamination. Therefore, workers at each plant are related to procedures for admission to employment and the principles of hygiene work and move around the plant, as well as training.

The last factor that affects the hygiene of production is a way of packing and the type and quality of packaging used, including the conditions of storage. To assess the level of hygiene in the meat processing plant a delicatessen meat was selected which was more sensitive and vulnerable to contamination due to revised structure by grinding and increased surface of the product. Also the temperature changed faster which could be translated into faster growth of microorganisms. Therefore it was concluded that the microbiological level of delicatessen meat can be treated as a material indicator (detector), hygiene of the plant.

Microbiological analysis in plant

According to obligatory legislation the principles of microbiological analysis in meat processing plants for raw meat products have changes over the years 2004–2007. Table 2 shows legal acts of microbiological parameters ranges for raw meat products from minced meat.

The plant carried out research in the direction of Stapylococcuss aureus in the period of 2005–2006 and next after cancelation of the appropriate regulation, these analysis were abandoned. In this work is not presented the results of these studies. Despite the abolition of the obligation to conduct research in the direction of the total number of microorganisms, in analysis plant was decided to continue these analyses because of information about the general contamination of the product obtained that way.

Table 2

The legal requirements for microbiological criteria for raw meat products from minced meat

Legal act	Microbiological criteria			
	Salmonella	*E. coli*	Total count of microorganisms	*Staphylococuus aureus*
Regulation of Polish Ministry of Agriculture, 2004	absent in 10g, n=5, c=0	m=5x101, M=5x102, n=5, c=2	m=5x105, M=5x106, n=5, c=2	m=102, M=5x103, n=5, c=2
Regulation (EC) 2073/2005	not detected in 10g, n=5, c=0	m=5x102, M=5x103, n=5, c=2	lack of reqiurements	lack of reqiurements
Regulation (EC) 1441/2007				

Source: own work based on obligatory legislation

The results of determinations of *E. coli* content in samples of delicatessen meat in the period 2005–2010 are shown in Table 3. *Escherichia coli* was found in a few samples of meat and in the amounts do not endanger the health of the consumer in the proper handling of meat. Only in 2007, *Escherichia coli* were identified in all tested samples. Reported amounts do not exceed the requirements for meat, however, ie. for *E. coli* was up to 100 cfu/g.

Table 3

The content of *E. coli* in samples of delicatessen meat in the period 2005–2010 years

Year	Number of studied samples	Number of samples in which *E. coli* detected	Number of *E. coli* in 1 g		
			Range	Average	Mediana
2005	85	22	1 x 101–1,1x 102	0.7x101	0
2006	140	0	0	0	0
2007	115	115	0.9x101–6,5x101	1x101	0.9x101
2008	145	0	0	0	0
2009	145	5	1.5x101–3.5x101	0.08x101	0
2010	65	5	4x101–6x101	0.4x101	0

Source: study of plant

In studies of Noveir et al. [2000] conducted in Turkish meat products (255 raw minced beef, 50 uncooked hamburger and 101 soudjouk samples) total in all samples was found – *E. coli* type 1 in number of 957, *E. coli* 0157 –3 and others *Escherichia* spp. in number of 54. There were not significant number. In other studies [cited by Dontorou et al. 2003] *E. coli* 0157:H7 in beef minced meat samples was found too. A few of raw meat samples examined were contaminated *E. coli* 0157:H7 (in Denmark – 0.3% of 1584 samples, in Netherlands – 1.1% of 571 samples; in Switzerland – 0 of 211 samples; in Greece – 0 of 64 samples).

Monitoring of *E. coli* is needed. For example *E. coli* O157:H7 was first recognized as a human enteric pathogen in 1982 when it caused two major outbreaks of hemorrhagic colitis in the USA. Since then, it has been responsible for hundreds of cases and outbreaks over the world and is considered to be one of the most important and potentially life-threatening

pathogens. *E. coli* 0157:H7 has the ability to cause hemorrhagic colitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and, in severe cases, death [cited by Dontorou et al. 2003].

Results of determinations of the content of *Salmonella* spp. in examined delicatessen meat are shown in Table 4. There were no *Salmonella* spp. in none sample of meat in the period 2005–2010, which is consistent with the standards.

According to Wong et al. [2009] uncooked pig meat is a potential route for the introduction of new clones of *Salmonella* and *E. coli* O157:H7 in New Zealand. In contrast, *E. coli* 0157:H7 infection has never been associated with consumption of food in New Zealand, although it has been detected in raw beef and raw pork and other meat (sheep meat, veal).

Table 4

The content of *Salmonella* sp. in samples of delicatessen meat in 2005–2010 years

Year	Number of studied samples	Number of samples in which *Salmonella* sp. detected	Number of *Salmonella* sp. in 1g	
			Range	Average
2005	85	0	0	0
2006	140	0	0	0
2007	115	0	0	0
2008	145	0	0	0
2009	145	0	0	0
2010	65	0	0	0

Source: study of plant

The results of determinations of the total number of microorganisms varied in different years. According to the requirements meat should contain the total number of microorganisms 2 of 5 samples in the range between m and M, where m=5 x 105, M=5 x 106. The most contaminated meat was in 2006, and only in that year in the two samples found exceed of acceptable levels of microorganisms.

Table 5

Content of bacteria aerobic mesophiles in samples of delicatessen meat in 2005–2010 years

Year	Number of studied samples	Number of samples in which bacteria aerobic mesophiles was detected	Number of bacteria aerobic mesophiles in 1 g (OLD in 1 g)		
			Range	Average	Mediana
2005	85	85	5 x 103–4.5 x 105	8 x 104	5 x 104
2006	140	140	3.8 x 103–5.2 x 107	10 x 105	5 x 105
2007	115	115	1.1 x102–1.6 x 105	2.3 x 104	1.1 x 104
2008	145	145	1.2 x 103–4.1 x 105	4.9 x 104	10 x 104
2009	145	145	4 x 103–2.3 x 105	4.7 x 104	2.8 x 104
2010	65	65	1 x 103–2.3 x105	6.4 x 104	4.3 x 104

Source: study of plant

In studied documentation of plant was found only simulations of withdrawal from the market. There was no records of real carried out the withdrawal. There no were also problems with the complaints. Analysis of the complaints focused on culinary meat (another part of the production plant). Delicatessen meat complaints concerned only incorrect color associated with the presence of curing salts and method of packaging (unseal). In other carried studies elements and clean of production area were found confirmation of a high level of hygiene of studied plant.

Conclusions

1. Based on microbiological monitoring of meat a significant variation of the microbiological quality over the each tested year and between years was observed. There were no Salmonella spp. in none sample of meat. Escherichia coli was found in a few samples of meat and in the amounts do not threaten the health of consumer in the proper handling of meat. The number of aerobic mesophilic bacteria ranged from 4x103 to 2.2x105.
2. Despite the fact that in produced meat no studied samples endanger the health of the consumer, conducting microbiological analysis is a necessity. This is due to Regulation (EC) 2703/2005 as amended by Regulation (EC) 1441/2007. The second reason is that, if such a hazard existed, there would have to withdraw whole production batch of product from the market.
3. Microbiological monitoring of meat systematically carried out also allows for evaluation of the hygienic condition of the plant. In the case of deterioration of the hygienic condition of the plant, e.g. caused by failure of staff would also result in low quality of meat products produced.

References

Desmarchelier P., Fegan N., Smale N., Small A., 2007. Managing safety and quality through the red meat chain, Meat Sci., 77, 28–35.

Dontorou C., Papadopoulou C., Filioussis G., Economou V., Apostolou I., Zakkas G., Salamoura A., Kansouzidou A., Levidiotou S., 2003. Isolation of Escherichia coli O157:H7 from foods in Greece, International Journal of Food Microbiology, 82, 273–279.

EFSA, 2008a. Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline on the prevalence of Salmonella in slaughter pigs. Part A. The EFSA Journal 135.

EFSA, 2008b. Overview of methods for source attribution for human illness from food-borne microbiological hazards. Scientific Opinion of the Panel on Biological Hazards. The EFSA Journal 764.

Noveir M.R., Dogan H.B., Halkman A.K., 2000. A note on Escherichia coli O157:H7 serotype in Turkish meat products, Meat Sci., 56, 331–335.

PN-EN ISO 6579, 2003. Mikrobiologia żywności i pasz. Horyzontalna metoda wykrywania Salmonella spp.

PN-ISO 16649-2, 2004. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby ß- glukoronidazo -dodatnich Escherichia coli. Część 2: Metoda płytkowa w temperaturze 44°C z zastosowaniem 5-bromo-4-chloro-3-indolilo ß-D-glukuronidu.

PN-EN ISO 4833-2004+Apl., 2005. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby drobnoustrojów. Metoda płytkowa w temperaturze 30 stopni C.

PZH, 2008. Meldunek 12/B/08 o zachorowaniach na choroby zakaźne i zatruciach.

Regulation Commission (EC) No 2073/2005 of 15th November 2005 on microbiological criteria for foodstuffs (Official Journal of the European Union, 22.12.2005, L338/1).

Regulation Commission (EC) No 1441/ 2007 of 5th December 2007 amending Regulation (EC) No 2073/ 2005 on microbiological criteria for foodstuffs (Official Journal of the European Union, 7.12.2007, L322/12).

Regulation of Polish Ministry of Agriculture, 2004. Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 25 maja 2004 r. w sprawie wymagań weterynaryjnych dla mięsa mielonego i surowych wyrobów mięsnych (Dz. U. 2004, 132, 1419).

Wong T.L., MacDiarmid S., Cook R., 2009. *Salmonella*, *Escherichia coli* O157:H7 and *E. coli* biotype 1 in pilot survey of imported and New Zealand pig meats, Food Microbiology, 26, 177–182.

Wong D.M.A. Lo Fo, Hald T., van der Wolf P.J., Swanenburg M., 2002. Epidemiology and control measures for *Salmonella* in pigs and pork, Livestock Production Science, 76, 215–222.

5

EFFECTIVENESS EVALUATION OF THE INTERNAL AUDIT IN SELECTED PLANT OF MEAT INDUSTRY

Introduction

The crucial element of competitiveness of each enterprise operating within the food sector is to ensure the quality of food, especially its health safety. In the era of globalization, the achievement of the desired quality of the product is not attainable without the implementation of the quality systems, which provide the repeatability of operations performed in the enterprise and protect against faults committed by employees [Knaflewska and Pospiech 2007] The mandatory quality assurance systems, emanating from the regulations of food law (i.e. GMP – Good Manufacturing Practice, GHP – Good Hygienic Practice, HACCP – Hazard Analysis and Critical Control Point) have a remarkable contribution to building the health safety of produced and marketed food. These systems are the core for the development of additional, non-mandatory systems of quality assurance and quality management (QACP – Quality Assurance Control Points, a quality management system according to the ISO 9000 standards, a food safety management system according to the ISO 22000 standards, TQM – Total Quality Management, BRC – British Retail Consortium, IFS – International Food Standard). A compelling interest in introducing systemic solutions is observed in recent years in Poland. It is associated with the reconstruction of the economy and activities aiming at integration with the global economy [Knaflewska and Pospiech 2007]. Nevertheless, the implementation of quality systems itself is insufficient. Efficient methods of verification should be developed in order to determine whether the implemented and operating system corresponds to the stipulated assumptions [Gajda 2008]. Verification of the quality assurance systems may be performed with the use of:

- internal or external audits,
- various tests of random sampling,
- surveys conducted among users of the system,
- analysis of the effectiveness of corrective and preventive actions,
- review of the customer complaints,
- review of the records documenting operating of the system,
- review and analysis of occurring irregularities [Czarnecka-Skubina and Kołożyn-Krajewska 2005, Trafiałek et al. 2010].

Audit is the standard method to verify the proper operating of quality assurance and management systems. It should be noted that the regulations of food law do not entail organizations of the food sector to conduct the audits. Choice of verification method appertains to the organization. In the case of non-mandatory systems (BRC, IFS, ISO 9000 or ISO 22000), audit is one of the obligatory requirements. According to the definition of the International Organization for Standardization (ISO), audit is a systematic, independent and documented

process for obtaining the evidence from audit and objective evaluation of this process in order to determine the extent of fulfilling the audit criteria [PN-EN ISO 9000, 2006]. Therefore, it is to determine whether the factual state is consistent with the requirements included in the legislation, standards, and procedures, instructions, known as the audit criteria. Subsequently, the audit is an effective tool for improving the quality management system in the enterprise, allowing for prompt and proper correction of the discrepancies occurring in the ongoing processes [Dzwolak 2009, Michalak 2009].

Audit is not synonymous with control; nonetheless, these processes are often equated due to the objective of ensuring consistency between the factual actions and the intended activities. The internal audit aims at examination of the existence and effectiveness of control solutions. In the long term view, audit contributes to the improvement of control; estimates and evaluates the performance of all control measures, while not being a control measure itself. It can be concluded that the audit monitors the current status and inspires future-oriented solutions. Therefore, acts in a preventive manner by proposing remedial measures [Kromer and Rychły-Lipińska 2009].

Companies, in order to improve the implemented quality management systems, focus their actions not only on the quality of the products or services, but also on the quality of work initiated in the framework of primary and secondary processes, including audit and post-audit actions. The present study was undertaken to attain the knowledge concerning the role of internal audit in the improving quality management systems. The aim of the present study was to assess the effectiveness of internal audits in selected meat processing plant.

Scope of the study

The current case study was conducted in one of the meat processing plants in Southeastern region of Poland. The organizations has implemented mandatory (required by the law) quality assurance systems, i.e.:

- GMP / GHP (Good Manufacturing Practice/Good Hygienic Practice),
- HACCP (Hazard Analysis and Critical Control Point),

and non-mandatory:

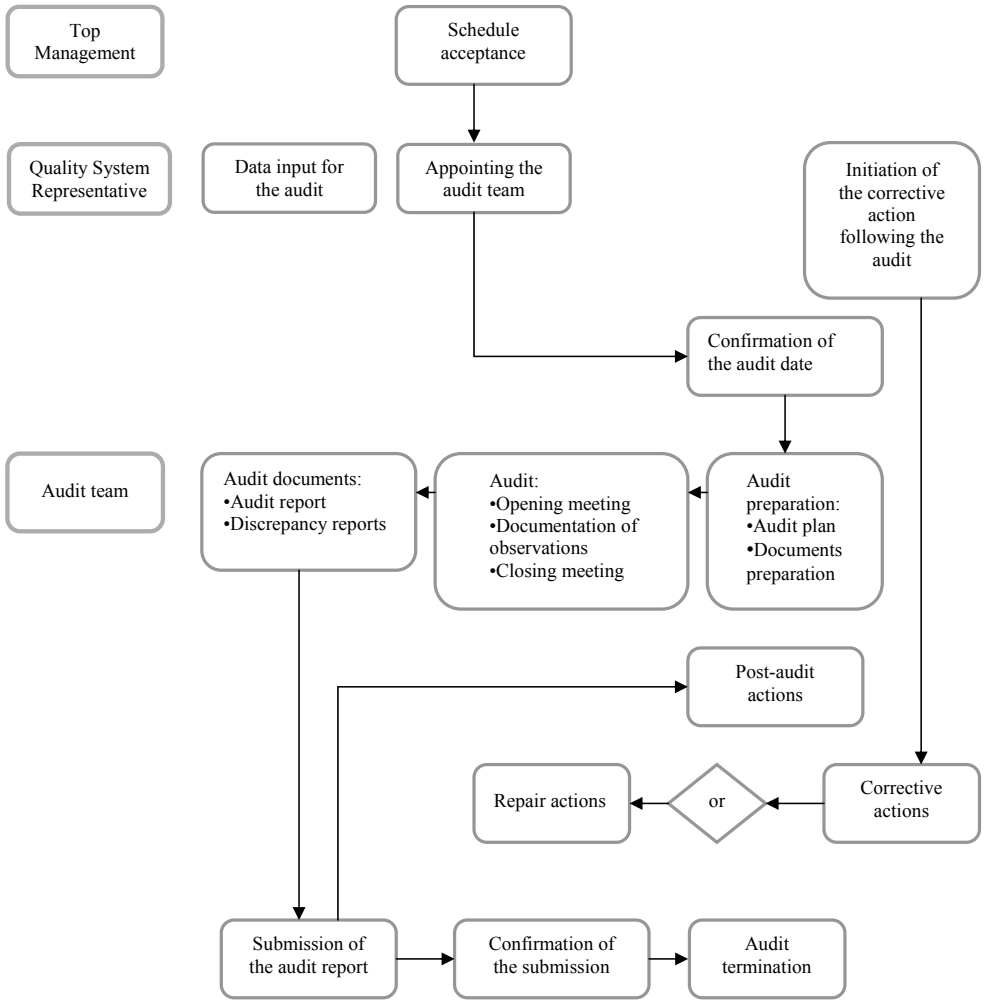
- The quality management system according to ISO 9000,
- Standard IFS (International Food Standard),
- Standard BRC (British Retail Consortium).

The implementation of quality systems in the organization has begun in the late 90s of the last century from fulfilling the requirements of the Codes of Good Manufacturing Practices and Hygiene. The quality management system according to the requirements of ISO 9000 was implemented in year 2004. The system within its scope includes all stages of production, from the purchase of slaughter animals, through the distribution system to the external customers, including the monitoring of their satisfaction degree. Furthermore, this system established the basis for the implementation of additional systems, i.e.: IFS and BRC.

The study material concerned the quality management system (QMS) based on ISO 9000 standards. The records obtained from the internal audits conducted in years 2008–2009 were analyzed for compliance with the actual back then PN EN ISO 9001, 2001. Audits were carried out in accordance with the annual program of internal audits and the applicable procedure of "Internal Audit" in all organizational units of the plant, namely:

- Department of Slaughter and Split,
- Department of Raw Materials,
- Technical Department,
- Final Products Storage,
- Department of Quality Systems,
- Technical Storage,
- Department of Human Resources,

Audits carried out in accordance with the steps outlined in Figure 1.



Source: own elaboration

Fig. 1. The stages of conducting the internal audit in organization

Analysis of the conducted internal audits

In years 2008–2009, 26 internal audits were conducted in the selected organization. The number of discrepancies between years and areas of the enterprise was variable (Tab. 1). Overall, nine discrepancies were detected, including five in 2008 and four in 2009.

Table 1

The number of discrepancies detected in individual departments of the organization

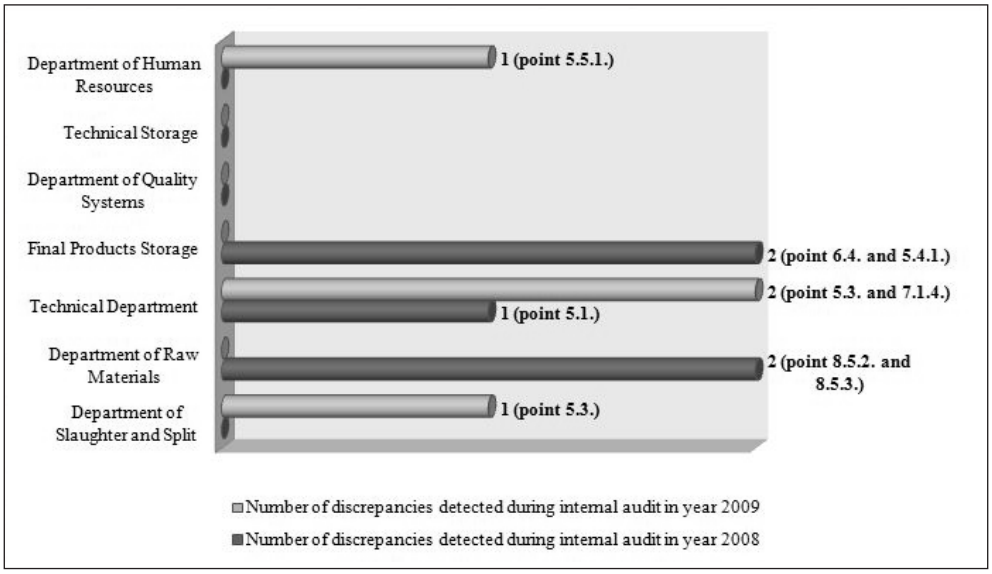
Source: own elaboration

Studied area	The number of discrepancies detected during internal audits	
	year 2008	year 2009
Department of Slaughter and Split	–	1
Department of Raw Materials	2	–
Technical Department	1	2
Final Products Storage	2	–
Department of Quality Systems	–	–
Technical Storage	–	–
Department of Human Resources	–	1

Audits that were carried out in two sections of the organization, i.e. in the Department of Quality Systems and Technical Storage, did not reveal any discrepancies. In both analyzed areas, the undertaken actions contributed to the proper functioning and the improvement of the quality management system. Audit reports from the Department of Quality Systems indicated on the proper supervision on the operation of quality management system. Any actions taken by the top management concerning corrective and remedial activities, supervision of nonconforming products and the documentation and records were conducted in accordance with the requirements of PN-EN ISO 9001, 2001 and system procedures. Additionally, audits did not reveal discrepancies in the Technical Storage. Activities within the scope of identifying the product, the purchase process and securing the product were performed accordingly to the established rules and processes.

Discrepancies in operating of QMS were not stated in the Department of Slaughter and Split and the Department of Human Resources in 2008. Requirements of PN-EN ISO 9001:2001 concerning the quality objectives, commitment of management, product realization, responsibilities and powers, supervision of the equipment for monitoring and measurement and nonconforming products were correctly implemented. The only caveat reported by the auditor performing the audit in the Department of Slaughter and Split was the supervision of the weights. The imprints regarding the weights validation were frangible. Two internal audits were conducted in the analyzed area in year 2009. The first one exposed that the QMS is functioning properly and is constantly being improved due to the great involvement of

employees from the production supervision. However, the next audit revealed a discrepancy relating to the lack of acquaintance with the formulation of quality policy among manual workers, reflecting the low involvement of employees in the improvement of production processes and low awareness of the activities undertaken by them [section 5.3. PN-EN ISO 9001, 2001] – Figure 2. The company organized staff training concerning the quality policy in the framework of corrective action. The auditor recommended that special attention should be given to training the new employees in order to prevent this discrepancy from occurring in the future. According to the auditor, immense commitment of supervisory staff in the improvement of the QMS is not always reflected in the increase of awareness among the production workers.



Source: own elaboration

Fig. 2. Number of discrepancies stated in years 2008–2009 in individual departments of meat processing plant concerning the main requirements of the standard

No significant discrepancies in the functioning of the QMS were stated in the Department of Human Resources. The supervision on the documents, records and staff training was conducted properly in this department. The only irregularity detected in this area in 2009 was the lack of extant descriptions of responsibility and competences for the newly established positions [point 5.5.1. PN-EN ISO 9001, 2001]. The irregularity was eliminated by supplying the missing information. Aside from that, the quality management system in regard to the ensuring the adequate human resources was operating properly and was consistent with the requirements of PN-EN ISO 9001, 2001.

Two internal audits were conducted in Final Products Storage in 2008. Irregularities in the functioning of the QMS were stated different areas in both cases. The first audit exposed a discrepancy in the control of the admission of the final product [point 6.4. PN-EN ISO 9001, 2001]. The corrective actions were carried out in the form of training for workers and proved to be an effective tool to eliminate irregularities, as the subsequent audits did not

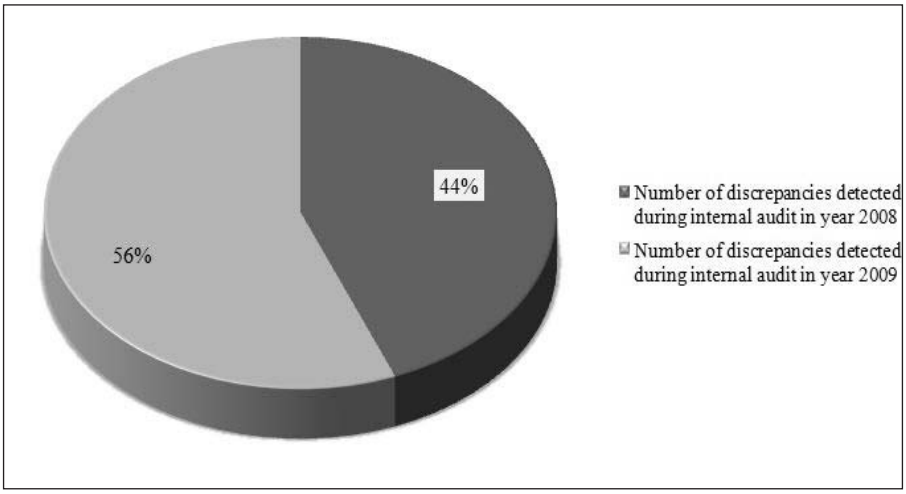
reveal this discrepancy. The lack of qualitative objectives was revealed during the second audit [point 5.4.1. PN-EN ISO 9001, 2001]. Development of objectives and training concerning the requirements for quality management system were introduced as a corrective action. Developing and implementing post-audit actions proved to be successful, as the audit conducted in 2009 has not shown any discrepancies in this department. Analysis of the audits conducted in the Department of Raw Materials allows concluding that the area requires a continuous, systematic supervision of the system documentation. The discrepancy detected in 2008 relating to the lack of control and recording of the implementation of corrective and preventive actions [point 8.5.2 and 8.5.3 of PN-EN ISO 9001, 2001] was eliminated through the personnel training regarding the proper method of documentation of corrective actions. Even though no discrepancies were found in 2009, the auditor concluded that the irregularity was repeated, which suggests that the performed corrective actions have not produced the expected results in the improvement of the QMS.

The worst situation was found in the Technical Department, where performed studies revealed discrepancies in regard to the point 5.1. PN-EN ISO 9001, 2001 in the form of lack of the quality objectives and lack of acquaintance of quality policy among workers. Both in 2008 and 2009 it was stated that the department has no set quality objectives, which means that the management of the area has not undertaken the recommended corrective actions and has not implemented the assigned goals for the previous period. Furthermore, irregularities regarding the lack of extant records were also found in 2009 in the supervision of machines and apparatus for monitoring and measurement. This clearly indicates that the department requires systematic monitoring and improvement, and above all – increase the involvement of managers and employees in the implementation of assigned tasks.

According to Michalak [2008] the internal audits conducted regularly bring a number of benefits tangible for the organization. These include i.a.:

- Obtaining valuable suggestions for the improvement of the management system.
- The possibility of improving documentation of the management system.
- Attaining the knowledge of improving the system of supervision or control.
- Improvement of the internal communication.
- Utilization of the information to measure processes.
- Increase of the pro-quality involvement and staff knowledge relating to their work.
- Identification of ineffective and inefficient operations.
- Obtaining hints for improving the efficiency of machinery and equipment.

Based on the study results, we can conclude that the internal audits provide valuable information and suggestions for the company regarding the improvement of the management system. These activities are most noticeable in most of the studied areas. In order to improve the quality management system, the organization has introduced a series of post-audit actions (staff training, position instructions, introduction of additional supervision or internal controls and improvement of the quality management system documentation). Implementation of these actions allowed partial elimination and subsequent reduction of the proportion of revealed discrepancies in the following year (Fig. 3). Results of audits were therefore a valuable source of information about the course of improving the quality management systems in the studied organization.



Source: own elaboration

Fig. 3. Percentage share of discrepancies detected during audits in individual years

In summary, audit is the fundamental management tool with primary objective to verify whether the system and processes are consistent with ISO 9000 and with the rules adopted by the company. The audit process confirms the correct implementation of processes and procedures actions within the company. Moreover, audit ensures that standards, regulations and company policy are properly implemented and retained.

References

Czarnecka-Skubina E., Kołożyn-Krajewska D., 2005. Sprawdzić i poprawić, cz. I. Bezp. Hig. Żywn., 3, 20, 22–26.

Dzwolak W., 2009. Audity w zarządzaniu jakością i bezpieczeństwem żywności. Przem. Spoż., 2, 32–35.

Gajda L., 2008. Audit wewnętrzny podstawą doskonalenia systemu zarządzania jakością, [w:] Koncepcje zarządzania jakością. Doświadczenia i perspektywy. Pod red. T. Sikory. Wyd. UE Kraków.

Knaflewska J., Pospiech E., 2007. Quality assurance systems in food industry and heath security of food. Acta Sci. Pol. Technol. Aliment, 6, 2, 75–85.

Kołożyn-Krajewska D., Sikora T., 2010. Zarządzanie bezpieczeństwem żywności. Teoria i praktyka. Wyd. C.H. Beck, Warszawa.

Kromer B., Rychły-Lipińska A., 2009. Audit wewnętrzny w ujęciu procesowym. Problemy Jakości, 8, 42–46.

Michalak A., 2008. Audity wewnętrzne – narzędzia doskonalenia, obowiązkowy standard, cz. 1. Problemy Jakości, 10, 16–22.

Michalak A., 2009. Audity doskonalenia systemów zarządzania. Problemy Jakości, 1, 21–24.

PN-EN ISO 9000, 2006. Systemy zarządzania jakością. Podstawy i terminologia.

PN-EN ISO 9001, 2001. Systemy zarządzania jakością. Wymagania.

Trafiałek J., Kołożyn-Krajewska D., Lucke F.K., 2010. Weryfikacja jako element doskonalenia systemu HACCP, [w:] Zarządzanie Jakością – Doskonalenie Organizacji. Tom II. Pod red. Tadeusza Sikory. Wyd. UE Kraków.

6

AUDIT OF FOOD DEPARTMENTS BASED ON SELECTED HEALTH CARE ESTABLISHMENTS

Introduction

Quality assurance of foods prepared in catering establishments is only possible with proper technological and hygiene proceedings. Besides the nutritional and sensory quality of food safety is also an important quality feature. Each available product on the market should have a sufficient quality and, above all, do not threaten the health of the consumer. To fill consumer expectations, the food business operator must implement a food safety system and procedures based on HACCP principles.

The sixth principle of the HACCP system refers to the need of verifying the implemented system, in order to assess the effectiveness of actions taken during the whole technological process. Verification is defined as activities other than monitoring the CCP, undertaken to determine the correctness of developed HACCP plan and degree of compliance with the functioning of the HACCP plan [Corlett 1998, *Codex Alimentarius* 2003]. The verification cover the whole HACCP system, all CCP and associated records. Verification includes activities such as: a review of records collected by the control of the CCP, the analysis of deviations from established critical limits, the analysis of customer complaints, laboratory testing of food samples, tools and working surfaces, internal audit, external audit, a review of the most common variances on the individual stages of production, review and evaluation of taken the corrective action, the assessment of the accuracy of critical limits and overview the changes implemented in the HACCP plan [Kołożyn-Krajewska and Sikora 2004, Kołożyn-Krajewska 2007, Trafiałek et al. 2010]. The method and frequency of verification procedures need to be specified [Gaze 2003].

One of the verification method is an audit. The primary aim of audit is to determine the effectiveness of the functioning of the system to ensure food safety for execution of specific quality targets and fulfillment of the requirements of law. The auditor is required to collect objective documents about the functioning system in practice by making observations and finding variances [Dzwolak 2003, Szkiel 2010].

The use of procedures based on HACCP principles is mandatory for all establishments producing and introducing foods on the market. Specific plants are medical facilities, including hospitals and nurseries. Hospital meals should be tasty, attractive, nutritious, and above all the safest and the highest quality care, free of any contaminants [Barrie, 1996]. Food-borne pollution is a serious problem in hospitals, because patients are more susceptible to poisoning than healthy people. Even a low number of pathogens can cause symptoms of poisoning and death of the patient [Reglier-Poupet et al. 2005]. Similar aspects are associated with feeding young children aged 1–3 years in day nursery.

In catering it can be observed a lack of systemic approach to solving problems related to ensuring food safety. In addition, in the most hospital food departments there are observed difficulties and irregularities in implementation of basic GHP principles, which are the base for implementation of HACCP system [Turlejska et al. 2006].

An implemented food safety ensuring system in the hospital food departments and nursery should be verified in accordance with the sixth principle of the HACCP system. The first review should take place immediately after the implementation of system, and the next one in a predetermined plan and in the event of changes or problems. The schedule of verification should include the area and frequency of verification [Sperber 1998].

The aim of this study was to assess the safety of foods produced in the selected food departments of hospitals and nurseries by verifying the HACCP system. Verification task was to demonstrate whether the system is complete, operates in practice according to agreed plan and if is effective in ensuring the safety of health produced food.

Materials and methods

Research materials were the documentation of selected food departments of three hospitals (hospital 1, 2, 3) and three nurseries (nursery 1, 2, 3) from the Mazovia region. Verification of system was based on the review and analysis: HACCP Book, system documentation, records of operations carried out, the analysis of the production process from receipt of goods, by the storage, production to expedition of final meals.

The scope of work included the preparation and conducting an audit survey in food departments to determine variances or weaknesses in the system, identifying corrective actions and report preparation.

The methodology of work was based on the verification of the HACCP system by audit. The legal basis for the conducting of audits is the Regulation (EC) 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene and Safety Act of Food and Nutrition [2006]. Basic rules of the audit includes PN-EN ISO 19011:2003 standard.

The checklists containing requirements for 12 steps of the implementation of the HACCP system as *Codex Alimentarius* [2003] were used. The list of control questions is a helpful and useful tool allowing for efficient verification of the system. The list should be prepared and tailored to the particular object [Ababouch 2000]. For this study the requirements are divided into eight parts: preliminary stages of implementation system and the 7 principles of HACCP. Requirements were evaluated in each part using the 4 point scale according to Table 1. For each part and each audited establishments, a final evaluation of the sum being the average ratings in each section was released.

Table 1

The way of estimation of audit requirements

Estimation	Description
2	Requirements are not fulfilled, the impact on food safety
3	The requirements are not fulfilled, could affect food safety
4	Not all requirements are fulfilled in the documentation and/or in the practical functioning of the HACCP system, but it does not affect food safety
5	All requirements are fulfilled in the documentation and/or in the practical functioning of the HACCP system

Source: own study

Results and discussion

On the basis of audits carried out an assessment of the implementation and operation of the HACCP system in each plant were done. Ratings of auditors for individual requirements are presented in Table 2, and an overall summary of audit results are presented in Table 3.

The food department of hospital number 2 was the best evaluated with 4.9 score. Minor auditor comments related to hazard analysis, determination of the CCP and establishment of critical limits. The current functioning of the system, including its verification, was correct. The auditors did not determine any variances in the practical operation of the system.

The food department of hospital No. 3 with 2.8 score was the worst estimated by the auditors. Variances were identified in the system documentation during implementation of the system, as well as during the current operations. The auditors had significant comments to hazard analysis which not included all types of hazards, not estimated significance of hazards, no analysis performed for all stages of production, raw materials, production environment, machinery, equipment and personnel. Critical Control Points in the number of 14 were incorrectly identified. Some of CCPs did not have substantial justification in that kitchen, for example CCP6 – chilling food (not done in this kitchen), CCP 7 – storage of semi-finished products in the cold (not stored in this kitchen), CCP9 – warmholding of hot food (not stored), CCP10 – heating to a temperature of consumption (not heated up), CCP13 – reheating and serving (not heated up). Moreover, in the hospital No 3 verification of the system was not conducted at all, and there were many irregularities in current documentation.

The problems with proper identification of the elements for monitoring were pointed by Panisello and Quantick [2001]. They conducted an extensive study of technical barriers in the implementation of HACCP, which are defined as all behaviors, attitudes and beliefs affecting negatively the idea of a proper understanding and application of the system. Among these barriers they mentioned illusion of control, which was based on wrong identification of hazard occurred during food production in the plant and conviction of the total control. According to these authors, an excessive number of items that require monitoring and too extended documentation were major barriers to the proper functioning of the system. Hielm et al. [2006] also pointed the problem of choosing the number of CCPs during the implementation of the HACCP system. However, Mortimore [2001] underlined the need of reduction the number of CCPs to simplify management of them.

Table 2

The results of audits conducted in the survey establishments

Reguirements	Nursery			Hospital		
	1	**2**	**3**	**1**	**2**	**3**
1	2	3	4	5	6	7
Realization of 5 initial stages according to *Codex Alimentarius*						
Is it set up a team for HACCP?	5	5	5	5	5	2
Is there a full description of the product?	4	4	5	5	5	3
Is an end-consumer identified?	4	4	5	5	5	5
Is a flow diagram of the technological process built?	5	5	5	5	5	4
Is the flow diagram verified on in the site?	2	2	5	5	5	2
Principle I of HACCP system: Hazard analysis						
Is a list of hazards associated with applying raw materials prepared?	5	5	5	5	4	2
Is the list of hazards completed and includes all types of hazards: biological (including microorganisms and pests), chemical and physical?	4	4	4	5	5	3
Were the causes of hazards and scale of hazards estimated?	5	5	5	5	5	2
Are there preventive action specified?	4	4	4	5	5	2
Is a list of hazards associated with all technological stages made?	5	5	5	5	5	3
Is the hazard analysis in relation to personnel done?	5	5	5	5	5	2
Is the hazard analysis in relation to building, machinery and equipment made?	3	3	3	5	5	2
Principle II of HACCP system: Critical Control Point (CCP) identification						
Are the CCPs determined?	5	5	5	5	5	5
Are all determined CCPs necessary?	4	4	5	5	4	3
Are CPs separated from the CCPs?	5	5	5	5	4	3
Was the identification CCP done with use of the decision tree compatible with *Codex Alimentarius?*	5	5	5	5	4	3
Principle III of HACCP system: Establishment of critical limits for CCP						
Are critical limits defined for all CCP?	5	5	5	5	4	3
Are determined critical limits correct and posses substantial justification?	5	5	5	5	5	4
Are the critical limits known to employees responsible for the CCP?	3	3	4	5	5	3
Principle IV of HACCP system: Monitoring of CCP						
Are CCP monitoring procedures/ instructions written?	5	5	5	5	5	3
Do employees responsible for the CCP know how to monitor parameters?	4	3	4	4	5	2
Do employees carry out monitoring in accordance with the given way of monitoring?	4	4	3	3	5	3
Do employees carry out CCP monitoring in accordance with the established frequency?	4	4	4	3	5	3

Table 2 cd.

1	2	3	4	5	6	7
Regularity of monitoring recording	4	4	4	3	5	3
Are the control and measurement devices calibrated?	3	3	5	5	5	2
Principle V of HACCP system: Corrective action						
Are corrective actions determined for all identified CCPs?	5	5	5	5	5	4
Are corrective action effective?	5	3	5	5	5	5
Are there written procedures/instructions of corrective actions?	5	5	5	5	5	2
Do the personnel responsible for the CCP know corrective actions?	5	3	5	5	5	2
Do employees take corrective actions when monitoring indicates a loss of control in the CCP?	3	3	4	3	5	2
Are there documents confirming the execution of corrective actions?	4	4	4	5	5	2
Are records of corrective action carried out systematically?	5	5	5	5	5	2
Principle VI of HACCP system: Verification of system						
Establishing method and frequency of verification	5	5	5	5	5	2
Are there written procedures/ instructions for verifying the HACCP system?	5	5	5	5	5	2
Is there a responsibility for verification of the system set?	5	5	5	5	5	2
Are verification activities documented?	2	2	3	3	5	2
Is verification led by established methods?	2	2	3	3	5	2
Is verification led in accordance with a determined frequency?	3	3	3	3	5	2
Are results of verification analyzed?	3	3	3	3	3	2
Principle VII of HACCP system: Documentation						
Is there a procedure for documentation of the HACCP system?	5	5	5	5	5	2
Are changes in system documentation supervised?	4	4	4	3	5	2
Is there a designated person for the supervision of documentation?	5	5	5	5	5	2
Does designated person carry the actual supervision of system documentation?	4	4	4	3	5	2
Are documents (procedures, instructions) clear and readable?	4	4	4	4	5	2
Are documents available for workers?	5	5	5	5	5	2
Do records of staff training in range of food hygiene exist?	5	5	5	5	5	5
Is there evidence that supervisors of HACCP system are trained with the system?	5	5	5	5	5	2
Do supervisors of system know the CCPs determined in plant?	3	4	5	4	5	2

Source: own study

Table 3

Summary of results of HACCP audits

Reguirements	Nursery			Hospital		
	1	2	3	1	2	3
Realization of 5 initial stages according to *Codex Alimentarius*	4	4	4	5	5	3,2
Principle I of HACCP system: Hazard analysis	4,4	4,4	4,4	5	4,9	2,3
Principle II of HACCP system: Critical Control Point (CCP) identification	4,75	4,75	5	5	4,25	3,5
Principle III of HACCP system: Establishment of critical limits for CCP	4,3	4,3	4,7	5	4,7	3,3
Principle IV of HACCP system: Monitoring of CCP	4	3,8	4,2	3,8	5	2,7
Principle V of HACCP system: Corrective action	4,6	4	4,7	4,7	5	2,7
Principle VI of HACCP system: Verification of system	3,6	3,6	3,9	3,9	5	2
Principle VII of HACCP system: Documentation	4,4	4,6	4,7	4,3	5	2,3
TOTAL	34.05	33.45	35.6	36.7	38.85	22
AVERAGE ASSESSMENT	4.3	4.2	4.5	4.6	4.9	2.8

Source: own study

Food departments in nurseries No 1, 2, 3 and in hospital No. 1 were assessed quite similarly. The auditors had a small comments on the development of a system, i.e. realization of 5 initial stages of HACCP system, hazard analysis, the designation of Critical Control Points and critical limits. However, in the practical operation of the HACCP system, starting from monitoring the CCP, corrective actions and verification system, auditors identified several variances or comments.

In all the nurseries and hospital No. 1, the Critical Control Point monitoring and system verification were estimated as the worst. At these plants procedures for the monitoring of the HACCP were developed and implemented, but not all employees knew how to monitor determined parameters and did not conduct monitoring in accordance with determined frequency. In the card of monitoring records were not done systematically and no one checked if employees fulfilled their obligations so, the verification of the current functioning of the HACCP system was not carried out.

Verification is the most efficient and reliable method for determining whether the HACCP system is functioning in the plant in accordance with determined plan [Sperber 1998, Kwiatek and Kudyba 2003, Staszewska and Janik 2004]. If the verification is not carried out at all, like in hospital No 3 or is done irregularly, such as in nurseries and hospital No. 1, it can be concluded that this is the reason for the lack of knowledge of the variances during current functioning of the system. Verification should be conducted at regular intervals. A careful check of the system must show that all measures and working methods are applied as planned [Notermans and Gallhof 1994]. Moreover, it is also important the commitment of

all employees in the functioning of the HACCP system. This will allow the proper flow of information and self-employees control [Eves and Dervisi 2005]. Many authors [Howes et al. 1996, Soriano et al. 2002, Youn and Sneed 2003, Henroid and Sneed 2004, Medeiros et al. 2004, Bas et al. 2006, Seamon and Eves 2006, Trafiałek and Kołożyn-Krajewska 2006, Yapp and Fairman 2006] indicate the staff, his knowledge, motivation and commitment as factors to assure proper implementation and functioning of the HACCP system.

Audits were conducted by development of the audit reports. The report is a confidential document containing the results of the audit, which will contribute to improving the system and preventing formation of variances in the future (Arter 2003]. The final report contains all observations and variances, which were collected during the audits. In each of the audit report, the auditors suggested applying corrective actions for removing variances identified during audits. It should be noted that in the case of prosecution of food poisoning of consumers, only proper documentation can be used to show proper proceeding during production [McSwane et al. 2003].

Conclusions

1. Only in one of six audited establishments auditors did not have major objections to the development and actual functioning of the HACCP system.
2. One of the audited hospitals obtained a negative assessment, and it can be assumed that production of food in the food department of this hospital causes a hazard to the patients.
3. The HACCP system in audited nurseries was implemented properly, but the current functioning of the system requires a lot of attention and supervision.
4. Based on data obtained from the audited health care establishment it can be concluded that the verification of system is a necessity, especially in institutions such as the surveyed ones, where people are sick with weakened immune systems (hospital), or small children, not fully shaped immunity of health (nursery). The verification of system should be considered as preventive actions of food poisoning caused by consumption of meals in public catering establishments.

References

Ababouch L., 2000. The role of government agencies in assessing HACCP. Food Control, 11, 137–142.

Arter D., 2003. Quality audits for improved performance. AS Quality Press, Milwaukie, 83–87.

Barrie D., 1996. The provision of food and catering services in hospital. Journal of Hospital Infection 33, 13–33.

Baş M., Ersun A.S., Kivanç G., 2006. The evaluation of food hygiene knowledge, attitudes, and practices of food handlers in food businesses in Turkey, Food Control, 17, 317–320.

Codex Alimentarius, 2003. Hazard Analysis and Critical Control Point [HACCP] System and Guidelines for its application. Food Hygiene Basic Text, FAO/WHO Roma.

Corlett D.A., 1998. HACCP user's manual. Aspen Publishers, Gaithersburg, Maryland.

Dzwolak W., 2003. Audit systemu HACCP. Bezp. Hig. Żywn., 10, 5, 21–22.

Eves A., Dervsi P., 2005. Experiences of the implementation and operation of hazard analysis critical control points in the food service sector. Hospitality Management, 24, 1–19.
Gaze R. (Ed.), 2003. HACCP, a practical guide. Campden & Chorleywood Food Research Association Group, Chipping Campden.
Hielm S., Tuominen P., Aarnisalo K., Raaska L., Maijala R., 2006. Attitudes towards own-checking and HACCP plans among Finnish food industry employees. Food Control, 17 (5), 402–407.
Henroid D., Sneed J., 2004. Readiness to Implement Hazard Analysis and Critical Control Point (HACCP) Systems in Iowa Schools. Journal of the American Dietetic Association, 104 (2), 180–185.
Howes M., McEwan S., Griffiths M., Haris L., 1996. Food handler certification by home study: measuring changes in knowledge and behavior. Dairy Food Environmental Sanitation, 3, 208–214.
Kołożyn-Krajewska D., Sikora T., 2004. Weryfikacja systemu HACCP. Bezp. Hig. Żywn., 1, 12, 26–27.
Kołożyn-Krajewska D. (Ed.), 2007. Higiena produkcji żywności. Wyd. SGGW, Warszawa.
Kwiatek K., Kudyba M., 2003. Czy i dlaczego weryfikować. Bezp. Hig. Żywn., 2, 7, 26–27.
McSwane D., Rue N., Linton R., 2003. Essentials of food safety and sanitation. Ed. Pearson Education. New Jersey, 169–196.
Medeiros L.C., Hillers V.N., Chen G., Bergmann P., Kendall V., Schoreder M., 2004. Design and development of food safety knowledge and attitude scales for consumer food safety education. Journal of American Dietetic Association, 104 (11), 1671–1677.
Mortimore S., 2001. How to make HACCP really work in practice. Food Control, 12, 209–215.
Notermans S., Gallhof G., 1994. Quantitative Risk analysis. As an element of quality assurance systems in food industry. Fleischwirtschaft, 74, 10, 1036–1045.
Panisello P.J., Quantick P.C., 2001. Technical barriers to Hazard Analysis Critical Control Point (HACCP). Food Control, 12, 165–173.
PN-EN ISO 19011, 2003. Wytyczne dotyczące auditowania systemów zarządzania jakością i/lub zarządzania środowiskowego.
Reglier-Poupet H., Parain C., Beauvais R., Descamps P., Gillet H., Le Peron J.Y., Berche P., Ferroni A., 2005. Evaluation of the quality of hospital food from the kitchen to the patient. Journal of Hospital Infection, 59, 131–137.
Regulation (EC) 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene (OJ. EU L 139, 30.4.2004, p. 1).
Safety Act of Food and Nutrition, 2006. Ustawa o bezpieczeństwie żywności i żywienia z dnia 25 sierpnia 2006 roku (Dz. U. 2006, 171, 1225 z późn. zm.).
Seamon P., Eves A., 2006. The management of food safety – the role of food hygiene training in the UK service sector. Hospitality Management, 25, 278–296.
Soriano J.M., Rico H., Moltó J.C., Manes J., 2002. Effect of introduction of HACCP on the microbiological quality of some restaurants meals, Food Control, t. 13, 253–261.
Sperber W.H., 1998. Auditing and verification of food safety and HACCP. Food Control, 9, 2–3, 157–162.
Staszewska E., Janik M., 2004. Weryfikacja systemu. Przegl. Piek. Cukier., 52, 11, 34–35.
Szkiel A., 2010. The use of techniques and tools of quality management systems improvement by food chain organizations, [w:] Zarządzanie jakością. Doskonalenie organizacji. Tom II, pod red. T. Sikora, Uniwersytet Ekonomiczny w Krakowie, Kraków 2010, (in Polish, English abstract), 496–515.
Trafiałek J., Kołożyn-Krajewska D., 2006. Implementacja systemu HACCP w małych zakładach produkujących żywność – wyniki badań prowadzonych w Polsce przed i po akcesji do Unii Europejskiej. Żywność. Nauka. Technologia. Jakość., 46, 1, 201–213.
Trafiałek J., Kołożyn-Krajewska D., Lücke F.K., 2010. Verification as an element of the HACCP system improvement, [w:] Zarządzanie jakością. Doskonalenie organizacji. Tom II, pod red.

T. Sikora, Uniwersytet Ekonomiczny w Krakowie, Kraków 2010, (in Polish, English abstract), 478–486.
Turlejska H., Pelzner U., Konecka-Matyjek E., 2006. Rola i znaczenie systemu HACCP w żywieniu zbiorowym w szpitalach. Zdrowie Publiczne, 1 (116), 152–155.
Yapp C., Fairman R., 2006. Factors affecting food safety compliance within small and medium-sized enterprises: implications for regulatory and enforcement strategies, Food Control, 17, 42–51.
Youn S., Sneed J., 2003. Implementation of HACCP and prerequisite programs in school foodservice. Journal of the American Dietetic Association, 103 (1), 55–60.

7

HACCP SYSTEM FUCTIONING EVALUATION MODEL BASED ON BISTRO BARS IN POLAND

Introduction

With the growing importance of food safety, there can be noticed an increasing interest of food safety management in food production, trade and services companies [Kijowski and Sikora 2003, Kołożyn-Krajewska and Sikora 2010, Luning et al. 2005].

For several years, in Poland, there has been noticed a growing trend for eating outside the home. Currently, consumers have a catering service not only to meet their basic nutritional needs, but offered services include a wider scope, as well as a growing group of customers. A good example are gas stations, where there are bistro bars are situated [Czarniecka-Skubina 2006, Nowicki and Sikora 2011a]. Catering companies, including bistros at petrol stations, in order to succeed in the market, in the company's strategy must take into account customer requirements and needs as well as implement a quality assurance system. Proper quality is a guarantee of regular customers and the ability to grow and profit [Nieżurawska 2001, Nowicki 2009].

This article presents analysis of the four synthetic variables vs. selected categories and also shows a functioning evaluation model of the HACCP system based on an example of selected fuel concern bistros.

Research material

The study was conducted in 2008 in nine provinces, using the survey method with a questionnaire developed for this testing. The selection of regions was not random but there had been selected all provinces in which there were located the most of the stations of selected company. In other provinces the number of stations was very small, so that is why they had not been tested. The study covered bistros of selected network of petrol stations in the number of 210 stations. The study subjects were employees of petrol stations.

There was implemented and maintained HACCP system in the tested bistro bars of the selected fuel company. The menu included: sandwiches, hot dogs, casseroles, and drinks such as coffee and tea. In addition to offering products there were also offered as ready-made supplied products and only served to the customers (cakes, tortillas, sandwiches).

Bistros' employees of fuel stations network were surveyed with an anonymous questionnaire designed to determine their level of knowledge, safety awareness and hygiene of food processing and serving. Respondents were selected randomly from among those who currently were working in bistros. The total number of respondents was 280 people and it

obtained a representative sample of the whole population. The correct completed questionnaires replied only 269 respondents. The study was conducted at the stations during the two consecutive months in order to ensure homogeneity of the respondents, due to the level of knowledge of employees and organizational standards in the bistros. The questionnaire survey intended for employees included 22 closed questions and one open-ended question, which allowed to obtain a precise answers. In addition, respondents answered questions categorized due to socio-economic characteristics.

The results obtained in the study were analyzed statistically using multiple methods of analysis of variables, both qualitative and quantitative, including descriptive statistics, statistical inference, and multivariate analysis methods [Kot et al. 2007].

The results

The characteristics of the social structure of the respondents are presented in Table 1. Among 269 persons who have properly filled out the survey, the biggest group were women (78%). Taking under consideration the educational level of respondents, the biggest group were people with high school education (46%). In terms of age of the largest group of bistros' employees were people below the age of 35 (74% in total).

Analyzing work experience, it was found that the vast majority of people has been working in bistros over 6 years, which may indicate that workers with such experience will have extensive knowledge in the health and safety of food production.

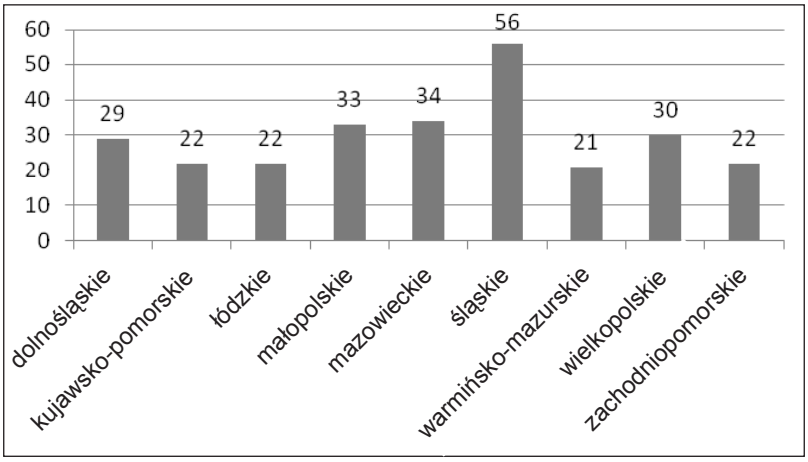
Table 1

Social structure of respondents.

Gender	Percentage [%]
woman	30
man	70
Age	Percentage [%]
below 25	23
26–35 years old	55
36–45 years old	15
46–55 years old	4
56–65 years old	2
above 65 years old	1
Education	**Percentage [%]**
primary	1
vocational	5
high school	25
high-bachelor	19
high-master	50

Source: own research

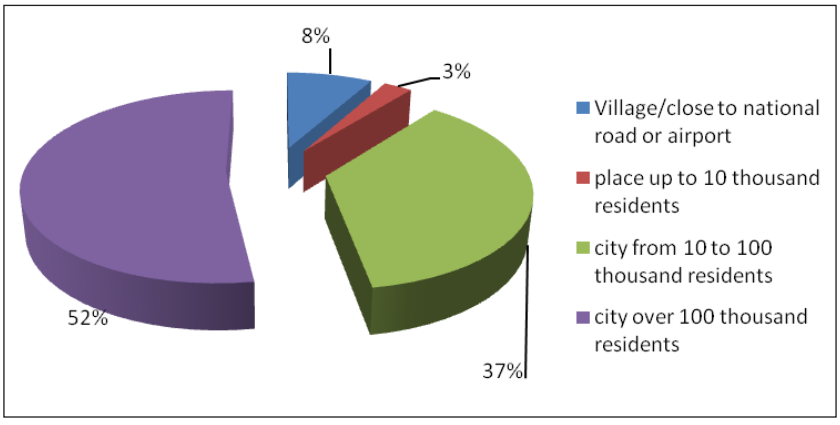
Employees of the bistros of selected gas stations were tested in 9 regions of Poland. Quantitative distribution of surveyed workers in the various provinces are presented on Figure 1. Employees have the largest representation in Slaskie region, because in this province there is situated a biggest number of fuel stations of the selected company. It is connected with the Katowice conurbation and a very high concentration of population in this area. In other provinces the number of surveyed respondents was lower, due to smaller number of petrol stations there.



Source: own research

Fig. 1. The number of surveyed employees i selected regions

As for the location of the particular stations of the test group because of the size of the city (Fig. 2), the vast majority (52%) was located in cities with more than 100 thousand residents. A large percentage of stations was also located in cities from 10 to 100 thousand. residents.



Source: own research

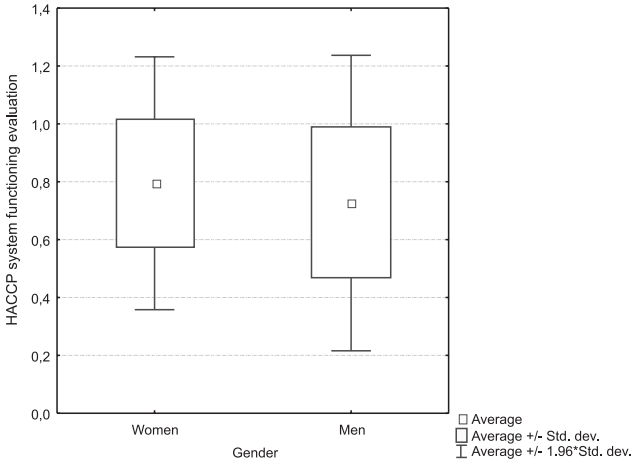
Fig. 2. Location of stations

Analyzing the responses of employees to individual questions in the questionnaire, they were grouped by theme that led to creating four variables that describe:

1. HACCP system functioning evaluation.
2. Knowledge about the HACCP system.
3. Knowledge about hazards and hygiene in food production.
4. Self-esteem of knowledge and preparation for work.

For the variable "HACCP system functioning evaluation" there were used answers to questions in the survey, in which workers responded to the functioning of the system at the station in which they are employed. The variable "Knowledge of the HACCP system", was created by grouping questions No: 3, 6, 7, 8, 9, 10, 11 and 12, containing answers to the various elements of the system. The questions: 15, 16, 17, 18 and 19 were used to create a variable describing the "Knowledge about hazards and hygiene in food production." In order to obtain information from employees to identify the "Self-esteem of knowledge and preparation for work" there was used fourth group of questions in the survey, which covered this subject. It should be stressed that this is a subjective assessment of the workers.

Analyzing synthetic variable assessing the functioning of the system by employees in relation to gender (Fig. 3), it has been observed that women evaluate system higher than man. However, the scope of their assessments is smaller than for men, which caused the average rating among men is lower.



Source: own research

Fig. 3. Box-whiskers plot chart describing HACCP system functioning in relation to the gender of respondents

A wider range of ratings awarded by men may be due to the fact that they do not clearly perceive the system and therefore are not sure of how it works and how complex the HACCP system is functioning in the enterprise. When performing the verification test to compare the average, a statistically significant difference in response rate due to sex of employees for this variable (p=0.056) was stated (Tab. 2). Although the significance level is slightly above the 0.05–0.0551 value obtained – it is so insignificant that it should lead to the significance of the difference in the levels of the compared variables. Condition is satisfied because $\alpha < 0.1$ indicates the significance of the difference.

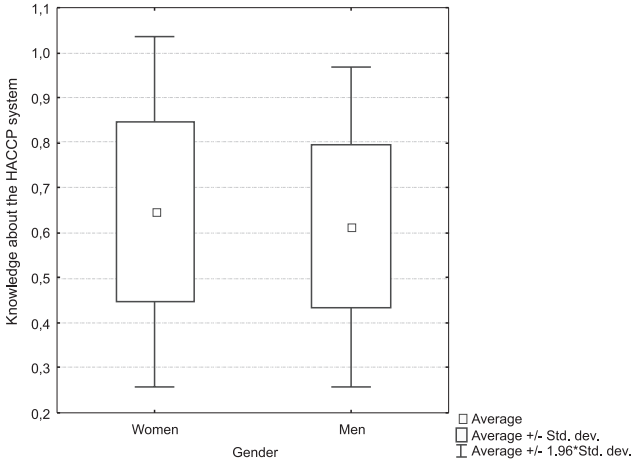
Table 2

Table summarizing the results of t-test to compare the average level of variables assessment of specific areas in relation to gender

Variable	T-tests, Grouping: Gender, Group 1: woman, Group 2: man										
	Average women	Average men	t	df	p	N valid women	N valid men	Std. dev. women	Std. dev. men	F variance ratio	p variance
System functioning assessment	0.794471	0.728070	1.920821	263	0.055835	208	57	0.222808	0.259982	1.361533	0.126501
Knowledge about the HACCP system	0.646635	0.614035	1.117758	263	0.264690	208	57	0.198625	0.181369	1.199336	0.426266
Knowledge about the hazards	0.279808	0.308772	–0.892198	263	0.373102	208	57	0.208922	0.245154	1.376922	0.113252
Self-esteem knowledge assessment	0.802945	0.751754	1.724386	263	0.085813	208	57	0.184796	0.242769	1.725833	0.006439

Source: own research

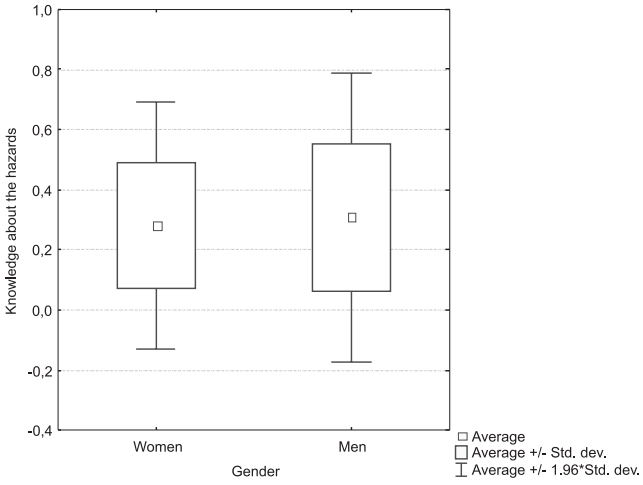
Taking into account the variable of employees knowledge about the HACCP system in relation to gender, similar as in the case of evaluation of functioning of the HACCP system, there were observed that women have a greater knowledge of the HACCP system than men (Fig. 4), whereby the scope of the results obtained and its limits for women is greater, suggesting that the imbalance in the level of knowledge concerning the HACCP system are higher among women than among men. However there was no statistically significant difference in average values (p=0.26).



Source: own research

Fig. 4. Box-whiskers plot chart describing employees knowledge of HACCP system in relation to the gender of respondents

Very interesting information provides the distribution of knowledge about the hazards variable in relation to employees' gender (Fig. 5), where a slightly higher level of knowledge about the hazards among men than among women was observed. For men the range of results and its limits are higher than among women, which reveals much greater differences in the level of knowledge about the hazards among men compared to women. However, there was no significance importance in the average levels of knowledge in this area (p=0.37).

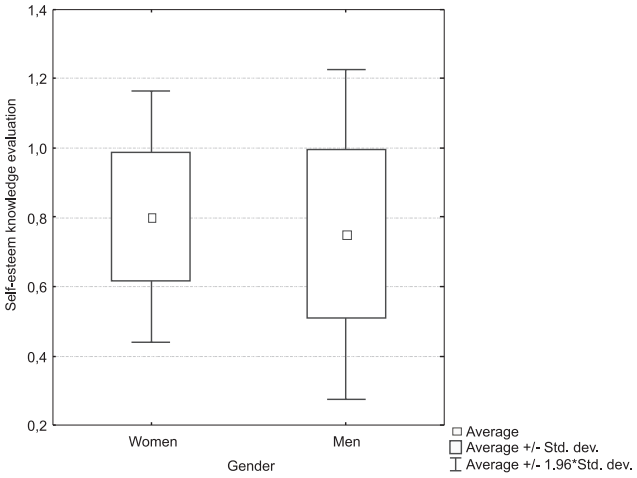


Source: own research

Fig. 5. Box-whiskers plot chart describing employees knowledge about hazards in relation to the gender of respondents

Taking under consideration the employees' self-esteem of knowledge in relation to a gender perspective, has proved once again that women assess their knowledge of food safety management, as well as their preparation for work higher than men (Fig. 6). This distribution also shows that women are more confident of their knowledge, because the limits of their response is much smaller than for men. During the test the significance of differences in average levels of knowledge among men and women to the level of p=0.08 was stated. In this case it also assumed that if the level of significance for the test is lower than 0.1 it still recognize the difference as significant.

The bistro bars of selected fuel concern were divided into two groups A and B, where bistros coded A were subjected to the process of rebranding, which had to change their image, and its scope included a new training cycle, introduced new products and changes related to the design and interior lay out. While bistros labeled B were sites before rebranding process. Rebranding is the process of economic development, which is characterized by a change of company name or product image or logo. In this case in the tested company, these changes were associated with repositioning of the offered products and refreshment of the company image [Nowicki and Sikora 2011b].



Source: own research

Fig. 6. Box-whiskers plot chart describing self-esteem of employees knowledge in relation to the gender of respondents

By analyzing the distribution of synthetic variables in relation to bistro type, there has been observed that in the bistro bar coded A, the evaluation of the system, the employees knowledge about the hazards, and self-esteem of knowledge (Fig. 7, 9, 10), is at a far higher level than in for bar coded B. This is due to rebranding process so that employees of bars coded A held for example a new training cycle. This allows them to be a better prepared to work in the bistro and have a more complete knowledge as evidenced by carried out studies. In case of health safety of food products offered, rebranding has brought a lot of benefits in terms of better functioning of the HACCP system, the higher the knowledge about the risks, as well as higher levels of self-esteem of knowledge among employees. By using T-test for verifying the significance of differences in average levels of variables, it was found that there are statistically significant differences in bistro type for the three synthetic variables: assessment of functioning ($p=0.03$), the level of knowledge about the risks ($p <0.001$) and self-esteem of knowledge ($p=0.02$) (Tab. 3). However, in the case of a variable describing the knowledge of the HACCP system in both types of bistro average level of knowledge and range values are shaped almost identically (Fig. 8).

Another element of the analysis was to construct a model in which it would be possible to assess the functioning of the system as depending on the level of knowledge, hazards awareness and competence of the employees of bistro. For realizing this goal the results of the survey were used and based on them the thematic variables were constructed.

There has also been proposed a linear model form of HACCP system functioning evaluation- Y_i, depending on the level of knowledge about the system – X_i, knowledge about the hazards – Z_i and self-esteem assessment of competence by an employee – W_i, which fulfilled the picture of theirs knowledge with the questions included in the variable about knowledge of the system.

$$Y_i = \alpha_0 + \alpha_1 X_i + \alpha_2 Z_i + \alpha_3 W_i$$

where:

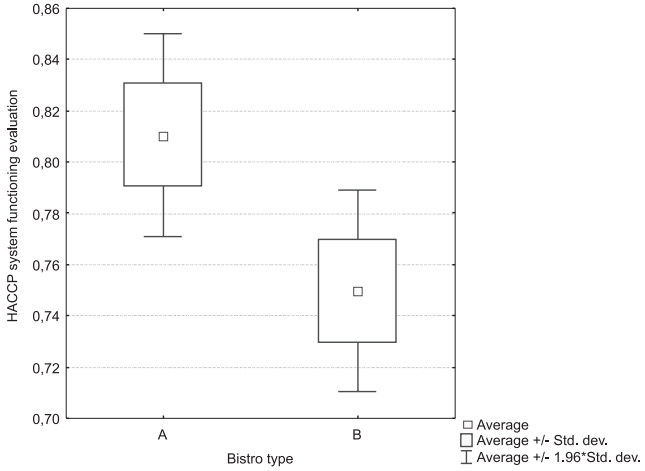
$\alpha_0 \ldots \alpha_3$ – estimated parameters of the model.

Table 3

Table summarizing the results of t-test to compare the average level of variables assessment of specific areas in relation to bistro type

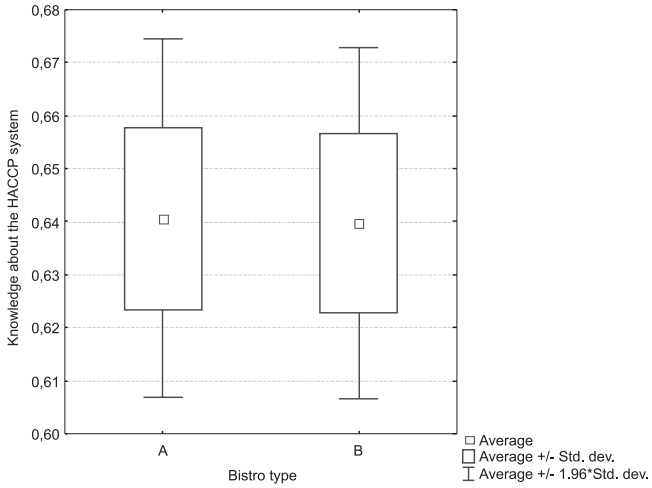
Variable	T-tests, Grouping: Bistro type, Group 1: A, Group 2: B										
	Average A	Average B	t	df	p	N valid A	N valid B	Std. dev. A	Std. dev. B	F variance ratio	p variance
System functioning assessment	0.810547	0.750000	2.129333	262	0.034159	128	136	0.227850	0.233730	1.052281	0.772530
Knowledge about the HACCP system	0.640625	0.639706	0.038133	262	0.969611	128	136	0.194452	0.196911	1.025448	0.887343
Knowledge about the hazards	0.334375	0.241176	3.557325	262	0.000445	128	136	0.200957	0.223266	1.234342	0.230982
Self-esteem knowledge assessment	0.819727	0.764246	2.278800	262	0.023484	128	136	0.185037	0.208911	1.274695	0.167436

Source: own research



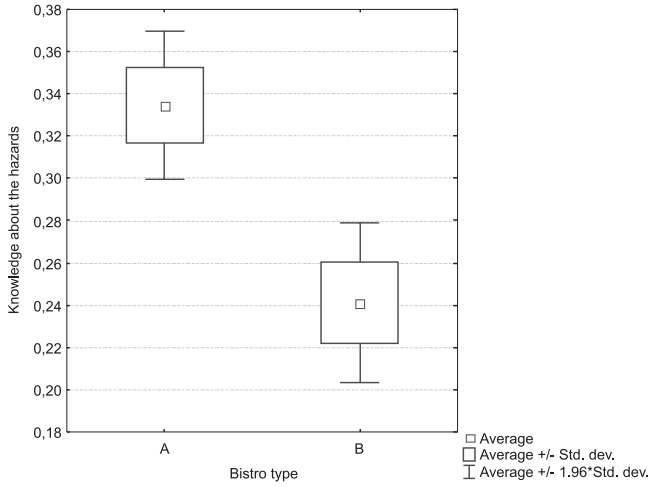
Source: own research

Fig. 7. Box-whiskers plot chart describing HACCP system functioning in relation to the type of the bistro



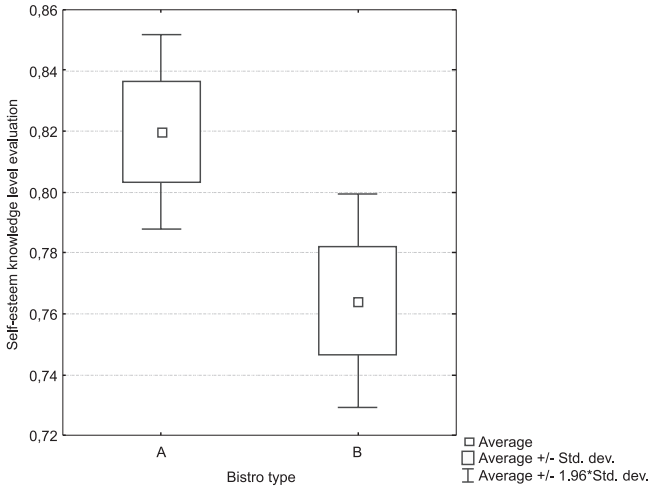
Source: own research

Fig. 8. Box-whiskers plot chart describing employees knowledge about HACCP system in relation to the type of the bistro



Source: own research

Fig. 9. Box-whiskers plot chart describing employees knowledge about hazards in relation to the type of the bistro.



Source: own research

Fig. 10. Box-whiskers plot chart describing self-esteem of employees knowledge in relation to the type of bistro

As a result of multiple regression model estimation, it was found that the variable X_i –level of knowledge about the system was not significant (Tab. 4). Therefore, it has been eliminated and re-estimation was conducted. Resulting in a satisfactory model to describe the degree of the system functioning using only two variables: the level of knowledge about the hazards and self-esteem of knowledge (Tab. 5).

Table 4

Summary of regression of the dependent variable assessment of the functioning of the system relative to the other quantitative variables

	BETA	Std. dev.	B	Std. dev.	t(5)	p level
Free Variant			0,024250	0,377475	0,06424	0,951266
Knowledge about the HACCP system	0.041940	0.249589	0.053061	0.315765	0.16804	0.873139
Knowledge about the hazards	-0.547615	0.251991	-0.725333	0.333769	-2.17316	0.081817
Self-esteem knowledge assessment	0.818639	0.239024	1.171649	0.342095	3.42492	0.018738

Source: own research

Table 5

Summary of regression of the dependent variable assessment of the functioning of the system relative to the other quantitative variables, after elimination of the variable knowledge about the HACCP system

	BETA	Std. dev.	B	Std. dev.	t(5)	p level
Free Variant			0.069866	0.240112	0.29097	0.780870
Knowledge about the hazards	-0.562154	0.216659	-0.744590	0.286971	-2.59466	0.040955
Self-esteem knowledge assessment	0.813016	0.216659	1.163602	0.310085	3.75252	0.009482

Source: own research

The analysis showed that the employees have more knowledge about the hazards in food production, the lower they estimate the system functioning. This relationship seems to be the most correct due to the fact that having a high knowledge of the hazards in food production and processing enables the employee to perceive the complex activities of the HACCP system functioning, and pay attention to details that for the employee with a low level of threat perceptions are not relevant, or even are not noticeable. From the other hand, employees who have high knowledge about the hazards, seeing for example irregularity arising from this fact automatically translate them to the fact that the system does not work completely and in their evaluation the system functioning will be lower.

Another statistically significant relationship is that, the employees estimate higher their knowledge and preparation to work, the HACCP system functioning is being estimated higher. This is probably due to the fact that employees that feel their knowledge as high and complete and are well prepared to work in the catering services directly associate this fact with the functioning of the system. Such thinking or approach appears to be correct from the standpoint of employee consciousness, but often may be fatal, because this is just subjective feeling that does not necessarily translate directly to the smooth functioning of the HACCP system.

The final form of the model is as follows:

$$Y_i = - 0.562* Z_i +0.813 * W_i$$

The resulting model is well suited for use in practice in order to evaluate the functioning of the system in the bistro located at the station.

Conclusions

- The conducted researches and its analyze developed a model describing the degree of the system functioning with two variables: the level of knowledge about the hazards Zi and self-esteem evaluation of knowledge level Wi. The model is as follows:

$$Y_i = - 0,562*Z_i + 0,813*W_i$$

- Statistical analysis of results showed that the employees have more knowledge about the hazards in food production, the lower they estimate the system functioning.
- There has also been proved relationship that the employees estimate higher their knowledge and preparation to work, the HACCP system functioning is being estimated higher.
- The bistro bars, which have undergone the rebranding process, the evaluation of the system, the level of knowledge about the risks in food production and self-knowledge and preparation for workers is much higher than in the bistros before the changes. Statistical verification showed significant relationships among the two groups with regard to individual bistros synthetic variables.

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References

Czarniecka-Skubina E., 2006. Jakość usługi gastronomicznej w aspekcie żywieniowym, technologicznym i higienicznym. Żywność. Nauka. Technologia. Jakość, 1(46) Supl., 24–34.

Kijowski J., Sikora T. (red.), 2003. Zarządzanie jakością i bezpieczeństwem żywności. Integracja i informatyzacja systemów. WNT, Warszawa.

Kołożyn-Krajewska D., Sikora T., 2010. Zarządzanie bezpieczeństwem żywności, Teoria i praktyka Wyd. C.H. Beck, Warszawa.

Kot S. M., Jakubowski J., Sokołowski A., 2007. Statystyka, Diffin.

Luning P.A., Marcelis W.J., Jongen W.M.F., 2005. Zarządzanie jakością żywności. Ujęcie technologiczno-menedżerskie. WNT, Warszawa.

Nieżurawska M., 2001. Jakość żywności a preferencje konsumentów. Przem. Spoż., 55, 12, 32–33.

Nowicki P., Sikora T., 2011a. Customer satisfaction of food quality and safety based on the example of bistro bars in Poland, Proceedings of the 5th International Quality Conference, Center for quality, Faculty of Mechanical Engineering, University of Kragujevac, Serbia, 20.05.2011, 619–624.

Nowicki P., Sikora T., 2011b. Wpływ procesu rebrandingu na funkcjonowanie systemu HACCP w przedsiębiorstwie, [w:] Wybrane aspekty zarządzania jakością pod red. M. Salerno-Kochan, Wydawnictwo Naukowe PTTŻ, 204–214.

Nowicki P., 2009. Poziom zapewnienia jakości świadczonych usług przez sieć barów bistro w opinii klientów, Żywność. Nauka. Technologia. Jakość, 2, 154–166.

8

FOOD SAFETY AND CONTROL – A NEW STUDY PROGRAM

Introduction

Faculty of Biotechnology and Food Sciences has bachelor and master degree program which are accredited named Food safety and control. The study program was based on the requirements of practice, inspection bodies and laboratories to train well-prepared students for domestic and foreign markets. Profile of this study program is broadly aimed to enforce the state and the private sphere, in food chain, in trade and a common canteen. Bachelor's theoretical basis is measured to obtain a general knowledge of chemistry, physics, mathematics, biology, plant and animal production, information technology and public health and food production. In follow-up post-secondary subjects such as biochemistry, microbiology, analytical chemistry, biophysical chemistry and the first training courses profiling – General Food Hygiene, Epidemiology and Food allergies, Microbiological methods, Food risks in food production and Sensory analysis of food.

The profile subjects of this program are Food Hygiene, Sanitation in Food, Predictive microbiology in the food science, Good hygiene practice in food processing, Food technology basics, Hygiene distribution and sale of food. On the master degree program is the study designed to manage security systems and food control. On this program are tought subject as Counterfeiting and authentication of food, Food chemistry, Food microbiology, Senzometrics and food informatics, Food safety, Food toxicology, Food safety health, Risk assessment, Technology of food animal and vegetable origin, Food control and legislation, Food mycology, GM Foods and Food Safety Authority. The graduates is the official food control, corporate control of food, corporate labs, audit firms, education and research.

The principle of the Bologna Process is a co-operation between nation states that each decide on its higher education policy. The objectives of Bologna Process are described by ten action lines [Nyborg 2004] on road towards the achievement of a European Higher Education Area. The goals defined in Bologna Process include the adoption of a comparable degree system with two main cycles, aimed at facilitating movement between countries and establishment of a system of credits as a condition for achieving the goal of increased mobility for students and academic and administrative staff in higher education. The promotion of quality assurance and increased inter-institutional cooperation is also an objective of the Bologna Process [Bologna Action Lines 2005].

The Prague Communiqué in 2001 set out directions and priorities for the next stages of the Bologna Process. The Prague Communiqué defined following actions lines: lifelong learning, higher education institutions and students and promoting the attractiveness of the European Higher Education Area.

The Berlin Communiqué in 2003 defined doctoral studies and synergy between the European Higher Education Area and the European Research Area [The Bologna Process 2004].

Implementation of Bologna Principles in the system of study in Faculty of Biotechnology and Food Sciences in Sua in Nitra

The Slovak Agricultural University is the only university of its kind in Slovakia. It has acuquired a unique national status. The Faculty of Biotechnology and Food Sciences, as a one of the six faculties, was established in 2002 and lunched its advisory services which would create conditions for the development of agriculture, food production and processing and biotechnology. The Faculty is dedicated to the objective technological and economic sciences specializing in modern process of food production, evaluation and processing and specializing in modern process of food production, evaluation and processing and specific products of biotechnologies. The main goal is the achieve the biological and technological integrity of the "agricultural product-food system".

Establishment of a system of credits (ECTS)

The Faculty realizes flexible system of study with credits evaluation according with ECTS rules. At creation of study programs appears necessity to define each educational activity, includes in programs, as a single (independent) until with goal, content, used forms of educations and method of evaluation. Because of an effective work at creation of study program oneself show applicable, that all the subjects were taught only one semester. Credit values of subjects were carried out under quantity of student work and no under the position of subjects from the view of study focus. Subjects, which teaching is realized by other faculties, have those credit value as a belong them in the Faculty of Biotechnology and Food Scien-ces (FBFS). Flexibility of content formation study program from the view of expectation educational ascent and carrying-capacity student firstly make allowance of assigned credits of individual subjects.

Implementation of ECTS required a lot of work in individual subjects, but makes educational process overlap of subject contents and reveal topicality content of subjects. Therefore, it is very important step was assigning a credit value to the other duties in study plans besides teaching subjects, e.g. practice, bachelor work and master thesis. Student of bachelor's degree has to receive 164 credits for compulsory theoretical and special subjects and optional subjects and 6 credits for practice and 10 for final bachelor's work.

On master degree student has to receive 90 credits for compulsory theoretical and special subjects and optional subjects and 10 credits for practice and 20 credits for final master's thesis. PhD student has to receive 60 ECTS for study part of study program, which consists first of all from lectures, seminars and individual study of professional literature. Student has to receive other 60 ECTS for her scientific part consists of individual research project of the student. This part of studying full-time form also includes performing pedagogical activity. The completion of PhD study consists of completing dissertation examination and the defence of a dissertation.

Bachelor's study program Food safety and control

Faculty of Biotechnology and Food Sciences of SUA of Nitra opens in year 2007 new bachelor's program – Food safety and control (Tab. 1). First absolvents were ended his study in 2010. On this study program is high interest from side of high school students, yearly appear of this study about 250 students.

Table 1

Engineer study program

Faculty of Biotechnology and Food Sciences										
Bachelor's Degree Program Study Program: Foods Safety and Control										
Code	**Obligatory Subjects**	**Hours per week**			**Semester – credits**					
		L	**T**	**S**	**1**	**2**	**3**	**4**	**5**	**6**
1	2	3	4	5	6	7	8	9	10	11
640P102	Biology of Animal Production	2	2	s	6					
442P101	Inorganic Chemistry	2	2	s	6					
441M109	Biophysics and Physical Properties of Foods	1	3	s	6					
481E104	Information and Commucation Technologies	2	2	s	6					
461E104	Mathematics	1	3	s		6				
442P107	Organic Chemistry	2	2	s		6				
421A115	Biology of Plant Production	2	2	s		6				
541P105	Public Health and Food Production	1	2	s		4				
640P101	Protection of Animals and Food Production	2	1	s		4				
541P302	Sensory Analysis of Foods	2	2	s			6			
421P201	Biochemistry	2	2	s			6			
462E101	Biostatistics	1	2	s			4			
541P201	General Food Hygiene	1	2	s			4			
421P202	Microbiology	1	3	s			6			
442P201	Analytical Chemistry	1	3	s				6		
541P204	Epidemiology and Food Alergy	1	2	s				4		
421P203	Microbiological Analysis of Food	1	2	s				4		
322P201	Theory and Methodology of Final Paper	2	1	z				2		
541P203	Risks of Food Production	2	2	s				6		
541P407	Sanitation in Food Industry	1	2	s					4	
541P306	Labelling and Packaging of Foods	2	2	s					6	
421P308	Prediktive Microbiology in Food Science	1	3	s					6	
541P308	Hygiene of Food Dictribution and Markets	1	2	s					4	
621P303	Evaluation of Animal Raw Material and Foodstuffs	1	3	s					6	
541P401	Food Hygiene	2	2	s						6
541P309	Basic of Food Technology	1	3	s						6
621P302	Evaluation of Plant Raw Materials and Foods	1	3	s						6
541P305	Good Hygiene Praxis in Food Industry	1	2	s						4
314P301	Final Report			z				2	4	4
911P302	Operational Training			z	2			4		
Total obligatory credits					26	26	26	28	30	26
Total obligatory elective and optional credits					4	4	4	2		4
Total credits per semester					**30**	**30**	**30**	**30**	**30**	**30**

Table 1 cd.

1	2	3	4	5	6	7	8	9	10	11
Code	**Obligatory Elective Subjects**	**Hours per week**			**Semester – credits**					
		L	**T**	**S**	**1**	**2**	**3**	**4**	**5**	**6**
090E101	Introduction to University Studies	1	0	z	1					
442P112	Inorganic Chemistry – Workshop	0	1	z	1					
222Exxx	Foreing Language	0	2,2	z,s	2	2	2	2		
442P102	Organic Chemistry – Workshop	0	1	z		1				
345P101	Practice – Workshop	2	0	z		2				
481P201	The Information Resources in Biology and Food Scien	0	2	z		2				
640P202	Cell Physiology	1	2	s			4			
442P301	Biophysical Chemistry	1	2	s			4			
541P307	Immunoanalysis in Biology and Food Science	1	2	s				4		
541M301	Basis of Food Processing Equipment	1	2	s					4	
345E305	Food Qulity Management	2	1	s					4	
422P301	Environmental Radioactivity	2	1	s					4	
421P307	Biologically Active Components in Food-stuffs	2	1	s						4
421P302	Biochemistry of Nutrition	1	2	s						4
422A304	Basics of Biosafety	2	1	s						4

s – exam, z – credited, L – lecture, T – tutorial, S – semester

Graduate's profile

Graduated student understands problems of general hygiene requirements for the conditions of food businesses, stores and shops, special hygienic requirements for hygiene in production of various foodstuffs, hygiene of storage, transportation and sale of food.

Student understands the basic legislative requirements in terms of food legislation of the SR and the EU, HACCP system, sanitary program, metrology program, ISO standards, food quality and food safety standards, epidemiology and prevention of alimentary diseases and allergies from food and rules of proper hygiene in practice. Students also:

- obtain and understand the principles of internal (company) system of hygiene control and food safety,
- obtains knowledge of the principles of HACCP system, documentation, verification and design remedial measures and verification in a HACCP,
- thoroughly get familiar with the principles of sanitation, sanitation program compiling,
- selecting sanitary equipment and methods of assessing the effectiveness of sanitation is able to provide minimum hygiene training staff, monitoring of compliance of hygiene direction in food services, vehicles and compliance with the principles of hygiene by workers responsible for developing of metrology program, the application of ISO standards and standards for quality and food safety (ISO 22 000),

- reviews and updates the specifications used for auxiliary raw materials, packaging, cleaning and disinfectant,
- using modern laboratory methods and apparatus for food, hygiene and sanitation control and to control the presence of allergens in food,
- analyzes the causes of unsatisfactory conditions of hygiene, microbial contamination, the spread of alimentary diseases, missing of control at critical points in production,
- organize work operations in sanitation and hygiene assurance, metrology, risk analysis, proposing corrective actions, verification of the HACCP system, validation and verification of processes, documentation

Application of a graduate

Graduates are applied in food businesses, sales and distribution of food as well as a manager for hygiene and sanitation, managers of risk management and managers of food control respectively. Other application of graduates is in the business of sanitation and deratization in food companies, the advisory and consulting businesses involved in the HACCP system, the introduction of ISO standards, the application of legislative and standards of quality and food safety and management systems of food safety. Further application found in school systems based on the food education, food research, private educational and consulting institutions, counselling centres, accredited labs, corporate labs and corporate control.

Characterization of a graduate of study program and knowledges

Graduate controlling principles of microbiological and chemical food safety both horizontally and vertically, principles of integrated approach from farm to table and from farm to table. Provides and manages food traceability, evaluation of information, analysis of information from the rapid alert system and return of products from market. Applies, develops and evaluates a system of risk analysis with the use of mathematical and predictive models in terms of the precautionary principle to protect human health and communication about safety of produced food. Master's degree programme is presented in Table 2.

Following completion of compulsory and compulsory optional courses of study schedule:

- obtain and apply the principles of traceability of raw materials and food in the whole chain of production and apply them to specific conditions of the food company,
- obtain and apply knowledges about the strategy of risk analysis on a scientific basis in terms of management evaluation and communication,
- applied knowledge of mathematical models, statistical evaluation of the phenomena and their application in control work,
- using modern analytical methods for food control, their authentication, to detect adulteration of food, respectively,
- professionally evaluates the legislation, on the basis of legislation formulates changes in food labeling, nutrition facts and health claims etc.,
- control food information systems and databases, manages and documents the internal control system in the enterprise within the food safety team and top management,

– analyzing, documenting and evaluating crisis situations to forecast potential risks at the enterprise level and in communication with the public and the media,
– applies engineering methods and processes for food preservation, and sensory, microbiological, physical-chemical analysis of food,
– performs prediction and prevention of alimentary diseases, toxicology and mycological examinations.

Table 2

Master's degree program

Faculty of Biotechnology and Food Sciences								
Master´s Degree Program Food Technology (Engineer´s Study)								
Study Program: Food Safety and Control								
Code	**Obligatory Subjects**	**Hours per week**			**Semester – credits**			
		L	**T**	**S**	**1**	**2**	**3**	**4**
1	2	3	4	5	6	7	8	9
421P305	Food Microbiology	1	3	s	6			
442P402	Food Chemistry	2	2	s	6			
541P417	Adulteration and Authentication of Foods	1	2	s	4			
541P430	Sensometrics and Informatics in Food Science	2	2	s	6			
721A401	Food Toxicology	2	2	s		6		
541P428	Risk Assessment	2	1	s		4		
541P414	Food Safety	2	2	s		6		
541P422	Accreditation and Certification in Food Industry	1	2	s		4		
541P427	Food Technology of Animal Products	1	3	s		6		
421P503	Genetic Modified Foods	2	2	s			6	
541P403	Food Control and Legislation	1	2	s			4	
541P501	Foodborne Diseases	1	2	s			4	
541P506	Food Mycology	1	2	s			4	
541P005	Food Technology of Plant Products	1	3	s			6	
911P501	Diploma Thesis			z				20
911P301	Practical Training			z				10
Total obligatory credits					22	26	24	30
Total obligatory elective credits					8	4	6	0
Total credits per semester:					**30**	**30**	**30**	**30**
Code	**Obligatory Elective Subjects**	**Hours per week**			**Semester - credits**			
		L	**T**	**S**	1	**2**	**3**	**4**
422P101	Chemistry of Waste	1	2	s		4		
442P303	Environmental Chemistry	2	1	s	4			
541P402	Hygiene of Nutrition and Alimentation	2	2	s		6		

Table 2 cd.

1	2	3	4	5	6	7	8	9
541P429	Nutrogenomics	2	2	s	6			
442P302	Foreign Matter in Food Chain	1	2	s			4	
721P201	Physiology of Human Nutrition	1	2	s			4	
342E308	Food Marketing	2	2	s			6	
621M409	Food Machinery	2	2	s			6	
541P508	Healthy Food Safety	2	2	s			6	

s – exam, z – credited, L – lecture, T – tutorial, S – semester

Application of a graduate

Graduates are applied in food businesses in the team of food safety, in company laboratories and in management, control and audit.

Find application in state and private laboratories to control food safety, advisory consulting services, in the field of accreditation systems in food business, legislative projecting, estimating of risks in food

Find wide application in the field of safety management in the distribution and business level, and also in public catering.

Find application in food education, state divisions, the foreign inspection institutions, in cooperation with the European food safety authority, scientific panels and working groups

Comparison of some study program in Europe

In UK for example at Queen's University of Belfast has bachelor program BSc (Hons) Food Quality, Safety & Nutrition. And this is short description: The Food Quality, Safety & Nutrition degree (3 and 4 year options) incorporates the study of food production and quality, food structure and composition, microbiology, hygiene, nutrition, diet and health, food processing and business management and entrepreneurship, in a comprehensive and integrated programme. With increasing concerns over food safety, greater health awareness and a growing knowledge of the effects of food production on the environment, the opportunities for graduates have become wider and more diverse. These programmes address the importance of the food sector in both the local and global contexts and reflect increasing public concern with food quality and safety. They have been specially designed for students who wish to combine study in the areas of the nature and composition of fresh and processed foods, how the body nourishes itself in health and disease and the nature of local and global food safety concerns. he teaching programmes have been developed in consultation with employers, industry, and subject benchmarks, to offer students a curriculum designed to enhance employability and graduate skills through subject knowledge and understanding combined with entrepreneurial and management skills.

This pathway is offered as both a 3 year and a 4 year programme. The 4 year programme includes a 46 week period of work placement during which students also carry out a project. The 3 year programme has a 16 week work placement.

Students must take the equivalent of at least 6 modules in each stage, including any compulsory modules

At stage zero are taught Introductory Biology 1, Introductory Chemistry 1 and Introductory Chemistry 2. At Stage 1 teach this subjects: Composition of Foods, Food Molecules and Macromolecules, Fundamental Nutrition, Human Physiology, Introductory Skills for Biosciences, Microorganisms, Introductory Food Chemistry.

At Stage 2 teach this subjects: Diet & Health, Food Appearance & Texture, Food Hygiene & Microbiology, Work Placement (FQN), Food commodities, Food Marketing, Food Policy, Food Processing & Packaging and Raw Material Production.

At Stage 3 teach this subjects: Business Innovation and Entrepreneurship, Clinical Nutrition, Food Quality & Safety, Professional Studies (FQN), Project (FQN), Current Issues in Food Safety and Nutrition, Food Product Development, Health Promotion and Empowerment and Psychology of Consumer Behaviour.

In Belgium at Ghent University – Department of Food Safety and Food Quality – is offering a three months International Training Program in Food Safety, Quality Assurance and Risk Analysis.

The purpose of this project is to train and disseminate knowledge and know how to participants, working for governmental, research or non profit organisations in the agri food chain and carry responsibility within the control of different aspects of food safety in developing countries. The main subjects, microbiological, chemical and physical hazards, in food safety are intensively reviewed. Participants will be trained to manage the possible measures of the hazards specified by (inter)national legislation, (*Codex Alimentarius*) guidelines, quality assurance standards/systems (e.g. Pre Requisite Programs, HACCP, ISO 22000, Global Gap). Risk Analysis, as basic methodology to take policy/management decisions, is an important subject in this training. Focus is made on both agricultural and industrial level. The training should take 4 months and will be organised every 2 years.

The program can be followed completely or per module.
The program consists of the following modules:

Module 1: Situating food safety in the international context and the development of (inter) national standards/legislation/guidelines
This introductory module discusses the principles of the different hazards of food safety, food safety versus food security and the international process of developing criteria for food safety resulting in guidelines as *Codex Alimentarius*, legislation.

Module 2: Knowledge of the agri food chain (agricultural sector and transformation sector)
The second module covers theory and practical lectures regarding good practices in the primary plant, animal sector and in aquaculture. Next to this, food chemistry and food microbiology for the processing sector is reviewed. The principles of analytical techniques for food microbiology and chemistry will be discussed and demonstrated.

Module 3: Food safety and related hazards
This module transfers the knowledge and know how related to food safety hazards (physical hazards; nutritional disorders and link towards food safety; chemical hazards (including pesticides, heavy metals, mycotoxins, processing hazards such as acrylamide, PAHs, etc.); allergens; (micro)biological hazards (e.g. bacteria, viruses, moulds)).

Module 4: Risk analysis
This module is setting up the risk analysis procedure for both microbiological and chemical food safety hazards: e.g. Principles and parts of risk analysis; Chemical risk analysis; Microbiological risk analysis; Sensitivity analysis; Risk management and risk communication. Theoretical knowledge will be translated in practical examples.

Module 5: Quality Assurance Systems to control food safety
This module focuses on the different measures to control food safety in the agri food chain (e.g. Principles of a Quality Assurance Systems; Pre Requisite Programs based on Codex Alimentarius principles and exercises; HACCP principles (theory and exercises); Quality assurance systems in primary sector; Quality assurance systems in the processing industry; Validation and verification of food safety management systems; Traceability and crisis management: principles and case studies) and the related visits to companies, institutes, etc.

Module 6: Control on food safety in the agri food chain
In this module the principles of inspection/audit are discussed and the organization of the European food safety agencies.

Module 7: Case study food safety problem in home country
In this last module, the participants should work out a case study from a particular food safety problem in their home country. Possible preventive measures in the food chain, control strategies and risk analysis application should be evaluated to tackle the problem. All collected knowledge during the project should be applied for the case study. A presentation and defense of the case study is forseen.

In Denmark at University of Copenhagen at Faculty of life sciences is program Foof technology with specialization. This is short description of this programme: Food safety is a key issue in modern society, both in relation to our health and to our possibilities of selling the food we produce, both in Denmark and abroad.

Many things affect food safety: Food trading has become global, we change eating habits and thus also preparation methods, and new health problems arise. At the same time, technology offers new methodologies.

This means that there are good perspectives in working on facing the challenges associated with food safety.

Structure of the MSc programme:

Year 1
Block 1
Hygiene and Sanitation
Int. Legislation and Quality Management
Block 2
Control of Food-borne Microorganisms
Chemical Food Safety
Block 3
Thematic Course: Microbiological and Chemical Food Safety

Year 2
Risk Analysis in Food Safety
Thesis (30 or 45 ECTS)
A block is 9 weeks long and equals 15 ECTS.

Competences

With an MSc degree in Food Science and Technology and the specialisation in Food Safety, you will be an expert on food safety. You have knowledge, skills and competences, which, among other things, ensure that you can:

Give a detailed account of food safety in the entire food production chain, including microbial and non-microbial hazards in relation to the safety of fresh and processed foods.

Apply and evaluate methods to monitor hygienic measures, apply HACCP through microbiological knowledge and apply the principles behind hygienic factory layout and equipment design.

Communicate the role of hygiene in food safety and spoilage.

Recommend food safety correcting actions and communicate these recommendations to decision-makers at each step in the animal production chain.

Incorporate cultural, societal, ethical and economic elements in food safety management.

Your special competences provide you with many career opportunities both nationally and internationally.

Conclusions

Study program Food safery and control is one of the standard program of study that is taught at the Agricultural universities in Europe and worldwide. Education experts in this area is in accordance with the requirements of the EFSA, FAO, WHO and other institutions related to food safety. Risks associated with production and consumption of food constantly threaten consumers, an example is the presence of entero-haemorrhagic E. coli. It is therefore necessary that in each country there should be effective food control system that operates effectively traceability and rapid alert system. To increase the quality of education should be improved especially practical laboratory teaching and practice of students.

References

Bologna Action Lines, 2005. www.bologna/bergen2005.
Nyborg P., 2004. Principles and Objectives of the Bologna Process. www.bologna/bergen2005.
The Bologna Process/Towards the European Higher Education Area. 2004 www.bologna/bergen2005.

CHAPTER 2

CHEMICAL AND TOXYCOLOGICAL HAZARDS IN FOOD PRODUCTION

1

CADMIUM AND LEAD INFLUENCE ON POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF PROSO-MILLET

Introduction

Proso (*Panicum miliaceum* L.) is important species from the highest genus *Panicum*. Proso was the one of the main cereals of Slavs. Proso is the oldest summer cereal used by human besides wheat and barley.

Evidence of its cultivation exists in China from as long ago as 5000 BC; from there it widened to India and across the Eurasian steppes, reaching eastern Europe approximately 4000 years ago [Lágler et al. 2005]. Millets grow under difficult ecological conditions and tolerate poor soils and a certain degree of drought better than any other cereal crops [Obilana 2003]. Hence, it is ideally suited for cultivation in areas with hot, dry and short summer seasons [Baltensperger 2002]. In North America, proso is the most commonly produced in the drier regions of the western Great Plains, where it is often employed as a rotational crop with winter wheat [Graybosch and Baltensperger 2009]. Proso was the one of the main cereals of Slavs. Its importance gradually decreased, particularly during 18th and 19th century. It was caused by the development of potato growing and import of rice but also higher consumption and popularity of bread and other bakery products [Kalinova and Moudry 2006]. Nowadays, it is utilised like human food mainly in developing countries. In Europe, the grain is usually used as feed for pets. The renewed interest in the proso exploitation for human food in developed countries was caused by health reasons. In developing countries the commercial processing of these locally grown grains into value-added food and beverage products is an important driver for economic development [Taylor 2004].

The caryopsis of proso millet is rich in protein, mineral substances, vitamins and its nutritive parameters are comparable or better then common cereals. The ration of nutrients is very similar to recommended ratio of protein, saccharide and lipids. The biological value of proso-millet is on the level of bean and wheat flour [Becker et al. 1994].

The protein content in proso caryopsis is comparable with maize. It has very wide range. Usually it ranges from 11.3 to 12.7%. The content is dependent on variety, the presence of water and nutrients in a soil and conditions during grain formation [Dendy 1995].

From stand of amino acid composition of millet proteins is deficient of lysine (3.68 %), threonine and tryptophan [Gondola et al. 2009].

However, they have high content of sulphur amino acids, which has a regeneration effects on the liver [Nagy and Ábrahám 2010]. Proso is a suitable foodstuff for patients with gluten–free diet because the specific prolamin fraction is under permitted level. The various mineral components millets grain vary widely, to a large extent reflecting the mineral composition of the soils and conditions where the plants grown. Millet is a good source of dietary calcium, magnesium and iron [Léderi 2010].

Millet is an edible component of the kernel and is a rich source of phytochemicals, such as dietary fibre and polyphenols [Hadimani and Malleshi 1993]. Cereal polyphenols are important phytochemicals with one or more aromatic rings, with hydroxyl groups in different patterns [Towo et al. 2003]. It has been established that the position and degree of hydroxylation are of primary importance in determining antioxidant activity of flavonoids. The o-dihydroxylation of the B-ring contributes to the antioxidant activity [Shahidi and Naczk 2004]. Millets contain phenolic acids, which are located in the pericarp, testa, aleurone layer, and endosperm [McDonough et al. 1986].

Phenolic acids consist of two classes: hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids are directly derived from benzoic acid and include gallic, *p*-hydroxybenzoic, vanillic, syringic, and protocatechuic acids,among others. The hydroxycinnamic acids have a C6–C3structure and include coumaric, caffeic, ferulic, and sinapic acids. In general, ferulic, *p*-coumaric, and cinnamic acids are the major phenolic acids in millets [McDonough and Rooney 2000].

It has been shown that phenolic compounds act mainly as reducing agents, hydrogen donors and singlet-oxygen quenchers during anti-oxidant mechanisms [Kang et al. 2006]. The ability of some phenolic compounds to act as potential antioxidants was confirmed by many authors [Kähkönen et al. 1999, Amin et al. 2004]. They have potential effect against some types of cancer; they stimulate the immune system, pass through the cardiovascular diseases and affect aging processes [Podsedek 2007, Chuah et al. 2008].

Heavy metals such as Pb and Cd can cause excessive production of reactive oxygen species, which can lead to oxidative stress [Prasad, 2004] and to damage biomolecules [Ekmekci et al. 2008]. The effect of heavy metals on plants resulted in growth inhibition, structure damage, a decline of physiological and biochemical activities, as well as function of plants [Hamid et al. 2010]. According to Sarwar et al. [2010] Cd toxicity can cause decreases in photosynthetic rate, chlorophyll content. As a result of stress, which causes heavy metals, there may be changes in the composition of lipids and membranes, resulting in a change of enzyme activity bound to membranes.

Stress-induced effects of heavy metals in plants tissues but may also activate the antioxidant system of cells [Keilig and Müller 2009].

The objectives of our work were: 1) to evaluate the influence of selected heavy metals (Pb and Cd) on polyphenols content and antioxidant activity of proso-millet, 2) to find correlations between the content of some heavy metals in soil and their accumulation in consumerist part of millet as well as 3) to determine the correlations between heavy metal content and total polyphenols contact and antioxidant activity in model conditions.

Materials and methods

The tested crop was proso millet (*Panicum miliaceum* L) variety Unikum. Priority of this variety is high malting quality and good adaptability.

In the model conditions of vegetation pot experiments the rate of some heavy metals accumulation in pseudocereals grains (tumbleweed, oat, millet) depending on the extent of soil contamination by specific elements that were applied to the soil in the form of solutions of their soluble salts was observed.

Heavy metals were added as follow:

Cd in the form $CdCl_2 \cdot 2.5\ H_2O$	Pb in the form $Pb(NO_3)_2$
variant A_1: NPK + 0 mg $Cd \cdot kg^{-1}$ of soil ,	variant A_2: NPK + 0 mg $Pb \cdot kg^{-1}$ of soil,
variant B_1: NPK + 4.6 mg $Cd \cdot kg^{-1}$ of soil,	variant B_2: NPK + 392 mg $Pb \cdot kg^{-1}$ of soil,
variant C_1: NPK + 9.1 mg $Cd \cdot kg^{-1}$ of soil,	variant C_2: NPK + 742 mg $Pb \cdot kg^{-1}$ of soil,
variant D_1: NPK + 13.5 mg $Cd \cdot kg^{-1}$ of soil,	variant D_2: NPK + 1092 mg $Pb \cdot kg^{-1}$ of soil ,

Six kilograms of soil was weighted into plastic bowl-shaped pots with average of 20 cm and height of 25 cm with foraminated bottom. Basic nutrients were added in the form of NPK fertilizer. The experiment was based on four replications.

Determination of heavy metals content

Content of risky elements was assessed after mineralization by dry way method of AAS atomic absorption spectrophotometry on apparatus Varian AA 240 FS.

Determination of risk heavy metals content in pseudocereals grains

The plants were harvested when fully ripe. Samples were homogenized and subsequently mineralized by wet way. The contents of risk elements in grain were determined by AAS method (AAS Varian DUO 240FS/240Z).

Determination of total polyphenol content (TPC)

The total polyphenol content was assessed by the method of Lachman et al. [2003] employing the reduction of a phosphowolframate-phosphomolybdate complex to blue products by phenolic compounds. The absorbance was measured at 765 nm using Shimadzu UV-1800 spectrophotometer (Japan). Results were expressed as mg gallic acid equivalents (GAE) per kg FW.

Determination of the total antioxidant activity (AOA)

The antioxidant activity of buckwheat extracts was measured in terms of radical-scavenging ability, using the DPPH method [Brand-Williams et al. 1995].

Aliquot amount of extracts (100 μL) was allowed to react with DPPH solution. The decrease in absorbance of DPPH was measured at 515 nm using Shimadzu UV-1800 spectrophotometer in intervals until the absorbance stabilised. Results were expressed as the percentage of inhibition of free radical by present antioxidants.

Results and discussion

Environmental pollution has various forms and one of them is also soil contamination by heavy metals. Heavy metals through food chain get into plants, animals and then also into human body. Their threat lies not only in their acute intoxication, which is scarce, but these substances also tend to accumulate and organism is gradually changed by their effects. They cause apparently not noticeable disorders, but these can result in metabolic disorders [Vollmannová et al. 2006]. The exposure of animals to heavy metals caused various alterations

in zootechnical parameters [Kalafová et al. 2009, Kolesarova et al. 2011] as well as imbalance in internal milieu [Capcarova et al. 2008, Kolesarova et al. 2010].

While agricultural production is the main source of foodstuffs, it is important to evaluate negative effetcs of risky elements on quality of agricultural products.

In this paper the ability of oat to accumulate the risk metals in the model conditions in relation to the total polyphenol substances content and antioxidant activity was verified. The results are presented in the Table 1. Graded addition of cadmium into the soil resulted in its higher content in grains.

Table 1

Content of heavy metals in proso-millets after Cd application into the soil

Variant	Heavy metals [mg.kg^{-1}]								
	Fe	Mn	Zn	Cu	Co	Ni	Cr	Pb	**Cd**
A_1	50.63	20.44	18.16	5.74	0.54	3.90	0.78	0.69	**0.34**
B_1	49.56	19.55	17.10	5.76	0.20	3.51	0.79	0.41	**0.40**
C_1	44.94	16.08	16.87	5.34	0.60	3.36	0.75	0.54	**0.52**
D_1	43.45	16.08	16.56	5.03	0.38	3.50	0.66	0.41	**0.70**

In each variant the maximum tolerable amount stated by *Codex Alimentarius* SR for cadmium (0.1 mgkg^{-1}) was exceeded. The highest content of cadmium was measured in the variant D1 and it was 2 times higher content of cadmium in comparison with the control variant. The strong correlation (R=0.788; P–value=2.869x10^{-4}) was between the applied amount of cadmium in soil and its content in the grain millet.

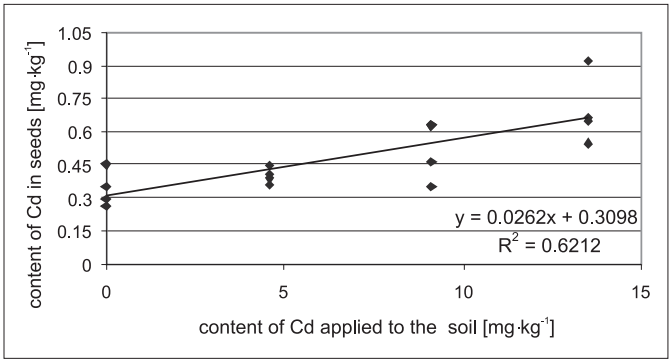

Fig. 1. Relation between cadmium content in millet grain and cadmium content in the soil

Lead is dangerous toxic metal that causes morphological and physiological changes in plants [Kambhampati et al. 2005]. In our model conditions of vegetation pot experiments the accumulation of this metal in millet grain and its effect on the antioxidant status of plants was observed.

Similarly to cadmium the gradual addition of lead to the soil caused overload of maximum tolerable amount for lead stated by *Codex Alimentarius* SR (0.2 mgkg^{-1}) (Tab. 2).

Table 2

Content of heavy metals in proso-millets after Pb application into the soil

Variant	Heavy metals [mg·kg^{-1}]								
	Fe	Mn	Zn	Cu	Co	Ni	Cr	**Pb**	Cd
A_2	33.90	9.72	21.16	7.48	0.08	0.75	0.68	**0.24**	0.09
B_2	27.04	8.52	24.08	6.10	0.05	0.7	0.65	**0.58**	0.13
C_2	27.73	9.57	17.92	4.50	0.05	1.03	0.60	**0.66**	0.16
D_2	27.42	8.20	16.23	4.70	0.18	1.25	0.48	**1.01**	0.23

The statistically significant positive correlation was noted between content of Pb applied to the soil and the content of Pb in millet grain (P-value=1.548x10^{-6}).

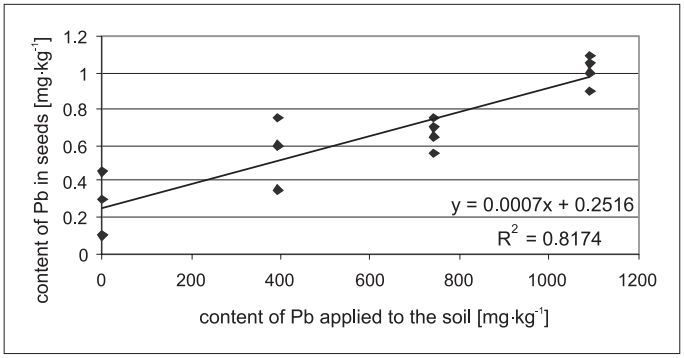

Fig. 2. Relation between lead content in millet grain and lead content in the soil

Heavy metals influenced also the content of polyphenolic substances and the antioxidant activity in the plats (Tab. 3–4).

Table 3

Content of total polyphenols (TP) and antioxidant activity (AOA) in millet grain after application of cadmium to the soil

Variant	TP [mg.kg^{-1} DM]	AOA [%]
A_1	1034.2	4.44
B_1	1152.4	5.50
C_1	1269.1	4.36
D_1	1197.3	4.53

Table 4

Content of total polyphenols (TP) and antioxidant activity (AOA) in millet grain after application of lead to the soil

Variant	TP	AOA
A_2	794.9	3.27
B_2	791.3	2.94
C_2	778.7	2.44
D_2	729.3	2.42

The reaction on the stress caused by toxic action of heavy metals is the production of various metabolites that might have diverse effects on the antioxidant system in plants. In our experiments the intentional application of cadmium to the soil caused increased production of total polyphenols. The highest increase was achieved in the variant C (1269.06 $mgkg^{-1}$). Similarly to our results Musilova [2009] found statistically significant positive correlation between accumulation of cadmium in the potato tubers and the content of total polyphenols. In our experiments the increasing concentration of lead in millet grain caused decrease of total polyphenolic substances. The highest decrease was found in the variant D (about 8.25% against the control variant). Statistically significant negative correlation was noted between the content of lead in millet grain and the content of total polyphenols (P-value = $3.38x10^{-3}$).

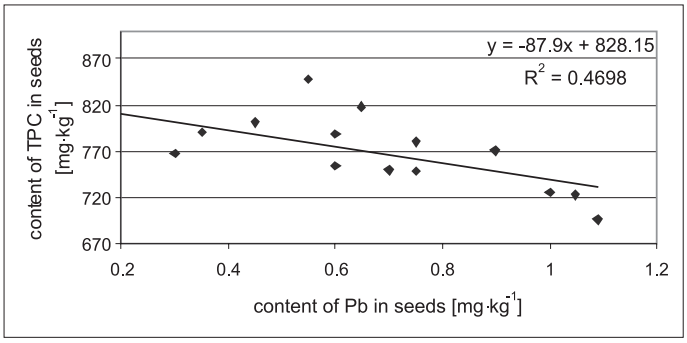

Fig. 3. Relation between total polyphenols content in millet grain and the content of accumulated lead

Kachout et al. [2009] indicated the increase of the antioxidant activity under the thumb of heavy metals. In our experiments intentional addition of heavy metals had no clear impact on the antioxidant activity. The mild increase of antioxidant activity in millet grain from value of control variant 4.42 to 5.50% was recorded.

Increase of lead concentration resulted in decreased antioxidant activity in millet grain. The highest decrease was measured in variant D (2.42 %). The statistically significant negative correlation (P-value=$2.36x10^{-5}$) between lead content in the millet grain and the antioxidant activity was recorded.

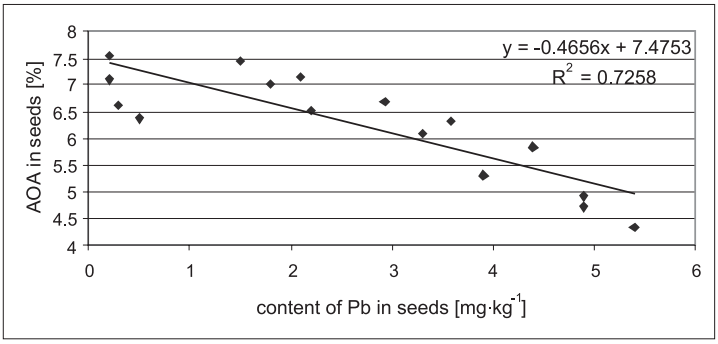

Fig. 4. Relation between antioxidant activity in millet grain and the content of accumulated lead

There are many disunited opinions on the effect of heavy metals and the antioxidant activity in the literature. Michalák [2006], Kachout et al. [2009] determined the increase of the antioxidant activity after heavy metals treatment. According to Hegedűs et al. [2001] the concentration of heavy metals can cause decrease in the antioxidant activity.

Conclusions

Millet grain contains polyphenolic compounds that act as antioxidants. These phenolic compounds are the component of defence mechanism and they are considered to be the bioindicators of environmental pollution. The plants vegetated in the stress conditions (heavy metals addition to the soil) can change the content of phenolic compounds as well as the activity of antioxidant enzymes. These changes are probably the response of the cells against increasing production of reactive oxygen species after long-continuing intoxication caused by heavy metals. For that reason it is important to monitor the content of risk elements in the environment.

Acknowledgements

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References

Amin I., Zamaliah M.M., Chin W.F., 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chem., 87, 581–586.

Baltensperger D.D., 2002. Progress with proso, pearl and other millets, [in:] J. Janick and A. Whipkey (Eds), Trends in New Crops and New Uses, ASHS Press, Alexandria, VA, 100–103.

Brand-Wiliams W., Cuvelier M. E., Barset C., 1995. Use of a free radical to evaluate antioxidant activity. Lebensm. Wiss. Technol., 28, 25–30.

Becker H.G., 1994. Buchweizen, Dinkel, Gerste,Hafer, Hirse und Reis- die Schäl- und Spelzgetreide und ihre Bedeutung f˙ur die Ernährung. AID-Verbrauchrdienst, 39, 123–130.

Capcarová M., Kolesárová A., Arpášová H., Massányi P., Lukáč N., Kováčik J., Kalafová A., Schneidgenová M., 2008. Blood biochemical dynamics and correlations in laying hens after experimental nickel administration. Int. J. Poultry Sci., 7, 538–547.

Chuah A.M., Lee Y.C., Yamaguchi T., Takamura H., Yin L.J., Matoba T., 2008. Effect of cooking on the antioxidant properties of coloured peppers. Food Chem., 111, 20–28.

Dendy D.A.V., 1995. Sorghum and Millets: Chemistry and Technology. St.Paul,Minnesota: American Association of Cereal Chemists, 406.

Ekmekci Y., Tanyolac D., Ayhan B., 2008. Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. J. Plant Physiol., 165, 600–611.

Gondola I., Boce L., Nag L., 2009. Alternativ lehetőségek a szántóföldi növénytermesztésben. Agrofórum, 20, 13–21.

Graybosch R.A., Baltensperger D.D., 2009. Evaluation of the waxy endosperm trait in proso millet (*Panicum miliaceum*). Plant Breeding, 128, 70—73.

Hadimani N.A., Malleshi N.G., 1993. Studies on milling, physico chemical properties, nutrient composition and dietary fibre content of millets. J. Food Sci. Technol., 30, 17–20.

Hamid N., Bukhari N., Jawaid F., 2010. Physiological responses of *Phaseolus vulgaris* to different lead concentrations. Pak. J. Bot., 42, 239–246.

Hegedűs A., Erdei S., Horváth G., 2001. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci., 160, 6, 1085–1093.

Kachout S.S., Mansoura A.B., Leclerc J.C., Mechergui R., Rejeb M.N. Ouregchi Z., 2009 Effects of heavy metals on antioxidant activities of Atriplex hortensis and Artiplex rosea. J. Food Agric. Environ., 7, 938–945.

Kalafová A., Massányi P., Chrenek P., Kováčik J., Lukáč N., Chrastinová Ľ., Schneidgenová M., Čupka P., Jurčík R., 2009. The effect of single nickel and combined nickel and zinc peroral administration on selected mineral blood parameters in female rabbits. Potravinárstvo, 3, 4, 26–29.

Kalinová J., Moudrý J., 2006. Content and Quality of protein in proso millet (*Panicum miliaceum* L.) varieties. Plant Foods for Human Nutr., 61, 45–49.

Kambhampati M.S., Begonia G.B., Begonia M.F.T., Bufford Y., 2005. Morphological and physiological responces of Morning glory (*Ipomoea lacunose* L.) grown in a lead- and chelate-amended soil. Int. J. Environ. Res. Public Health, 2, 299–303.

Kang K.A., Zhang R., Piao M.J., Park S., Park J., Kim J.S., Kang S.S., Hyun J.W., 2006. Screening of antioxidant and anticancer effects from flavonoids. Cancer Prev. Res., 11, 235–239.

Keilig K., Müller L., 2009. Effect of flavonoids on heavy metal tolerance in Arabidopsis thaliana seedlings. Botanical Studies, 50, 311–318.

Kähkönen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Pihlaja K., Kujala T.S., Heinonen M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47, 3954–3962.

Kolesarova A., Capcarova M., Roychoudhury S., 2010. Metal induced ovarian signalling. Nitra, Slovak Agricultural University, 2010, 135.

Kolesarova A., Capcarova M., Sirotkin A.V., Medvedova M., Kalafova A., Filipejova T., Kovacik J., 2011. In vitro assessment of molybdenum-induced secretory activity, proliferation and apoptosis of porcine ovarian granulosa cells. J. Environ. Sci. Health, part A, 46, 170–175.

Lachman J., Hejtmánková A., Dudjak, E., 2003. Content polyphenolic antioxidants and phenolcarboxylic acids in selected parts of yacon, [in:] Proceed. of Vitamins 2003 – Přírodní antioxidanty a volné radikály. Univerzita, Pardubice, 89–97.

Lágler R., Gyulail G., Humphreys M., Szabó Z., Horvath L., Bittsanszky A., Kiss J., Holly L., Heszky L., 2005. Morphological and molecular analysis of common millet (*P. miliaceum*) cultivars compared to a DNA sample from the 15th century (Hungary). Euphytica 146, 77—85.

Léderi I., 2010. Az alternatív növények élelmezési jelentősége. In Az alternativnövények szerepe az Észak-alföldi Régióban. Debreceni Egyetem Agrár és Gazdálkodástudományok Centruma, Kutatóintézetek és Tangazdaság, Nyíregyháza, 107–131.

McDonough C.M., Rooney L.W., Earp C.F., 1986. Structural characteristics of Eleusine coracana (finger millet) using scanning electron and fluorescence microscopy. Food Microstructure, 5, 247–256.
McDonough C.M., Rooney L.W., 2000. The millets, [in:] Kulp, K., Ponte Jr., J.G. (Eds.), Handbook of Cereal Science and Technology. Marcel Dekker, Inc., New York, 177–201.
Michalák A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol. J. Environ. Stud., 15, 523–530.
Musilova J., 2009. Vzťah vybraných rizikových kovov k nutričným komponentom ľuľka zemiakového (Solanum tuberosum L.). Habilitačná práca, SPU, 152.
Nagy L., Ábrahám E.B., 2010. Köles. Az alternatív növények szerepe az észak alföldi Régióban, 2, 46–253.
Obilana A.B., 2003. Overview: Importance of millets in Africa. AFRIPRO. Proceed. Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa (Belton PS and Taylor JRN, eds.). Pretoria, South Africa, 26–43.
Podsedek A., 2007. Natural antioxidants and antioxidants capacity of Brassica vegetables: A review. LWT--Food Sci. Technol., 40, 1–11.
Prasad M.N.V., 2004. Phytoremediation of metals and radionuclides in the environment. The case for natural hyperaccumulators, metal transporters, soil amending chelators and transgenic plants, Heavy Metal Stress in Plants: From biomolecules to Ecosystems. Springer-Verlag. Heidelberg. Narosa New Delhi, 345–392.
Sarwar N., Malmi S.S., Zia M.H., Naeem A., Bibi S., Farid G., 2010. Role of mineral nutrition in minimizing cadmium accumulation by plants. J. Sci. Food Agric., 90, 925–937.
Shahidi F., Naczk M., 2004. Phenolics in food and nutraceuticals. CRC Press, Boca Raton, London, New York, Washington, D.C., 421–426.
Taylor J.R.N., 2004. Grain production and consumption: Africa, [in:] Wrigley, C., Corke, H., Walker, C.E. (Eds.), Encyclopedia of Grain Science. Elsevier, London, 70–78.
Towo E.E., Svanberg U., Ndossi G.D., 2003. Effect of grain pretreatment on different extractable phenolic groups in cereals and legumes commonly consumed in Tanzania. J. Sci. Food Agric., 83, 980–986.
Vollmannová A., Timoracká M., Melicháčová S., Harangozo Ľ., Jomová K., 2006. The relationship of heavy metal contents in soils to content of chemoprotective compounds in soya bean. In Zem v pasci? Analýza zložiek životného prostredia. Zvolen TU, 2006, 744–748.

2

MERCURY CONCENTRATION IN SELECTED FISH FROM POGORIA LAKES LOCATED IN DĄBROWA GÓRNICZA REGION

Introduction

Poland is characterized by one of the European countries with the highest mercury (Hg) emission either to air or water. According to various sources, in the year 2000 world mercury emission was on the level of 21.9 Mg/year, an average for the European countries 23.9 Mg/year, for Poland 26.0 Mg/year [Pacyna et al. 2008]. In 2008 the NILU (Norwegian Institute of Air Research) reported mercury emission in Poland on the level of 25.7 Mg/year. With this amount Poland is ranked on the second placed in Europe, after Russia [Panasiuk et al. 2010]. The Upper and the Lower Silesia are two the most mercury polluted regions in Poland [Szpadta 1994, Kabata-Pendias and Pendias 1999, Gworek and Rateńska 2009, Dutchak and Varygina 2010].

The main sources of mercury emission are energetic sector, including coal combustion, and other industries dealing with mercury during production. Global mercury emission from anthropogenic sources is presented on Figure 1.

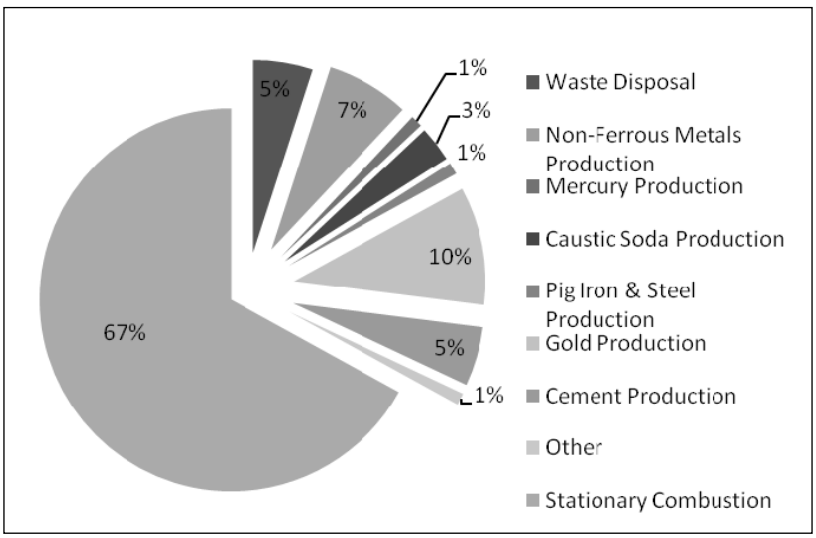


Fig. 1. Global emissions of total Hg from anthropogenic sources in the year 2000 [Pacyna et al. 2008]

Bioaccumulation of mercury compounds in organisms comprising in the food chain can be very dangerous. Mercury can be transferred within the food chain, thus this pollutant can easily get to the final food products from the raw materials, including fish, collected from regions characterized by high trend to heavy metal bioaccumulation [Dobrowolski et al. 1999]. Quality control of fish, especially species preferred by anglers, in Poland is not as strict and regular as in the United States of America [LEPR 2008]. Such situation becomes more threat for an increasing number of people fishing and their relatives consuming caught fish. In reference to a huge informative action led in the Great Lakes Region of the USA and Canada, Poland should monitor and prepare details reports of the heavy metal, including mercury, accumulation in fish. On the basis of collected data specific bulletins containing fish species, fish size and the heavy metals level, are published. Moreover, the American legislative body based on the epidemiological-toxicological research established limits of specific fish species daily intake for small children and pregnant women [LEPR 2008].

Methylmercury, an organic form of mercury is known from its high bioaccumulation and the highest toxicity [Górecki et al. 2010] causing a mass poisoning, when consume the contaminated meat and seafood products, which are main sources of this compound. Discharge of waste materials from the synthetic materials production plants characterized by high mercury concentration in Minamata Bay (Japan) in ’60 of XX century raised the content of the metal in fish and sea creatures [Dobrowolski 1978, Futatsuka 1988, Tsuda at al. 2009]. Consumption of the contaminated sea foods resulting in serious human intoxication, incidence of a Minamata disease and death of hundreds of people [Harada 1988, 2004]. Environmental medicine considers mercury and its derivatives as highly toxic and risky materials for human health and it recommends continuous monitoring of the level of mercury and methylmercury in food products.

Many studies were carried out to control the Hg concentration in fresh fish, seafood and fish products around the world [Amundsen et al. 1997, Falandysz et al. 2000, Dusek et al. 2005, Polak-Juszczak 2007, Arribere et al. 2008, Sackett et al. 2010, Miklavcic et al. 2011].

In Poland monitoring of mercury in food is still not complete, especially in the Upper Silesia region. Among others Dąbrowa Górnicza as a center of the intensive industry activities connected with coal combustion, coke and energy production is characterized by the highest Hg emission [Mniszek and Zielonka 1995, Czaplicka et al. 2009].The biggest industrial plant in Dąbrowa Górnicza is former steelwork Huta Katowice belongs now to Arcelor Mittal Poland S.A., and it does not monitor the mercury emission and concentration in environment. In years 2008–2009 the Hg monitoring was carried out by Institute of the Primary Environmental Engineering PAN on behalf of the Province Inspector of Environmental Protection in Katowiceand revealed still high mercury emission in the region. The level of mercury concentration in the air in Dąbrowa Górnicza in 2008 and 2009 was 2.92 $\mu g/m^3$ (from April to September) and 4.04 $\mu g/m^3$ (from October to March). Mercury deposition from April 2008 to September 2009 ranged between 31 and 33 $\mu g/m^2$ [Czaplicka et al. 2009]. The structure of the mercury emission is showed on Figure 2. Therefore, it is reasonable to take under consideration monitoring of the Hg level in fish from Pogoria lakes.

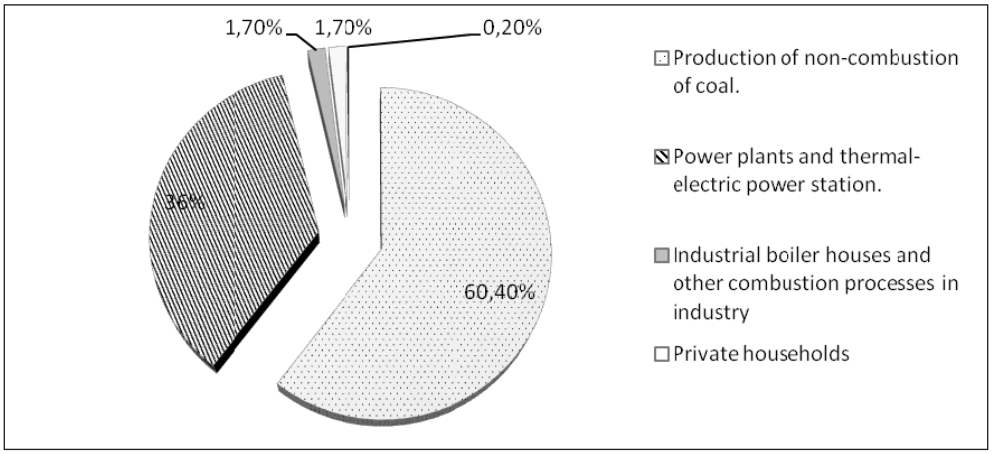


Fig. 2. Structure of the Hg emission in Dąbrowa Górnicza [Górecki et al. 2010]

Mercury gets into lakes water together with atmospheric falls and surface and dirt waters. Mercury and its derivatives occur in water in relatively low concentration, in oceans and seas around 0.005 µg/l, in rivers around 0.01 µg/l [Kabata-Pendias and Pendias 1999]. In water mercury exists in many different forms depending on the oxidative-reduction conditions; in the oxidative environment $HgCl_4^{2-}$ and $HgOH^+$, when the reductive CH_3HgS^- and HgS_2^-, in changing conditions CH_3HgCl and HC_3Hg^{2+} predominate. Mercury compounds can be transformed in water mainly due to microorganisms activity and the photochemical reactions, what led to the surface evaporation of the element [Kabata-Pendias and Pendias 1999]. Thus in surface water Hg is accumulated in sediments where it is absorbed by clay minerals, organic materials, ferrichydroperoxides. The contaminated sediments are dangerous for all organisms living close to bottom of reservoir. The Hg trapped in sediments can be again released to water due to chemical and biochemical reactions, as well as mechanical movement induced by natural processes, transport or dredging. In recent years Bojakowska et al. [2010] stated higher mercury concentration in regions suffered from flood, which transported sediments from the contaminated area to other places.

Mercury does not play any biological role in plants, animals and humans, however it is found in all animal tissues. Higher levels of Hg compounds are present in sea organisms (from 0.3 to 3.0 ppm), comparing to land animals (from 0.02 to 0.1 ppm). In vertebrates the highest mercury concentration is analyzed in firm tissues and organs (body liquids, milk, hair, fur, feathers, organs), that is why they are a good indicators for estimating the environment pollution. Studies by Kabata-Pendias and Pendias [1999] and Szkoda et al. [2010] revealed that long mercury, especially in organic form, intake caused neurological changes in animals, like reproduction disorders in fish. Fish are characterized by up to 90% of CH_3Hg^+ content in relation to the total mercury concentration. The highest mercury level are found in predators like (swordfish, tuna, shark, merlin, pike), which are on the top of nutrition pyramid in water [Westöö 1973]. Moreover, higher Hg concentration are found in the long-living fish and those with high mobility [Zillioux et al. 1993, Akerman and Balk 1998].

Polish Ministry of Health in specific regulation [Dz. U. 2007, no 61, pos. 417] established the max permissible mercury concentration in water for human consumption

(0.001 mg/l). Moreover, chemical parameters of the analytical methods like systemic terror (20%), standard deviation (10%) and detectable limit (20%) are regulated by Dz. U. 2006, no 123, pos. 858. European Union Water Directive (2000/60/WE) established the basic rules of the EU co-operation in the area of the water policy and also recommended mercury as "a priority toxic substance", which emission should be stopped or totally eliminated. According to the EU Regulation 882/2004 and Directive 96/23/WE, as well as Polish Ministry of Agriculture Regulation [Dz. U. 2006, no 147, pos. 1067], it is advised to monitor the chemical and biological residues level, including toxic metals, in all slaughtered animal species (in diffe-rent tissues including meat and liver), as well as in milk, eggs, honey and animal feed. The analytical extent is actualized every year according to the obligatory standards for the safety of human consumption, international organizations requirements and food importers. The maximum concentration of mercury in food products obligatory within European Union is given in the EU Regulation WE 1881/2006 and further novelization. However, max Hg concentration is stated only for fish and fish products. The highest permissible level of mercury for fresh water fish equal 0.5 mg/kg, for sea fish 1.0 mg/kg, and for diet supplements 0.1 mg/kg. Provisional Tolerable Weekly Intake (PTWI) for mercury established by FAO/WHO is 5 µgHg/kg body weight, and for methylmercury 1.6 mg/kg body weight in one week (by Jonit Expert Committee on Food Additives) [Szkoda et al. 2010].

The objective of this study was to control mercury concentration in selected fish from Pogoria lakes due to the intensive increase of rod fishing in Dąbrowa Górnicza region.

Materials and methods

The experiment was carried out on the selected four fish species the most frequently caught by rod-fishing in Pogoria lakes:

- Ide (*Leuciscusidus*),
- Bream(*Abramis brama*),
- Perch (*Percafluviatilis*),
- Roach (*Rutilusrutilus*).

Fish were caught in two water reservoirs: Przeczyce on 10thMay 2010 and PogoriaIII on 3rdMay 2010. Immediately after draft fish were frozen and transferred to the laboratory. Thawed fish were measured and weighted, then three fish with an average size from each group were selected for further analyses according, excluding ide from PogoriaIII (only one fish was caught). Selected fish were washed with deionized water and biometric parameters (length up to 0.5 cm and weight up to 0.1 g) were measured. Following, liver, hart, abdominal muscles, breast and abdominal fins were collected. Obtained materials were then homogenized, sealed in plastic bags and kept at the temperature – 20°C.

Mercury concentration in prepared fish materials was analyzed in Mercury Analyzer MA – 2000 connected with the personal computer (PC) equipped with data analyses software. Scheme of the mercury measuring system MA-2 is presented on Figure 3.

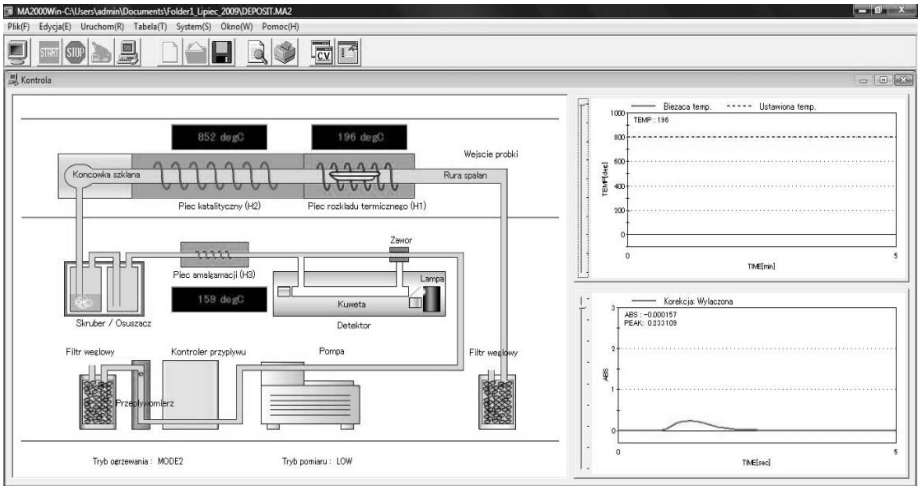


Fig. 3. Scheme of the mercury measuring system (MA-2 analyzer, Testchem Co)

Analyzed material is thermally degraded in H1 oven, following by atomization in H2 oven when mercury fumes are absorbed by golden collector. Formed amalgam is then heated and atomic mercury is released. Mercury concentration is analyzed using cold mist technique by the atomic absorption method at wavelength 253.7 nm in absorption chamber. Before the next analysis golden collector is chilled and in scrubber all gases are purified. An initial heating of the golden collector is performed in order to avoid any influences on the analysis.

All the previously prepared fish materials (50–100 mg; heart and liver samples were smaller due to the size of those organ) were put inside roasted porcelain containers and two previously roasted (750°C for more than 3 h) additives (which remove all interfering substances) B (activated aluminum oxide) and M (sodium carbonate and calcium hydroperoxide) were added. Scheme of the sample and additives B and M layout in the porcelain container is presented on Figure 4.

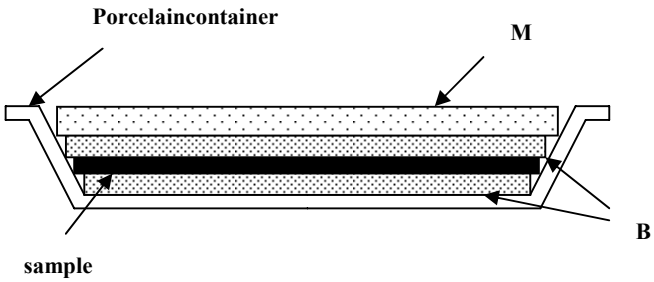


Fig. 4. Scheme of the porcelain container with sample and additives B and M

Analysis of mercury concentration in fish muscle tissue and organs was performed at "mode 3" destined for oils and plastic. Sample was heated in two stages, initially at 350°C for 4 min and next at the temperature 800°C for 6 min, at "low" mode (0–20 ng) for 5 sec.

Mercury concentration was expressed as parts per billion (ppb). Repeatability of the analyses was expressed as a standard deviation and a relative standard deviation. The former calculated according to following formula:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{śr})}{n\text{-}1}}$$

Results and discussion

Results collected in the study showed that mercury concentration in muscles of the selected fish caught by rod-fishing in two Pogoria lakes did not exceed permissible level for fresh water fish flesh i.e. 500 µg/kg [WE 1881/2006] and ranged between 8–85 µg/kg (Fig. 5).

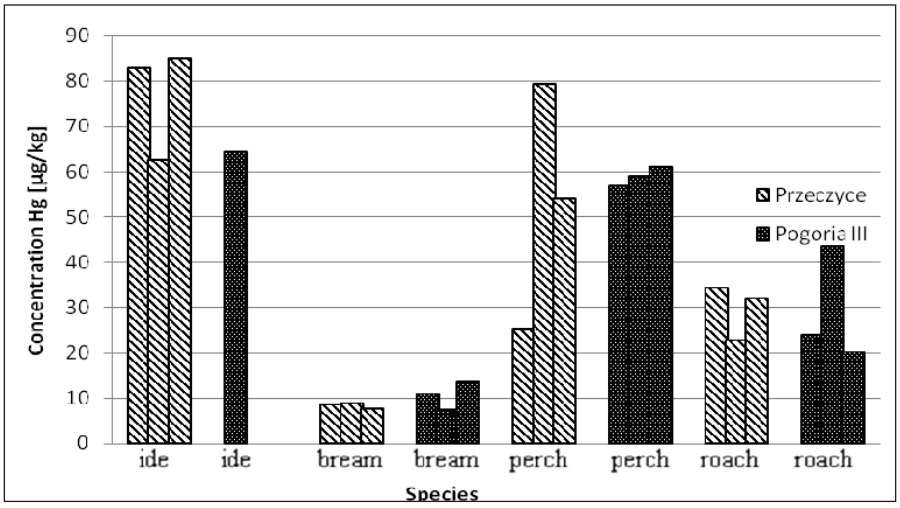


Fig. 5. The mercury concentration in muscles of selected fish species from Pogoria Lakes

The highest mercury concentration in muscles tissue was analyzed in ide (more than 63 µg/kg) and perch (more than 50 µg/kg) from both Pogoria lakes. The lowest Hg level was stated for bream muscles (less than 14 µg/kg) despite the place of rod-fishing. Muscle samples collected from the selected fish common in Pogoria and Przerzyce reservoirs were characterized by the highest analytical stability amongst all analyzed animal tissues, when measured mercury concentration. Relative standard deviation ranged between 1–19% (Fig. 6). Fish organs, like liver and heart, contained generally much lower mercury compounds comparing to muscle tissue (up to 50 µg/kg and 60 µg/kg for liver and heart, respectively) (Fig. 7, 9). Similarly to muscles, livers form ide and perch from both water reservoirs were characterized by higher Hg concentration, in relation to bream and roach. The lowest mercury concentration was found in bream heart (up to 10 µg/kg). No differences in mercury content in bream, roach and ide heartswere noticed when analyzed fish from two Pogoria

lakes. Hearts collected from perch caught in PogoriaIII were contaminated by mercury for more than 50% in relation to the same fish species from Przerzyce lake. Analytical stability of fish organs, expressed by relative standard deviation, was much lower than muscle samples and did not exceed 48% (Fig. 8, 10). Mercury concentration was also analyzed in breast and abdominal fins, however, they are not consumed by people but their chemical composition can affect the health status of the whole fish.

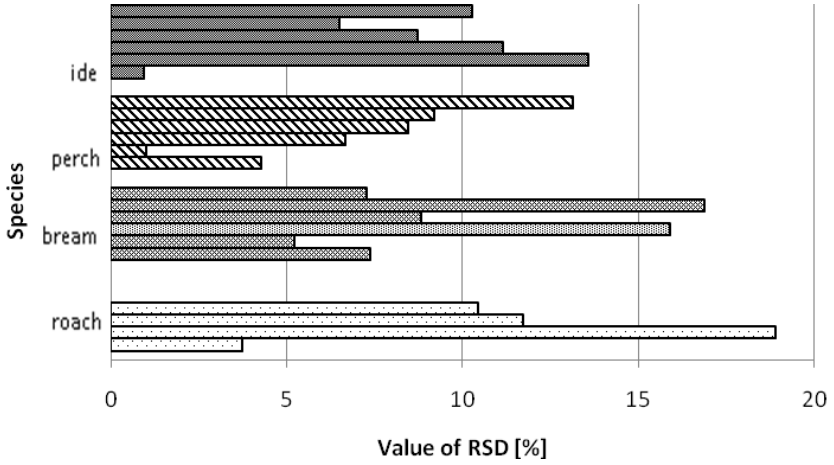


Fig. 6. Relative standard deviation for Hg concentration in muscles of selected fish species from Pogoria Lakes

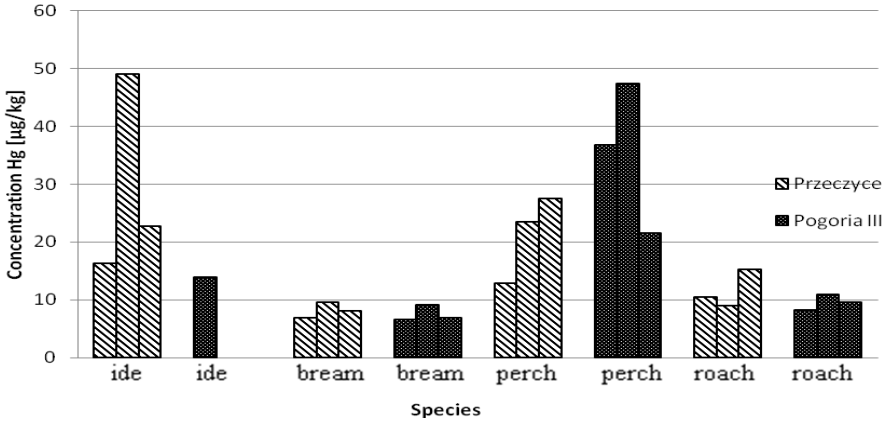


Fig. 7. The mercury concentration in liver of selected fish species from Pogoria Lakes

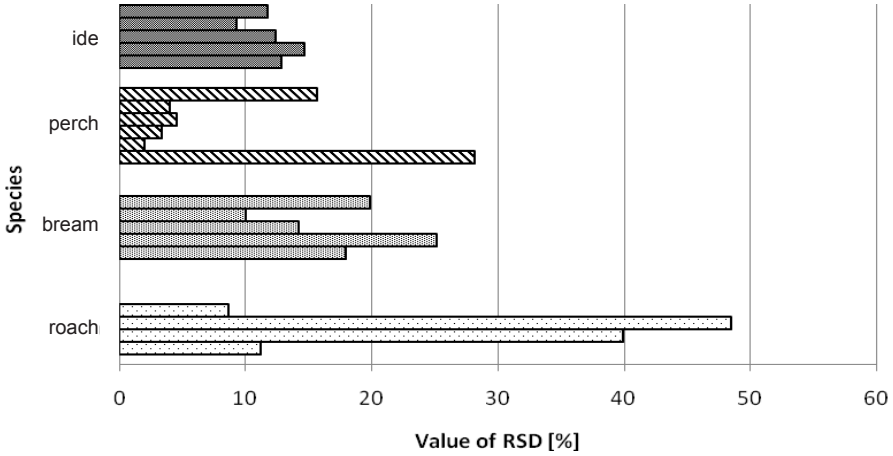


Fig. 8. Relative standard deviation for mercury concentration in liver samples of selected fish species from Pogoria Lakes

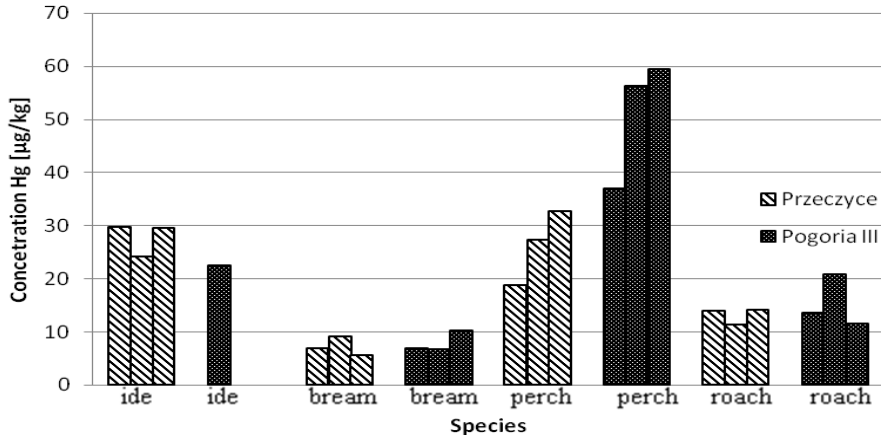


Fig. 9. The mercury concentration in heart of selected fish species from Pogoria Lakes

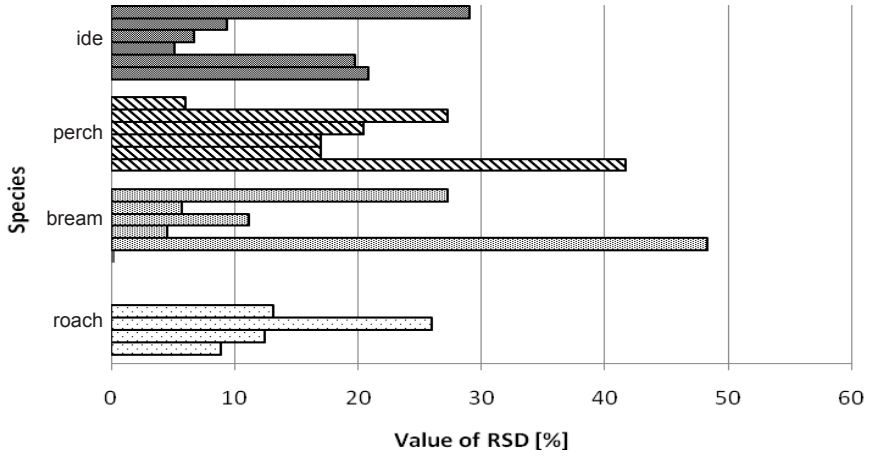


Fig. 10. Relative standard deviation for Hg concentration in hearts of selected fish species from Pogoria Lakes

The obtained results revealed that fins were characterized by the lowest mercury content among all analyzed fish parts (3–13 μg/kg) (Fig. 11). Similarly, to other results presented in this work, perch fins were the most contaminated, whilst bream and roach were characterized by much lower mercury content. Relative standard deviation for Hg analysis in fish fins ranged between 0.1–27% and was the highest for roach. As it was mentioned above mercury content in fish caught in Pogoria lakes did not exceed level recommended by European Union legislative body [WE 1881/2006]. Thus, despite some threats connected with high mercury emission in Upper Silesia region it can be stated that fish caught by rod-fishing in Pogoria lakes may not carry a risk for consumer health. However, a continuous monitoring of mercury contamination of fish around a year should be carried out [Michalska 2010]. Moreover, it would be highly recommend to spread mercury monitoring on other water reservoirs and also widen it for other compounds like selenium, copper, lead, cadmium etc.

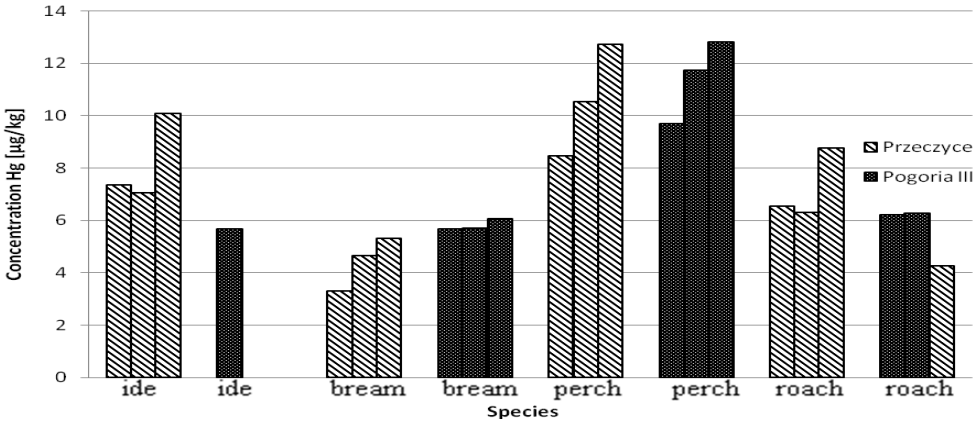


Fig. 11. The mercury concentration in fins of selected fish species from Pogoria Lakes

Analysis of the collected results showed that mercury is accumulated mainly in muscle tissue of all the selected fresh water fish species. Organs, like liver, heart and fins, had much lower ability to accumulate mercury compounds. Similar observations were previously reported by Svobodova et al. [1999] for perch, roach and bream from Czech Republic reservoirs, despite that the analyzed Czech fish were characterized by more than 50 times higher mercury content in comparison to fish from presented study. Higher mercury contamination was also reported by Falandysz et al. [2000] for roach, bream and perch from the Lower Vistula River. This reflects that the selected fresh water fish from Pogoria lakes are not affected by mercury and can be consumed by people.

The best repeatability of the collected results was stated for fish muscle tissue. Whilst, quite high differences in mercury concentration analysis in liver and heart could be explained by chemical composition, especially relatively high moisture content, which influenced sample density and ability to evaporation during analysis. Some discrepancies were also observed for mercury content in fins, which could be affected by not uniform Hg deposition in breast and abdominal fins, and it could be avoid by slight modification of the analytical procedure on the stage of the experimental material preparation.

The most important findings of this work was that none of the analyzed fresh water fish from Pogoria lakes were contaminated by high concentration of mercury. Moreover, there were no differences in Hg content in fish from both experimental water reservoirs, i.e. Przeczyce and PogoriaIII, which are situated around 10 km and 4 km from steelwork, respectively. Sackett et al. [2010] showed that there is a relation between mercury concentration in fish flesh and distance from coal-fired power plants. However, the result of our study did not confirm this statement for Dąbrowa Górnicza region. So, such a low level of mercury concentration in fish, despite the place of rod-fishing designated those animals for human consumption without risk of heavy metal intoxication. This is also important from economical point of view because in recent years many new fish species like oilfish (*Ruvettus pretiosus*), Nile perch (*Lates niloticus*), panga catfish (*Pangasius* spp.) were imported to the Polish market. Some of those new species were characterized by a very low heavy metals content [Polak-Juszczak 2007], what caused a decrease of the traditional Polish fish consumption.

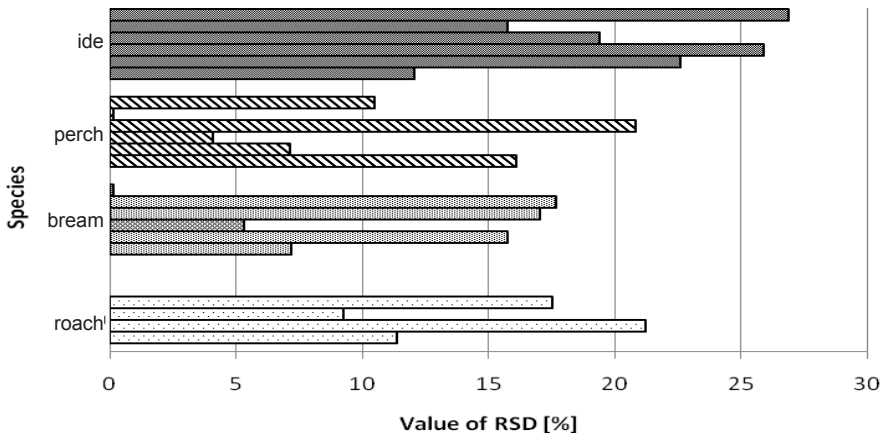


Fig. 12. Relative standard deviation for Hg concentration in fins of selected fish species from Pogoria Lakes

Comparison of the relative mercury concentration in the selected fish organs and muscles showed that the lowest deviation in mercury content data was obtained for bream (up to 56%) (Fig. 13).This revealed the most uniform Hg deposition in bream in relation to other species. The highest discrepancies were observed for ide (up to 90%), following by perch (up to 80%) and roach (78%).

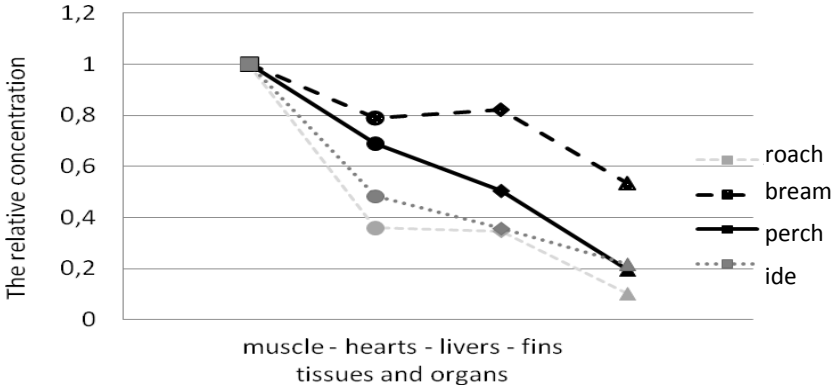


Fig. 13. Comparison of Hg concentrations in organs and muscles of selected fish species from Pogoria Lakes

Within all analyzed fresh water fish species present in Pogoria lakes ide was characterized by the highest mercury content in muscle tissue. This could be partly explained by age and size of fish due to ides analyzed in the study had a body weight twice as big as other species, which was connected with an age of the animal. Generally, fish species selected for the study have comparable body weight at the same age, when living in similar environmental conditions. That is why fish age can be directly related to body weight. Apart from the highest mercury contamination of ide muscles, mercury deposition in fish flesh was also higher in comparison to other species. The positive correlation between fish size and mercury content was also reported by Burgess and Hobson [2006] for yellow perch and it was explained by longer exposition on the mercury contaminated environment. When fish were similar in size and body weight, predators usually had higher mercury content in muscle tissue and organs [Westöö 1973, Nair 1974, Akerman and Balk 1998, LEPR 2008]. This was also confirmed in the presented study, as Hg concentration in meat from perch was the highest within all analyzed fish similar in size from Pogoria lakes.

The obtained results can indicate an improvement of the quality of water in Pogoria lakes from Dąbrowa Górnicza region, regarding to mercury contamination, despite some contrary data recently published by Michalska [2010] and Zyśk et al. [2010]. Presented study was carried out as a screening research, thus the collected results are preliminary and can be a good base for further studies. Therefore, it is necessary to continuously monitor the mercury concentration in fish in order to eliminate any risk of negative impact of this compound connected with fish consumption on human health.

References

Amundsen P.A., Staldvik F.J., Lukin A.A., Kashulin N.A., Popova O.A., Reshetnikov Y.S., 1997. Heavy metal contamination in freshwater Fish from the border region between Norway and Russia. Sci. Total Environ., 201, 211–224.

Arribere M.A., Ribeiro Guevara S., Bubach D.F., Arcagni M., Vigliano P.H., 2008. Selenium and mercury in native and introduced fish species of Patagonian Lakes, Argentina. Biol. Trace Elem. Res., 122, 42–63.

Akerman G., Balk L., 1998. Descriptive studies of mortality and morphological disorders in early life stages of cod and salmon originating from the Baltic Sea; Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea. American Fisheries Society Symp., 2, 41–61.

Bojakowska I., Górecka E., Karmasz D., 2010. Mercury in recent sediments of selected rivers and lakes in Poland – monitoring studies: years 1991–2009. Oceanological Hydrobiol. Stud., 39 (suppl. 1), 51–64.

Burgess N.M., Hobson K.A., 2006. Bioaccumulation of mercury in yellow perch (*Perca flavescens*) and common loons (*Gavia immer*) in relation to lake chemistry in Atlantic Canada. Hydrobiol., 567, 275–282.

Czaplicka M., Szmyd E., Grzegorczyk M., Pyta H., Zajusz R., Kubica B., Smolarski M., 2009. Ocena stopnia zanieczyszczenia powietrza atmosferycznego rtęcią, z uwzględnieniem jej fizycznej i chemicznej specjacji w świetle Dyrektywy 2004/107/WE., IPIŚ PAN, Katowice.

Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC.

Dobrowolski J.W., 1978. New concept of nutritional prevention of Minamata disease. J. Const. Med., 42, 99–108.

Dobrowolski J., Dominik J.W., Wagner A., Pardos M., Benninghoff Ch., 1999. Testy biologiczne i badania chemiczne w monitoringu ekologicznym środowiska wodnego. Chemia środowiskowa, ćwiczenia i seminaria, Szczepaniec-Cięciak E., Kościelniak P., Wyd. U. J., 2, 405–433.

Dutchak S., Varygina M., 2010. Meteorological Synthesizing Centre-East, http://www.msceast.org/.

Dusek L., Svobodova Z., Januskova D., Vykusova B., Jarkovsky J., Smid R., Pavlis P., 2005. Bioaccumulation of mercury in muscle tissue of Fish in the Elbe River (Czech Republic): multispecies monitoring study 1991–1996. Ecotoxicol. Environ. Safety, 61, 256–167.

Dz. U. 2006, 123, 858. Obwieszczenie Marszałka Sejmu Rzeczypospolitej Polskiej z dnia 12 czerwca 2006 r. w sprawie ogłoszenia jednolitego tekstu ustawy o zbiorowym zaopatrzeniu w wodę i zbiorowym odprowadzaniu ścieków.

Dz. U. 2006, 147, 1067. Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 28 lipca 2006 r. w sprawie sposobu postępowania z substancjami niedozwolonymi, pozostałościami chemicznymi, biologicznymi, produktami leczniczymi i skażeniami promieniotwórczymi u zwierząt i w produktach pochodzenia zwierzęcego.

Dz. U. 2007, 61, 417. Rozporządzenie Ministra Zdrowia z dnia 29 marca 2007 r. w sprawie jakości wody przeznaczonej do spożycia przez ludzi.

Falandysz J., Chwir A., Wyrzykowska B., 2000. Total mercury contamination of some Fish species in the firth of Vistula and thelower Vistula river, Poland. Pol. J. Environ. Stud., 9, 335–339.

Futatsuka M., 1988. Epidemiological aspect of methylmercury poisoning in Minamata; For truth and justice in the Minamata Disease Case, Proceed.Int.Forum on Minamata Disease, Ed. by Shigeti TSURU et al.

Górecki J., Iwanicha A., Macherzyński M., Styszko K., Gołaś J., 2010. Oznaczenie metylortęci techniką chromatografii gazowej z detekcją przy zastosowaniu atomowej spektroskopii fluorescencyjnej (GC-AFS). Rtęć w środowisku: identyfikacja zagrożeń dla zdrowia człowieka Ed. L. Falkowska, Gdańsk, Fundacja Rozwoju Uniwersytetu Gdańskiego, 297–303.

Gworek B., Rateńska J., 2009. Migracja rtęci w układzie powietrze – gleba – roślina. Ochrona środowiska i zasobów naturalnych, 41, Instytut Ochrony Środowiska, Warszawa, 614–623.
Harada M., 1988. The intrauterine methylmercury poisoning known as "Congenital Minamata Disease" – A 20 years serial investigation and its recent problems; For truth and justice in the Minamata Disease Case, Proceed.Int. Forum on Minamata Disease, Ed. Shigeti Tsuru et al.
Harada M., 2004. Minamata disease. Ed. Kumamoto Nichinichi Shinbun Culture and Information Center, Tokyo.
Kabata-Pendias A., Pendias H., 1999. Biogeochemia pierwiastków śladowych. PWN, Warszawa.
LEPR, Lake Erie Protection & Restoration Plan 2008; Priority: Toxic pollutants; Goal: Reduce the loadings of persistent bioaccumulative toxics, in particular mercury, to Lake Erie. Prep. by Ohio Lake Erie Commission, Toledo.
Michalska A., 2010. Analiza zawartości rtęci w środowisku na terenie województwa śląskiego. Problemy Ekologii, 14, 4, lipiec-sierpień, 165–169.
Miklavcic A., Stibilj V., Heath E., Polak T., Snoj Tratnik J., Klavz J., Mazej D., Horvat M., 2011. Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market. Food Chem., 124, 711–720.
Mniszek W., Zielonka U., 1995. Wskaźniki emisji rtęci do powietrza atmosferycznego. Ochrona powietrza i problem odpadów, 29, 6, 178–181.
Nair K.P., 1974. Mercury Concentration in fish. Seafood Export J., 6, 56–60.
Pacyna J.M., Pacyna E.G., Steenhuisen F., Wilson S., 2008. Global anthropogenic emissions of mercury to the atmosphere, [in:] The Encyclopedia of Earth, 1–4, 29 August 2008.
Panasiuk D., Pacyna J.M., Głodek A., Pacyna E.G., Sebesta L., 2010. Analiza kosztów i korzyści dla zdrowia ludzkiego i środowiska związanych z redukcją emisji rtęci w Polsce. Raport GIOŚ, 9–15.
Polak-Juszczak L., 2007. Chemical characteristics of fishes new to the Polish market. Acta Sci. Pol. Piscaria, 6, 23–32.
Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
Sackett D.K., Aday D.D., Rice J.A., Cope W.G., Buchwalter D., 2010. Does proximity to coal-fired plants influence fish tissue mercury? Ecotoxicol., 19, 1601–1611.
Svobodova Z., Dusek, L., Hejtmanek M., Vykusova B., Smid R. 1999. Bioaccumulation of mercury in various Fish species from Orlik and Kamyk water reservoirs in the Czech Republic. Ecotox. Envi. Safety, 43, 231–240,
Szkoda J., Kmiecik M., Żmudzki J., 2010. Rtęć w żywności i paszach – urzędowe badania kontrolne. Rtęć w środowisku: identyfikacja zagrożeń dla zdrowia człowieka Ed. L. Falkowsa, Gdańsk, Fundacja Rozwoju Uniwersytetu Gdańskiego, 215–218.
Szpadta R.,1994. Zanieczyszczenia środowiska rtęcią i jej związkami. Powiatowy Inspektorat Ochrony Środowiska. Biblioteka Monitoringu Środowiska, 119, Warszawa.
Tsuda T., Yorifuji T., Takao S., Miyai M.,Babazono A., 2009. Minamata disease: Catastrophic poisoning due to a failed public health response. J. Publ. Health Policy, 30, 54–67
Westöö G., 1973. Methylmercury as percentage of total mercury in flesh and viscera of salmon and sea trout of various ages. Science, 181, 567–568.
Zillioux E.J., PorcellaD.B., Benoit J.M., 1993. Mercury cycling and effects in fresh water wetland ecosystems. Environm.Toxicol. Chem., 12, 2245–2264.
Zyśk J., Wyrwa A., Roustan Y., Pluta M., 2010. Integrated impact analysis of mercury emitted from the Polish power sector: Rtęć w środowisku: Identyfikacja zagrożeń dla zdrowia człowieka; Ed. L. Falkowska, Gdańsk, Fundacja Rozwoju Uniwersytetu Gdańskiego, 51–56.

3

INFLUENCE OF CADMIUM AND ZINC ACCUMULATION IN POTATO TUBERS ON THEIR CONTENT IN DIFFERENT FRACTIONS OF SACCHARIDES

Introduction

Potato tubers are generally considered for plant product that have importance especially in human nutrition and also for processing industry. In human nutrition they provide mainly protection, bulk and increases satiety. In natural state they contain approx. 75% of water, while the main deal of dry matter is formed by starch (approx. 75%). Potatoes are the source of proteins, mineral substances, vitamins, polyphenols and minimal amount of lipids.

Saccharides come to existence in nature in cells of photoautotrophic organisms by assimilation of aerial carbon dioxide in presence of water and with utilization of energy of day light changed in photosystems on chemical energy. They are stable substances of all cells. Glucose is the most common saccharide [Velíšek 2002].

Content of saccharides in potatoes is dependent on variety and physiological state of tuber, while the composition of individual saccharides can change during development of the crop and storage of tubers. Monosaccharides glucose and fructose are the main saccharides present in tuber, both in equal composition: 0.15–1.5 %, and non-reducing disaccharide sucrose (0.4–6.6 %) [Vreugdenhil et al. 2007].

Table 1

Content of saccharides in potato [g.kg-1 fresh matter] [USDA 2009]

total saccharides	sucrose	glucose	fructose	maltose	lactose	galactose	starch
7.8	1.7	3.3	2.7	0.0	0.0	0.0	154.4

Higher amount of saccharides, especially sucrose are present in unripe tubers. In first day after harvest, the content of sucrose in unripe tuber declines, while the content of fructose and glucose increases [Muray et al. 1998]. Brandt et al. [2009] reported increased concentration of sucrose from 0.083 to 0.140% and glucose from 0.019 to 0.079% in relation to growth phase, while its remain content was affected by conditions of storage – amount of glucose increased with length of storage on maximum and in dependence from temperature of storage it declined. Content of starch increases during ripening of potatoes in comparison to fruit [Velíšek 2002].

Highly localized browning is one of the signs of frozen potatoes, that is characterized by darker color of tissues and these defects often affect quality of potato products. Content of reducing saccharides contributes to brown color. Accumulation of reducing saccharides can be caused by stress from high temperatures during growing of tuber and can be supported by lack or abundance of nitrogen [Bethke et al. 2009a, 2009b].

High content of reducing saccharides is undesirable by potatoes processing on special products. In fried potato chips and French fries as aftermath of high content of reducing saccharides is an enhanced reaction with amino acids with formation of brown products [Míča 1986].

Not only outer quality but also internal merit is main factors for producers as well as for consumers of potatoes, except yield of tubers. The quality of potatoes that includes their hygienic safety is influenced by many factors. It is not given only by components crucial in human nutrition, but also by unacceptable substances. Also heavy metals rank to the mentioned group and exceeding of the level of their hygienic norm can decide about suitability of potato tubers for consumption. Typical specificity of foreign matters is that in low concentrations they can exert stimulating effect on growth of plants. They frequently act as toxic in high doses [Hlušek et al. 1998a], although from the standpoint of the yield forming the potatoes are included to group of plants that are tolerant to increased levels of foreign matters in soil [Hlušek et al. 1996].

Soil is the starting place of heavy metals entering plants and afterwards to food chain. In spite of the fact that after 1990's there was recorded mild decline of agricultural soil contamination in Slovak Republic, at present times there are 25000 hectares of contaminated soils mainly situated in so-called loaded areas.

Cadmium ranks among most serious contaminants of the environment. Combustion of oil fuels, carbon, wastes and application of unsuitable superphosphate fertilizers are the main sources of contamination by Cd. Cadmium has higher availability than lead, copper and zinc from the point of view of transport to plants. It can affect plants as stress factor manifesting physiological changes that can result in inhibition of growth or even extinction of plant.

From the health point of view cadmium belongs to heavy metals and their toxic traits are manifested by relatively low concentrations. Cadmium accepted by foodstuffs is cumulated especially in kidneys, less in liver and other organs, while it causes disorders of cardiovascular system and central nervous system, damage of kidneys and liver, causes anemia and oncological diseases [Bencko et al. 1995]. Potatoes, together with cereals and leafy vegetable are main sources of cadmium.

Content of cadmium in plants was in range from 0.01 to 0.22 $mg \cdot kg^{-1}$. Soil type and soil reaction affect content of cadmium in plants. Cadmium is more available to plants in acidic soils than in soils with alkali reaction. Calcium is a 'protector of environment' in this situation. Content of organic substances in soil plays also an important role. Plants cultivated in soil with low content of organic substances have especially higher content of cadmium. Definite and uniform opinions on transport of cadmium in plants do not exist, but higher content of cadmium is observed in roots in comparison to upper biomass [Tomáš et al. 2007].

Zinc in optimal concentration interval ranks among essential elements for plant and also for animal organism. It is accumulated mainly in roots; in higher concentrations in plants is phytotoxic [Tomáš et al. 2007]. Zinc and cadmium can compete in bond places in soil system [Bystrická and Tomáš 2009]. Zinc plays a role in protein and saccharides metabolism, is a part of more than 60 metaloenzymes, forms bonds with nucleic acids [Dąbrowski and Sikorski 2005].

High contents of zinc in plant tissues inhibit functions of proteins containing other metals, which are substituted by zinc [Broadley et al. 2007, Malakouti et al. 2007]. Its excessive content affects transpiration, photosynthesis and enzymatic activity [Rout and Das 2003].

Supposed toxic concentration is from 150 to 200 mg $Zn \cdot kg^{-1}$ of dry matter of plant material [Sauerbeck 1989].

Content of zinc in potato tubers depends on level of soil contamination with this element. Moreover, its high content could be awaited in potatoes cultivated in localities with high concentration of Zn in soil (300 mg $Zn \cdot kg^{-1}$). Higher accumulation of Zn in peels could be problematic, thus the consummation of peeled potatoes is recommended [Musilová et al. 2009].

Emissions from industry and application of sludge, agrochemicals in soil are main anthropic sources of zinc in the environment.

Materials and methods

Soil samples as well as samples of plant materials were taken from 4 sampling sites in locality of Hontiansky and Banskoštiavnický region (Terany, Hontianske Nemce, Prenčov-1, Prenčov-2), situated in vicinity of Štiavnica River draining main part of Štiavnické vrchy in Slovak Republic (SR).

Soil

Characteristics of locality – Combined negative influence of soil contaminating with risky metals and influence of anthropogenic geochemical anomalies are occurring in some localities of SR. Extremely high contents of zinc (even 1000 $mg \cdot kg^{-1}$) occurs in geochemical anomaly in Štiavnické vrchy, in flat lands and terraces of Štiavnická river. Reference value A (total content) is 140 $mg \cdot kg^{-1}$, clark content value is 71 $mg \cdot kg^{-1}$ [Tomáš et al. 2007].

Average contents of cadmium in humus horizons of fluvial soils situated in vicinity of mentioned river are in range approx. 0.9 $mg \cdot kg^{-1}$, while some fold they exceed reference value for valid hygienic limit A_1 (0.3 $mg \cdot kg^{-1}$, Tab. 1, 2), assessed maximal concentrations Cd have been above 3 $mg \cdot kg^{-1}$, while they approximate to indicate value of hygienic limit B, which detached the category of contaminated soil [Styk 2001].

Important transport medium of heavy metals is Štiavnica River that originates in the area of Štiavnické vrchy. Leakages from mining shaft and piles have been getting into mentioned river and thus substantial amount of risky elements that by floods contaminated closest alluvial soil [Bajčan et al. 2007]. Contents of some monitored elements significantly exceeded legislatively given limit values, what these soils rank among risky, while contamination was analytically proved from this standpoint [Tóth et al. 2005]. It is important to monitor transfer of heavy metals from contaminated soil into agricultural crops in mentioned area.

Sampling and preparation of samples – soil samples were taken in soil horizon 0–0.2 m according to the exact method into pedological probe GeoSampler fy. Fisher. Sampling was carried out as single from three sampling sites in each locality. After their air-drying and grinding with soil grinding machine *VEB Thurm ZG 1* on fine earth I (average 2 mm particle size) soil samples were sieved through sieves with average 0.125 mm (fine earth II). Fine earth I was used on an assessment of soil reaction, contents of nutrients and contents of mobile forms of Cd, Zn. Fine earth II was used on an assessment of pseudototal contents of Cd, Zn.

Used analytical methods – agrochemical characteristics of soil was assessed in taken soil samples (active soil reaction pH/H_2O and changeable soil reaction (pH/KCl), contents

of nutrients (P, K, Ca, Mg) were assessed by method of Mehlich II and the contents of heavy metals: pseudototal contents of risky metals in extract of *aqua regia* [Law no. 220/2004] and the contents of mobile forms of risky metals [Law 220/2004 – critical values in relation to soil – plant] in soil extract NH_4NO_3 (c=1 mol.dm^{-3}). Analytical method of the determination of contents of macroelements and risky elements was flame atomic spectrometry (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA)

Soil taken from individual sites could be characterized as medium heavy, sandy-loamy, with high or very high contents of nutrients and with slightly weak by acid and acid soil reaction (Tab. 2).

Table 2

Content of nutrients in mg·kg-1 in soil and soil reaction

Locality	pH/H_2O	pH/KCl	P	K	Ca	Mg
Terany	8.55	6.40	338.32	474.5	2675	459.5
Hontianske Nemce	7.51	6.15	460.19	6874.5	3520	591.0
Prenčov 1	7.45	5.45	236.38	794.5	2615	384.5
Prenčov 2	7.51	5.74	598.85	1032.5	3190	497.5

Contents of heavy metals in soil were compared with limit values according to Law no. 220/2004 for expression of range of soil contamination.

Contents of Cd, and Zn were in comparison to limit values, assessed by Law no. 220/2004, some fold higher, significant increasing in soil samples taken from locality Prenčov, where the content of cadmium was over 16.7-times higher than limit value for this element and the content of zinc 10.4-fold were higher in comparison to limit values. Critical values, assessed for zinc were over 1.6–1.87-times higher in soil taken from locality Prenčov-1, Prenčov-2 (Tab. 3).

Table 3

Content of heavy metals in mg·kg-1 in soil

Locality	Extraction reagent	Cd	Zn	Extraction reagent	Cd	Zn
Terany	*Aqua regia*	1.64	212	NH_4NO_3 (c = 1 mol.dm^{-3})	0.032	0.125
Hontianske Nemce		1.22	184		0.035	0.155
Prenčov-1		11.72	1555		0.076	3.735
Prenčov-2		10.26	1385		0.068	3.20
	Limit value	**0.7**	**150.0**	**Critical value**	**0.1**	**2.0**

Exceeded limit/critical value

Plant

Sampling and preparation of samples – potatoes were harvested in full ripeness from the same sampling sites as the soil samples. Potatoes of Adora variety (very early variety – oval tubers, yellow color of skin, and beige color of flesh) were used. Sample of a fresh matter was prepared by homogenizing of all potato tubers taken from one sampling site. Contents of

Cd and Zn in potato tubers were assessed in four repetitions after mineralization of the sample by wet way method.

Analytical methods

Assessment of cadmium and zinc content in edible parts of potato tubers: fresh potato tubers were used for analyses of heavy metals contents (2nd day after sampling). Mineralization of samples was carried out by microwave digestion in the microwave MARS X-press (CEM USA)**.** Contents of heavy metals were assessed in a filtrate of mineralizate and after filling with distilled water till mark in 50 cm^3 by AAS method; content of Cd was assessed at wavelength 228.8 nm (detection limit 0.001 $\mu g.cm^{-3}$), content of Zn was assessed at wavelength 213.9 nm (detection limit 0.0006 $\mu g.cm^{-3}$). The contents of heavy metals were assessed after homogenization of samples and after mineralization by wet way method of AAS.

Assessment of different fractions of saccharides:

- fractions containing soluble saccharides (SS) and insoluble saccharides (IS) were isolated by sequential analysis from lyophilised a defatted sample by gradual extraction (E) and coagulation (Z) by following reagents: E: dist. water, NaCl (c=1 $mol.dm^{-3}$) in phosphate puffer (c=0,1 $mol.dm^{-3}$), 70 % ethanol, NaOH (c=0,05 $mol.dm^{-3}$); Z: lead acetate, tannin:
- extraction of sample in distilled water and then filtration – insoluble portion Z1 and filtrate F1 was gained, from which coagulum was separated after adding of $Pb(NO_3)_2$ with filtration, containing part of proteins, and filtrate F2, which was put to assessment of risky metals in SS;
- extraction and washing of insoluble portion Z1 NaCl – filtrate F3 was gained and the residue Z2;
- extraction and washing of residue Z2 with ethanol, shaking and vacuum filtration – filtrate F4 was gained and the residue Z3, which was designed for further extraction;
- precipitation and separation of another share of proteins by tannins from F4 – filtrate F5 was gained, which contains in ethanol-soluble saccharides, after connecting F5 and F2 the solution was vacuum concentrated on 50 cm^3 and used directly for determination of contents of risky metals in SS;
- extraction, washing Z3 NaOH and vacuum filtration – filtrate F6 and residue Z4, which presents non-soluble saccharides and ash;

IS and ash were mineralized with concentrated HNO_3 and the contents of risky metals in mineralizate were assessed. Individual fractions were separated by modified method of Osborne [Michalík 1982], contents of heavy metals were assessed by AAS method (Varian AA DUO 240FS/240Z).

Results and discussion

Contents of Cd and Zn in potato tubers

Foreign matters present stress factor for most of plants and by enter into food chain pose threat for human organism. Affecting of foreign elements on plants is considerably variable. Except of cumulating in plant their high content in soil could be manifested by depression effect on its growth [Hlušek et al. 2001]. From hygienic point of view it is important, if they are accumulated in parts that are used for consumption.

The highest accumulation of cadmium in soil occurs in layer 0–50 mm from surface and its concentration declines with rising depth. With regard on the fact they can not be degraded and present long-lasting threat for soil environment. Soil contamination has been significantly manifested on its cumulating in potato tubers, where beside samples taken from locality Terany hygienic limit values given by FC SR was exceeded (Hontianske Nemce: 1.1-fold, Prenčov-1: 3.6-fold, Prenčov-2: 2.6-fold).

Increased content of cadmium in soil was manifested by its increased concentration in potato tubers in such range that it was some fold exceeded the limit value assessed by valid legislation. Lowering of the contents of heavy metals in potato tubers could be partly achieved by processing of potatoes. In case of lead mainly peeling of potatoes is applied, while the leakage affects also the content of other elements [Míča and Vokál 1996].

Content of cadmium does not pose any threat in natural conditions for growth and development of potatoes, although when soil is contaminated, it can cumulate in potato tubers and by their regular consumption with increased content of Cd it could have toxic influence on human organism.

Increasing of zinc content in soil in locality Prenčov about critical value was not manifested by its increasing in potato tubers, because the content of zinc in tubers depends on its content in available form in soil, what is in consistency with results of Hlušek et al. [1998b]. The highest content of Zn (6.87 $mg.kg^{-1}$ FW) was in tubers of potatoes from site Prenčov 2 (Tab. 3).

Important criteria of potatoes quality evaluation include not allowed exceeded hygienic norms of foreign matters concentrations from the standpoint of human nutrition.

Contents of fractions SS and IS

Contents of saccharides fractions were assessed also in samples of potatoes grown in localities Hontiansky and Banskoštiavnický region (Terany, Hontianske Nemce and Prenčov). Their values were in range from 0.32 to 2.33% of water-soluble saccharides and from 12.74 to 16.56 % of non-soluble saccharides (Tab. 4).

Reducing monosaccharides glucose and fructose and non-reducing disaccharid sucrose formed the highest portion of water-soluble saccharides. Dynamics of changes in levels of saccharides depends on variety, but by achieving of ripening the ratio of sucrose to reducing saccharides reached minimal value [Vreugdenhil et al. 2007].

High content of reducing saccharides is undesirable by processing of potatoes on special products. During thermal processing of potatoes (frying, roasting, drying) two types of reactions occur, which caused so-called non-enzymatic browning, i.e. caramelization and Maillard reaction. Specific degradation of saccharides (caramelization), in which also sucrose is

by highly degree degraded, is observed in lower rate. Maillard reaction is more important for color changes between reducing saccharides and amino acids. α-amino-compounds are rarely the limiting factor and thus the intensity of color changes are in relation especially to the content of reducing saccharides. From the standpoint of practical utilization reactions of non-enzymatic browning are especially important for fried products. Eliminating of color changes depends on selection of suitable raw material on processing. Recommended maximal content of reducing saccharides in potatoes on production might not be higher than 0.2% and contrary for preservation of certain color might not be lower than 1 % [Míča 1986, Hamouz et al. 2000].

Daniels-Lake et al. [2009] assumed that browning in chips is a consequence of interaction of trace levels of ethylene with accumulated CO_2 from respiration of potatoes.

Contents of Cd and Zn in fractions SS and IS

Increased contents of Cd and Zn in fresh matter were in consistency with increased contents of these heavy metals in saccharides fractions (Cd SS: 0.020–0.067; IS: 0.017 –0.039 mg·kg^{-1} FW, Zn SS: 1.429–2.999; IS: 0.404–0.750 mg·kg^{-1} FW). The most significant difference in cumulating of HM in saccharides fraction was evaluated between contents of Cd in SS from localities Terany and Prenčov-1 (Tab. 4).

Table 4

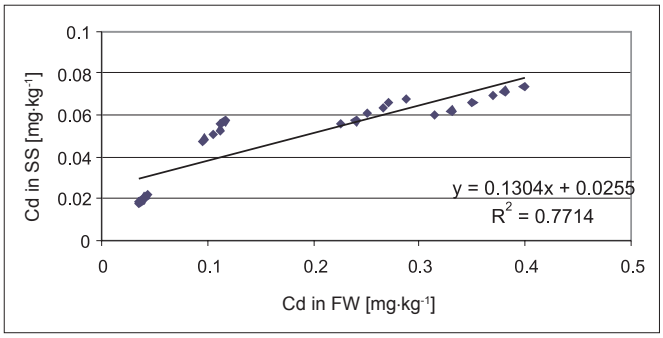
Contents of saccharides fractions (% FW) and content of Cd, Zn (mg·kg^{-1} FW) in fractions SS and IS of potatoes from Hontiansky and Banskoštiavnický region (mg·kg^{-1})

locality	Cd in FW	Zn in FW	SS	Cd in SS	Zn in SS	IS	Cd in IS	Zn in IS
Terany	0.039	5.289	2.33	0.020	1.771	14.54	0.017	0.517
Hontianske Nemce	0.106	6.100	1.06	0.052	2.999	16.56	0.023	0.404
Prenčov-1	0.357	4.939	1.04	0.067	2.006	12.74	0.039	0.750
Prenčov-2	0.256	6.868	0.32	0.062	1.429	14.32	0.032	0.621
PK SR*	**0.1**	**10.0**						

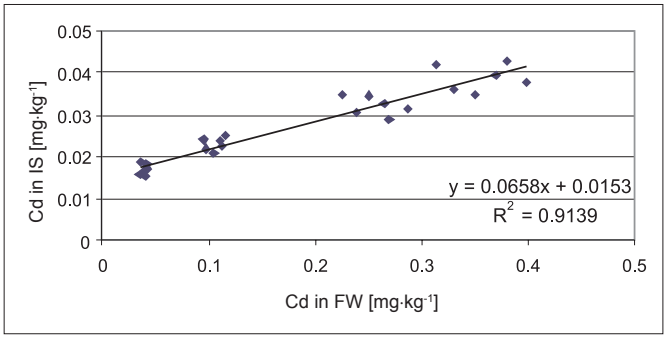
* Exceeded limit

There is strong positive correlation between the content of cadmium in FW and in saccharides (SS: R=0.878; IS: 0.956). Variability of cadmium content cadmium in dependence on content in fresh matter of potatoes was shown on regression line explained on 77.1% in fraction SS (IS: 91.4%). While the value F (SS $1.686 \cdot 10^{-8}$; IS $3.387 \cdot 10^{-13}$) was lower than significance level α (0.05, and 0.01), the proper regression line in all cases is suitable for explanation of dependence. The equation of particular regression line is mentioned in Graphs 1, 2.

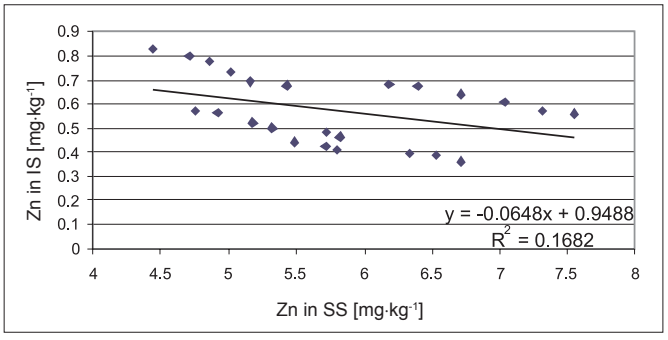
Cumulating of zinc had not statistically significant influence on its cumulating in fraction SS (R = 0.000; P-value = 0.930), there is a weak by negative correlation (R = 0.410; P-value = $4.651 \cdot 10^{-2} < \alpha$) between contents of Zn in FW of potato tubers and in IS (Graph 3).



Graph 1. Statistical dependence of Cd content on fraction SS from amount of Cd accumulated in potato tubers [mg·kg^{-1} FW]



Graph 2. Statistical dependence of Cd content in fraction IS from amount of Cd accumulated in potato tubers [mg·kg^{-1} FW]



Graph 3. Statistical dependence of Zn content in fraction IS from amount of Zn accumulated in potato tubers [mg·kg^{-1} FW]

Cultural crops (sometimes also cultivars) differ from their requirements and sensitivity on microelements. There are also differences in their ability to uptake these elements from soil that is manifested in mineral composition of individual plant organs. Their content in potato tubers is very significant from the nutritional as well as hygienic point of view. While there will be higher values, these elements might negatively affect the plant and also human health [Hlušek et al. 1998b].

Conclusions

Potatoes cultivated in soils with higher contents of risky elements could be their source in food chain.

Cadmium accumulation in potatoes was manifested by its increased content in fractions SS and also IS. This content increased in potatoes from 0.020 to 0.067 $mg \cdot kg^{-1}$ FW in fractions SS and from 0.017 to 0.039 $mg \cdot kg^{-1}$ FW in fraction IS from following localities Terany < Hontianske Nemce < Prenčov-2 < Prenčov-1. There is strong positive correlation between the content of cadmium in FW and in saccharides ($P < 0.01$).

In spite of that content of zinc in soil exceeded in all four localities the limit value (> 150 $mg \cdot kg^{-1}$ soil) and in two localities the critical value (> 2 $mg \cdot kg^{-1}$ soil), this fact was not manifested by its higher accumulation in potato tubers (the content of Zn did not exceed hygienic norm 10 $mg \cdot kg^{-1}$ FW in samples, defined in PK SR). Any relation was not confirmed between Zn content in fresh matter and in fraction SS, content of Zn in fraction SS increased from 1.429 to 2.999 $mg \cdot kg^{-1}$ FW as following Prenčov-2 < Terany < Prenčov-1 < Hontianske Nemce. Weak negative correlation ($P < 0.05$) between Zn content in fresh matter of potato tubers in fraction IS was evaluated and Zn content increased from 0.404 to 0.750 $mg \cdot kg^{-1}$ FW as following: Hontianske Nemce < Terany < Prenčov-2 < Prenčov-1.

The intake of food is one of the possibilities of entering mentioned risky elements into human organism. When there is important moiety of foodstuffs with their increased cumulating, its consumption could pose the risk for human health. Potatoes are in our diet of our population represented by important range and thus it is necessary to monitor thoroughly the entering of risky metals into tubers, as well to specify the cumulating of heavy metals in individual components of potato tuber. Such knowledge might be the basis for further research in the field of breeding, as well as food processing.

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References

Bajčan D., Lahučký L., Stanovič R., Árvay J., 2007. Hygiena poľnohospodárskych plodín dopestovaných na metalicky zaťažených aluviálnych pôdach. IX. Banskoštiavnické dni 2007, 33–38.

Bencko V., Cikrt M., Lener J. 1995. Toxické kovy v životním a pracovním prostředí člověka. Grada Publishing, 281.

Bethke P.C., Sabba R., Higgins C., Bussan A.J., 2009a. Stem End Defect in Chipping Potatoes from Production Fields and Temperature-Controlled Greenhouses. American Journal of Potato Research, 86, 136.

Bethke P.C., Sabba R., Bussan A.J., 2009b. Sugar End Defect in Stressed Burbank Potatoes Grown Under Controlled Environment Conditions. American Journal of Potato Research, 86, 136.

Brandt T.L., Olsen L., Kleinkopf G.E., 2009. Effect of Growing Season on "Russet Burbank" Storage Tuber Quality. American Journal of Potato Research, 86, 138.
Broadley M., White P.J., Hammond J.P., Zelko I., Lux A., 2007. Zinc in plants. New Phytology, 173, 677–702.
Bystrická J., Tomáš J., 2009. Antagonistic relations of lead and cadmium with selected micronutriens. Ochrona Środowiska i Zasobów Naturalnych (environmental protection and natural resources), 40, 165–170.
Dąbrowski W.M., Sikorski Z.E., 2005. Toxins in Food. CRC PRESS LLC, 245.
Daniels-Lake B., Prange R., Bishop S., Hiltz K., 2009. Blocking the CO_2 – Ethylene Interaction with 1-Methylcyclopropene. American Journal of Potato Research, 86, 139.
Hamouz K., Vokál B., Lachman J., Čepl J., 2000. Influence of environmental conditions and way of cultivation on the reducing sugar content in potato tuber. Plant Production, 46, 1, 23–27.
Hlušek J., Richter R., Růžička A., 1996. Vliv různého hnojení na výnos a kvalitu brambor. Bramborářství, IV, 2–5.
Hlušek J., Jůzl M., Zrůst J., 1998a. Iron, manganese and copper content in tubers of very early potato varieties. Rostlinná výroba, 44, 1–5.
Hlušek J., Jůzl M., Zrůst J., 1998b. Kvalita brambor v závislosti na odrůdě, hnojení a lokalitě pěstování. Úroda, 6, 21–25.
Hlušek J., Zrůst J., Jůzl M., 2001. Obsah kadmia, arsenu a berylia v rostlinách brambor při rozdílných hladinách těchto prvků v půdě. Bramborářství, IX, 9–12.
Law no. 220/2004. Criteria for identification of risky spots of soil contamination and methodology procedures of their evaluation.
Malakouti M.J., Bybodi A., Ranjbar R., Nouri, A.A., 2007. The Role of Zinc (Zn) and Potassium (K) on the Reduction of Nitrate (NO_3) and Cadmium (Cd) Contaminants in Potato and Onion. Zinc crops 2007, Improving crop production and human health. 24–26. May, Istanbul, Turkey.
Míča B., 1986. Kvalita brambor. Kvalita stolních a konzumních brambor a její ovlivnění, Havlíčkův Brod, Škobárny o. p., 141.
Míča B., Vokál, B., 1996. Technologická hodnota brambor. Bramborářství, IV, 5–8.
Michalík I., 1982. Návody na cvičenia z biochémie a fyziológie rastlín. (2nd ed.). VŠP, Nitra, 164.
Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W., 1998. Harperova biochemie. 2. české vydanie. Nakladatelství a vydavatelství H&H, 872.
Musilová J., Poláková Z., Tóth T., 2009. Zinok – rizikový alebo prospešný mikroelement v zemiakoch? Potravinárstvo, 3, 4, 51–54.
Rout G.R., Das P., 2003. Effect of metal toxicity on plant growth and metabolism, I. Zinc. Agronomie, 23, 3–11.
Sauerbeck D., 1989. Die transfer von Schwermetallen in die Pflanzen. Dechema Arbeitsgruppe, Frankfurt n/Mohan., 281–316.
Styk J., 2001. Problém ťažkých kovov (kadmium, olovo, meď, zinok) v pôdach Štiavnických vrchov a ich príjem trávnymi porastmi. VÚPOP, Bratislava, 136.
Tomáš J., Hronec O., Tóthova M., Adamišin P., Andrejovský P., 2007. Poškodzovanie pôd a rastlín ľudskými činnosťami. SPU, Nitra, 110.
Tóth T., Tomáš J., Lazor P., Chlpík J., Jomová K., Hegedűsová A., 2005. Rizikové prvky v pôdach a plodinách Štiavnického regiónu. ChemZi, 1, 285–286.
USDA National Nutrient Database for Standard Reference, 2009, http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl
Velíšek J., 2002. Chemie potravin I. OSSIS – Tábor. 344.
Vreugdenhil D., Bradshaw J., Gebhardt Ch., Govers F., MacKerron D.K.L., Taylor M.A., Ros H.A., 2007. Potato biology and biotechnology. Advances and perspectives. Elsevier – Amsterdam – Boston – Heidelberg – London – New York – Ooxford – Paris – San Diego – San Francisco – Singapore – Sydney – Tokyo, 857.

4

ASSESSMENT OF VARIATION IN ACRYLAMIDE CONCENTRATION IN FRENCH FRIES PREPARED IN FOOD SERVICE ESTABLISHMENTS USING A TECHNO–MANAGERIAL APPROACH

Introduction

Acrylamide is a probable human carcinogen [IARC 1994] and its presence in a range of fried and oven-cooked foods [Tareke et al. 2000, 2002] has raised considerable health concern world-wide [FAO/WHO 2005]. Highest concentrations have been identified in potato based products such as French fries [DiNovi 2006, Claeys et al. 2010]. In addition, dietary intake studies observed large differences in acrylamide concentrations both between single foodstuffs (different brands) within particular food categories and within batches of products processed under the same conditions [Dybing and Sanner 2003, Konings et al. 2003].

The above-mentioned findings underpin that there is much uncertainty about actual concentrations of acrylamide in French fries. This variation is the result of the sum of variations of all individual factors [Lewontin 2006] that influence acrylamide formation. Reducing too high concentrations and large variation in acrylamide in French fries therefore requires insight in the contribution of individual sources of variation to final variation. Previously, it has been discussed that variation in food quality and safety is due to variation in product properties and technological conditions as well as variation in decision-making behaviour of people involved in the food production system [Luning and Marcelis 2006, 2007]. Many technological studies have provided profound insight in which product properties and processing conditions influence the formation of acrylamide, like frying time and temperature [Mottram et al. 2002, Becalski et al. 2003]. However, many of these influencing factors are also affected by how people deal with them in daily practice. Variable and or inadequate decision-making on these factors may result in large variation in the actual concentrations of acrylamide in French fries at the time of consumption. For example, a competent food handler at a frying station who exercised control to avoid over-frying, was capable to reduce acrylamide concentration in French fries by a factor of 2–3 [Fiselier et al. 2004]. It shows that the way people exercise control of the technological conditions can affect the variation in acrylamide concentration. Differences in decision-making behaviour of people may thus contribute to uncertainty about the actual concentrations of acrylamide in French fries at the time of consumption.

French fries are widely prepared in food service establishments (FSE) and their preparation is apparently a crucial factor because acrylamide is formed towards the end of the frying process [Fiselier et al. 2006, Amrein et al. 2007]. Furthermore, Al-Kahtani [1991] showed that frying practices among various types of FSE differed considerably in terms of frying conditions (temperature-time regime), frying equipment selection (design, capacity, heating system and material of fabrication), and quality control during frying. Many studies that were done under controlled laboratory conditions established the relationship between

various food properties (e.g. concentration of reducing sugars and amino acids) and processing conditions (e.g. frying temperature-time regimes and pH adjustment with organic acids) on acrylamide formation [Mottram et al. 2002, Jung et al. 2003]. However, there is restricted insight into the contribution of actual frying practices in FSE (e.g. using different raw materials, applying different frying temperature-time regimes and using different frying equipment) to the variation in acrylamide concentration in French fries for consumption. Moreover, to judge the actual risk of acrylamide and to develop appropriate control measures, require insight into the distribution profiles of acrylamide concentrations [FAO/WHO 2007].

The objectives of this research were to identify the technological and people related factors that contribute to high and variable concentration of acrylamide as well as to obtain insight into the actual variation in acrylamide concentrations in French Fries prepared under typical conditions in food service. In a literature study, an insight into the contribution of major product properties and technological conditions to large variation in acrylamide concentrations was obtained. The insight was used to assess the critical points of control in French fries preparation in FSE and used a 'food quality decision model' to describe which type of control decisions can effectively lower acrylamide concentrations and reduce its variation at the critical points. Subsequently, the typical control situation in FSE in practice was examined and discussed. In an observational study, three types of FSE, i.e. chain fast-food services (CFS), institutional caterers (IC), and restaurants (R) were selected to reflect the common types of FSE. The actual practices at receiving, thawing and frying during the preparation of French fries were observed to get an insight into the typical frying practices in these FSE types. The set and the actual frying temperature and frying time were recorded and acrylamide and reducing sugars concentrations were analysed.

Technological factors contributing to variation in acrylamide concentration

Receiving supplied materials

The first step of French fries production is the receipt of raw materials, which is usually in the form of frozen par-fried potato stripes. In French fries, reducing sugars are the limiting factor in the formation of acrylamide [Amrein et al. 2004] and various researchers observed a strong correlation between acrylamide formation and the reducing sugar available in potatoes [Becalski et al. 2004, Williams 2005]. The concentrations of reducing sugars vary widely among potato varieties, which may result in variation in the actual formation of acrylamide. To illustrate, Amrein and co-authors [2003] found that the concentration of glucose ranged from 0.04 to 2.7 mg/g, with the lowest values found in samples of variety Lady Claire and Marlene, and the highest in Naturella and Nicola. Considerable differences in reducing sugars among potatoes of the same cultivar suggested that other factors, such as storage, may have an even stronger influence on variation [Biedermann et al. 2002]. Moreover, also climatic conditions can result in variable concentrations of reducing sugars within cultivars. For example, higher reducing sugar concentrations were found in cultivars Eba (+165%), Bintje (+146%), and Agria (+113%) tested in 2003 as compared to the samples from 2002, due to the extraordinary hot and dry conditions in that year [Amrein et al. 2004]. The concentrations of reducing sugars may vary over storage time as well, and its concentration at a specific time may not always reflect concentrations present at earlier or later time periods [Vivanti et al. 2006]. For example, a mean value of 0.82 mg/g Fresh Weight (FW) was found after

harvesting and may increase to 1.26 mg/g FW after storage at 8°C [Matthaus et al. 2004]. Based upon above studies we may conclude that frozen par-fried potato stripes potentially can have high concentrations in reducing sugars with a considerable variation between potatoes, which can contribute to high and variable acrylamide concentrations if no specific measures are taken earlier in the chain.

Storage of frozen par-fried potato stripes

The frozen par-fried potato stripes are subsequently stored at -18°C until use. At frozen state, the molecular mobility strongly reduces and rates of all chemical and biochemical reactions slow down. In fact, almost no chemical activity was observed [Archer 2004]. Therefore, this step does not seem to contribute to variation.

Thawing

The sacks with frozen French fries are removed from the freezer storage, prior to the deep-frying process. The sacks of French fries usually stand for two or more hours at room temperature, until the French fries have thawed to a point where they can be deep fried [Glenn et al. 1993]. To our knowledge, no studies have been performed on the effect of thawing at room temperature for duration of minimum 2 hours on changes in glucose and fructose, and or the formation of acrylamide.

Frying

Deep-frying of par-fried potato stripes is the most important processing step. The formation of acrylamide starts at temperatures slightly above 120°C, and reaches a maximum around 170–180°C, depending on the model system studied and the duration of heating [Mottram et al. 2002, Claeys et al. 2005]. Pedreschi and co-authors [2004] reported that the acrylamide concentration of potato slices was about 500 µg/kg after frying for 7 min at 150°C as opposed to about 4500 µg/kg after frying for 3.5 min at 190°C. However, at higher temperatures (180 and 200°C), the drastic increase was followed by a fast decrease due to degradation of acrylamide [Knol et al. 2005]. The increase in acrylamide concentration with increasing frying temperature followed an exponential function [Matthaus et al. 2004, Gokmen et al. 2006], which implies that small deviations in frying temperature can have large consequences for acrylamide formation. Therefore, frying temperature is expected to be an important factor that determines the extent of variation in acrylamide concentrations.

Frying time is also important, because frying temperature and time determine the kinetics of acrylamide formation [Knol et al. 2009]. Williams [2005] found that most acrylamide formation occurs during the early stages of frying at the higher temperatures (175°C), but sufficient quantities of precursors still remained for acrylamide formation at the early stage when frying at lower temperature (150°C for 3 minutes). Romani and co-authors [2008] observed that the increase of time became a key factor in acrylamide formation in French fries after around 4 minutes of frying when the temperature of potato surface reached 120°C and the temperature of oil bath reached 120°C. Above studies clearly demonstrated the profound effect of time as an important factor to variation, because minor deviations in frying time can result in large variation (and high values) of acrylamide concentrations in French fries.

Different authors have observed an increased acrylamide formation rate at low water content, whereas with increasing water content the reaction rate decreased [De Vleeschouwer

et al. 2007]. This can possibly be explained by the temperature: moisture gradient within the food, which is dependent on reaction time and on distance from the centre of the French fries [De Vleeschouwer et al. 2008]. As a result, the formation of acrylamide is concentrated at the crust region rather than core region [Franke et al. 2005, Gokmen et al. 2006].

Holding

Once the French fries are in the holding bin, they will be distributed to the consumers as per their orders [Glenn et al. 1993]. French fries are consumed hot and no systematic study on the effect of holding step influencing the formation of acrylamide has been reported [Castle 2006]. It is unlikely that the holding step has an effect because the temperature is much below the temperature at which formation and degradation takes place.

Overall, we can conclude that the variable concentrations of reducing sugars in the par-fried potato strips at receipt can be expected to be a major source of variation of acrylamide formation, if no specific measures are taken. Moreover, mainly the deviations in actual frying regimes (due to set temperature and time) will contribute to variable and high acrylamide concentrations, if no specific measures are taken during frying.

Control decisions to reduce acrylamide in French fries preparation

The next step in our study was to analyse the major decisions that can be taken in FSE to control the variation and level of acrylamide concentration. The major acrylamide control points in FSE include the receipt of raw materials and the frying practices. To systematically analyse the control decisions, we used a 'food quality decision model' that distinguishes decisions on technological and administrative (organisational) conditions to create the circumstances to prevent undesirable product properties and or people actions, and decisions on dynamics of the food and human systems to reduce actual variation [Luning and Marcelis 2007].

Management typically takes decisions on technological and administrative conditions by selecting appropriate (technological and people) resources to create an adequate technological and managerial infrastructure for the production of desired products. These are often mid or long-term decisions. In addition, management takes short-term decisions on food dynamics by putting requirements on product properties, to ensure adequate materials/products, and on the control of human dynamics by directing their actions through providing specific information, gaining commitment, and/or by giving detailed direct instructions. Additionally, food handlers typically take daily control decisions on out of tolerance situations of product properties and process conditions, and on subsequent corrective actions [Luning and Marcelis 2007, Luning et al. 2009].

Control decisions at receipt affecting acrylamide concentration

At receipt, the initial concentration and variation in reducing sugars in the raw materials can be controlled via management decisions on incoming materials (i.e. setting strict product specifications on reducing sugars), and decisions on supply sources (i.e. selecting preferred suppliers). Various researchers considered the selection of appropriate varieties (with lower concentrations of reducing sugars) as a simple and efficient measure to reduce the extent of variation of acrylamide concentrations in French fries production [Fiselier and Grob 2005, Lindsay and Jang 2005].

Management decisions on the organisation are crucial for adequate control of raw materials. They concern, first of all, acquiring food handlers with appropriate competences and skills, providing training to maintain and or improve existing competences and skills, and providing procedures for homogenous decision-making on purchasing and receiving [Naing et al. 2007, Seaman and Eves 2006]. In addition to these resource-type decisions, also the daily control decisions are important for the raw material quality. These are assigning competent people for the purchasing and receiving tasks, and instructing them on how to purchase and check raw materials, and correction of inadequate behaviour. In this way, it is controlled that only appropriate raw materials are accepted for French fries preparation.

Control decisions at frying affecting acrylamide concentration

As analysed in the technological section, control should be focused on the frying time-temperature regime [CIAA 2006, Romani et al. 2008]. Actual frying can be controlled via different types of decisions. Control includes a clear specification of product properties, in this case the colour of the French fries [Grob et al. 2003, CIAA 2006], and management decisions on the frying resources, i.e. selecting frying equipment (e.g. with advanced automated frying temperature regimes that allow a lower temperature at the end of frying), and setting process parameters (e.g. stipulating correct temperature-time regimes). To illustrate, the actual frying temperature mainly depends on the capability of the heating power of the fryer to remain near the adjusted frying temperature [Fiselier et al. 2006]. Palazoglu and Gokmen [2008] showed that advanced equipment with controlled temperature programs can reduce acrylamide concentrations in French fries more than half (58%).

Decisions on the organisation concern minimally required competences, skills, and training of food handlers and procedures on frying practices (i.e. prescribing how to deal with thawing time, remaining portions, sort out fines, oil conditions, etc). Daily control decisions include assigning competent people to frying tasks, and giving them instructions about the exact final colour, frying time, and portion size, and correcting inadequate behaviour. Various studies have provided evidence for certain optimal frying regimes realising French fries with good culinary properties while being low in acrylamide [Grob et al. 2003, Fiselier and Grob 2005]. It has been recommended to keep acrylamide concentrations low by ending the frying process before the on-set of browning [Fiselier et al. 2006]. However, the appropriateness of the selected frying regime should be validated for the actual circumstances in the specific FSE [Luning et al. 2009] to assure that good quality and low acrylamide concentration can be really met.

Control situation in FSE in practice

Examining the current situation in FSE revealed that they have restricted possibilities to control their raw materials. The selection of potato varieties specifically for production of frozen par-fried potato stripes for French fries preparation is not yet widely practiced [Grob 2007]. Although manufacturers of frozen par-fried potato stripes usually select potato varieties with lower concentrations of reducing sugars (long before acrylamide became a subject) by a frying test to avoid strong browning of the finished product [Grob 2005], frozen par-fried potato stripes still can contain high reducing sugars. For example, in a survey, Fiselier and Grob [2005] found still samples with high concentrations of reducing sugars (2 out of 49 samples contained 2.8 g/kg and 3.5 g/kg, respectively) although the average

concentration amounted to 0.67 g/kg. The possibilities for selecting appropriate suppliers seem to be still limited. Moreover, small and medium FSE's commonly purchase frozen par-fried potato stripes from different sources on local markets even without knowing what reducing sugars are. This means that they just deal with the given concentrations and variation in reducing sugars in their purchased frozen par-fried potato stripes, which put demands on the capability of their frying equipment and the appropriateness of frying protocols and compliance to them.

Materials and methods

Analysis of fructose and glucose

The procedure was adapted from Vivanti and co-authors [2006]. Briefly, after extraction with mobile phase consisting of acetonitrile/water (80:20, v/v) and addition of maltose as an internal standard, the supernatant was filtered and injected into a Waters HPLC instrument equipped with a refractive index (R.I.) detector.

Analysis of acrylamide

The scheme described by Becalski and co-authors [2005] was generally followed. After aqueous extraction, using $^{13}C_3$-labelled acrylamide as internal standard, the acrylamide extract was further cleaned-up by solid phase extraction. The extract was analysed using Gas Chromatography-Time of Flight-Mass Spectrometry (GC-TOF-MS).

Statistical analysis

An analysis of variance (ANOVA) was used to explore the mean differences in the $\log_e$ transformed acrylamide concentration among the three FSE types while also investigating the variation due to different establishments, sampling days and frying batches. A mixed model was used with FSE types as fixed factor and establishment, sampling day and frying batch as random factors. All factors are nested within the pre-mentioned sampling level. The significance of the differences among the three FSE types was determined using Tukey's HSD test. The obtained means of $\log_e$ transformed acrylamide concentration were back-transformed to an original scale of measurement. Bivariate correlations analysis and standard multiple linear regression analysis were used to analyse the data. The p-Value less than or equal to 0.05 was considered significance. Statistical analyses were performed using the SPSS version 16.0 (SPSS Inc., Chicago, IL.).

Results and discussion

In a literature study, the initial concentration of reducing sugars and the actual frying condition (time-temperature) are identified as the major technological factors that influence acrylamide formation. Food handler controls these influencing factors in their daily practices in FSE and inadequate control may leads to variable and high acrylamide concentration in French fries. The receipt of raw materials and the frying practices are identified as the control

points to reduce the variation and concentration of acrylamide. However, examining the current situation in FSE revealed that management's decision to control the appropriate supplies and suppliers is restricted because potatoes with low concentration of reducing sugars are not readily available [Fiselier and Grob 2005]. Moreover, small and medium FSE's commonly purchase frozen par-fried potato strips from different sources on local markets without knowing the concentration of reducing sugars. The situation put demands to reduce the variation and concentration of acrylamide on other influencing factors such as the adequate frying facilities, the appropriate frying protocols and the compliance to the protocols.

In an observational study, we found the mean concentration of acrylamide in French fries among the three FSE types was not significantly different. The mean concentration of acrylamide was 231 μg/kg for the CFS, 254 μg/kg for the IC, and 354 μg/kg for the R. Although the mean concentration of acrylamide among the three FSE types was not significantly different, the least variation in acrylamide concentration was found in French fries prepared in the chain fast-food service (CFS) as compared to the institutional caterers (IC), and the restaurants (R). The coefficient of variation in acrylamide concentration was lower in the CFS (21%) than in the IC (31%), and in the R (55%). The concentration of acrylamide in French fries ranged from 150 to 392 μg/kg (a 2.6-fold difference) for the CFS, 151 to 505 μg/kg (a 3.3-fold difference) for the IC, and 152 to 1023 μg/kg (a 6.7-fold difference) for the R. The finding indicates that the fryer setting in CFS such as a low and uniform frying temperature of 177°C and a short and narrow range of frying time (150–165 seconds) allow an adequate control of acrylamide formation. Acrylamide concentration of as high as 1023 μg/kg was obtained in the restaurant due to the usage of frying pan, which makes it impossible to control the frying temperature and time.

Our next approach was to examine whether acrylamide formation correlates with any of the influencing factors. A small correlation between frying time and acrylamide concentration was found ($r=0.104$, $n=360$, $p<0.05$), which is contradicting with previously published studies who reported a linear relationship between frying time and acrylamide concentration [Matthaus et al. 2004, Gokmen and Senyuva 2006]. The observation could be possibly explained with what has been found by Romani and co-authors [2008] who reported that the increase of time became a key factor in acrylamide formation in French fries only after around 240 seconds of frying (frying temperature of 180°C). In this observational study, most of IC and CFS (with exception of R) reported frying time ranged between 150–240 seconds that were shorter than the identified critical time, which explains the small correlation between the two variables.

The higher mean acrylamide concentration in R could be explained by a wide range of measured frying temperature (148–215°C) with a mean temperature of 185°C. These observations show the pronounced effect of frying temperature on formation of acrylamide as repeatedly demonstrated by various researchers [Grob et al. 2003, Matthaus et al. 2004]. Furthermore, unlike frying time, there was a strong correlation between frying temperature and acrylamide concentration ($r=0.596$, $n=360$, $p<0.05$). Also, our results are consistent with studies conducted to survey frying conditions in FSE [Al-Kahtani 1991, Morley-John et al. 2002]. For example, Morley-John and co-authors [2002] reported that a wide range of temperature, i.e. 136–233°C with a mean temperature of 182°C was used by the independent fast-food services (similar to R in this study) as well as by the fast food outlets in New Zealand to fry French fries.

In our study, no significant correlation between reducing sugars and acrylamide concentration was found (r=0.102, n=360, p=0.053). The finding is contradicting to studies that have shown a strong correlation between acrylamide formation and the reducing sugar available in potatoes [Becalski et al. 2004, Williams 2005]. The finding could be possibly explained with what has been found by Williams [2005] who reported that sufficient quantities of precursors still remained for acrylamide formation when frying at lower temperature (150^{o}C for 3 minutes) unlike frying at the higher temperatures (175^{o}C) where precursors are used effectively to generate acrylamide. Furthermore, this is an observational type of study and thus further research is necessary under carefully controlled conditions in FSE to further investigate this initial observation.

It was unexpected to find a moderate, negative correlation between thawing practice and acrylamide concentration (r=-0.482, n=360, p<0.05), which implied that thawing practice contributes significantly to the reduction of acrylamide concentration in French fries. The finding however is consistent with Tuta and co-authors [2010] who recently have shown that thawing of par-fried potato strips reduced the acrylamide formation by 89% (frying temperature of 180°C).

In a standard multiple linear regression model, all influencing factors (except sucrose) are making a significant unique contribution to the prediction of acrylamide formation (r2=0.492, p<0.05). It was expected for non-reducing sugars, i.e. sucrose not to make a statistically significant contribution, which is in line with those of previously published studies [Amrein et al. 2003, Williams 2005]. The influencing factor of frying temperature makes the largest unique contribution (beta=0.513), although thawing practice also made a statistically significant contribution (beta=-0.368). Both reducing sugars (beta=-0.101) and frying time (beta=0.143) make less of contributions. This supports the findings of various researchers who observed that these influencing factors contribute significantly to the formation of acrylamide [Grob et al. 2003, Becalski et al. 2004, Matthaus et al. 2004, Amrein et al. 2006, Tuta et al. 2010].

Conclusions

Our literature analysis revealed that glucose and fructose concentration (in the par-fried potato stripes), and the actual frying conditions (as affected by frying equipment capability, portion size, setting of frying parameters) are the major technological factors in acrylamide formation, and inadequate control of these factors can result in high concentrations of acrylamide. The current situation in small and medium FSE's indicates that management control of resources (i.e. decisions on appropriate supplies and suppliers, adequate frying facilities, and adequate personnel) is yet restricted, which creates conditions for variable acrylamide formation. However, the question remains if even under appropriate technological and managerial conditions for production of French fries with lower acrylamide concentrations, variable daily decisions may be still a considerable source of variation. To our knowledge, little is known about the quantitative contribution of variable decision-making of food handlers to acrylamide concentrations in French fries.

Our observational study concluded that due to variable frying practices, the French fries prepared by different types of FSE had different distributions profiles of acrylamide concentrations. The variation in actual frying temperature contributed the most to the variation in

acrylamide concentrations, followed by the variation in actual frying time; no obvious effect for reducing sugars. The least variation in acrylamide concentration was found in French fries prepared in the chain fast-food service (CFS) as compared to the institutional caterers (IC), and the restaurants (R), which indicates that CFS practised a better quality control in setting a low and a uniform frying temperature of 177°C and realising a short and a narrow range of frying time (150–165 seconds). The better quality control in setting a lower and a uniform frying temperature and time to fry French fries is expected to be a practical solution for high and variable acrylamide concentration in French fries. This observational study provides directions for further research that include investigating the effects of technological (focused on raw material properties) and managerial control interventions (focused on food handlers) on the variation of acrylamide concentrations in French fries prepared under FSE circumstances.

References

Al-Kahtani H.A., 1991. Survey of quality of used frying oils from restaurants. JAOCS, 68 (11), 857–862.

Amrein T.M., Andres L., Escher F., Amado R., 2007. Occurrence of acrylamide in selected foods and mitigation options. Food Additives and Contaminants, 24, 13–25.

Amrein T.M., Bachmann S., Noti A., Biedermann M., Barbosa M.F., Biedermann-Brem S., Grob K., Keiser A., Realini P., Escher F., Amadó R., 2003. Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. J. Agric. Food Chem., 51 (18), 5556–5560.

Amrein T.M., Limacher A., Conde-Petit B., Amado R., Escher F., 2006. Influence of thermal processing conditions on acrylamide generation and browning in a potato model system. J. Agric. Food Chem., 54 (16), 5910–5916.

Amrein T.M., Schonbachler B., Rohner F., Lukac H., Schneider H., Keiser A., Escher F., Amadò R., 2004. Potential for acrylamide formation in potatoes: data from the 2003 harvest. European Food Research and Technology, 219 (6), 572–578.

Archer D.L., 2004. Freezing: an underutilized food safety technology? Int. J. Food Microb., 90 (2), 127–138.

Becalski A., Lau B.P.Y., Lewis D., Seaman S.W., 2003. Acrylamide in foods: Occurrence, sources, and modeling. J. Agric. Food Chem., 51(3), 802–808.

Becalski A., Lau B.P.Y., Lewis D., Seaman S.W., Hayward S., Sahagian M., Ramesh M., Leclerc Y., 2004. Acrylamide in french fries: Influence of free amino acids and sugars. J. Agric. Food Chem., 52 (12), 3801–3806.

Becalski A., Lau B.P.Y., Lewis D., Seaman S.W., Sun W.F., 2005. Determination of acrylamide in various food matrices, [in:] Chemistry and safety of acrylamide in food M. Friedman & D.S. Mottram (Eds.), Springer Science+Business Media, Inc.

Biedermann M., Noti A., Biedermann-Brem S., Mozzetti V., Grob K., 2002. Experiments on acrylamide formation and possibilities to decrease the potential of acrylamide formation in potatoes. Mitteil. Lebensmittel. Hyg., 93 (6), 668–687.

Castle L., 2006. Analysis of acrylamide in foods, [in:] Acrylamide and other hazardous compounds in heat-treated foods, K. Skog & J. Alexander (Eds.), Cambridge, England: Woodhead Publishing Limited.

CIAA, 2006. The CIAA Acrylamide Toolbox. http://www.ciaa.be/documents/brochures/CIAA_Acrylamide_Toolbox_Oct2006.pdf

Claeys W.L., Baert K., Mestdagh F., Vercammen J., Daenens P., de Meulenaer B., Maghuin-Rogister G., Huyghebaert A., 2010. Assessment of the acrylamide intake of the Belgian population and the effect of mitigation strategies. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 27 (9), 1199–1207.

Claeys W.L., De Vleeschouwer K., Hendrickx M.E., 2005. Kinetics of acrylamide formation and elimination during heating of an asparagine-sugar model system. J. Agric. Food Chem., 53 (26), 9999–10005.

De Vleeschouwer K., Van der Plancken I., Van Loey A., Hendrickx M.E., 2007. Kinetics of acrylamide formation/elimination reactions as affected by water activity. Biotechnology Progress, 23(3), 722–728.

De Vleeschouwer K., Van Der Plancken I., Van Loey A., Hendrickx M.E., 2008. Investigation of the influence of different moisture levels on acrylamide formation/elimination reactions using multiresponse analysis. J. Agric. Food Chem., 56 (15), 6460–6470.

DiNovi M., 2006. US FDA/CFSAN. The 2006 Exposure Assessment for Acrylamide. Retrieved from http://www.cfsan.fda.gov/~dms/acryexpo/acryex4.htm

Dybing E., Sanner T., 2003. Risk assessment of acrylamide in foods. Toxicol. Sci., 75 (1), 7–15.

FAO/WHO, 2005. Joint FAO/WHO Expert Commitee on Food Additives, Sixty-fourth meeting, Summary and Conclusions Retrieved from http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

FAO/WHO, 2007. Joint FAO/WHO Food Standards Programme CODEX Committee on Contaminants in Foods. Proposed draft code of practice for the reduction of acrylamide in food, ftp://ftp.fao.org/codex/cccf1/cf01_15e.pdf

Fiselier K., Bazzocco D., Gama-Baumgartner F., Grob K., 2006. Influence of the frying temperature on acrylamide formation in French fries. Europ. Food Res. and Technol., 222 (3–4), 414–419.

Fiselier K., Gama Baumgartner F., Fiscalini A., Biedermann M., Grob K., Imhof D., 2004. Good manufacturing practice (GMP) for French fries low in acrylamide: results of a pilot project. Mitteil. Lebensmittel. Hyg., 95 (2), 127–134.

Fiselier K., Grob, K., 2005. Legal limit for reducing sugars in prefabricates targeting 50 µg/kg acrylamide in French fries. Europ. Food Res. Technol., 220 (5–6), 451–458.

Franke K., Sell M., Reimerdes E.H., 2005. Quality related minimization of acrylamide formation-an integrated approach, [in:] Chemistry and Safety of Acrylamide in Food. M. Friedman & D. Mottram (Eds.), [in:] Springer Science+Business Media, Inc. New York.

Glenn O.R., James W.F., Robert F.M., Richard L.K., Thomas P.K., Ronald N.P., 1993. United States Patent No. 5189944.

Gokmen V., Palazoglu T.K., Senyuva H.Z., 2006. Relation between the acrylamide formation and time-temperature history of surface and core regions of French fries. J. Food Engin., 77 (4), 972–976.

Gokmen V., Senyuva, H.Z., 2006. Study of colour and acrylamide formation in coffee, wheat flour and potato chips during heating. Food Chem., 99 (2), 238–243.

Grob K., 2005. Reduction of exposure to acrylamide: achievements, potential of optimization, and problems encountered from the perspectives of a Swiss enforcement laboratory. Journal of AOAC International, 88 (1), 253–261.

Grob K., 2007. Options for legal measures to reduce acrylamide contents in the most relevant foods. Food Additives and Contaminants, 24 (suppl. 1), 71–81.

Grob K., Biedermann M., Biedermann-Brem S., Noti A., Imhof D., Amrein T., Pfefferle A., Bazzocco D., 2003. French fries with less than 100 µg/kg acrylamide. A collaboration between cooks and analysts. Europ. Food Res. Technol., 217 (3), 185–194.

IARC, 1994. Some Industrial chemicals, Lyon: IARC. IARC Monograph on the evaluation for carcinogenic risk of chemicals to human, 60, 435–453.

Jung M.Y., Choi D.S., Ju J.W., 2003. A novel technique for limitation of acrylamide formation in fried and baked corn chips and in french fries. J. Food Sci., 68 (4), 1287–1290.
Knol J.J., Van Loon W.A.M., Linssen J.P.H., Ruck A.L., Van Boekel M.A.J.S., Voragen A.G.J., 2005. Toward a kinetic model for acrylamide formation in a glucose-asparagine reaction system. J. Agric. Food Chem., 53 (15), 6133–6139.
Knol J.J., Viklund G.A.I., Linssen J.P.H., Sjoholm I.M., Skog K.I., van Boekel M.A.J.S., 2009. Kinetic modelling: A tool to predict the formation of acrylamide in potato crisps. Food Chem., 113 (1), 103–109.
Konings E.J.M., Baars A.J., van Klaveren J.D., Spanjer M.C., Rensen P.M., Hiemstra M., van Kooij J.A., Peters P.W.J., 2003. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food Chem. Tox., 41 (11), 1569–1579.
Lewontin R.C., 2006. The analysis of variance and the analysis of causes. Int. J. Epidemiol., 35 (3), 520–525.
Lindsay R.C., Jang S., 2005. Chemical intervention strategies for substantial suppression of acrylamide formation in fried potato products. In Chemistry and safety of acrylamide in food M. Friedman & D. S. Mottram (Eds.), Springer Science+Business Media, Inc. 393–404.
Luning P. A., Marcelis W.J., 2006. A techno-managerial approach in food quality management research. Trends in Food Science & Technology, 17 (7), 378–385.
Luning P.A., Marcelis W.J., 2007. A conceptual model of food quality management functions based on a techno-managerial approach. Trends in Food Science & Technology, 18(3), 159–166.
Luning P.A., Marcelis W.J., Rovira J., Van der Spiegel M., Uyttendaele M., Jacxsens L., 2009. Systematic assessment of core assurance activities in a company specific food safety management system. Trends Food Sci. Technol., 20 (6–7), 300–312.
Matthaus B., Haase N.U., Vosmann K., 2004. Factors affecting the concentration of acrylamide during deep-fat frying of potatoes. Eur. J. Lipid Sci. Technol., 106(11), 793–801.
Morley-John J., Swinburn B.A., Metcalf P.A., Raza F., Wright H., 2002. Fat content of chips, quality of frying fat and deep-frying practices in New Zealand fast food outlets. Australian and New Zealand Journal of Public Health, 26(2), 101–106.
Mottram D.S., Wedzicha B.L., Dodson A.T., 2002. Acrylamide is formed in the Maillard reaction. Nature, 419 (6906), 448–449.
Naing N.N., Zain M.M., Hamzah W.M., Mat H.A., Abdullah N., Bakar M.H.A., 2007. A study on effectiveness of health education program on knowledge, attitude and practice (KAP) of food handlers towards foodborne diseases and food safety. International Medical Journal, 14(4), 253–260.
Palazoglu T.K., Gokmen V., 2008. Reduction of acrylamide level in French fries by employing a temperature program during frying. J. Agric. Food Chem., 56 (15), 6162–6166.
Pedreschi F., Kaack K., Granby K., 2004. Reduction of acrylamide formation in potato slices during frying. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 37(6), 679–685.
Romani S., Bacchiocca M., Rocculi P., Rosa M.D., 2008. Effect of frying time on acrylamide content and quality aspects of French fries. Eur. Food Res. Technol., 226 (3), 555–560.
Seaman P., Eves A., 2006. The management of food safety-the role of food hygiene training in the UK service sector. International Journal of Hospitality Management, 25(2), 278–296.
Tareke E., Rydberg P., Karlsson P., Eriksson S., Tornqvist M., 2000. Acrylamide: A cooking carcinogen? Chem. Res. Tox., 13(6), 517–522.
Tareke E., Rydberg P., Karlsson P., Eriksson S., Tornqvist M., 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem., 50 (17), 4998–5006.

Tuta S., Palazoglu T.K., Gokmen V., 2010. Effect of microwave pre-thawing of frozen potato strips on acrylamide level and quality of French fries. J. Food Eng., 97 (2), 261–266.
Vivanti V., Finotti E., Friedman M., 2006. Level of acrylamide precursors asparagine, fructose, glucose, and sucrose in potatoes sold at retail in Italy and in the United States. J. Food Sci., 71 (2), C81-C85.
Williams J.S.E., 2005. Influence of variety and processing conditions on acrylamide levels in fried potato crisps. Food Chem., 90 (4), 875–881.

5

SAFETY OF SOIL AND AGRICULTURAL PRODUCTION IN VICINITY OF INDUSTRIAL ENTERPRISES IN MIDDLE POVAZIE REGION

Introduction

The atmosphere of northern hemisphere was and still is contaminated by exhalates from energetics, transport, agriculture and especially from industry. The change of soil reaction caused by chemical reactions of acid components of these exhalates with the other components of atmosphere constitutes one of the global environmental problems. The problem of the soil acidification in our country is connected with destruction of organic and mineral soil components, decreasing of basic cation concentration, imobilization of nutrients, decreasing of soil buffer capacity and mobilization of toxic elements. Ten selected heavy metals (Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, Zn and As) with legislative given maximal allowed soil contents in three various soil types [Law 220/2004] rank among risky and potentially toxic elements.

The Slovak Republic covers 49,037.17 km^2, of which 49.3 % is agricultural land and 40.97% percent is forested land. The state of the environment is differentiated and is caused by social and economic activities. Loaded areas represent approximately 13% of the SR territory.

The soil as a starting place for input of risk substances into a human food chain is continually monitored in our country [Hegedüsová 1999, Tóth et al. 2004, Hronec et al. 2006]. The necessity to monitor the soil contaminant contents is especially important in vicinity of industrial sources from the aspect of food safety and quality assurance.

The aim of this work is to asses the status of soil hygiene and the hygiene of agricultural production from the point of Cd, Pb, Ni, Zn, Cu, Cr, Co, Fe and Mn contents in chosen locality of Middle Povazie region in vicinity of three important industrial enterprises: the rubber corporation in Puchov, the cement mill in Ladce and glass works in Lednicke Rovne. The potential influence of these emitting sources on the soil and agriculture plant contents of risk elements is investigated.

Materials and methods

Soil samples were taken from the site Vrchy with the acreage 9.4 ha, located in cadastre area managed by the agricultural co-operative in village Mestečko. There are three important industrial enterprises: Puchov rubber plant – the continuator of well-known trademark Matador as it took up the tradition of the first tyre manufacturer in former Czechoslovakia, the oldest Slovak cement mill Ladce – producent of portland cement and Rona Glassworks Lednicke Rovne – an important world producer of domestic glass. The distances of the

investigated locality from the rubber corporation, the cement mill and glass works are 7 km, 16 km and 7 km respectively. Soil type: FMG (Fluvi-Eutric Gleysol) and KMm (Eutric Cambisol), soil class: loamy soil. The sampling sites determination was done by covering of borders of the key site by raster, their distances inside the site presented sampling sites. Site borders were gained with navigation apparatus GPS MAP 60 Cx GARMIN (GPS). After data transfer about position and above sea level into the program OziExplorer the borders were adapted and covered by raster with density of lattice of 5 seconds. Sampling places with the accuracy ± 2 meters were determined with GPS. The site borders were defined by 6 points, their above sea level ranged from 241.1 to 330.4 m. Sampling sites are presented on Figure 1. After localization of sampling point we had done taking of the soil on this place by valid methods from two horizons (A: 0–0.2 m; B: 0.3–0.45 m) with pedological probe GeoSampler fy. Fisher. Pseudototal content of Cd, Pb, Ni, Zn, Cu, Cr, Co, Fe and Mn including all of the forms besides residual fraction of metals was assessed in solution of *aqua regia* and content of mobile forms of selected heavy metals in soil extract of NH_4NO_3 (c = 1 mol.dm^{-3}). Gained results were evaluated according to Law 220/2004. Analytical method was flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA).

The samples of flax seeds were collected from the same sampling points as the soil samples. After their dryining and regulation the plant samples were decomposited with using of HNO_3 in the microwave digestion instrument MARRS X-PRESS. The solutions were analyzed by flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA). Gained results were evaluated according to Food Codex of the Slovak Republic.

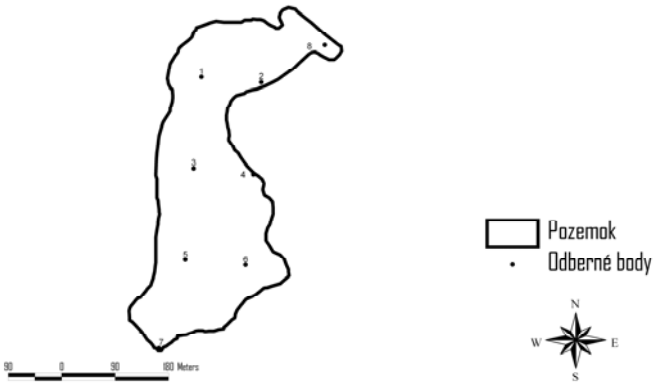

Fig. 1. Sampling sites in locality Vrchy

Results and discussion

In Table 1 the values of the determined soil reaction and heavy metal content in the soil horizon A are presented. The determined content of heavy metals in soil extract by *aqua regia* includes all of metal forms present in soil with exception of residual silicate residual fraction. Gained results were evaluated according to Law 220/2004.

Table 1

Soil reaction and heavy metal contents [mg·kg^{-1}] in soil horizon A (soil extract by aqua regia)

No.	Sampling point	pH (KCl)	Soil extract by aqua regia [mg·kg^{-1}]								
			Cd	Pb	Ni	Zn	Cu	Cr	Co	Fe	Mn
1.	1A	4.23	0.68	27.4	27.4	72.6	0.68	27.2	17.8	759.6	45.4
2.	2A	3.80	0.86	19.2	26.8	58.2	0.86	30.0	13.4	786.6	36.4
3.	3A	3.92	0.60	21.6	32.8	68.4	0.60	36.4	16.2	1111	42.4
4.	4A	3.70	0.74	26.4	25.4	55.5	0.74	30.0	18.0	815.8	54.4
5.	5A	3.65	0.76	31.4	30.8	68.8	0.76	30.4	24.0	1047	68.6
6.	6A	3.64	0.86	27.8	27.6	70.0	0.86	28.8	21.4	905.4	68.6
7.	7A	6.74	0.94	29.6	91.6	71.2	0.94	46.6	24.4	1447	63.6
8.	8A	4.01	0.98	25.4	28.2	72.2	0.98	31.2	18.8	905.6	44.4
Average		4.21	0.80	26.10	36.33	67.11	0.80	32.58	19.25	972.2	53.0
Min		3.64	0.60	19.20	25.40	55.50	0.60	27.20	13.40	759.6	36.4
Max		6.74	0.98	31.4	91.60	72.6	0.98	46.60	24.40	1446.6	68.6
SD		1.04	0.13	4.02	22.46	6.54	0.13	6.26	3.80	227.7	12.7
Hygienic limit*		–	**0.7**	**70.0**	**50.0**	150.0	60.0	70.0	15.0	–	–

* Law 220/2004

The soil reaction in the observed locality was extremely acid till neutral. Soil reaction has a major effect on the uptake of many risky elements, the most of them become more available to plants as pH decreases. Slovakia as well as other countries of the northern hemisphere are oppressed with the problem of the soil acidification. This problem is caused by the cooperation of natural and anthropic factors. In soils with a low pH value the humus matter destruction, an enhanced heavy metal mobility and their bioavailability and enhanced damage of risk elements input into agricultural plants are significant. The influence of soil acidity is described in works of many autors [Blume 1990, Torma 1998]. In these works the enhanced mobility of Pb, Cd, Cr and Ni in the soils with pH value under 4,5 is proved. The liming is one of the possibilities of these negative phenomena elimination. This treatment performs an imobilization, neutralization and also structural function.

In most of the soil sampling sites the determined Cd and Co content in in soil extract by *aqua regia* exceeded the limit value given by Law 220/2004 (by 5–40% and 8–63% respectively). In one sampling point also the Ni content by 83% exceeded maximal allowed value given by the legislative. Cadmium is heavy metal with an anknown essential biological function and belongs to the most toxic pollutants of the environment [Das et al. 1997]. The accumulation of Cd in soils has become a major concern as environmental viewpoint and food production [Rahmanian et al. 2011]. Some studies have found that Co is also relatively toxic to plants when given in high doses [Osman et al. 2004]. However there is little information that can be used to quantify the effect of soil properties on the expression of Co toxicity in different plant species and the different studies are difficult to compare. Li et al. [2009] confirmed that soil properties greatly influence the expression of Co by tomato, oilseed rape and barley grown on a range of soils varying widely in chemical and physical properties and solubility of Co is a key factor influencing its toxicity to plants.

Table 2

Heavy metal contents [mg·kg^{-1}] in soil horizon A (soil extract by NH4NO3 , c = 1 mol·dm^{-3})

No.	Sampling point	Soil extract by NH_4NO_3 , c = 1 mol·dm^{-3} [mg·kg^{-1}]								
		Cd	Pb	Ni	Zn	Cu	Cr	Co	Fe	Mn
1.	1A	0.03	0.12	0.30	0.46	0.08	0.03	0.10	0.90	28.25
2.	2A	0.06	0.15	0.52	0.38	0.08	0.03	0.12	0.34	25.35
3.	3A	0.05	0.15	0.38	0.23	0.08	0.03	0.14	0.70	29.05
4.	4A	0.07	0.18	0.82	0.50	0.09	0.03	0.26	0.54	16.75
5.	5A	0.07	0.20	0.96	0.65	0.11	0.05	0.22	0.52	51.35
6.	6A	0.07	0.18	0.92	0.25	0.11	0.04	0.17	0.53	52.95
7.	7A	0.04	0.30	0.18	0.59	0.12	0.07	0.16	0.96	37.80
8.	8A	0.04	0.17	0.61	0.65	0.09	0.06	0.13	0.62	1.05
Average		0.05	0.18	0.59	0.46	0.09	0.04	0.16	0.64	30.32
Min		0.03	0.12	0.18	0.23	0.08	0.03	0.10	0.34	1.05
Max		0.07	0.30	0.96	0.65	0.12	0.07	0.26	0.96	52.95
SD		0.02	0.06	0.29	0.17	0.02	0.02	0.05	0.21	17.25
Hygienic limit*		**0.1**	**0.1**	**1.5**	**2.0**	**1.0**	–	–	–	–

* Law 220/2004

In Table 2 contents of mobile forms of selected heavy metals in soil extract by NH_4NO_3 (c=1 mol·dm^{-3}) are presented. Gained results were evaluated according to Law 220/2004. The limit values of risk elements according this legislative norm are considered to be critical values of agricultural soil in relationship to the plant. In all sample sites the maximal available soil content of mobile Pb forms was by 20–200% exceeded.

The values of the soil reaction pH/KCl in the observed locality in horizon A are presented in Figure 2.

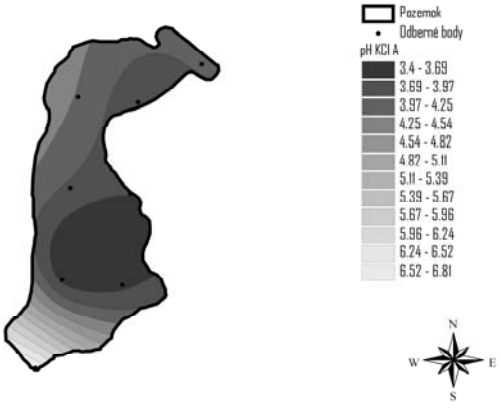

Fig. 2. Values of the soil reaction pH/KCl in locality Vrchy (A horizon)

The Cd content in soil extract by aqua regia was in 6 sampling sites of soil horizon A than hygienic limit value given by Law 220/2004 (Fig. 3). It means, that soil contamination by Cd was analytically confirmed. Also the Co content in soil extract by aqua regia was in 7 sampling sites of soil horizon A higher than hygienic limit value given by Law 220/2004 (Fig. 4). It means, that soil contamination by Co was analytically confirmed, too.

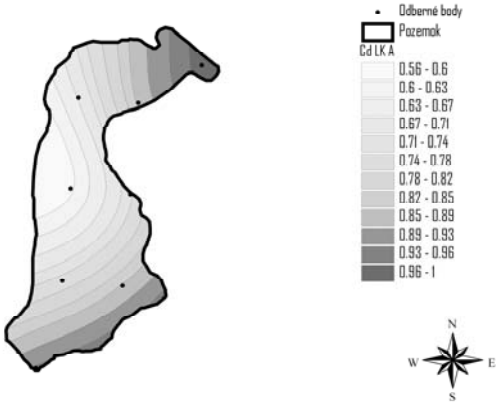

Fig. 3. Cd content in soil extract by aqua regia in locality Vrchy (A horizon)

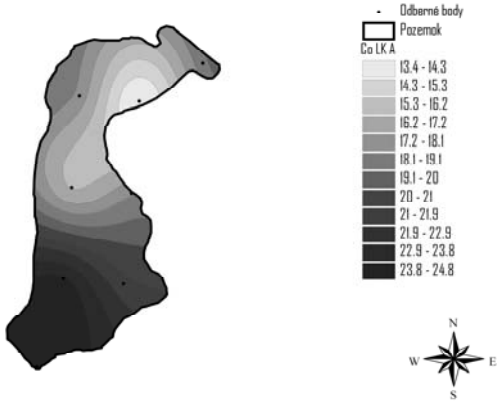

Fig. 4. Co content in soil extract by aqua regia in locality Vrchy (A horizon)

The high concentration of heavy metals in soils is usually reflected by higher concentrations of metals in plants, and consequently in animal and human bodies [Buszewski et al. 2000]. Soil properties are important factors modifying metal bioavailability and toxicity and should be considered during the ecological risk assessment of metals in contaminated soils [Bradham et al. 2006].

The Pb, Ni, Zn, Cu, and Cr soil contents in horizon A determined in soil extract by aqua regia are below their hygienic limits.

The legislative critical value in relationship to grown plant determined in soil extract of NH_4NO_3 was in all sampling sites of horizon A only for Pb soil content (Fig. 5).

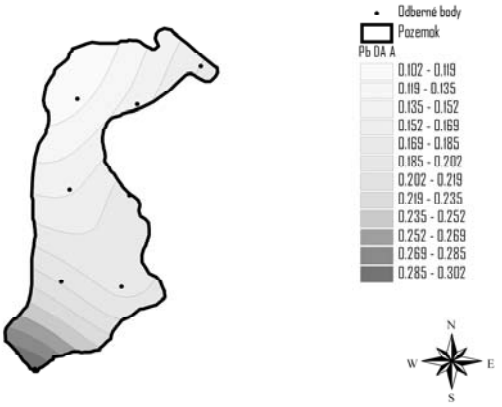

Fig. 5. Pb content in soil extract by NH_4NO_3 in locality Háje (A horizon)

The similar situation was observed also in horizon B (Tab. 3). Also in horizon B was the determined value of the soil reaction extremely acid till neutral. From observed heavy metals Cd, Co and Pb can be considered as risky elements. The Cd content in soil extract by aqua regia was in soil horizon B in 7 sample points by 9–51% higher than hygienic limit value given by Law 220/2004. In one point the determined value was on the level of hygienic limit. It means, that soil contamination by Cd was analytically confirmed.

Also the Co content in soil extract by aqua regia was in 7 sampling sites of soil horizon B by 7–56% higher than hygienic limit value given by Law 220/2004. It means, that soil contamination in horizon B by Co was analytically confirmed, too.

Table 3

Soil reaction and heavy metal contents [mg·kg^{-1}] in soil horizon B (soil extract by aqua regia)

No.	Sampling point	pH (KCl)	Soil extract by aqua regia [mg·kg^{-1}]								
			Cd	Pb	Ni	Zn	Cu	Cr	Co	Fe	Mn
1.	1B	4.32	0.76	23.6	27.2	69.8	10.10	28.0	16.2	845.6	47.0
2.	2B	3.79	0.96	19.8	29.0	71.5	13.50	29.8	13.8	832	32.6
3.	3B	5.07	0.70	27.8	31.6	55.8	17.00	35.0	16.0	1102	39.4
4.	4B	3.58	0.90	24.8	24.2	35.6	10.90	29.8	18.2	823.2	51.2
5.	5B	3.68	0.80	30.8	29.6	65.2	14.40	29.2	23.0	1049	68.2
6.	6B	3.63	0.96	27.2	28.0	66.8	12.40	29.8	19.6	883.2	66.8
7.	7B	7.06	1.06	28.6	90.8	70.2	15.60	41.2	23.4	1421	58.6
8.	8B	3.88	1.06	24.0	26.2	72.8	11.90	28.6	19.0	877.6	44.2
Average		4.38	0.90	25.83	35.83	63.5	13.23	31.43	18.65	979	51.0
Min		3.58	0.70	19.80	24.20	35.6	10.10	28.00	13.80	823	32.6
Max		7.06	1.06	30.8	90.80	72.8	17.00	41.20	23.40	1421	68.2
SD		1.19	0.14	3.46	22.32	12.5	2.36	4.49	3.36	206	12.8
Hygienic limit*		–	**0.7**	70.0	50.0	150.0	60.0	70.0	15.0	–	–

* Law 220/2004

The legislative critical value determined in soil extract of NH_4NO_3 was in horizon B only for Pb soil content in all sample points exceeded (Tab. 4). The enhancement was in interval 30–160% above the maximal available value given by the legislative.

Table 4

Heavy metal contents [mg·kg^{-1}] in soil horizon B (soil extract by NH4NO3 , c = 1 mol·dm^{-3})

No.	Sampling point	Soil extract by NH_4NO_3, c = 1 mol·dm^{-3} [mg·kg^{-1}]								
		Cd	Pb	Ni	Zn	Cu	Cr	Co	Fe	Mn
1.	1B	0.03	0.13	0.26	0.58	0.08	0.03	0.09	0.46	28.25
2.	2B	0.05	0.16	0.58	0.65	0.09	0.02	0.13	0.86	28.35
3.	3B	0.05	0.25	0.24	0.62	0.09	0.04	0.14	0.80	31.90
4.	4B	0.06	0.18	0.78	0.35	0.09	0.03	0.20	0.66	65.20
5.	5B	0.07	0.24	1.00	0.65	0.14	0.05	0.23	0.61	53.35
6.	6B	0.05	0.14	0.69	0.65	0.09	0.03	0.14	0.63	46.90
7.	7B	0.04	0.26	0.16	0.58	0.11	0.06	0.14	0.60	1.95
8.	8B	0.05	0.21	0.87	0.67	0.11	0.06	0.17	0.45	29.35
Average		0.05	0.19	0.57	0.59	0.10	0.04	0.15	0.63	35.66
Min		0.03	0.13	0.16	0.35	0.08	0.02	0.09	0.45	1.95
Max		0.07	0.26	1.00	0.67	0.14	0.06	0.23	0.86	65.20
SD		0.01	0.05	0.32	0.10	0.02	0.02	0.05	0.14	19.31
Hygienic limit*		**0.1**	**0.1**	**1.5**	**2.0**	**1.0**	**–**	**–**	**–**	**–**

*Law 220/2004

High contents of heavy metals in the soil represent a potential risk for the human health. In soil and in agricultural plants grown on the metallic contaminated soil the synergic and antagonistic effects between different risk elements [Bystrická et al. 2008], but also between heavy metals and some nutritive components of plant foods are confirmed [Vollmannová et al. 2007, Musilová et al. 2009a, 2009b].

The heavy metal dynamism in soils is affected by static and dynamic factors. Maternal rock, seasons, climatic and geomorphological conditions of given locality and its elevation are the most important static factors. The soil reaction, humus quality and its content, the sorption capacity, redoxpotential, microbial activity, granularity, moisture and temperature of soil rank among important dynamic factors. In soils with a low pH value the humus matter destruction, an enhanced heavy metal mobility and their bioavailability and enhanced damage of risk elements input into agricultural plants are significant. The influence of soil acidity is described in works of many autors [Blume 1990, Torma 1998]. In these works the enhanced mobility of Pb, Cd, Cr and Ni in the soils with pH value under 4,5 is proved. The liming is one of the possibilities of these negative phenomena elimination. This treatment performs an imobilization, neutralization and also structural function. Results from monitoring of soil hygienic state in various regions of the Slovakia are presented in works of many authors [Linkeš et al. 1997, Makovníková 1998]. High concentrations of cadmium were found especially in regions with geochemical anomalies. Cadmium from phosphorus fertilisers did not have any clear effect on the pollution of the soils. The influence of

P-fertilization is very low and only evident after a long-term period (50 and more years). High values of lead were also determined especially in areas with geochemical anomalies, mostly in the mountainous regions. The lead, originating from the traffic along the highways, had no significant effects on soil pollution [Kobza 2005]. The transfer of soil pollutants into the plants causes many physiological disorders. The degree of heavy metal mobility, activity and bioavailability and consequently plant uptake is influenced by many factors such soil reaction, temperature, redox potential, cation excahnge capacity of solid phase, competition with other metal ions, ligation by anions, composition and quantity of the soil solution [Trangmar et al. 1985, Wopereis et al. 1988].

Flax (*Linum usitatissimum* L.) is an important crop especially in the north and northwest China. Comprehensive utilization of flax includes besides technological purposes (textile and paper industry) also seed oil for human healthcare and nourishment or feedstuff rich in protein, dietary fiber and other components valuable for animals. Its high content of alpha linolenic acid has made the ancient flax seed become our modern miracle food. Flax seeds may aid lowering total cholesterol and LDL cholesterol and also reducing the risk of a heart attack. According Rubilar et al. [2010] flax is considered a functional food or source of functional ingredients, because it contains alpha-linolenic acid, lignans and polysaccharides (other than starch), all of which have positive effects in disease prevention. In 2010 flax was grown on 2101 hectars of agricultural soil of the Slovak Republic [Tibenska 2010]. Also in observed locality Vrchy flax was the grown agricultural plant.

The samples of flaxseeds were collected from the same sampling sites of the investigated locality as the soil samples. Our results confirmed the ability of flaxseeds to accumulate high contents of risky heavy metals such as Cd, Pb, Cr and Ni (Tab. 5). Despite of low content of Cr and Ni in analysed soil samples from all sampling sites flaxseeds can accumulate also dangerous amount of these heavy metals.

Table 5

Heavy metal contents [mg·kg^{-1}] in seeds of flax (*Linum usitatissimum* L.)

No.	Sampling point	Content of heay metals [mg·kg^{-1}]						
		Cd	Pb	Zn	Cu	Cr	Ni	Co
1.	1	0.99	2.30	44.10	13.70	1.40	3.90	1.30
2.	2	0.88	1.30	45.80	15.50	1.60	3.70	0.60
3.	3	0.84	1.50	44.80	15.30	1.70	3.70	0.90
4.	4	0.94	1.40	44.70	15.10	1.10	3.90	1.00
5.	5	1.00	1.80	45.00	15.30	1.00	3.90	1.20
6.	6	0.97	2.00	45.30	15.60	1.40	4.10	0.80
7.	7	0.95	2.20	45.30	15.30	1.60	4.10	0.90
8.	8	0.93	1.90	44.50	15.40	1.70	3.90	0.70
Average		0.94	1.80	44.94	15.15	1.44	3.90	0.93
Min		0.84	1.30	44.10	13.70	1.00	3.70	0.60
Max		1.00	2.30	45.80	15.60	1.70	4.10	1.30
SD		0.05	0.37	0.53	0.60	0.27	0.15	0.24
Hygienic limit*		**0.5**	**1.0**	**80.0**	**25.0**	**0.5**	**2.0**	–

* Food Codex of the Slovak Republic

The legislative value given by Food Codex of the Slovak Republic for Cd content in foodstuffs determined in the samples of flaxseeds was in all sampling points exceeded (Fig. 6). The enhancement was in interval 68–100% above the maximal available value given by the legislative. Also Angelova et al. [2004] confirmed that flax is the crop that most strongly absorbs and accumulated heavy metals from the soil.

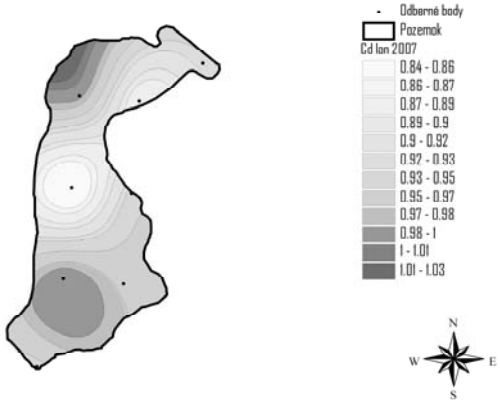

Fig. 6. Cd content in seeds of flax in locality Vrchy

The determined Pb content in flaxseeds in all sampling sites was by 30–130% higher than hygienic limit value given by Food Codex od the Slovak Republic (Fig. 7).

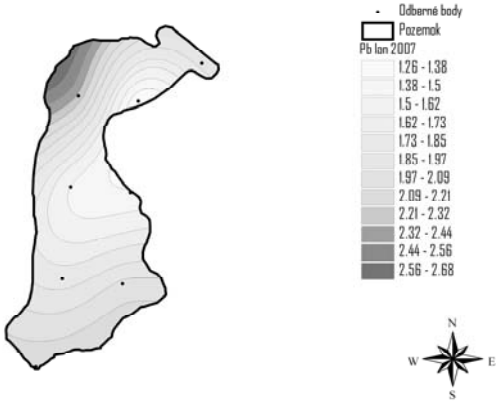

Fig. 7. Pb content in seeds of flax in locality Vrchy

Chromium is considered to be essential to a part of the living organisms, but in increased concentration is toxic. Some Cr compounds are recognized as human carcinogens. The quantities of Cr in the soil which are actually dangerous for the plants depend on its biological availability for them [Gauglhofer 1986]. The determined Cr content in flaxseeds in all sampling sites was by 100–240% higher than hygienic limit value given by Food Codex of the Slovak Republic (Fig. 8).

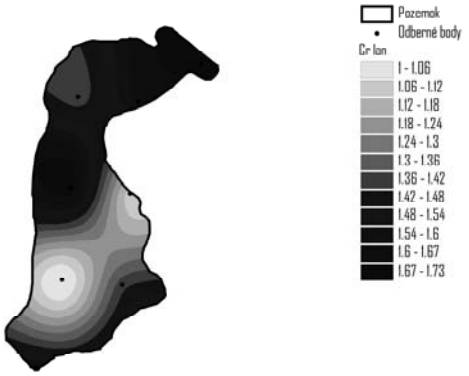

Fig. 8. Cr content in seeds of flax in locality Vrchy

According Hazlett et al. [1983] bioavailability of Ni from the soil to the plant is influenced by physical factors (texture, temperature and water content), chemical factors (pH, organic substances, redox potential) and biological factors (plant species variability, microbial activity). The determined Ni content in flaxseeds in all sampling sites was by 85–105% higher than hygienic limit value given by Food Codex od the Slovak Republic (Fig. 9).

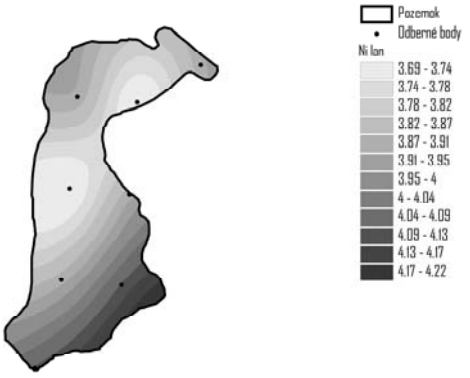

Fig. 9. Ni content in seeds of flax in locality Vrchy

It means, that seeds of flax from this locality are not suitable for human nutrition. The analysed flax can be used only for technical purposes. The consumption of flaxseeds from the investigated locality constitutes the risk for the human health because of high toxicity of present risky heavy metals. The results confirm the potential risk of environmental pollution sources influence on the food raw materials safety. It is necessary to monitor the heavy metal content in the soils as well as in the agricultural plants grown in vicinity of industrial enterprises in Middle Povazie region.

Conclusions

Our results confirm that low pH/KCl values, enhanced Cd, Co and Pb soil contents and enhanced Cd, Pb, Cr and Ni amounts in seeds of flax grown in observed locality in vicinity of the most important environmental pollution sources can indicate the potential causal connection with industrial activity in region of Middle Povazie. The results also confirm the necessity of risk metal monitoring in the soil as well as in the agricultural production in vicinity of potential pollution sources because of food chain safety assurance.

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References

Angelova V., Ivanova R., Delibaltova V., Ivanov K., 2004. Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). Industrial Crops and Products, 19, 3, 197–205.

Blume H.P. (Ed.), 1990. Manual of Soil Protection. Ecomed. Hamburg, 686.

Bradham K.D., Dayton E.A., Basta N.T., Schroder J., Payton M., Lanno R.P., 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. Environmental Toxicology and Chemistry, 25, 3, 769–775.

Buszewski B., Jastrzębska A., Kowalkowski T., Górna-Binkut A., 2000. Monitoring of Selected Heavy Metals Uptake by Plants and Soils in the Area of Toruń, Poland. Polish Journal of Environmental Studies, 9, 6, 511–515.

Bystrická J., Musilová J., Tóth T., 2008. The possibilities of cadmium uptake lowering by seeds of legumines with the application of Zn^{2+} into the soil. In Slovak J. of Animal Science, Nitra, 41, 4, 197.

Das P., Samantaray S., Rout G.R., 1997. Studies on cadmium toxicity in plants: a review. Environ. Pollut., 98, 29.

Gauglhofer J., 1986. Environmentlqa Aspets of Tanning with Chromium. J. Soc. Leather Technol. Chem., 70, 11–13.

Hazlett P.W., Rutherford G.K., van Loon G.W., 1983. Meta contaminants in surface soils and vegetation as a result of nickel/copper smelting at Coniston, Ontario, Canada. Reclamat. Reveg. Res., 2, 123–137.

Hegedüsová A., 1999. Heavy metals (Cd. Pb. Hg) in soils of the South Slovakia and hygienic state of cultivated vegetables. Dissertation Work. Nitra, 20–30.

Hronec O., Holobradý K., Tóth T., 2006. Old alkaline environmental loads of soils in Slovakia. Acta regionalia et environmentalica, Nitra, 3, 2, 32–35.

Kobza J., 2005. Soil and plant pollution by potentially toxic elements in Slovakia. Plant Soil Environ., Prague, 51, 6, 243–248.

Li H.F., Gray C., Mico C., Zhao F-J., McGrath S.P., 2009. Phytotoxicity and bioavailability of cobalt to plants in a range of soils. Chemosphere, 75, 979–986.

Linkeš V., Kobza J., Svec M., Ilka P., Pavlenda P., Barancikova G., Matuskova L., 1997. The monitoring of soils of the Slovak Republic. SFRI. Bratislava, 128.

Law no. 220/2004. Criteria for identification of risky spots of soil contamination and methodology procedures of their evaluation.

Makovníková J., 1998. Distribution of Cd, Pb, Cu and Zn in basic soil types of Slovakia and its evaluation in relationship to input of these metals into the plant. Dissertation Work. SFRI, Bratislava.

Musilová J., Bystrická J., Tomáš J., Poláková Z., Melicháčová S., 2009a. Changes of Vitamin C Content in Relation to the Range of Accumulation of Cd, Pb and Zn in Potato Tubers. Czech J. Food Sci., 27, Special Issue, 192–194.

Musilová J., Tóth T., Árvay J., 2009b. Contents of Heavy Metals in Different Saccharides Fractions of Potato Tubers. Czech J. Food Sci., 27, Special Issue, 382–385.

Osman M.E.H., El-Naggar A.H., El-Sheekh M.M., El-Mazally E.E., 2004. Differential effects of Co^{2+} and Ni^{2+} on protein metabolism in Scenedesmus obliquus and Nitzschia perminuta. Environ. Toxicol. Phar. 16, 169–178.

Rahmanian M., Habib K., Younes R.D., Mir Hasan R.S.M., 2011. Effects of heavy metal resistant soil microbes inoculation and soil Cd concentration on growth and metal uptake of millet, couch grass and alfalfa. African Journal of Microbiology Research, 5(4), 403–410.

Rubilar M. Gutiérrez C., Verdugo M., Shene C., Sineiro J., 2010. Flaxseeds as source of functional ingredients. J. Soil Sci. Plant Nutr. 10, 3, 373–377.

Tibenská H., 2010. Olejniny. VÚEPP Bratislava, 54.

Torma S., 1998. The suistanable fertility of the soil and antierosion protection. SFRI. Bratislava, 211–218.

Tóth T., Tomáš J., Vollmannová A., Lahučký L., Ducsay L., Varga L., 2004. Kontaminácia pôd v okolí chemického podniku Chemko Strážske. In Chem. Listy, 98, 11, 1063.

Trangmar B.B., Yost R.S., Uehara G., 1985. Application of Geostatistics to Spatial Studies of Soil Properties. Adv. Agron. Elsev. Sci., 38, 45–94.

Vollmannová A., Urminská D., Melicháčová S., Harangozo Ľ., Timoracká M., 2007. Polyphenolic compounds with antioxidant activity in buckwheat grown in the metallic burden soil. In Synthetic and Natural Compounds in Cancer Therapy and Prevention. International Conference ERDF of European Union, 28.–30.3.2007, Bratislava, 84.

Wopereis M.C., Gascuel-Odoux C., Bourrie G., Soignet G., 1988. Spatial Variability of Heavy Metals in Soil on a One-hectare Scale. Soil Sci. Wolters Kluwer, 146, 2, 113–118.

CHAPTER 3

MICROBIOLOGICAL HAZARDS IN FOOD PRODUCTION

1

CLOSTRIDIUM PERFRINGENS OCCURRENCE IN PARTICULAR LINKS OF FOOD CHAIN

Introduction

Transmission of pathogens in food chain may cause both animal and human diseases. Knowledge about microorganism occurrence and toxic properties on every food chain links may be helpful for risk assessment and risk management. *Clostridium perfringens* is recognized as most important animal pathogen among anaerobic sporulating bacteria responsible for enterotoxemia of many warm-blooded organisms [Songer 1996]. Animal morbidity ranges from 15 to 50 percent and mortality may come up to 100%. Significance of the anaerobe for food animal morbidity increased following the termination of antimicrobial growth promoters, effectively decreasing the morbidity caused by opportunistic bacteria. *C. perfringens* is also recognized as third etiologic factor of human food poisoning and food infections both in United States of America and in country belonging to Organization for Economic Co-operation and Development [Brynestad and Granum 2002]. The production of the major toxins is a base for classification criterion into one of the five toxotypes (A – E). All type A strains produce α toxin, type B α, β, and ε toxins, type C α and β toxins, type D α and ε toxins, and type E α and ι toxins [Sawires and Songer 2006]. C. perfringens is capable of producing many additional toxins or enzymes, inter alia β2 toxin (cpb2) and enterotoxin (cpe), whose part in the pathogenesis is confirmed [Garmory et al. 2000]. The toxin production ability and level of produced toxins decide about pathogenicity of the bacterium. Not less important than gene activity is immune decreasing of host, which may lead to disease. Taking into account that pathogenicity of *C. perfringens* is conditioned by presence of toxin genes the study were undertaken for assessment of toxin genes occurrence in strains isolated from particular food chain links. Toxin genes were detected from feed, swine faeces, food of pork origin and human faeces isolates by multiplex polymerase chain reaction (mPCR).

Materials and methods

Isolates for the present study were obtained from feed samples taken from feed processing plants or imported batches by Veterinary Inspectors during official controls and from samples sent to our laboratory by private farmers. The animal isolates derived from 5 swine farms from 2 to 150 sows in the herd, located not far than 50 kilometers from our laboratory. Samples of food of animal origin (n=37) contained raw pork meat (n=26), ready-to-eat smoked sausages and meat products (n=9) and powdered soups containing meat (n=2). Meat and meat products were derived from local retail and soups from army reserves. Human

isolates were collected from the different age patients in local hospital. The study is performing since 2007 year until 2010 in Department of Hygiene of Animal Feeding Stuffs (National Veterinary Research Institute in Pulawy).

Table 1

Kind of analysis and number of studied samples

Kind of analysis \ Kind of studied sample	Feeds	Swine faeces	Pork origin food	Human faeces	Total
C. perfringens number	100	100	109	100	409
C. perfringens presence in 1 g sample	–	–	109	–	109
C. perfringens toxotype identification	105	404	37	323	839

Twenty gram of feed and food or one gram of faeces was spread on *C. perfringens* number on tryptose sulfite cycloserine agar and incubated for 24 h at 37 ± 1°C. Twelve obtained isolates from each positive sample were tested for the presence of *cpa*, *cpb*, *cpb2*, *etx*, *iap* and *cpe* toxin genes which encode respectively for alpha toxin, beta toxin, beta2 toxin, epsilon toxin, iota toxin and enterotoxin by using mPCR method. The primers, reagent concentrations and reaction conditions were as protocol published by Baums et al. [2004] except that TrueStart *Taq* DNA Polymerase (Fermentas) was used instead of Taq DNA Polymerase (Fermentas). The PCR products were identified by UV transilluminator, (Chemi-Smart 3000, Vilber Lourmat, France) following electrophoresis through a 2% agarose gel containing 1 μg/ml of ethidium bromide. The size of amplicons was compared with O'Gene Ruler 100 bp DNA Ladder (Fermentas).

Results and discussion

The presence of *C. perfringens* at the higher level than 1.0 x 10^1 cfu/g was detected in 68% of feed samples, 92% of swine faeces, 4% of food samples, and 67% of human faeces. Feed contamination level ranged from 1.0 x 10^1 cfu/g to 9.5 x 10^2 cfu/g (Fig. 1) and from 1.0 x 10^1 cfu/g to 1.2 x 10^7 cfu/g in swine faeces (Fig. 2). Among food of animal origin samples, the presence of the anaerobes was detected in 8.1% of them. The contamination level of samples classified as positive ranged from 1.0 x 10^1 cfu/g to 3.2 x 10^1 cfu/g (Fig. 3). *C. perfringens* occurrence level in human faeces extended from 1.0 x 10^1 cfu/g to 7.3 x 10^7 cfu/g, but nearly half of positive samples ranged from 10^4 to 10^6 cfu/g (Fig. 4). The analysis of *C. perfringens* occurrence in the studied chain links revealed, that the highest level of these anaerobes were in swine and human faeces (10^7 cfu/g). However, nearly 40% of swine and human faeces samples contained no more than 10^2 cfu/g. The bacteria were seldom noticed in food of animal origin and compound feeds samples. Additionally, it was observed that the number of *C. perfringens* in faeces was strongly depended on farm hygiene (positive correlation). The way of sampling was also important. When faeces were sampled from live animals and transported as quick as possible to laboratory in cool temperature or frozen till analyses, *C. perfringens* number did not exceed 10^4 cfu/g, what is typical for healthy animals. In cases, when the content of the intestines from dead animals was analyzed, the obtained results were

significantly higher (ranged from 10^6 to 10^8 cfu/g), what is typical for animals affected by *C. perfringens* caused enteritis. On the contrary, the level of *C. perfringens* in swine faeces was positively correlated with herd size and age of animals. The anaerobes were detected in one-day piglet faeces but their level did not exceed 10 cfu/g. Additionally, more than 1.0 x 10^1 cfu/g were found in raw pork meat. No more than 1.0 x 10^1 cfu/g anaerobes were found in raw pork meat and ready-to-eat pork meat products (white sausage, "zwyczajna" sausage, head cheese, poultry sausage). In contrast to ready-to-eat meat products, which contained only spores, vegetative cells and spores of *C. perfringens* were isolated from raw meats. Statistical analysis of patient age and number of anaerobes in human faeces did not confirm linear dependence between both parameters. Sex of patient also did not influence the number of anaerobes in human faeces.

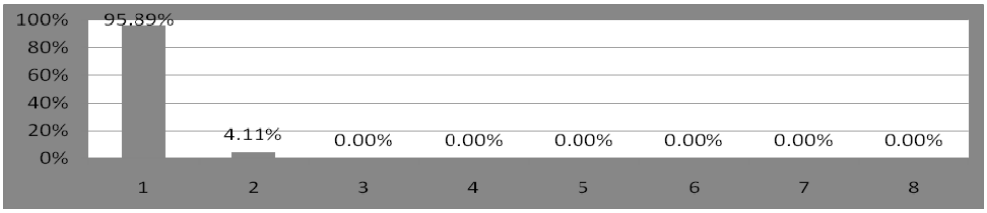

Fig. 1. *C. perfringens* number in compound feeding stuffs

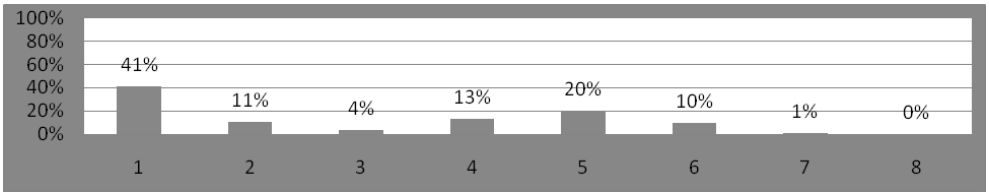

Fig. 2. *C. perfringens* number in swine faeces

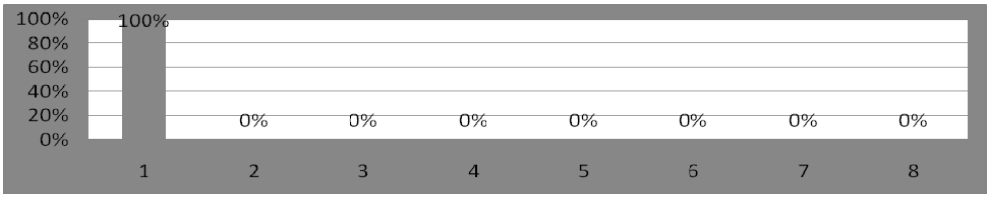

Fig. 3. *C. perfringens* number in pork origin foods

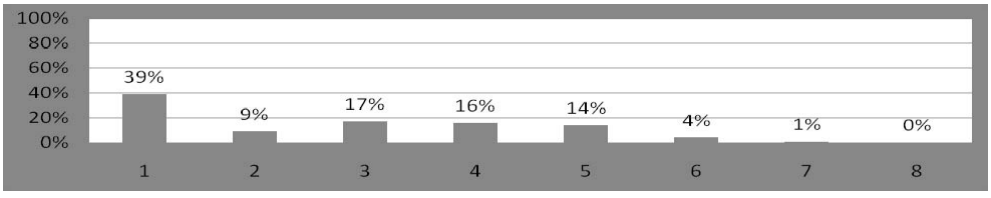

Fig. 4. *C. perfringens* number in human faeces

It should be mentioned that the lowest limit of detection for *C. perfringens* enumeration method was 10 cfu/g, what means that in samples classified like negative, anaerobes may occurred on level less than 10 cfu/g. The obtained results for *C. perfringens* number were analyzed according to distributive series. Figs 1–4 present bacterial number in logarithm scale (*e.g.* $\log_{10}$=3 means range from 10^3 to 9,999 cfu/g; $\log_{10}$=4 means range from 10^4 to 99,999 cfu/g, *etc.*).

In the midst of all studied isolates for toxic type and subtype identification, type A strains dominated, among them the percentage of subtype β2 strains varied considerably. The occurrence frequency of subtype β2 strains was comparable to the occurrence frequency of type A strains among feeds and swine faeces (Figs 5–8). Isolated *C. perfringens* food strains were classified as type A. Subtype β2 strains in food of animal origin (8%) were relatively rarely detected. Any of them contained enterotoxin gene. The presence of enterotoxin gene was demonstrated only in 12 isolates (1.4%) from among 839 studied strains. Enterotoxic strains occurred in compound feeds, swine and human faeces. There were also isolated 14 strains possessed only β2 toxin-encoding gene, what was observed in feed isolates.

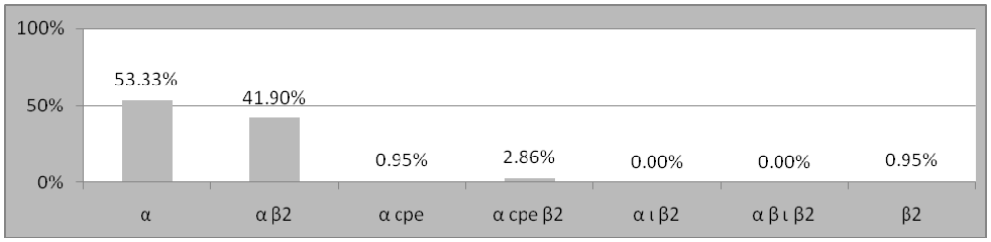

Fig. 5. Occurrence of *C. perfringens* toxotypes in compound feeding stuffs

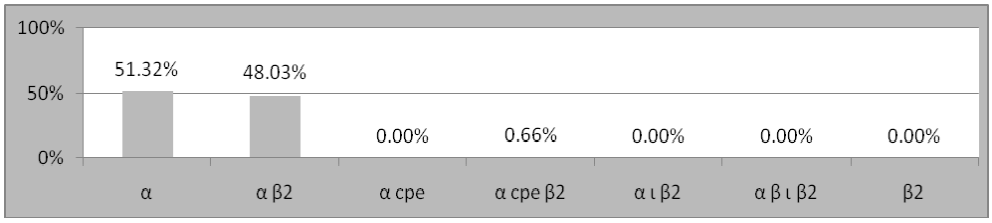

Fig. 6. Occurrence of *C. perfringens* toxotypes in swine faeces

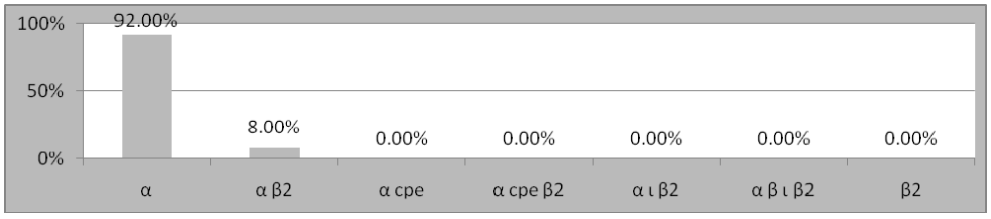

Fig. 7. Occurrence of *C. perfringens* toxotypes in foods of animal origin

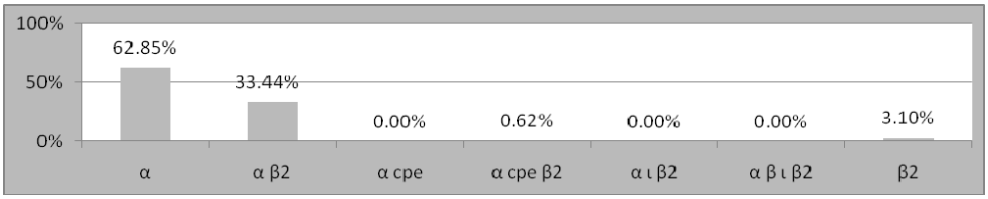

Fig. 8. Occurrence of *C. perfringens* toxotypes in human faeces

Detected levels of type A *C. perfringens* strains did not exceed physiological number of these strains in the intestines of mammals [Johansson et al. 2006, Sipos et al. 2003]. Additionally, the obtained results and results of the earlier study confirm good microbiological quality of feed samples with reference to *C. perfringens* in Poland. In default of Polish performance standards regarding *C. perfringens* number in food of animal origin, the obtained results were compared to American criterion in relation to food of animal origin. On February 2001, the United States Department of Agriculture, Food Safety and Inspection Service established performance standards for *C. perfringens* to a maximum of 1-$\log_{10}$ (a factor of 10 cfu/g) to all ready-to-eat and all partially cooked meat and poultry products on a basis of conducted a quantitative risk assessment. In regard to these performance standards, the studied food of animal origin samples revealed good microbiological quality. Slightly crossing 10 cfu/g was noticed only in raw meat samples, what before consumption are usually subjected thermal treatment.

The presented study confirmed *cpe* occurrence in 1.4% of isolates from different samples (feeds; swine and human faeces). There were no *cpe*-positive strains in the analyzed food of animal origin samples. However, the panmictic nature of *C. perfringens* orders to be careful during meal preparation. Taking into account literature data regarding to average enterotoxin-positive strains occurring in food of animal origin (5%), infection dose of *C. perfringens* for humans (10^6–10^8 cfu) and assuming that "zwyczajna" sausage contamination level amount to 10 cfu/g, then healthy adult man should eat 2 tons of sausage to disease might occurre. Supposing more probable scenario, the disease may appear after consuming 200 g portion of sausage contaminated by 10^5 cfu/g anaerobes. There are many potentialities, which may occurre in reality and variables may be the percentage of occurring enterotoxin-positive strains or size of the infective dose. Besides, each above mentioned factor is occurred in fact like set of data (e.g. various levels of food contamination, different immunity) what influence on seeking final value. Furthermore, above calculations regard only "food-human" food chain fragment and do not concern probability of diseases occurrence in animals, what is determined by other *C. perfringens* toxotypes and predisposing factors [Garmory et al. 2000].

Acknowledgements

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References

Baums C.G., Schotte U., Amtsberg G., Goethe R., 2004. Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. Vet. Microbiol., 100, 11–16.

Brynestad S., Granum P.E., 2002. *Clostridium perfringens* and foodborne infections. Int J. Food Microbiol., 74, 195–202.

Garmory H.S., Chanter N., French N.P., Bueschel D., Songer J.G., Titball R.W., 2000. Occurrence of *Clostridium perfringens* beta2-toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidemiol. Infect., 124, 61–67.

Johansson A., Aspan A., Bagge E., Baverud V., Engström B.E., Johansson K.E., 2006. Genetic diversity of *Clostridium perfringens* type A isolates from animals, food poisoning outbreaks and sludge. BMC. Microbiol., 6, 1–34.

Sawires Y.S., Songer J.G., 2006. *Clostridium perfringens*: Insight into virulence evolution and population structure. Anaerobe, 12, 23–43.

Sipos W., Fischer L., Schindler M., Schmoll F., 2003. Genotyping of *Clostridium perfringens* isolated from domestic and exotic ruminants and swine. J. Vet. Med. B Infect. Dis. Vet. Public Health, 50, 360–362.

Songer J.G., 1996. Clostridial enteric diseases of domestic animals. Clin. Microbiol. Rev., 9, 216–234.

2

COMPARATIVE STUDY OF DIFFERENT STABILIZATION PROCEDURES FOR MILK SAMPLES USED IN PROFICIENCY TESTING FOR *LISTERIA MONOCYTOGENES*

Introduction

Risk assessment is a systematic process of identification and evaluation of hazards resulting from microbiological contaminations. *L. monocytogenes* is a widespread pathogen which may be found in foods including raw milk and milk products. This microorganism is responsible for many outbreaks with high mortality rates. Since the recognition of *L. monocytogenes* as a food-borne pathogen, there have been rapid advances in the development of suitable methods for its isolation and identification [Sutherland and Porritt 1997, Gasanov et al. 2005]. Commission Regulation (EC) No 2073/2005 [Anonymus 2005a] has laid down microbiological criteria for *L. monocytogenes* detection and enumeration.

The control of pathogenic bacteria present in foods and scientific data concerning their behavior, are closely linked to analytical methods used and the way they are implemented. National and international proficiency testing schemes are organized to assess the capacity of the laboratories to conduct correctly microbiological analyses [Gnanou Besse et al. 2007]. The Department of Hygiene of Food of Animal Origin of National Veterinary Research Institute acting as the National Reference Laboratory organizes the PT for official veterinary and commercial laboratories. In 2011 the provider organized 5 rounds of proficiency testing by interlaboratory comparisons. Proficiency tests were organized according to ISO/IEC 17043:2010 [Anonymus 2010]. The International Standard specifies general requirements technical and management for the competence of providers of proficiency testing schemes and for the development and operation of proficiency testing. These requirements are intended to be general for all types of proficiency testing schemes, and they can be used as a basis for specific technical requirements for particular fields of application. This International Standard has been prepared to provide a consistent basis for all interested parties to determine the competence of organizations that provide proficiency testing. As a result, both parts of ISO/IEC Guide 43:1997 have been replaced. This International Standard has preserved and updated the principles for the operation of proficiency testing described in ISO/IEC Guide 43 and has retained in Annexes A to C information on typical types of proficiency testing schemes, guidance on appropriate statistical methods, selection and use of proficiency testing schemes by laboratories, accreditation bodies, regulatory bodies, and other interested parties.

Regular participation in proficiency testing (PT) or interlaboratory comparison provides independent verification of the analytical competence of the laboratory and is one way to monitor the validity of the research carried out in the process of quality control. PN-EN ISO/IEC 17025:2005 requires laboratories to have quality control procedures ensuring continuous monitoring of the validity of test results delivered to clients. Accordingly, the accreditation

and evaluation of laboratories on the basis of the PN-EN ISO/IEC 17011:2006 require the laboratories that are accredited or seeking accreditation, participation in proficiency testing, as one of the main results of control quality research. The Polish Centre for Accreditation (PCA) has concluded a policy regarding the use of PT and a collaborative study in the processes of accreditation and laboratories mentioned in the DA-O5.

In relation to official food control laboratories under regulation (EC) No 882/2004, requires accreditation for compliance with ISO 17025 standard and thus part in proficiency tests as a basic demonstration of competence.

Samples used in PT need to be homogeneous in order to be sure that if a laboratory has a result varied from that of other laboratories its incompatibility may be attributed to its procedure of analysis but not to the sample. The organization of the homogeneity control of samples prepared for proficiency testing schemes is a challenge for the PT organizers and the success depends on many parameters.

To organize these studies, it is necessary to prepare artificially contaminated samples, such as raw and pasteurized milk with a contamination level sufficiently stable and homogeneous during transportation to the participating laboratories. *L. monocytogenes* may grow at a refrigeration temperature. That is why appropriate temperature is not sufficient to maintain a constant concentration of *L. monocytogenes* during transportation of the samples to the participating laboratories

The problem of preservation of milk samples is common in many countries because the diagnostic laboratories are generally far away from dairy farming communities, thus the transport of samples to the laboratory inadequate [Dunham and Kroger 1985]. In such situations, it is necessary to explore other methods of milk sample preservation including the use of chemicals. Recently, scientists have used various milk preservatives (hydrogen peroxide, sodium azide, bronopol, potassium dichromate, boric acid, Milkofi x, azidiol, ortobor acid) to overcome this problem [Ng-Kwai-Hang and Hayes 1982, Hanus et al. 1992a, 1992b, Heeschen et al. 1994, Saha et al. 2003]. We selected one reagent – sodium azide (azidiol) which is frequently used to preserve milk samples for routine analysis [Rapp and Münch 1984, Barcina et al. 1987].

Finally, considering a high resistance of *L. monocytogenes* to temperatures below 0°C [Lou and Youself 1999], the possibility to use a freezing method was also evaluated to stabilize the concentration of *L. monocytogenes* in the samples. In particular, the stability of our samples of milk was examined in the conditions of transportation during Proficiency Testing.

Materials and methods

Bacterial strains and preparation of inocula

The experiments were carried out with one freeze dried test strain of *L. monocytogenes* ATCC 19111 from the National Veterinary Research Institute collection. The strain was resuscitated and then incubated for 18 h at 37°C in Brain Heart Infusion broth (BHI, OXOID) before use. The final BHI culture contained about 10^9 CFU ml^{-1}. All dilutions were prepared in Maximum Recovery Diluent (OXOID). To determine the inoculum level, appropriate dilutions were enumerated on TSAYE (OXOID).

Contaminations levels

Levels of contaminations used for enumeration of *L. monocytogenes* samples:

- High level – $1x10^4$ CFU mL^{-1}
- Low level – $1x10^3$ CFU mL^{-1}

Samples used for detection of *L. monocytogenes*:

- Uncontaminated
- Contaminated at a low level ($1x10^2$ CFU mL^{-1}).

Milk samples

Raw milk from a local dairy and pasteurized one bought from a local distributor we used. The absence *L. monocytogenes* in the raw and pasteurized milk was previously checked according to the Standard EN ISO 11290-1 methods [Anonymous 1996]. The matrix was also checked for the presence of antibiotics by using (Delvotest SP-NT, DSM Food Specjalitest B.V.).

Preservation procedures

Considering the assays with bacteriostatic agent, milk samples artificially contaminated with *L. monocytogenes* were kept for up to 8 days at a constant temperature between 2–8°C. During the last interlaboratory trial the temperature of samples at reception in some laboratories was up to 8°C. Refrigeration temperatures were achieved by adding cooling contributions into the package during transportation.

The bacteriostatic agents were added and mixed with milk before contamination. Sodium azide (azidiol), a chemical bacteriostatic agent, at a concentration of 0.02% (POCH) were used. Sodium azide was added to 1 L of milk and then the samples were spilt into 30 ml vials.

According to the freezing preservation procedure, milk samples artificially contaminated with *L. monocytogenes* were stored at −23°C for 8 days. To follow the evaluation of total background microflora levels, samples without any *L. monocytogenes* contamination were also stored in the same conditions. In the case of raw and pasteurized milk and the two preservation methods, samples were analyzed at 1, 3, 6, and 8 days after contamination. Each time the analysis involved three samples from the test level. In the case of frozen milk the high level of contamination was applied.

Microbiological analysis

The evolution of *L. monocytogenes* levels was monitored using the EN ISO 11290–1 [Anonymous 1996] and EN ISO 11290-2 [Anonymous 1998]. The ISO 11290 method has a two-stage enrichment process: the food sample is first enriched in half Fraser broth (bioMerieux) for 24 h, then an aliquot is transferred to full strength Fraser (bioMerieux) for further enrichment. Fraser broth also contains the selective agents acriflavin, naladixic acid and esculin, which allow detection of β-D-glucosidase activity by *Listeria*, causing a blackening of the medium. Both the primary and secondary enriched broths are plated on Oxford (bioMerieux) and ALOA (bioMerieux) agars.

Statistical evaluation

Statistical analysis of the experience included the average value and standard deviation of three samples analyzed every third day for a period of 8 days.

The statistical calculation for the final homogeneity and stability of the samples sent to participants of proficiency testing was performed according to ISO 13528:2005, Annex B [Anonymous 2005b].

One of the options provided by ISO 13528 point 6.5 to calculate the target standard deviation is the following equation, based on the precision of the method.

Results and discussion

The presented study enabled us to select preservation procedures, which could be used to stabilize the bacterial level of artificially contaminated milk samples during time indicated for carrying out the analysis including transportation of the samples. These studies have been conducted prior to final preparation of the samples used for Proficiency Testing of *L. monocytogenes*. The results are presented in Figs. 1, 2 and Table 1. The results obtained show that the use of sodium azide (azidiol) was effective in stabilizing the contamination level of *L. monocytogenes* in raw and pasteurized milk samples. The average values of the results of the samples fixed with this preservative analyzed in the intervals of 3 days for the period of 8 days were stable. The addition of azydiol enabled stabilization of *L. monocytogenes* concentration between 2–8°C at all used levels. The sodium azide has been described and used by Gnanou Besse et al. [2007] for the preservation of pasterized milk samples for bacteriological analysis. In their studies samples were incubated for up to 14 days at a constant temperature of 8°C or 12°C. However, at the low contamination level (10^2 CFU ml^{-1}), the enumeration of *L. monocytogenes* was difficult in the samples containing sodium azide because the colonies were small, heterogeneous, and sometimes appeared 72 h after incubation. Similar studies were conducted by Seškēna and Jankevica [2007] for raw milk stored at 4 and 20°C. They found, that the using of sodium azide for raw milk preservation could provide stable milk quality for 96 h. Similar results were obtained by Gonzalo et al. [2004] who observed that sodium azide can be used successfully in preserving milk samples for bacteriological analysis.

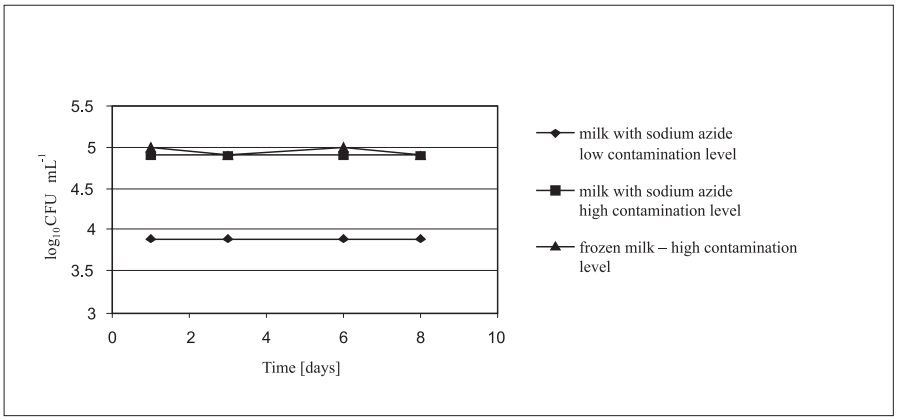

Fig. 1. Evolution of *L. monocytogenes* population levels in pasteurized milk samples within 8 days

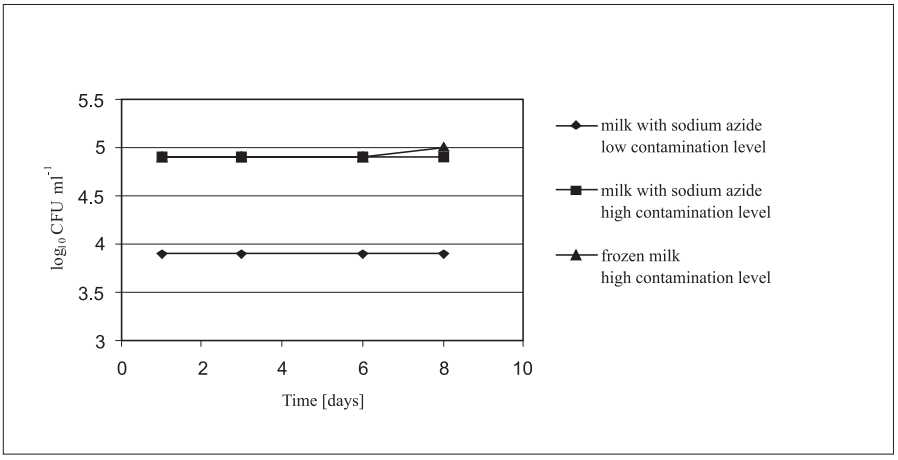

Fig. 2. Evolution of *L. monocytogenes* population levels in raw milk within 8 days

Furthemore, the presented studies showed that a freezing method was able to stabilize the concentration of *L. monocytogenes* in the artificially contaminated samples. In the case of frozen samples, the difference among average values of the results between 1st and 8th day from contamination amounted 0.1 $\log_{10}$ and fitted to the precision of the method. It has proved that the samples were stable. The same results were obtained in the case of both raw and pasteurized milk. Gnanou Besse et al. [2007] examined that without any bacteriostatic agent, a storage at −23°C was able to stabilize the concentration *L. monocytogenes* in the pasteurized milk. Freezing was convenient for milk samples contaminated with *L. monocytogenes*. According to the bibliographic review performed by Lou and Yousef [1999], the bacteria may survive to temperatures below 0°C, depending on the strain, temperature, and medium. In particular, the milk matrix seems to provide a good protection against freezing [Lou and Yousef 1999]. It can be noted that potential stress caused by freezing did not prevent the growth of *L. monocytogenes* and its recovery on selective agar.

Table 1

Final results of the experiment

Level of contamination [CFU mL^{-1}]	D_1	D_3	D_6	D_8	range of results (log_{10})	Sd*	Range D_{1min} – D_{8max}
MILK WITH SODIUM AZIDE							
Pasteurized milk							
$1x10^3$ CFU mL^{-1}	3.83; 3.89; 3.87	3.83; 3.88; 3.91	3.86; 3.89; 3.84	3.85; 3.90; 3.86	3.83–3.91	0.03	0.07
$1x10^4$ CFU mL^{-1}	4.83; 4.94; 4.90	4.95; 4.89; 4.89	4.84; 4.90; 4.84	4.92; 4.88; 4.90	4.83–4.95	0.04	0.09
Raw milk							
$1x10^3$ CFU mL^{-1}	3.89; 3.86; 3.95	3.89; 3.91; 3.82	3.87; 3.89; 3.85	3.88; 3.93; 3.99	3.82–3.99	0.03	0.13
$1x10^4$ CFU mL^{-1}	4.87; 4.92; 4.95	4.83; 4.92; 4.88	4.88; 4.85; 4.94	4.94; 4.83; 4.86	4.83–4.95	0.04	0.07
FROZEN MILK							
Pasteurized milk							
$1x10^4$ CFU mL^{-1}	5.00; 4.95; 4.96	4.95; 4.98; 4.91	4.91; 4.91; 4.89	4.95; 5.00; 5.03	4.89–5.03	0.02	0.08
Raw milk							
$1x10^4$ CFU mL^{-1}	4.92; 4.95; 4.97	4.94; 4.90; 4.86	4.97; 5.01; 4.97	4.85; 4.88; 4.88	4.85–5.01	0.02	0.04

*Standard deviation D – day analyse

In our proficiency testing, after analyzing our results discussed above, we used pasteurized milk as a matrix with addition of sodium azide at the concentration of 0.02% as a preservative. The efficiency of this method of stabilization the contamination level of *L. monocytogenes* in milk samples confirms results of final homogeneity and stability study. A final homogeneity study was performed one day after the shipment to the participants, indicated as a day to begin the analyses by the participants. The procedure of homogeneity testing was performed according to Annex B of ISO 13528:2005 and it consisted comparison between samples standard deviation s_s with the standard deviation for proficiency assessment σ. Samples are considered to be homogeneous if $s_s \leq 0.3\sigma$. The standard deviation (σ) calculated from the results obtained from the participants was 0.12 at the low level and 0.17 at the high level of contamination and 0.3σ was 0.04 and 0.05 so the homogeneity criterion was met and the samples are considered homogeneous (Tab. 2 and 4). The procedure of stability testing was also performed according to Annex B of ISO 13528:2005 and it included comparison of the average of the results obtained in the homogeneity check with the average of the results obtained in stability check. In our study, 3 samples in duplicate were analyzed twice – before sending samples to the participants and then on a day of samples shipment to the participants. Results of these studies are shown on Tables 3 and 5.

Table 2

Homogeneity testing according ISO 13528

Level of contamination [CFU mL^{-1}]	$1x10^3$ CFU mL^{-1}	$1x10^4$ CFU mL^{-1}
Mean-x.,.	3.90	4.86
Sample mean standard deviation-S_X	0.02	0.01
Intra-sample mean standard deviation-S_W	0.03	0.01
Inter-sample mean standard deviation-S_S	0.01	0.01
0.3σ	0.04	0.05
$S_S \leq 0.3\sigma$	Homogeneous	Homogeneous

Table 3

Stability testing according ISO 13528

Level of contamination [CFU mL^{-1}]	$1x10^3$ CFU mL^{-1}	$1x10^4$ CFU mL^{-1}
Mean of the measurements obtained in the homogeneity check-x.,.	3.90	4.86
Mean of the results obtained in the stability check -y.,.	3.89	4.87
$\lvert x.,. - y.,.\rvert$	0.00	0.01
0.3σ	0.04	0.05
$\lvert x.,. - y.,.\rvert \leq 0.3\sigma$	Stable	Stable

Table 4

Homogeneity testing

Level of contamination [CFU mL^{-1}]	range of results (log_{10})	mean (log_{10})	Sd*
$1x10^3$ CFU mL^{-1}	3.86 – 3.95	3.90	0.03
$1x10^4$ CFU mL^{-1}	4.83 – 4.89	4.86	0.01

*Standard deviation

Table 5

Stability testing

Level of contamination [CFU mL^{-1}]	first analyse (D_0)**			second analyse (D_7)***			Range D_{0min} – D_{7max}	
	range of results (log_{10})	mean (log_{10})	Sd*	range of results (log_{10})	mean (log_{10})	Sd*	range of results (log_{10})	Sd*
1x10^3 CFU mL^{-1}	3.87–3.94	3.90	0.03	3.87–3.93	3.89	0.02	0.06	0.01
1x10^4 CFU mL^{-1}	4.83–4.87	4.86	0.03	4.86–4.90	4.87	0.02	0.07	0.01

*Standard deviation **D_0 – day first analyse ***D_7 – day second analyse

References

Anonymous, 1996. Microbiology of food and animal feeding stuffs – horizontal method for detection and enumeration of *Listeria monocytogenes*, Part 1. Detection Method. International Standard ISO 11290-1. International Organization for Standarization, Geneva.

Anonymous, 1998. Microbiology of food and animal feeding stuffs – horizontal method for detection and enumeration of *Listeria monocytogenes*, Part 2. Enumeration Method. International Standard ISO 11290-2. International Organization for Standardization, Geneva.

Anonymous, 2005a. Commission Regulation (EC) No. 2073/2005 of 5 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L 338, 1–26.

Anonymous, 2005b. ISO13528 Statistical methods for use in proficiency testing by interlaboratory comparisons. International Organization for Standarization, Geneva.

Anonymous, 2010. ISO/IEC 17043 Conformity assessment. General requirements for proficiency testing. International Organization for Standarization, Geneva.

Barcina Y., Zorraquino M.A., Pedauye J., Ros G., Rincon F., 1987. Azidiol as a preservative for milk samples. Ann Vet., 3, 65–69.

Dunham J.R., Kroger M., 1985. Milk Preservatives. Dairy Herd Improvement, Kansas, 2.

Gasanov U., Hughes D., Hansbro P.M., 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: FEMS Microbiology Reviews, 29, 851–875.

Gnanou Besse N., Cauquil A., Vignaud M-L., Barre L., Deperrois V., Voitoux E., Obabaka M-B., Lombard B., 2007. Comparative study of different milk samples preservation procedures for bacteriologic examination, Food Anal. Methods, 1, 36–42.

Gonzalo C., Boixo J.C., Carriedo J.A., San Primativo F., 2004. Evaluation of rapid somatic cell counters under different analitycal conditions in ovine milk. J. Dairy Sci., 87, 3623–3628.

Hanus O., Gencurova V., Gabriel B., Zvackova I., 1992a. Comparison of the effectiveness of Milkofix, a preservative preparation, with traditional preservative agents in the determinations of somatic cell count in milk samples using a fluoro-optic-electronic method. Vet. Med., 37, 91–99.

Hanus O., Gencurova V., Gabriel B., Zvackova I., 1992b. Testing Milkofix, a new preservative preparation for milk samples used for infrared analysis of milk components II. Verification of its preservative effects in relation to infrared analysis. Vet. Med., 37, 33–43.

Heeschen W.H., Ubben E.H., Rathjen G., 1994. Somatic cell counting in milk: the of flow cytometry of somatic cell counting (somacount) and comparison with the results obtained with the fluorescens optical principle (fossomatic 360). Bentley Instruments, INC, Minnesota, 33.

Lou Y., Yousef A., 1999. Characteristics of *Listeria monocytogenes* important to food processors. In: Ryser ET, Marth EH (eds) Listeria, listeriosis and food safety. M. Dekker, New York, NY, 131–224.

Ng-Kwai-Hang K.E., Hayes J.F., 1982. Effects of potassium dichromate and sample storage time on fat and protein by Milko-Scan and on protein and casein by a modified Pro-Milk Mk II method. J. Dairy Sci., 65, 1895–1899.

Rapp M., Münch S., 1984. Neuntwicklung von flüssing Konservierungsmitteln für Milchproben. Deutsche Molkerei Zeitung, 39, 1264–1272.

Saha B.K., Ali M.Y., Chakraborty M., Islam Z., Hira A.K., 2003. Study on the preservation of Raw milk with hydrogen peroxide (H_2O_2) for rural dairy farmers. Pakistan J. Natur., 2, 36–42.

Sešķēna R., Jankevica L., 2007. Influence of chemical preservatives on the quality and composition indices of raw milk samples Acta Universitatis Latviensis, vol. 723, Biology, 171–180.

Sutherland P.S., Porritt R.J., 1997. *L. monocytogenes*. In: Foodborne microorganisms of public health significance, 5th ed., AIFST (NSW Branch) Food Microbiology Group, 333–378.

3

ASSESSMENT OF INHIBITORY PROPERTIES OF CHOSEN SALTS OF PHENOLIC ACIDS FOR ELIMINATION OF *ESCHERICHIA COLI* O157:H7

Introduction

Hydroxicinnamic acids are found in different herb spices commonly added to food products. They are polyphenolic compounds which possess antimicrobial and antioxidant activity. This is the reason why they gain a lot of interest from scientists and are very popular as natural food preservatives. This study concentrates on the assessment of antibacterial activity of salts of cinnamic, *p*-coumaric, ferulic and caffeic acids against *Escherichia coli* O157:H7. Phenolic compounds possess the antimicrobial activity against foodborne pathogenic and spoilage bacteria. They are able to inhibit the growth of *E. coli*, *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*. They also possess antioxidant activities [Cvikrova et al. 1993].

It is commonly known that there are physiological processes in human organism which unavoidably cause the production of free radicals as by-products. From one hand, they have a positive effect on energy production, phagocytosis, stimulation of cell growth and synthesis of biologically significant compounds. From the other side, free radicals constitute a danger for people's body due to the fact that the process of oxidation leads to the appearance of free radicals which may lead to the cell membrane disintegration, as well as membrane protein damage and DNA mutation. Such a process can cause the development of many diseases. Phenolic compounds which are both antimicrobial and antioxidant substances may delay, inhibit, and even prevent the oxidation processes by binding free radicals [Tori et al. 1995]. Plants are a rich source of antimicrobial and antioxidant biologically active substances which not only neutralize the free radicals and prevent the chronic diseases but are also commonly used as substances successfully eliminating foodborne pathogens from food [Pueppke and Broughton 1999, Devi and Reddy 2002).

The consumption of natural antioxidants diminishes a risk of cancer, cardiovascular disease, diabetes and diseases related to ageing. In food products lipid oxidation of fats causes not only chemical spoilage but also produces free radicals including peroxy radicals responsible for carcinogenesis, autogenesis and ageing [Irisarri et al. 1996]. Membrane lipids are known to be especially sensitive to oxidation due to the presence of high polyunsaturated fatty acids [Fedorova et al. 2002]. Phenolics are antioxidants possessing the redox properties, which enable them to play a role of reducing agents, hydrogen donors, free radical scavengers. They are able to bind metal preventing the inhibition of lipid peroxidation. Their importance in the human diet and antimicrobial activity has been well examined. It is known that some phenolic compounds possess different physiological activities such as anti-inflammatory, antiallergic, anticarcinogenic, antihypertensive, antiarthritic as well as antimicrobial activities [De Vries et al. 1997, Seneviratne and Jayasinghearachchi 2003].

As foodborne pathogens are a serious danger for human health, it is reasonable to use the antimicrobial properties of phenolic substances. The paper concentrates on examining the inhibitory features of salts of the chosen phenolic acids against *E. coli* O157:H7. *Escherichia coli* are a group of bacteria, which constitute inhabitants of the intestines of all humans and most animals. Most species do not lead to any disease. However, *E. coli* O157:H7 is a serotype which expresses the 0-antigen 157 and the H-antigen 7 of *E. coli*. The *E. coli* O157:H7 serotype possesses the enterohemorrhagic properties. It contains at least one virulence attribute. They can involve the ability to produce shiga-like toxin(s) (SLT also known as verotoxins or VT), adherence factor(s) and enterohemolysin. The adherence factors make it possible for the microorganisms to attach to and colonize intestinal host cells. *E. coli* O157:H7 usually produces verotoxines which provoke the disease. Even a very small infective dose containing 50–100 bacteria may easily contribute to the public health significance. Such a dose is the smallest in comparison to most other ford-borne pathogens.

The disease provoked by *E. coli* O157:H7 is called hemorrhagic colitis and its symptoms involve severe cramping (abdominal pain) and diarrhea (watery and bloody), as well as vomiting and low grade fever. The disease usually takes 8 days. Very severe complications caused by *E. coli* O157:H7 can appear in 15% of cases and they concern much more frequently the very young and the elderly. Such complications include HUS and thrombotic thrombocytopenic purpura (TTP). HUS mainly touches infants and young children and means the renal failure and the hemolytic anemia. HUS leads to the acute renal failure in children and the mortality rate amounts even to 10%. Other potential complications involve the unnecessary surgical intervention, coma or seizures, pancreatitis, and diabetes mellitus [Griffin 1995].

Materials and methods

Pure substance

The material consisted of the chosen salts of phenolic acids such as lithium, sodium and potassium salts of cinnamic, *p*-coumaric, caffeic and ferulic acids. The 1, 2, 3, 4 and 5% water solutions of each substance were prepared to check their antimicrobial activity towards *E. coli* O157:H7.

Microbial strain

The strain *E. coli* O157:H7 ATCC 8739 used for microbiological analysis was obtained from ATCC collections (the American Type Culture Collection, USA).

Maintenance and preparation of cultures

Culture of *E. coli* O157:H7 was isolated from bovine faeces and maintained on tryptone soy broth agar (TSBA) slants at 4°C (bioMérieux, Warszawa, Poland).

Preparation of liquid bacterial culture in tryptic soy broth

A 16 h old culture inoculated in tryptone soy broth (bioMérieux, Warszawa, Poland) at temperature 37^0C was taken for further experiment. The optical density of this culture after

inoculation was determined at 625 nm (Ultraspec III, Pharmacia, Sweden). The incubation was stopped when the optical density achieved a value in the range of 0.8–1.0. The culture suspensions were diluted to an absorbance of 0.1 and used as such for the antimicrobial tests.

Placing the culture dilution on a plate with medium

The medium used for further experiment was Columbia agar with sheep blood (bioMérieux, Warszawa, Poland) on Petri dishes (Ø 10 cm).

A 1 ml of a 16 h culture diluted to achieve an absorbance of 0.1 was placed on the surface of Petri dishes (Ø 10 cm) and was allowed to remain in contact with medium for 20 minutes at room temperature.

Agar-well diffusion method

The antimicrobial activity of the samples was assayed by the Agar-well diffusion method [Perez et al. 1990].

Six equidistant wells were made in each Petri dish using sterile cork borers (Ø 7 mm). An amount of 0,05 ml of each salt at five different water solutions was added to each well and one well was filled with 0,05 ml of tryptone soy broth as a control sample using a pipettor (Eppensdorf). The Petri dishes were incubated at 37°C for 24 h.

Measuring the inhibition zone diameter

At the end of the incubation period, inhibition zones which appeared on the medium Petri dishes were calculated in millimeters. The experiment was repeated ten times. The mean values were presented with an accuracy of 0,1 mm.

Results and discussion

The paper presents the antimicrobial activity of twelve salts of phenolic acids which are characterized by different chemical structures against Gram-negative *E. coli* O157:H7. The salts indicated the essentially various inhibitory activities against the examined bacteria. A significant difference in the antimicrobial activities of twelve phenolic substances was observed. The antimicrobial properties of the chosen phenolic salts and their potential usage in the food-pathogen elimination from food were estimated by the measurement of growth inhibition zone diameter. The antimicrobial activities of salts used in the experiment against *E. coli* O157:H7 are presented in Figure 1–3.

Figure 1 shows the antibacterial properties of lithium, sodium and potassium salts of water solution of cinnamic acid against *E. coli* O157:H7.

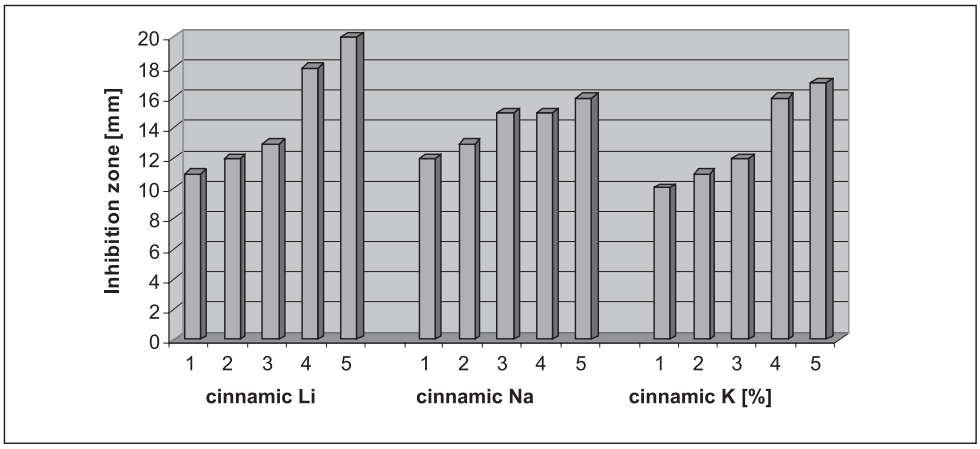

Fig. 1. Relationship between diameter of inhibition zone [mm] and the active substance concentration [%] of lithium, sodium and potassium salts of cinnamic acid against *E. coli* O157:H7

From the achieved results it can be indicated that the salts of cinnamic acid are characterized by a relatively a wide activity against Gram-negative bacteria. It can be stated that lithium, sodium and potassium salts of cinnamic acid are proved to possess comparably high inhibitory activity towards *E. coli* O157:H7. The inhibition zone diameter for a lithium salt of cinnamic acid amounted to 20 mm at 5% concentration of an active substance, 18 mm at 4% concentration, 13 mm at 3% concentration, 12 mm at 2% concentration and 11 mm at 1% concentration. A very strong antibacterial activity towards *E. coli* O157:H7 was observed in case of a sodium salt of cinnamic acid. The inhibition zone diameter for this salt amounted to 16 mm at 5% concentration of an active substance, 15 mm at 4% concentration, 15 mm at 3% concentration, 13 mm at 2% concentration and 12 mm at 1% concentration. A potassium salt of cinnamic acid also proved to be quite influential against *E. coli* O157:H7. The inhibition zone diameter for this salt amounted to 17 mm at 5% concentration of an active substance, 16 mm at 4% concentration, 12 mm at 3% concentration, 11 mm at 2% concentration and 10 mm at 1% concentration.

Figure 2 shows the antimicrobial activities of lithium, sodium and potassium salts of *p*-coumaric acid towards *E. coli* O157:H7.

It was examined observed that the salts of *p*-coumaric acid also possessed a relatively high inhibitory activity against *E. coli* O157:H7 except for a lithium salt of this acid. However, their activities were slightly lower in comparison to the salts of cinnamic acid especially in case of a potassium salt. It was observed that a sodium salt of *p*-coumaric possessed the relatively highest inhibitory activity against *E. coli* O157:H7. The inhibition zone diameter for this salt amounted to 12 mm at 5% concentration of an active substance, 11 mm at 4% concentration, 10 mm at 3% concentration, 10 mm at 2% concentration and 9 mm at 1% concentration. A slightly lower activity was indicated by a potassium salt of this acid. The inhibition zone diameter for this salt amounted to 12 mm at 5% concentration of an active substance, 11 mm at 4% concentration, 11 mm at 3% concentration, and this salt did not indicate any inhibitory activity at its 1% and 2% water solutions. A lithium salt of *p*-coumaric acid was completely ineffective against *E. coli* O157:H7.

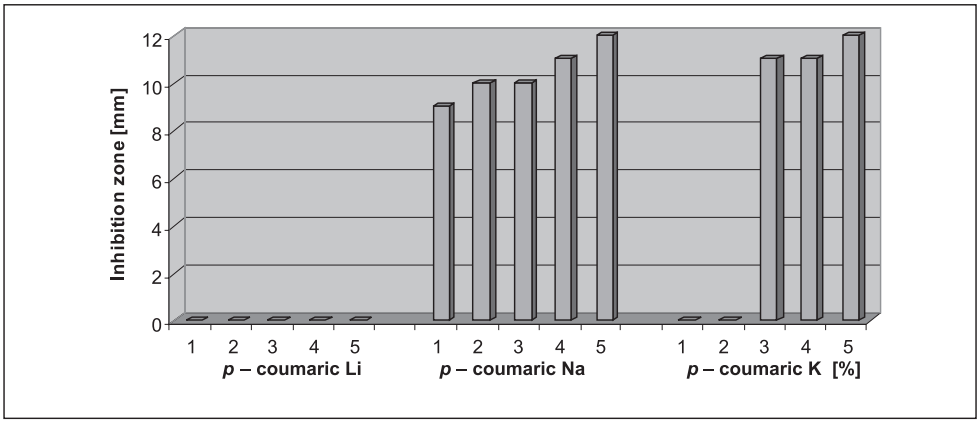

Fig. 2. Relationship between diameter of inhibition zone [mm] and the active substance concentration [%] of lithium, sodium and potassium salts of *p*-coumaric acid towards *E. coli* O157:H7

Figure 3 shows the antimicrobial activities of lithium, sodium and potassium salts of ferulic acid towards *E. coli* O157:H7.

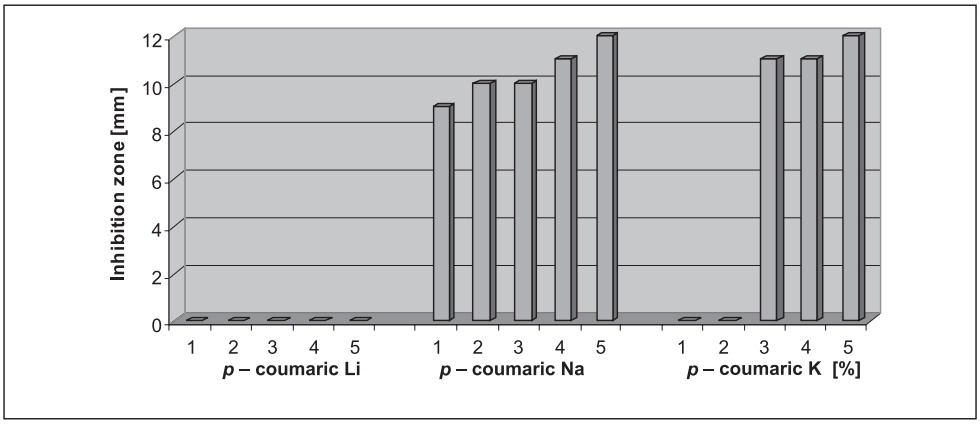

Fig. 3. Relationship between diameter of inhibition zone [mm] and the active substance concentration [%] of lithium, sodium and potassium salts of ferulic acid towards *E. coli* O157:H7

It can be observed that lithium and sodium salts of ferulic acid did not possess any antibacterial influence on the growth inhibition of the examined strain, while a potassium salt does. Its 5% water solution gives the inhibition zone amounting to 12 mm, and its 4% water solution gives the inhibition zone amounting to 11 mm and this is the minimal inhibitory concentration which causes the growth inhibition. The salts of caffeic acid were completely ineffective against *E. coli* O157:H7.

The results of many investigations showed that spices and their essential oils are known to have antimicrobial activity. The antibacterial features of phenolic compounds are strongly associated with the elimination of foodborne pathogenic and spoilage bacteria from food.

They are successfully used in the elimination or inhibition of the growth of *B. subtilis*, *B. cereus* and *S. aureus* in food. They are also known to inhibit the growth of Enterobacter spp. *and Listeria monocytogenes*. Some phenolic compounds are able to inhibit and even eliminate the growth of *Escherichia coli* and *Yersinia enterocolitica*. However, it is known that Gram-positive bacteria are usually more sensitive to phenolic compounds in comparison to Gram-negative bacteria. The mechanism of antibacterial activity of flavonoids is dependent on their ability to inhibit the synthesis of DNA and RNA as well as other related macromolecules [Harborne 1991]. It is also known that phenolic compounds containing more than three -OH groups show better antibacterial activity [Djordjevic et al. 1987]. Many phenolic compounds present in herb spices possess antimicrobial activity. They show an activity particularly towards foodborne pathogens such as *S. aureus*, *B. subtilis* and *E. coli* [Chakraborty et al. 2007].

Phenolic compounds have enjoyed a huge popularity among scientists because they are proved to possess a huge number of beneficial biological properties, which potentially can influence the human health positively [Lohar et al. 2006]. It can be observed that there is a growing number of diseases related to the consumption of contaminated food. People are usually infected by foodborne pathogens. This is a reason why more and more examination should be carried out on how to avoid cross contamination of food. Phenolic compounds are considered to be very effective against pathogenic microorganisms which might be resistant to other methods of their elimination [Bradford 1976, Kar and Mishra 1976, Ride 1983]. It means that the search for new products containing antimicrobial features is justified [Campbell and Ellis 1992]. They should be active against Gram-positive (*B. cereus*, *B. subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *E. coli*) as well as antifungal (*Candida albicans*, *Cryptococcus neoformans*).

The appearance of bacterial resistance towards different factors seems to be a result of the presently applied antibiotics which leads to many bacterial infections. Herbs and spices quite easily produce substances to protect themselves from insects, herbivores and microorganisms [Rossum et al. 1995]. The positive side is the fact that the natural antimicrobial agents are present in the plant extracts and they involve a mixture of compounds containing phenols, acids, esters, aldehydes [Blum et al. 2000]. Fortunately it is not easy for them to acquire resistance against bacteria in comparison to the synthetic antibiotics which constitute a single compound. Such compounds are highly effective towards the elimination of bacteria, fungi, yeast and parasites from food.

Developing countries are concentrating a lot of attention on the food safety, because the consumption of food possesses a huge influence on public health and trade competitiveness. The societies are becoming more and more concerned about the public health consequences doming from the consumption of unsafe food and the risk of foodborne and zoonotic diseases In India for example, it is identified that 20% of deaths among children under five are caused by diarrheal diseases. Despite the fact that the pharmaceutical companies have produced a huge number of new antimicrobial drugs, the resistance of foodborne bacteria towards these drugs has increased a lot [Singleton et al. 1999, Staman et al. 2001]. Bacteria are known to possess the genetic ability to transmit and gain resistance towards drugs. This is why the application of natural antimicrobial agents commonly known as phenolic substances attracts a lot of attention among scientists. Some plants are known to produce bioactive compounds which are able to inhibit or eliminate the growth of certain microorganisms in the food environment [Redmond et al. 1986, Bekkara et al. 1998]. Such substances do not indicate any

toxicity for people and should be used as natural preservatives. Such plant extracts contain substances such as tannins and phenolic compounds. They gained a status of safe preservatives.

Conclusions

It can be summarized that the chosen salts of phenolic acids are known to have different inhibitory properties against *E. coli* O157:H7. It encourages the scientists to carry out much more further experiments to develop the most effective methods enabling to eliminate the risk of pathogen appearance in food products. It can be observed that an essential correlation between antibacterial activity and the concentrations of active phenolic substance content exists. It is reasonable to establish the minimal inhibitory concentration of phenolic substance which causes the elimination of the foodborne pathogen from food. Such natural phenolic compounds may constitute an effective alternative for chemical preservatives in the process of guaranteeing the healthy safety and prolonging shelf-life of food products.

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References

Bekkara F., Jay M., Viricel M.R., Rome S., 1998. Distribution of phenolic compounds within seed and seedlings of two Vicia faba cvs differing in their seed tannin content, and study of their seed and root phenolic exudations. Plant Soil, 203, 27–36.

Blum U., Stamen K.L., Flint L.J., Shafer S.R., 2000. Induction and selection of phenolic acid-utilizing bulk-soil and rhizosphere bacteria and their influence on phenolic acid phytotoxicity. J. Chem. Ecol., 26, 2059–2078.

Bradford M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem., 72, 248–254.

Campbell M.M., Ellis B.E., 1992. Fungal elicitor-mediated responses in pine cell cultures: cell wall-bound phenolics. Phytochemistry, 31, 737–742.

Chakraborty D., Mandal S.M., Chakraborty J., Bhattacharyya P.K., Bandyopadhyay A., Mitra A., Gupta K., 2007. Antimicrobial activity of leaf extracts of Basilicum polystachyon (L) Moench. Indian J. Exp. Biol., 45, 744–748.

Cvikrova M., Nedelnik J., Eder J., Binarova P., 1993. Changes in pattern of phenolic acids induced by culture filtrate of Fusarium oxysporum in alfalfa plants differing in susceptibility to the pathogen. J. Plant Physiol., 142, 1–5.

Devi M.C., Reddy M.N., 2002. Phenolic acid metabolism of groundnut (Arachis hypogaea L.) plants inoculated with VAM fungus and Rhizobium. Plant Growth Regul. 37, 151–156.

De Vries R.P., Michelson B., Poulsen C.H., Kroon P.A., Van Den Heuvel R.H.H., Faulds C.B., Williamson G., 1997. The fae A genes from Aspergillus niger and A. tubingensis encode ferulic acid esterases involved in degradation of complex cell wall polysaccharides. Apel. Environ. Microbiol., 63, 4638–4644.

Djordjevic M.A., Redmond J.W., Batley M., Rolfe B.G., 1987. Clovers secrete specific phenolic compounds which either stimulate or repress nod gene expression in Rhizobium trifolii. EMBO J., 6, 1173–1179.

Fedorova M., Mortel J., Matsumoto P.A., Cho J., Town C.D., VanderBosch K.A., Gantt J.S., Vance C.P., 2002. Genome wide identification of nodule specific transcripts in the model legume Medicago tranculata. Plant Physiol., 130, 519–537.

Griffin P.M., 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*, [in:] Blaser M.J., Smith P.D., Favdin J.I., Greenberg H.B., Guerrant R.L., Eds. Infections of the Gastrointestinal Tract. New York: Raven Press Ltd., 739–761.

Harborne J.B., 1991. The chemical basis of plant defense, [in:] Palo R.T., Robbins C.T. (eds.) Plant defenses against mammalian herbivory. CRC Press, New York, 45–59.

Irisarri P., Milnitsky F., Monza J., Bedmar E.J., 1996. Characterization of rhizobia nodulating Lotus subbliforus from Uruguayan soil. Plant Soil, 180, 39–47.

Kar M., Mishra D., 1976. Catalase, peroxidase, and polyphenol oxidase activities during rice leaf senescence. Plant Physiol., 57, 315–319.

Lohar D.P., Sharopova N., Endre G., Penuela S., Samac D., Town C., Silverstein K.A.T., Vander Bosch K.A., 2006. Transcript analysis of early nodulation events in Medicago tranculata. Plant Physiol., 140, 221–234.

Perez C., Paul M., Bazerque P. 1990. Antibiotic assay by agar-well diffusion method. Acta Biologiae et Medicine Experimentalist, 15, 113–115.

Pueppke S.G., Broughton W.J., 1999. Rhizobium sp. Strain NGR234 and R. fredii USDA257 Share Exceptionally Broad, Nested Host Ranges. MPMI, 12, 293–318.

Redmond J.W., Batley M., Djordjevic M.A., Innes R.W., Kuempel P.L., Rolfee B.G., 1986. Flvones induce expression of nodulation genes in Rhizobium. Nature, 323, 632–635.

Ride J.P. 1983. Cell walls and other structural barriers in defence, [in:] Callow J.A. (Eds.) Biochemical Plant Pathology. Wiley, Chichester, 215–236.

Rossum D., Schuuramns F.B., Gillis M., Muyotcha A., van Versveld H.W., Stouthamer A.H., Boogerd F.C., 1995. Genetic and phenetic analyses of Bradyrhizobium strains nodulating peanut (Arachis hypogaea L.) roots. Apel. Environ. Microbiol., 61,1599–1609.

Seneviratne G., Jayasinghearachchi H.S., 2003. Phenolic acids: Possible agents of modifying N2-fixing symbiosis through rhizobial alteration. Plant Soil, 252, 385–395.

Singleton V.L., Orthofer R., Lameula-Raventos R.M., 1999. Analysis of total phenols and other oxidation substrates and oxidants by means of Folin-Ciocalteau reagent. Methods Enzymol., 299, 152–178.

Staman K., Blum U., Louws F., Robertson D., 2001. Can simultaneous inhibition of seedling growth and stimulation of rhizosphere bacterial populations provide evidence for phytotoxin transfer from plant residues in the bulk soil to the rhizosphere of sensitive species? J. Chem. Ecol., 27, 807–829.

Tori S.D., Dearing M.D., Kursar T.A., 1995. Extraction of phenolic compounds from fresh leaves: a comparison of methods. J. Chem. Ecol., 21, 117–125.

4

EFFECT OF A MIXING TEA AND *KALANCHOE* ON GROWTH OF *STAPHYLOCOCCUS AUREUS*

Introduction

Staphylococci sensitivity to variety of substances included in plants have been the subject of numerous studies. Among plant substances and plants themselves tested in relation to *Staphylococcus aureus* have been plant oils, pits and seeds extracts, pieces of tubers, leaves, dried plants, lavender herbs, flower petals, fruit and succulent tissues [Zhang and Lewis 1997, Shoran et al. 1998, Yoshida et al. 1999, Preuss 2000, Steinka 2003, 2005, Burt 2004, Knowles et al. 2005]. Biostatic impact of different parts of phytoncides on pathogenic bacteria is crucial for the hygiene matters. Biostatic impact of essential oils extracted from plants and water extracts obtained from herbs has been well known for many years. In recent years studies concerning the influence of phytoncides derived from different teas on antibacterial activity have been the center of attention [Hara 2003, Hatano et al. 2003, 2005, Jeong 2004, Stapelton et al. 2004, Tylor et al. 2005].

However, there is not much data on effectiveness of biostatic impact of tea infusions and other plants on *Staphylococcus aureus.* The aim of this research was to evaluate biostatic impact of infusions of different teas with *Kalanchoe sp*. and citrus fruit on *Staphylococcus aureus.*

Materials and methods

The subject of research were infusions of black, green, red teas and white teas, and *Kalanchoe* sp. leaves. The mixture was prepared in following proportions 4: 4: 4: 1: 0,25 and then 500 mL of boiling water was being added. Obtained infusion was cooled down after 10 minutes to temperature of 22° C and then samples were taken and put into sterile laboratory flasks and test tubes of 200 mL, 5 mL, 10 mL and 1 mL capacity. Then adequate quantities of *Staphylococcus aureus* were added to the containers with mixture samples to receive inoculum of 0,5% (A), 1% (C), (B) 50% and such in which the mixture would constitute 1% (D, E) in relation to stock breed *Staphylococcus.*

For investigation *Staphylococcus aureus ATTC 23934* was used. Two types of infusions were prepared: 1 – with *Kalanchoe* additive, 2 – with *Kalanachoe* and lemon juice including 0,26±0,4 g of citric acid.

Samples for research were taken after 30, 60 and 120 minutes of collective incubation and then spread on Baird-Parker RPF agar base. Mathematical objectivation of phenomena using equations were carried in Microsoft Office Excel 2003.

Results

The results of the carried research showed that the prepared mixtures characterized similar degree of biostatic activity in relation to the tested bacteria. Reduced number of *Staphylococcus aureus* amounted from 1,08 log cfu/mL to 4,02 log cfu/mL. The number of these bacteria decreased depending on the ratio between the volume of Staphylococci suspension and volume of infusions.

The maximum reduction of the number of *Staphylococcus aureus* of 4,02 log cfu/mL was stated in infusion where 0,5% of *Staphylococcus* was added to the infusion. The quadratic equation:
$Y = 0{,}898x^2\text{-}5{,}73x + 10{,}7$ pictured the variability of the staphylococci population at these proportions of the infusion and staphylococci suspension (tab.1). In dilutions where proportion between staphylococci and infusion was 1:1, slight reduction of the number of bacteria at a level of 1,08 log cfu/mL was observed.

At 1% of staphylococci in a mixture the reduction number of bacteria had been described by the following linear equation:

$$Y = 0{,}95x + 6{,}36 \text{ for } C_1.$$

Table 1

Parameters of the equations describing the biostatic effect of mixtures in relation to S*taphylococcus aureus*

Type of mixture	Type of equation Equation parameters	
Without citric fruit additive		
A_1	ax^2-bx+c	a-0.898, b-5.731, c-10.708, r^2- 0.935
B_1	ax^2-bx+c	a-0.177, b-1.216, c-7.062, r^2 0.939
C_1	ax^2-bx+c	a-0.49, b-3.398, c-8.805, r^2 0.901
	-ax+b	a—0.948, b-6.355, r^2 0.742
With citric fruit additive		
A_2	ax^2-bx+c	a-0.948, b-5.897, c-10.823, r^2 0.930
B_2	-ax+b	a-035, b-6.225, r^2 0.827
C_2	ax^2-bx+c	a-0.6, b- 3.902, c-9.245, r^2 0.945
	-ax+b	a-0.902, b-6.245, r^2 0.698

Between 30th and 60th minute of interaction between the infusion and staphylococci suspension slight increase of the number of these cocci was observed. Only 74.2% of variation in the number of *Staphylococcus aureus* could be explained by the time of the infusion impact. Observed drifts in the number of bacteria suggest that at such size of the cocci population added to the infusion, the behaviour of staphylococci did not indicate any important biostatic activity of ingredients included in the aquatic extract.

The degree of staphylococci reduction varied slightly for the times of 30 min, 60, and 120 min. The highest biostatic activity of infusions was observed in the 30th minute of incubation with staphylococci regardless of the presence or not of citric acid (Fig. 1, 2).

Observed, in the presence of acid, reduction of the number of staphylococci after 120 minutes was only of 0,30–0,57 log cfu mL higher in comparison with the 30 minutes time.

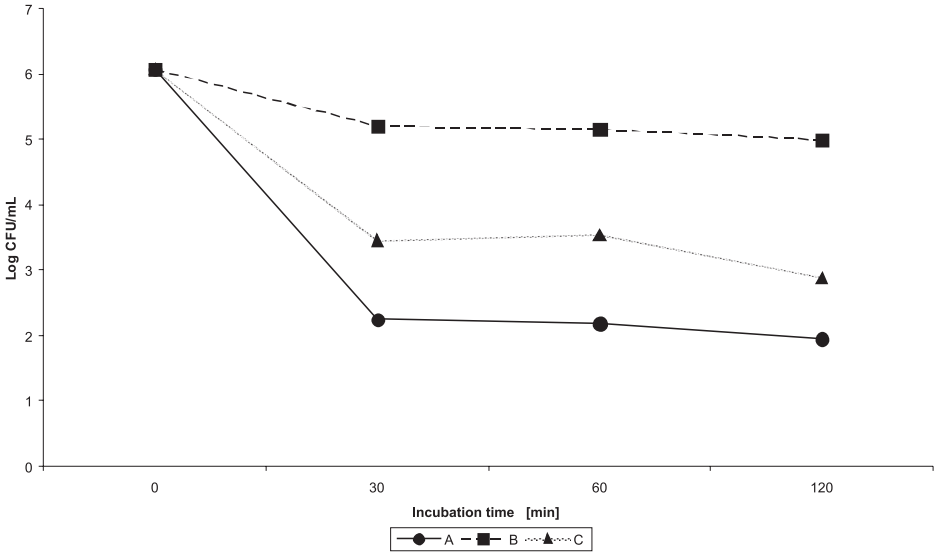


Fig. 1. Influence of infusion on the Staphylococcus aureus in mix without citric acid

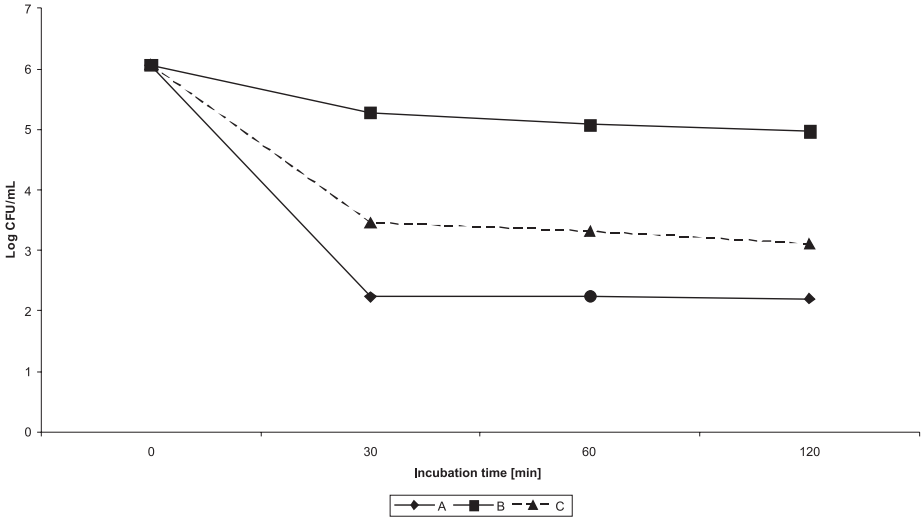


Fig. 2. Influence of infusion on the Staphylococcus aureus in mix with citric acid

Addition of citric acid caused lower degree of inhibiting the population of *Staphylococcus aureus* in comparison to infusions without this additive. These values varied significantly only at the minimal concentration of used infusion (B, C).

The equations used to describe changes in the number of bacteria were of linear character (Tab.1).

$$y = 0, 902x + 6,245 \text{ for } B_2 \text{ and}$$

$$y = 0, 35x + 6,225 \text{ for } C_2.$$

The coefficients of determination equations indicated that more than 30% of reduction in the number of population was not due to the working time of infusion on staphylococci if the infusion contained 1% of staphylococci. 17.3% of the variation in the number of these bacteria was irrespective to the working time of infusion if the proportion between the infusion and staphylococci suspension was 1: 1. Observed differences in the efficiency of the infusions' activity depended not so much on the time of activity but on the level of staphylococci population. The presence of citric fruit in infusions did not intensify biostatic impact of infusions on staphylococci.

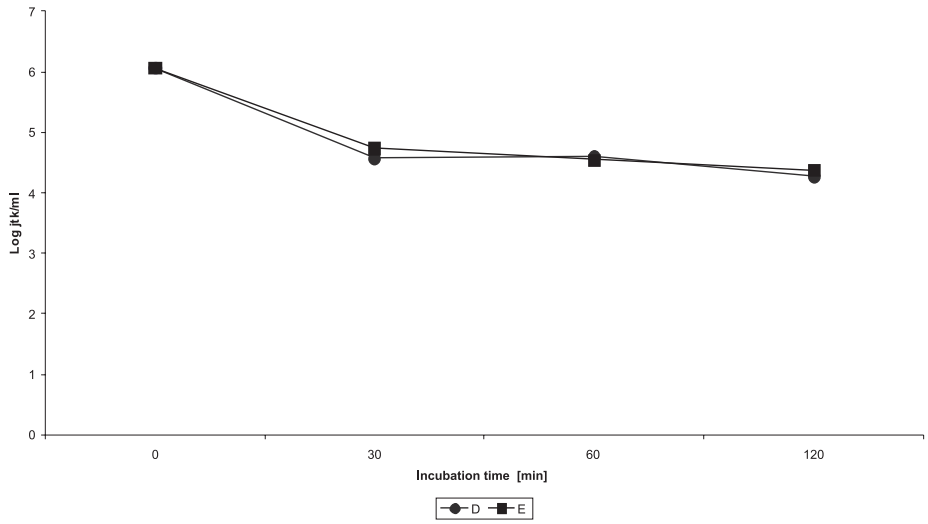


Fig. 3. Influence of 10% infusion on Staphylococcus aureus suspension

It was stated that the presence of 1 ml of infusion in contact with 10 ml of the stock breed staphylococci caused reduction in the number of micro-organisms at a level from 0,99 to 1,09 log cfu/ml.

Discussion

The examined infusion included four types of teas, *Kalanchoe* leaves and citric acid. In teas there are several types of phytoncides and their concentration depends on the type of dried material. Among phytoncides, that were present in tested teas should be mentioned such compounds as epicatechin, epigallocatechin, catechin gallate – according to data provided by reference books covering the subject of relevant biostatic meaning in relation to the micro-flora. Also, included in the teas caffeine could reveal minimum biostatic activity towards staphylococci.

There is little data on biostatic properties of mixed tea infusions. The data shows that, when separately assessed, black and green teas reveal average antibacterial activity towards *Staphylococcus aureus* MRSA [Tylor et al. 2005].

However, much of the earlier data allows the conclusion that epicatechin and catechin gallate are responsible for the anti- staphylococci activity of teas [Yam et al. 1997, Hara 2003].

Other tests on a model system with semi-syntetic catechins and epicatechins showed, that these compounds cause minimum inhibition of staphylococci [Stapleton et al. 2004]. In these studies synergistic effect of biostatic substances included in the mixture that was used as the base for the infusion and citric acid from lemons did not occur. So far reference books' data has not taken into account the researches on biostatic efficiency of multi-component plant mixtures. Until now, carried biostatic researches on binary models do not apply to dried compound infusions of leaves and citric fruit. [Steinka 2005].

From the data presented by Hammer et al. [1999] it appears that for the inhibition of *Staphylococcus aureus* the minimum inhibitory concentration MIC for the essential oils from lemon is 2% v/v. The content of acids in citric fruits' pulp explains biostatic impact of lemons, in which citric acid concentration in juice is 51,87– 60,32 gL^{-1}. The impact of lemon juice itself on staphylococci was evaluated in our earlier studies [Steinka and Kukułowicz 2008]. As far as synergistic impact of *Kalanchoe* and tea mixture on staphylococci was stated, as no synergistic impact of plant mixture – citric fruit against *Staphylococcus aureus* population was not observed. This might have been the effect of too low concentrations of citric acid in the mixture. In the tested mixture infusions of staphylococci and citric acid its concentration did not exceed 0,4 g. Other studies proved, that effective inhibition of staphylococci in the presence of lemon juice occurs if fruit quantity allows the presence of 1.03–1,51 g of citric acid. Confirmation of this thesis may be the fact, that inhibitory impact of the lemon juice itself on staphylococci was effective in model conditions, what proved our earlier studies [Steinka and Kukułowicz 2008]. Those studies revealed that observed dynamics of inhibition of *Staphylococcus aureus* population development by lemon juice was the highest in relation to other citric fruit juices and decreased the staphylococci population by 1,85 cfu/cm^3 during the 2 hour incubation time. Recorded in these tests reduction level of the mixture containing citric acid from the fruit was of about 2 log cfu/lower than for infusions without lemon additive. This could also be caused by interactions between organic acids present in the lemon juice and phytoncides present in dried tea leaves and *Kalanchoe* leaves.

Conclusions

1. Citric acid additive in quantity for 0,8g/L reduces biostatic impact of tested compound plant mixture on staphylococci.
2. Selected mixture of black, white, green and red teas with Kalanchoe effectively inhibits the development of the population of staphylococci.
3. Biostatic effect is intense within 30 minutes of incubation, it is less dependent on further collective incubation time and more dependent on the number of staphylococci.

References

Anesini E., Perez C., 1993. Scrining of plants used in argentine folk medicine for antimicrobial activity. J. Ethnopharmacol., 39, 119–128.

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

Hammer K.A., Garson C.F., Rile T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. J.Appl. Microbiol., 86, 985–990.

Hara Y., 2003. Green tea: health benefist and applications. Marcel Dekker NY, USA.

Hatano T., Kusuda M., Shiota S., Tsuchyiya T., Yoshida T., 2003. Theasinensis A, a polyphenol formed from (-)-epigallacatechin gallate, suppresses antibiotic resistance of methicillin – resistant Staphylococcus aureus. Planta Med., 69, 11, 984–989.

Hatano T., Kusuda M., Shiota S., Tsuchyiya T., Yoshida T., 2005. Effect of tannins and related polyfphenols on methicillin – resistant *Staphylococcus aureus*. Phytochemistry, 66, 17, 2047–2055.

Jeong W.S., Kong A.T., 2004. Biological properties of monomeric and polymeric catechins: green tea catechins and procyanidins, Pharmaceutical Biology, 42, 84–93.

Knowles J., Roller S., Murray D.B., Naidu A.S., 2005. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella* enterica serovar typhimurium, Appl. Environ. Microbiol., 71, 2, 797–803.

Preuss H.G., 2000. Botanicals as therapeutics. http://nabc.cals.cornell.edu/pubs/nabc_14.pdf

Shoran B.G., Purrington S.T., Breidt F., Fleming H.P., 1998. Antimicrobial properties of sinirgin and its hydrolysis products, J. Food Sci., 63, 4, 621–624.

Stapelton P.D., Shah S., Anderson J.C., Hamilton-Miller M.J., Taylor P.W., 2004. Modulation of beta-lactam resistance in Staphylococcus aureus by catechins and gallates. Int. J. Antimicrob. Agents, 23, 5, 462–467.

Steinka I., Wilczyńska A., Stankiewicz J., 2003. Attempts towards the application of natura plant preparation of lactic acid cheese curd In order to inhibit microflora reinfecting the product. 18th ICFMH Symposium Lillehammer-Norway, 18–23.08.2002.

Steinka I., 2005. Comparison of biostatic action of Sorbus aucuparia and Aloe arborescens under model conditions and in food, The IX International Congress Actual Problems of creation of new medicinal preparations of nutral origin Phytopharm. St. Petersburg June 22–25, 381–383.

Steinka I., Kukułowicz A., 2008. Biostatyczne oddziaływanie miazgi i roztworów roślinnych na gronkowce. Bromatologia i Chemia Toksykologiczna, XLI, 2, 191–196.

Tylor P.W., Hamilton-Miller J.M.T., Stapleton P.D., 2005. Antimicrobial properties of green tea catechins. Food Sci. Technol. Bull., 2,71–81.

Yam T.S., Shah S., Hamilton-Miller J.M.T., 1997. Microbiological activity of whole and fractionated crude extracts of tea (Camillia sinensis), and of tea components. FEMS Microbiol. Lett., 152, 169–174.

Yoshida H., Katsuzaki H., Ohta R., Ishikawa K., Fukuda H, Fujino T., Suzuki A., 1999. An organosulfur compound isolated from oil-macerated garlic extract, and its antimicrobial effect. Bioscience, Biotechnol. Biochem., 63, 3, 588–590.

Zhang Y., Lewis K., 1997. Fabatines: new antimicrobial plant peptides. FEMS Microbiol. Lett. 149, 59–64.

5

THE QUALITY ASSESMENT OF THE MEDIUM GROUND GRILL SAUSAGES

Introduction

Raw meat intended for culinary preparation in conditions with limited standard of sanitary (e.g. garden party) should characterized by particularly high microbiological quality. If it possible, should be also characterized by minimum advanced of proteolytic modifications of proteins as well as hydrolytic and oxidative changes of lipids [Derewiaka and Obiedziński 2007]. Manufacturers have to ensure the high microbiological standard of meat and meat products cause of possibility of improper preserving while cooling or freezing. Also there is a possibility of ineffective heat treatment while preparing meat for consumption by consumer in non-standard conditions. The advancement of protein degradation causing a risk of the formation of carcinogenic N-nitrosamines. This risk is particularly great when sausages containing nitrates (III) were heat treatment at high temperature (e.g. grilling) [Szmańko 1984, Szmańko and Dworecka 2007]. Advanced changes in the distribution of fat are factors stimulating the further oxidation process in lipids, but also effect production of carcinogenic oxysterols [Derewiaka and Obiedziński 2007].

To ensure the highest quality of meat the storage and preparing conditions have to be provided. Optimal storage conditions associated with the culinary preparation of grilled meat ensured also (nutritional as well as sanitary safety of consumers and their satisfaction.

Materials and methods

The experimental material consisted of medium ground grill sausages produced in industrial conditions in three production batches. The grill sausages were packed in vacuum after 24-hour cooling storage (for 5 samples of sausages in each package). During the packaging was used elimination of 90% of the air or inert gas atmosphere (80% N_2, 20% CO_2). The grill sausages were stored in the refrigerator at 3±1°C or in a state of deep cooling (at near cryoscopic temperature -3±1°C). Samples were storage for 0 (control-K), 7, 14, 21, 28 days.

In experimental material physical and chemical analyses were conducted. Acidity (pH value) was measured with MICROCOMPUTER CP 55 pH meter. The free amine groups content was evaluated with method described by Kuchro et al. [1983] with the modifications of Chrzanowska et al. [1983]. In order to lipids status analyses, such as peroxide value, acid number and TBA experimental grill sausages were heated in dryer at 50°C for 12 h. Following lipids extraction was performed by the addition of petroleum ether and shaking for 12 h

at room temperature. Obtained lipids, after ether removal, were subjected to further analyses: peroxide value [PN-ISO 3960:1996], acid number [PN-ISO 660:1998/Az1:2000], level of thiobarbituric reactive substances (TBA) according to Pikul et al. [1989].

Analyses of microbiological status included: general number of aeorobic bacteria [PN--EN ISO 4833:2004], molds and yeasts [PN-ISO 21527-1:2009], number of lactic acid bacteria [PN-ISO 15214:2002], the present of *Salmonella* [PN-EN ISO 6579:2003], *Escherichia coli* [PN-ISO 4832:2007], coagulase-positive *Staphylococcus* [PN-EN ISO 6888-1:2001], pathogenic bacteria [PN-EN ISO 7937:2005].

Statistical analysis of the results was carried out using STATISTICA 9.0 software where averages, standard deviations, least significant differences and estimation of differences between mean values at $p < 0.05$ were calculated.

To the mark the various experimental groups used the following shortcuts: K – control; 3V, 3M,-3V,-3M (7, 14, 21, 28) – packed in vacuum grill sausage (V) or in modified atmosphere (M), stored at 3°C and -3°C for 7, 14, 21 or 28 days.

Results and discussion

The pH value of not stored meat was measured at level 6.25 and was characteristic for heat-treated and not ripened meat products (Tab. 1) [Szmańko 1984, Szmańko et al. 2007].

Acidity of meat products (measured as pH value) depends on the chemical content of raw material and the curing mixture also is associated with the natural pH value of meat [Neer and Mandigo 1977, Słowinski and Mroczek 1994].

Further the storage periods were generated systematic decrease in pH value of grill sausages. Regularity observed in the whole experiment was slowly progressive acidification of the grill sausages stored at near cryoscopic temperature. From 21 days of storage more dynamic acidification of vacuum packed sausages was observed.

Decreasing of the pH value in stored meat products is generally reported in the literature [Szmańko 1998; Szmańko et al. 2005, 2006a, 2006b]. Also, in the literature can be found reports of more dynamic changes in the level of pH in products stored in vacuum packages [Wasiliewa et al. 1979, Xiong and Anglemier 1989].

By analyzing the main effects the method of packaging and storage temperatures have no effect on pH value of the experimental meat products.

During the storage time of experimental sausages was observed the systematic formation of free amino groups (Tab. 2). The changes in proteins of stored meat are generally reported in the literature [Szmańko et al. 1986, 1988, Szmańko and Sieniakowski 1991, Szmańko 1998]. After 21 days of storage in all four groups of experimental sausages (3V, 3M, -3V, -3M) a significant growth of free amino groups was observed.

Table 1

The pH value of the grill sausage, n=18

Experimental group			Parameters				
Storage temperature [°C]/ Packaging method			3		-3		NIR
			vacuum	map	vacuum	map	
Storage period [days]	K	$\bar{x}$	6.25^{D}	6.25^{D}	6.25^{B}	6.25^{A}	–
		sd	0.03	0.03	0,03	0.03	
	7	$\bar{x}$	6.04^{abCD}	5.90^{aC}	$6.18b^{AB}$	6.06^{abA}	0.203
		sd	0.07	0.10	0,03	0.18	
	14	$\bar{x}$	5.70^{aBC}	5.69^{aB}	6.04^{bAB}	6.06^{bA}	0.305
		sd	0,19	0.06	0.17	0.24	
	21	$\bar{x}$	5.45^{aAB}	5.61^{abAB}	5.95^{bAB}	5.98^{bA}	0.397
		sd	0.21	0.04	0.27	0.25	
	28	$\bar{x}$	5.25^{aA}	5.43^{aA}	5.87^{bA}	5.90^{bA}	0.342
		sd	0.36	0.19	0.30	0.34	
NIR			0.377	0.183	0.359	0.418	
$\bar{x}_{p\ (n=90)}$			5.74	5.78	6.05	6.04	
NIR			0.534		0,212		
$\bar{x}_{t\ (n=180)}$			5.75^{a}		6.05^{b}		
NIR			0.247				

$\bar{x}$ – mean value for the experimental group sd – standard deviation

$\bar{x}_{t}$ – mean values for grill sausages storage at the same temperature

$\bar{x}_{p}$ – mean values for identically packaged grill sausages at the same temperature

a, b, c – mean values in the same row denoted by different capital letters are significantly differ at level of $p \leq 0.05$

A, B, C… – mean values in the same columns denoted by different capital letters are significantly differ at level of $p \leq 0.05$

Table 2

The free amino groups of the grill sausages [μg Gly/g protein], n=18

Experimental group			Parameters				
Storage temperature [°C]/ Packaging method			3		-3		NIR
			vacuum	map	vacuum	map	
Storage period [days]	K	$\bar{x}$	4 034^{A}	4 034^{A}	4 034^{A}	4 034^{A}	–
		sd	286	286	286	286	
	7	$\bar{x}$	4 039^{A}	4 051^{A}	4 065^{A}	4 026^{A}	328
		sd	291	280	266	301	
	14	$\bar{x}$	4 748^{AB}	4 767^{AB}	4 743^{AB}	4 724^{AB}	659
		sd	582	591	537	579	
	21	$\bar{x}$	4 932^{B}	4 963^{B}	4 997^{AB}	4 974^{B}	663
		sd	585	532	556	626	
	28	$\bar{x}$	5 027^{B}	4 943^{B}	4 940^{A}	4 880^{AB}	642
		sd	484	528	541	658	
NIR			846	842	830	939	
$\bar{x}_{p\ (n=90)}$			4 556	4 551	4 556	4 527	
NIR			442		447		
$\bar{x}_{t\ (n=180)}$			4 553		4 541		
NIR			301				

$\bar{x}$ – mean value for the experimental group sd – standard deviation

$\bar{x}_t$ – mean values for grill sausages storage at the same temperature

$\bar{x}_p$ – mean values for identically packaged grill sausages at the same temperature

a, b, c – mean values in the same row denoted by different capital letters are significantly differ at level of $p \leq 0.05$

A, B, C… – mean values in the same columns denoted by different capital letters are significantly differ at level of $p \leq 0.05$

Due to the degree of protein degradation the raw material can be divided into two groups. One constituted of a samples stored for 21 days, which characterized by the advancement of smaller changes. The second group consisted of samples with more advanced changes, storage for 21 and 28 days. The applied methods of packaging and storage temperatures had no effect on the dynamics of changes in proteins.

The hydrolytic changes of fat contained in experimental sausages were progressed with the expiry of their time of storage (Tab. 3 and 4). Depending on the advanced of lipids hydrolytic changes the experimental products can be divided into two groups. Up to 14 days

of storage samples of grill sausages were characterized by a lower level of the acid number. Others were characterized by significantly higher advanced of hydrolysis changes in fat. The applied methods of packaging and storage temperatures had no effect on this parameter.

Table 3

The acid value of the grill sausage [mg 0,1N KOH/1 g total fat], n=18

Experimental group			Parameters				
Storage temperature [°C]/ Packaging method			3		-3		NIR
			vacuum	map	vacuum	map	
Storage period [days]	K	$\bar{x}$	1.11^{A}	1.11^{A}	1.11^{A}	1.11^{A}	–
		sd	0.05	0.05	0.05	0.05	
	7	$\bar{x}$	1.27^{AB}	1.25^{AB}	1.24^{AB}	1.25^{AB}	0.212
		sd	0.16	0.15	0.15	0.17	
	14	$\bar{x}$	1.51^{AB}	1.50^{AB}	1.49^{AB}	1.47^{AB}	0.431
		sd	0.34	0.32	0.33	0.31	
	21	$\bar{x}$	1.87^{BC}	1.84^{BC}	1.83^{BC}	1.81^{BC}	0.354
		sd	0.27	0.26	0.25	0.26	
	28	$\bar{x}$	2.44^{C}	2.41^{C}	2.39^{C}	2.37^{C}	0.351
		sd	0.58	0.56	0.57	0.54	
NIR			0.697	0.678	0.679	0.667	
$\bar{x}_{p\ (n=90)}$			1.64	1.62	1.61	1.60	
NIR			0.767		1.086		
$\bar{x}_{t\ (n=180)}$			1.63		1.61		
NIR			0.567				

$\bar{x}$ – mean value for the experimental group sd – standard deviation

$\bar{x}_{t}$ – mean values for grill sausages storage at the same temperature

$\bar{x}_{p}$ – mean values for identically packaged grill sausages at the same temperature

a, b, c – mean values in the same row denoted by different capital letters are significantly differ at level of $p \leq 0.05$

A, B, C… – mean values in the same columns denoted by different capital letters are significantly differ at level of $p \leq 0.05$

After 28 days of storage in samples of grill sausages occurred hydrolytic changes. The level of these changes was not generated health hazard.

A similar dynamics of hydrolytic rancidity of stored sausages in other studies was observed [Szmańko et al. 1984, 2004, 2006b].

Table 4

The peroxide value of the grill sausage [ml Na2S2O3/ 1 g total fat], n=18

Experimental group			Parameters				
Storage temperature [°C]/ Packaging method			3		-3		NIR
			vacuum	map	vacuum	map	
Storage period [days]	K	$\bar{x}$	0.81^{A}	0.81^{A}	0.81^{A}	0.81^{A}	–
		sd	0.03	0.03	0.03	0.03	
	7	$\bar{x}$	1.11^{A}	1.07^{A}	1.09^{abB}	1.04^{aA}	0.078
		sd	0.05	0.02	0.06	0.04	
	14	$\bar{x}$	1.25^{BA}	1.16^{abA}	1.22^{abB}	1.12^{aA}	0.108
		sd	0.07	0.07	0.06	0.05	
	21	$\bar{x}$	2.22^{aB}	2.04^{aB}	1.95^{aC}	1.88^{aB}	0.532
		sd	0.44	0.43	0.33	0.31	
	28	$\bar{x}$	3.52^{bC}	3.45^{abC}	3.26^{abD}	3.11^{aC}	0.374
		sd	0.36	0.33	0.18	0.13	
NIR			0.555	0.529	0.372	0.325	
$\bar{x}_{p\ (n=90)}$			1.78	1.70	1.67	1.59	
NIR			0.594		0.404		
$\bar{x}_{t\ (n=180)}$			1.74		1.63		
NIR			0.404				

$\bar{x}$ – mean value for the experimental group sd – standard deviation

$\bar{x}_t$ – mean values for grill sausages storage at the same temperature

$\bar{x}_p$ – mean values for identically packaged grill sausages at the same temperature

a, b, c – mean values in the same row denoted by different capital letters are significantly differ at level of $p \leq 0.05$

A, B, C… – mean values in the same columns denoted by different capital letters are significantly differ at level of $p \leq 0.05$

Popular grill sausages consisted of more than 30% of fat and up to 40% mechanically recovered meat, which are subjected to hydrolytic and oxidative rancidity. Oxidative status of lipids is particularly important because some products of rancidity process could negative influence on human health [Feiner 2006, Romans et al. 1994].

The experimental conditions of grill sausages storage generated systematic growth of oxidation changes. Between the experimental groups differentiation in the intensity of these changes were found after 14 days period of storage. The most dynamic oxidation in the vacuum packed grill sausages stored in cold conditions was observed. However, measured fats numbers not exceed acceptable standards.

After 21 days a positive impact of stored at near cryoscopic temperature was observed in lower changes in measured parameters. These trends were also confirmed in the literature [Szmańko 1984, Szmańko et al. 2004]. Next 7 days of storage of grill sausages increased the level of oxidation changes to degree of health hazard.

By analyzing the main effects (mean values of various experimental groups without periods of storage or method packaging) had no the method packaging and storage temperatures have no effect on the advanced of oxidation changes of fat.

Similarly as in case of the acid and peroxide value the long storage time of grill sausage resulted in dynamic changes in formulation of malonic aldehyde. In all four experimental groups after 7 days of storage statistically significant changes were recorded (Tab. 5). Almost within each experimental group a further period of storage generated a significant increase in the content of thiobarbituric acid reactive substances. Only between attempts 3M7 and 3M14 differences were not significant. Surprising was the absence of diversity of malonic aldehyde content in the grill sausages in each group after 28 days of storage.

Table 5

The TBA value of the grill sausage [mg malonic aldehyde/kg of products], n=18

Experimental group			Parameters				
Storage temperature [°C]/ Packaging method			3		-3		NIR
			vacuum	map	vacuum	map	
Storage period [days]	K	$\bar{x}$	0.41^{A}	0.41^{A}	0.41^{A}	0.41^{A}	–
		sd	0.03	0.03	0.03	0.03	
	7	$\bar{x}$	0.64^{B}	0.59^{B}	0.62^{B}	0.62B	0.069
		sd	0.06	0.08	0.04	0.60	
	14	$\bar{x}$	0.84^{cC}	0.63^{aB}	0.77^{bC}	0.82^{bcC}	0.064
		sd	0.05	0.06	0.07	0.07	
	21	$\bar{x}$	1.18^{D}	1.15^{C}	1.14^{D}	1.11^{D}	0.139
		sd	0.11	0.11	0.12	0.13	
	28	$\bar{x}$	1.51^{E}	1.52^{D}	1.50^{E}	1.51^{E}	0.079
		sd	0.09	0.06	0.07	0.06	
NIR			0.132	0.127	0.128	0.137	
$\bar{x}_{p\,(n=90)}$			0.92	0.86	0.89	0.90	
NIR			0.206		0.203		
$\bar{x}_{t\,(n=180)}$			0.88		0.89		
NIR			0.210				

$\bar{x}$ – mean value for the experimental group sd – standard deviation

$\bar{x}_t$ – mean values for grill sausages storage at the same temperature

$\bar{x}_p$ – mean values for identically packaged grill sausages at the same temperature

a, b, c – mean values in the same row denoted by different capital letters are significantly differ at level of $p \leq 0.05$

A, B, C… – mean values in the same columns denoted by different capital letters are significantly differ at level of $p \leq 0.05$

Grounded raw material is also subjected to microbiological contamination and analysis of it microbiological purity is particularly important in prevention of consumer health.

The significant differences in the level of microbiological contamination of grill sausages within individual experimental groups were observed after 7 days of storage (Tab. 6). The largest number of microorganisms was found in the experimental group of sausages 3M7. The grill sausages packaged in vacuum were less microbiological infected. Also storage at lower temperatures was effective in inhibition of microorganism's growth. The positive influences of storage, especially at temperature near cryoscopic was confirmed by the results of previous studies [Szmańko et al. 1990, 2004].

Table 6

The total microorganism count of the grill sausages [jtk/g], n=18

Experimental group			Parameters				
Storage temperature [°C]/ Packaging method			3		-3		NIR
			vacuum	map	vacuum	map	
Storage period [days]	K	$\bar{x}$	733^{BC}	733^{A}	733^{B}	733^{BC}	–
		sd	289	289	289	289	
	7	$\bar{x}$	540^{aAB}	$2\,200^{cD}$	$666a^{bB}$	830_{aA}	211
		sd	217	352	251	171	
	14	$\bar{x}$	933^{bC}	767^{aAB}	833^{bB}	666^{bC}	259
		sd	205	189	104	252	
	21	$\bar{x}$	353^{aA}	$1\,163^{bB}$	350^{aA}	300^{aA}	218
		sd	157	220	87	58	
	28	$\bar{x}$	366^{aA}	$1\,606^{bC}$	376^{aA}	447^{aA}	224
		sd	115	1 160	143	127	
NIR			233	255	218	154	
$\bar{x}_{p\,(n=90)}$			585^{a}	$1\,294^{b}$	592	595	
NIR			501		248		
$\bar{x}_{t\,(n=180)}$			939^{b}		593^{a}		
NIR			416				

$\bar{x}$ – mean value for the experimental group sd – standard deviation

$\bar{x}_{t}$ – mean values for grill sausages storage at the same temperature

$_{p}$ – mean values for identically packaged grill sausages at the same temperature

a, b, c – mean values in the same row denoted by different capital letters are significantly differ at level of $p \leq 0.05$

A, B, C… – mean values in the same columns denoted by different capital letters are significantly differ at level of $p \leq 0.05$

After 28 days of storage the total microorganism content in samples of meat products of experimental groups: 3V,-3V and -3M were lower almost twice time than in control samples (Tab. 7). Only in samples of experimental group 3M was not observed reduction of the amount of microorganisms. Reducing the level of microbial contamination, not only grill sausages but also of meat stored at temperature near cryoscopic were observed also in previous studies [Szmańko et al. 1990, 2007].

Table 7

The total microorganism content in different production batch of the grill sausages [jtk/g]

Storage temperature [C°]	Packaging method	Production batch	Period storage [days]				
			K	7	14	21	28
3	Vacuum	I	900	400	400	260	500
		II	400	320	1 500	600	300
		III	900	900	3 000	200	300
	Map	I	900	300	500	2 500	300
		II	400	4 200	700	90	4 100
		III	900	2 100	1 100	900	420
-3	Vacuum	I	900	400	400	450	410
		II	400	1 300	900	300	220
		III	900	300	1 200	300	500
	Map	I	900	710	400	400	520
		II	400	500	700	300	300
		III	900	1 400	900	300	520

After 14 days of storage in cold conditions was observed presence of lactic acid bacteria in samples of experimental sausages (Tab. 8). These bacteria were determined in grill sausages packaged in vacuum and inert gases. Increasing number of lactic acid bacteria was associated with a decrease of the pH value in these products. Sustained time of storage (21 and 28 days of storage) had effects in almost doubled number of these microorganisms in samples of meat products packaged in vacuum.

The propagation of microorganisms in vacuum packaged meat products is generally reported in the literature [Wasiliewa et al. 1979, Xiong and Anglemier 1989].

In the experimental grill sausages the presents of other groups of microorganisms i.e. molds, yeasts, aerobic coli form bacteria (in 0.1 g), pathogenic bacteria (in 0.1 g), Salmonella bacteria (in 25 g) and *Staphylococcuss* was not observed.

Table 8

The number of lactic acid bacteria in different production batch of the grill sausages [jtk/g]

Storage temperature [Cº]	Packaging method	Production batch	Period storage [days]				
			K	7	14	21	28
3	Vacuum	I	0	0	0	20	50
		II	0	0	30	70	80
		III	0	0	0	0	0
	Map	I	0	0	80	20	210
		II	0	0	0	0	0
		III	0	0	0	0	0
-3	Vacuum	I	0	0	0	0	0
		II	0	0	0	0	0
		III	0	0	0	0	0
	Map	I	0	0	0	0	0
		II	0	0	0	0	0
		III	0	0	0	0	0

Conclusions

Storage conditions had an effect on measured parameters of experimental grill sausages. Storage in temperature near cryoscopic (-3ºC) had a positive effect on oxidation and microbiological status of samples under investigation.

The applied packaging methods had no influence on the stability of grill sausages stored at 3ºC and -3°C.

The maximum period of storage of grill sausages packaged in vacuum or inert gases (due to the advanced fat oxidation) at chilling conditions and at near cryoscopic point, should not exceed 21 days.

Due to the negative changes of the grill sausages store at chilling conditions application of antioxidants, e.g. of natural origin, in the production of these kinds of products should be considered.

References

Chrzanowska J., Kołaczkowska M., Polanowski A., 1993. Production of exocellular proteolytic enzymes by various species of *Penicillium*. Enz. Mikrob. Techn., 15, 140–143.

Derewiaka D., Obiedziński M., 2007. Modelowe badania nad utlenianiem steroli. Żywność. Nauka. Technologia. Jakość., 5 (54), 335–345.

Feiner G., 2006. Meat Products Hanbook. CRC Press.
Kuchro C.N., Rahilly J., Fox P.F., 1983. Assessment of proteolysis in cheese by reaction with trinitrobeneze sulphonic acid. Irish J. Food Sci. Techn., 7, 129–133.
Neer K.L., Mandigo W.R., 1977. Effect of salt, sodium tripoliphosphate and frozen storage time on properties of flaked, cured pork product. J. Food Sci., 42, 738–742.
Pikul J., Leszczyński D., Kumerrow F., 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agric. Food Chem., 37, 1309–1313.
PN-ISO 3960, 1996. Oleje i tłuszcze roślinne oraz zwierzęce. Oznaczanie liczby nadtlenkowej.
PN-ISO 660, 1998/Az1, 2000. Oleje i tłuszcze roślinne oraz zwierzęce. Oznaczanie liczby kwasowej.
PN-EN ISO 6888–1, 2001. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby gronkowców koagulazo-dodatnich (*Staphylococcus aureus* i innych gatunków). Część 1: Metoda z zastosowaniem pożywki agarowej Baird-Parkera.
PN-ISO 15214, 2002. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby mezofilnych bakterii fermentacji mlekowej. Metoda płytkowa w temperaturze 30°C.
PN-EN ISO 6579, 2003. Mikrobiologia żywności i pasz. Horyzontalna metoda wykrywania *Salmonella spp*.
PN-EN ISO 4833, 2004. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby drobnoustrojów. Metoda płytkowa w temperaturze 30°C.
PN-EN ISO 7937, 2005**.** Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby *Clostridium perfringens*. Metoda liczenia kolonii.
PN-ISO 4832, 2007. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby bakterii z grupy coli. Metoda płytkowa.
PN-ISO 21527-1, 2009. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby drożdży i pleśni. Część 1: Metoda liczenia kolonii w produktach o aktywności wody wyższej niż 0,95.
Romans J., Castello W., Carlom C., Greaser M., Jones K., 1994. The Meat We Eat. Interstate Publisher, Inc.
Słowiński M., Mroczek J., 1994. Wpływ obniżonej ilości soli kuchennej na właściwości technologiczne, sensoryczne i stan mikrobiologiczny farszów mięsnych. Przem. Spoż., 3, 86–89.
Szmańko T., 1984. Wpływ przechowywania w stanie zamrożonym szynek wieprzowych nie puszkowanych na wybrane parametry fizykochemiczne, obraz histologiczny oraz poziom lotnych N-nitrozoamin. Zeszyty Naukowe AR Wrocław, Technologia Żywności. III, 149, 23–43.
Szmańko T., 1998. Ocena efektywności przechowywania wędzonek w temperaturze bliskiej krioskopowej oraz w stanie zamrożonym (badania modelowe). Zeszyty Naukowe AR Wrocław, Rozprawa 334, CLIV, 1–124.
Szmańko T., Duda Z., Ogonowska D., 1986. The quality of uncanned ham as influenced by long-term storage at cryoscopic temperature. Recent advances and developments in the refrigeration of meat by chilling. Proc. International Institute of Refrigeration. Bristol, Commission, 2, 329–337.
Szmańko T., Duda Z., Kajdan L., Kubis B., 1988. Storage of selected sort of processed meat product at cryoscopic temperature – an attempt at energy conservation. Changes in proteins, amino acids balance and in vitro digestibility of cured smoked raw pork-loin. Acta Alimen. Pol., 2, 145–156.
Szmańko T., Duda Z., Kuba J., 1990. Changes of selected quality parameters of cured, smoked raw pork-loin during storage at near cryoscopic temperature, Proc. 36th ICoMST, Cuba Havana III, 819–826.
Szmańko T., Duda Z., Szczepański J., Dworecka E., 2004. Zmiany przechowalnicze tłuszczu oraz zanieczyszczenie mikrobiologiczne wędzonek w zależności od warunków przechowywania. Żywność. Nauka. Technologia. Jakość, 2, (39), 46–58.

Szmańko T., Malicki A., Cichoń A., Brużewicz S., Dworecka E., 2005. Quality of sopocka pork loin wrapped directly post thermal treatment or after chilling and stored at near cryoscopic temperature. Pol. J. Food Nutr. Sci., 14/55, 111–116.

Szmańko T., Malicki A., Nawrat A., Brużewicz S., Dworecka E., 2006a. Shelf-life of homogenized sausage depends on the moment it was placed at near cryoscopic temperature. EJPAU. Vet. Med., 9, 1, 1–10.

Szmańko T., Malicki A., Nowara M., Brużewicz S., Dworecka E., 2006b. Ocena trwałości wędzonek powierzchniowo traktowanych substancjami bakteriostatycznymi, przechowywanych w temperaturze bliskiej krioskopowej. Acta Sci. Pol. Med. Vet., 6,11–24.

Szmańko T., Dworecka E., 2007. Problem rakotwórczych N-nitrozoamin w przetworach mięsnych. Prace Inst. Zarz. i Inż. Rol. PWSZ w Sulechowie. Tech. Żyw. i Żyw. Człow., 1, 83–94.

Szmańko T., Korzeniowska M., Kołodziejczyk J., 2007. The effect of plasma and myogel addition on the quality of homogenised sausages. Pol. J.Food Nutr. Sci., 2007, 57, 4 (C), 529–534.

Szmańko T., Sieniakowski S., 1991. Gefrierlagerung von geräuchertem Schweinefleisch. Veränderungen der Sarkoplasmaproteine und ausgewählter physikalisch-chemischer Eigenschaften bei Lagerung in Gefrierpunktnähe. Fleischwirtschaft, 71, 11, 1337–1340.

Wasiliewa L., Balandina G., Kanykina L., 1979. Isledowanija kacziestwa ochłażdionnych naturalnych połufabrikatow iz świniny pri chranieni w usłowijach wakuuma. Proc. 24th EMMRW, Budapeszt , Hangary, 5, 23, 395–397.

Xiong Y., Anglemier A., 1989. Gel electrophoretic analysis of the protein changes in ground beef stored at 2ºC. J. Food Sci., 54, 2, 287–291.

6

COMPARISON OF SALMONELLA ENTERITIDIS SURVIVAL IN PROBIOTIC RIPENING SOFT CHEESE WITH THE GROWTH CURVES PREDICTED BY THE PATHOGEN MODELING PROGRAM V. 6.0

Introduction

Predictive microbiology is based on the assumption that the response of a microbial population to environmental factors is repetitive and that past observations and studies may be used to predict the behaviour of microorganisms in food. Growth of microorganisms is thus a function of food as an environment and in each environment it is possible to determine a finite number of factors influencing physiological reactions of microorganisms. Models include such factors, as: temperature, pH, water activity (a_w), contents of sodium nitrite or organic acids, as well as atmosphere (conventional, modified or vacuum). Based on data collected under controlled conditions, mathematical relations are formulated determining impact and interactions of different variables [Buchanan 1993].

This enables explanation of many microorganisms' behaviour in food and by monitoring of the said parameters it is possible to determine product quality and its sanitary status. Understanding microbial population behaviour during production process and storage through the use of appropriate mathematical relations between microbial growth and external conditions enables precise determination of microorganisms' survival and growth [McClure et al. 1994, Ross and McMeekin 1994, Ilnicka-Olejniczak and Horecka 1995, Kołożyn-Krajewska 1995].

Predictive microbiology offers numerous practical advantages in food microbiology and therefore attracts increasing interest from researchers worldwide. In spite of complexity of many food systems, predictive microbiology may be successfully applied and it will definitely help to understand ecology of microorganisms in food as well. It is also used in HACCP system, in particular for threat analysis and determining parameter limits at critical control points. It may also become a very important tool for designing new products as it allows for estimating potential threats, proposing preservation methods, as well as determining storage possibilities and conditions [Ross and McMeekin 1994, Buchanan 1991].

Development of predictive microbiology, analytical methods, data collection techniques and the obligation to implement state-of-the-art quality assurance systems contributes to the development of electronic databases and expert systems. The databases may be used for routine work by food manufacture and marketing supervisory authorities or food manufacturers to facilitate decision making during new product launch, occurrence of potential pathogen growth risk or growth of microorganisms causing product deterioration under specific manufacture conditions [Cole 1991, Walker and Jones 1993].

Intensive research is in progress worldwide to improve known and develop new mathematical models of microbial behaviour and study results for many raw materials and foods

are being collected in extensive databases. One should suppose that as a result of this activity new, user-friendly and generally available computer software will soon be developed to allow achievements of predictive microbiology to be implemented in day-to-day work of food manufacturers and food manufacture and marketing supervision authorities. At the moment there are numerous less known and two popular commercially available computer models: Food MicroModel and Pathogen Modelling Program [Kołożyn-Krajewska 1994, Whiting and Buchanan 1997].

In the USA (USDA ARS) the "Pathogen Modelling Program" was developed. The programme comprises five groups of models:

- individual growth models for single genera and species of bacteria,
- complex growth models for several genera of bacteria,
- inactivation and survival models,
- probabilistic models for *Clostridium botulinum*,
- irradiation inactivation models.

Individual growth models were developed for the following bacteria: *Aeromonas hydrophila, Bacillus cereus,, Clostridium perfringens, Escherichia coli* O157:H7*, Listeria monocytogenes, Salmonella spp., Shigella flexneri, Staphylococcus aureus* and *Yersinia enterocolitica*. Complex models were developed for the same group of bacteria with the exception of *Clostridium perfringens*. Upon input of the following food environment conditions: temperature, pH, NaCl contents and $NaNO_2$ contents, specification of storage conditions (with or without oxygen) and initial contamination the programme generates the time (in hours or minutes) needed to reach a specified bacteria growth rate.

Inactivation models were developed for non-proteolytic *Clostridium botulinum* strains. The result is the time necessary to achieve target inactivation with specified thermal treatment temperature (from 70 to 90^{o}C) and initial bacteria number.

Survival models were developed for *Escherichia coli* O157:H7*, Listeria monocytogenes, Salmonella spp.* and *Staphylococcus aureus.* The time necessary to achieve intended decrease in number of bacteria is estimated for the assumed conditions: temperature, pH, NaCl, $NaNo_2$ and lactic acid contents.

Probabilistic model for *Clostridium botulinum* was developed to determine the time to toxigenesis separately for types E, F and non-proteolytic B in fish. The result is the time necessary to generate toxins and the probability of occurrence of such an event (P_{max}).

Irradiation models were developed for: *Salmonella typhimurium* in poultry meat, *Escherichia coli* O156:H7 in minced beef and general microflora in chicken legs. The number of microorganisms remaining after irradiation with specified dose at specified temperature is estimated.

Pathogen Modelling Program is a predictive computer programme for modelling growth of various pathogenic bacteria, including *Salmonella* spp., however data used to develop the programme were based on bacterial growth on liquid growing media, rather than real foods. Bacterial growth in food is subject to many conditions that may not be taken into the account when using microbial growing media.

Salmonella spp. rods belong to the group of intestinal bacteria. All species and strains are regarded as potentially pathogenic for humans. According to current state of knowledge, *Salmonella* spp. include about 2 500 serotypes [Szewczyk 2001].

Salmonella spp. bacteria are G(-) non-sporulating pathogens. Most of them have the ability to move, owing to peritrichal cilia. They produce no capsules. The bacteria can be

numbered among relative anaerobes or aerobes with cell dimensions of 0.5–0.7 x 2–4 μm [Doleżko-Marciniak 1986, Czajkowska 2003].

In humans, *Salmonella* rods cause such diseases as: typhoid fever, paratyphoid fevers, alimentary intoxications (salmonelloses) and sepsis [Szewczyk 2001]. The diseases caused by *Salmonella* spp. rods, described as salmonelloses, are induced mainly by the *Salmonella enteritidis* species. The risk of falling ill depends on individual susceptibility, bacillus type and the number of bacterial cells ingested with food. The minimal number of bacteria necessary for the development of pathological symptoms and signs is 10^5 cells/gram of product. Typical symptoms and signs of the alimentary intoxication can be observed six to 72 hours following the invasion of the gastrointestinal system by *Salmonella* spp. rods. The disease is manifested with sudden watery diarrhoea, dull abdominal pain, nausea and vomiting. The diarrhoea containing mucus and/or blood and also headache, fever (39ºC), chills and general weakness can last for several days. Typically, the disease is mild and lasts from one to seven days. In clinically more severe courses, sepsis may develop, leading to inflammatory conditions of the liver, spleen and lymph nodes and ultimately even to death. The distribution of *Salmonella* spp. is limited to the intestine. However, in patients with asthenia due to chronic diseases, these pathogens can migrate and can be the cause of infections of e.g. urinary and respiratory systems and the skin [Szewczyk 2001, Pijanowski et al. 2004, Ozga-Michalski 2006].

Salmonella spp. rods are widespread in nature. The principal reservoir of *Salmonella* spp. bacteria is the gastrointestinal tract of humans and animals. Consumption of infected food is the main cause of intoxications caused by these microorganisms. The food products being the principal source of these pathogens include eggs and milk and their derivatives (mayonnaise, cream, ice-cream, baby soups, hard processed cheese). *Salmonella* spp. bacteria are characterised by a high resistance to drying, therefore they can be expected in such products as milk and powdered eggs. *Salmonella* spp. bacteria can also develop in meat products, such as grilled meat, hamburgers, underdone poultry, cured meat containing raw or smoked meat, and steak tartare, fish spreads, meat aspics, pastes or meat dumplings. Under conditions meeting their physiological requirements, i.e. warmth, moisture, presence of protein, these bacteria can multiply outside human organism for many months [Czajkowska 2003, Ozga-Michalski 2006].

In order to prevent salmonelloses, the basic rules of hygiene must be observed. *Salmonella* spp. bacteria are resistant to freezing and drying, at lower temperatures their growth is inhibited by antagonistic microflora, while high temperature poses a threat to these microorganisms. *Salmonella* spp. rods die at 60–65ºC. In industry, 65–77ºC temperature range is used [Jałosińska 2006].

Many scientific reports demonstrate the susceptibility of *Salmonella* rods to presence of lactic acid bacteria, including probiotic strains. In presence of the strains the bacterium grows much slower and the population tends to decline. Probiotic microorganisms belong to the group of microorganisms broadly used in food industry referred to as lactic acid bacteria (LAB) [de Vrese and Schrezenmeir 2008]. The significance of the organisms in food industry consists of their ability of anaerobic fermentation of carbohydrates with lactic acid as the main metabolite [Libudzisz 2008]. The acid is usually generated in concentration of 0.6–3% as a result of decomposition of monosaccharides, disaccharides, as well as oligo- and polysaccharides. Lactic acid fermentation is a natural food preservation method based mainly on increased acidity resulting from the production of lactate by LAB. It must be noted that

bacteria from the abovementioned group are highly resistant to low pH, therefore can successfully grow in acidified products. Conversely, high acidity tolerance is absent in majority of pathogenic microorganisms that prevents the growth of such organisms in fermented food environment. Another way in which LABs prevent pathogenic microorganisms' growth is the production of antagonistic substances, such as bacteriocins and hydrogen peroxide [Gawęcki and Libudzisz 2006]. Lactic acid bacteria also produce a number of substances responsible for products' characteristic sensory features. Acetic acid, ethanol, diacetyl and acetaldehyde are responsible for specific and identifiable aroma of fermented food [Libudzisz 2008].

There are many *Lactobacillus* and *Bifidobacterium* strains with confirmed probiotic properties [Zaręba 2008]. One of them is the *Lactobacillus acidophilus* LA-5 isolated and patented by the Danish company Christian Hansen. This strain is used worldwide in manufacture of probiotic fermented milk drinks and diet supplements [Kun and Salminen 2009]. In Poland the probiotic strain *L. acidophilus* LA-5 is used to enhance such products as: Jogurt Polski (yoghurt by Mlekovita) and Lazur Złocisty (cheese by Spółdzielnia Mleczarska Lazur). Probiotic properties of the said strain were subject of many studies and described in detail. Thanks to their resistance to low pH in the stomach and bile acids, the bacteria are capable of surviving passage through the gastrointestinal tract. An important role is also played by the bacteria's resistance to digestive enzymes that allows the microorganisms to reach intestines and adhere to the mucosa [Kun and Salminen 2009]. Survival of the strain in the gastrointestinal tract was confirmed by studies in course of which orally administered cells were later isolated from faeces [Saarela et al. 2007].

LA-5, like the majority of representatives of *Lactobacillus*, in course of glucose fermentation generate lactic acid, acetic acid and hydrogen peroxide (H_2O_2). These metabolites make the intestinal environment less favourable for growth of potentially pathogenic and putrefactive organisms. LA-5 strain supplements also have impact on fermentation processes in the colon [Jiang and Savaiano 1995]. The strain generates bacteriocin CH5 characterised by broad scope of antibacterial action, as well as inhibition of certain types of yeast and mould. An example of such action is the antagonism against such pathogens, as *Salmonella typhimurium* and *Campylobacter jejuni* [Kun and Salminen 2009]. Studies demonstrate that *Lactobacillus acidophilus* LA-5 can also prevent enterohemolytic infections with *E. coli* serotype O157:H7 strain [Medellin-Peña and Griffiths 2009].

The objective of the present study was to compare survival of a mixture of three reference strains of *Salmonella enteritidis* (ATCC 1592, ATCC 13076, ATCC 2419) in a probiotic maturing blue cheese (Lazur cheese with the addition of probiotic *Lactobacillus acidophilus* LA5 strain) stored in the temperature of 10^0C and 15^0C with growth curves forecasted with Pathogen Modelling Program v. 6.0.

Materials and methods

The material used for the study was the probiotic maturing blue cheese (Lazur cheese with the addition of probiotic *Lactobacillus acidophilus* LA5 strain) infected with a mixture of three reference strains of *Salmonella enteritidis* (ATCC 1592, ATCC 13076, ATCC 2419). The study included: infecting sterile-cut 10g cheese samples with the mixture of three strains of *Salmonella enteritidis* that were earlier revived and grown on BHI broth and nutritive agar to achieve input suspension of $8.2x10^5$ cfu/ml (0.1 ml of mixture per sample); determination

of the number of *Salmonella enteritidis* in infected samples before storage and in the input suspension; storage of infected samples in 10°C and 15°C for 9 days and determination of the number of rods of the studied strains every 24 hours. The growth of *Salmonella enteritidis* in the said temperatures was also estimated using the predictive computer programme. For computer estimation of *Salmonella* spp. growth the Pathogen Modelling Program v. 6.0. was used. Pathogen growth curves obtained in course of author's microbiological studies were compared with curves obtained using the computer simulation.

Microbiological studies

Microbiological analyses to determine numbers of the studied *Salmonella enteritidis* strains in the product were carried out in accordance with the standard PN-EN ISO 6579:2003/A1:2007:

In each study period (24 hours) to the Stomacher bags (used to store 10g product samples) 90 ml of dilution liquid was added (Maximum Recovery Diluent, Merck). Product with liquid was homogenised for 2 minutes at standard speed in Stomacher 400 apparatus to achieve the first tenfold dilution of the studied product. Then a geometric series of dilutions was carried out and 0.5 dm^3 from three consecutive dilutions was surface inoculated on two dishes in parallel. BPLS agar modified was used as the medium to meet requirements of the abovementioned standard. Dishes were incubated for 24–48 hours in 37°C. *Salmonella enteritidis* growth had the form of red colonies surrounded by a bright red zone. For colony count dilutions generating 30–300 colonies per dish were used. The average number of colonies on three parallel dishes from the same dilution was assumed as the result.

Predicting *Salmonella enteritidis* growth

For computer estimation of Salmonella spp. growth the Pathogen Modelling Program v. 6.0. was used (USDA ARS NAA, USA).

Growth of the studied pathogen was simulated at temperatures used in the experiment (15°C and 10°C). For computer estimation of *Salmonella* spp. growth own experimental product data were used (initial pH and initial infection of the product with the tested strains).

Results and discussion

In this study, the growth was observed of a mixture of three *Salmonella enteritidis* reference strains (ATCC 1592, ATCC 13076, ATCC 2419) in a probiotic ripening blue cheese (Lazur cheese with addition of probiotic *Lactobacillus acidophilicus* LA5 strain), stored for nine days at two temperatures: 10ºC and 15ºC. Both at 10ºC and 15ºC a decrease was seen of the number of *Salmonella enteritidis* rods from the first day of the storage. In the case of 10ºC temperature a reduction occurred of the studied bacterial strain count from the initial level of 4.01 log cfu/g to 3.07 log cfu/g on the last day of the storage, while that reduction at 15ºC occurred from the initial level of 3.66 log cfu/g to 2.65 log cfu/g (Tab. 1). In the case of 10ºC temperature value, the storage duration exerted a more significant effect on the survival rate of the studied pathogen (a more intense drop of the bacterial count) than in the case of 15ºC temperature value. This was confirmed by the statistical data: for 10ºC temperature, the R^2 determination coefficient was 0.83, $p<0.0001$, while for 15ºC temperature value, R^2 was 0.76, $p<0.0001$.

At 10ºC, the count of *Salmonella enteritidis* bacteria decreased most dramatically to day 4 of the storage (from 4.01 log cfu/g to 3.44 log cfu/g), then the decrease was much lower, while at 15ºC the most intense reduction of the count of the studied strain cells was seen on the first day (from 3.66 log cfu/g to 3.30 log cfu/g) and the last day (from 2.96 log cfu/g to 2.65 log cfu/g) of the storage (Tab. 1; Fig. 1, 2).

Table 1

Salmonella enteritidis [log cfu/g] survival in probiotic maturing blue cheese depending on storage temperatures (average values)

Storage temperature	Storage time									
	0	1	2	3	4	5	6	7	8	9
10ºC	4.0104	3.7773	3.5680	3.4357	3.2966	3.2779	3.2623	3.2468	3.2073	3.0671
15ºC	3.6591	3.2977	3.2721	3.2491	3.1563	3.0787	2.9981	2.9965	2.9610	2.6518

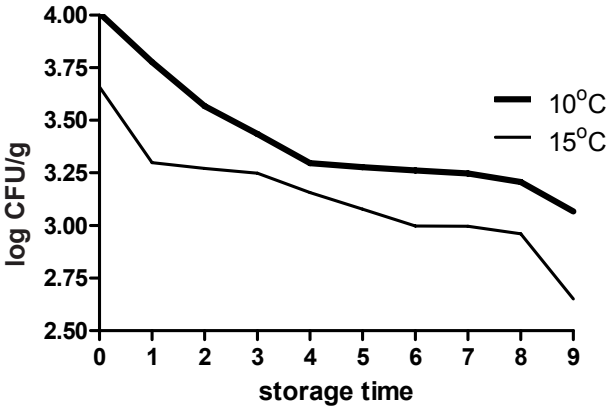


Fig. 1. *Salmonella enteritidis* survival curves in probiotic maturing blue cheese stored at 10 and 15°C

The computer "Pathogen Modelling Program" was applied for comparing the growth of *Salmonella enteritidis* strains used in own studies (where the dairy product – probiotic blue ripening cheese served as the model medium) with the growth of *Salmonella* spp. estimated following the above mentioned computer programme. which was developed on the basis of studies on pathogens' growth on microbiological liquid media. Own empirical data concerning the composition of the product. and own results of microbiological analyses concerning the growth of *Salmonella enteritidis* in the studied product were used for the estimation. The following values were accepted: pH – 7.44; NaCl concentration – 4%; log of the initial *Salmonella enteritidis* count at 10ºC – 4.01; log of the initial *Salmonella enteritidis* count at 15ºC – 3.66.

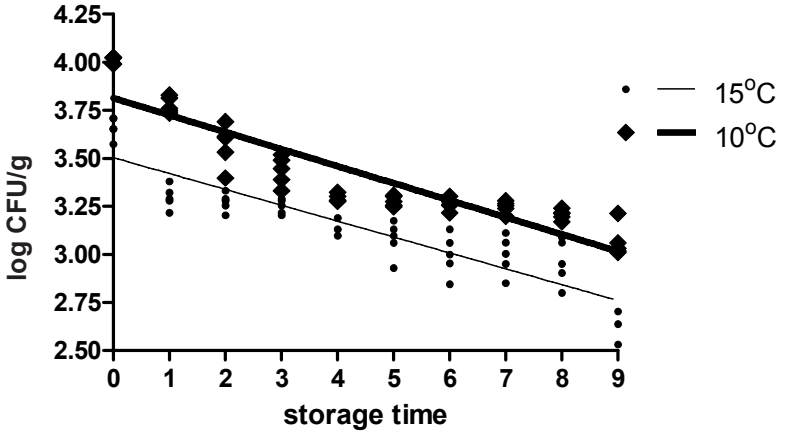


Fig. 2. Linear regression curves demonstrating changes in the number of *Salmonella enteritidis* bacteria in probiotic maturing blue cheese stored at 10 and 15°C

Particular attention should be paid to the great differences in the growth of the studied pathogen found in own studies. compared with the growth estimated on the basis of the Pathogen Modelling Program. At both storage temperature values. in the own studies a drop of the studied bacterium count occurred from the beginning of the storage period. while the computer programme forecast a growth of the same bacterium under the same conditions. For example. in the case of 10ºC temperature value at the end of the storage (9th day) a drop of bacterial count to 3.07 log cfu/g occurred in own studies. while the programme forecast an increase to about 6 log cfu/g (Fig. 3, 4). An even greater difference was seen in the case of 15ºC storage temperature value. On the 9th day a drop was obtained in own studies. to 2.65 log cfu/g. while the programme forecast an increase to 9 log cfu/g and that was the maximal population level in the given conditions (Fig. 5, 6).

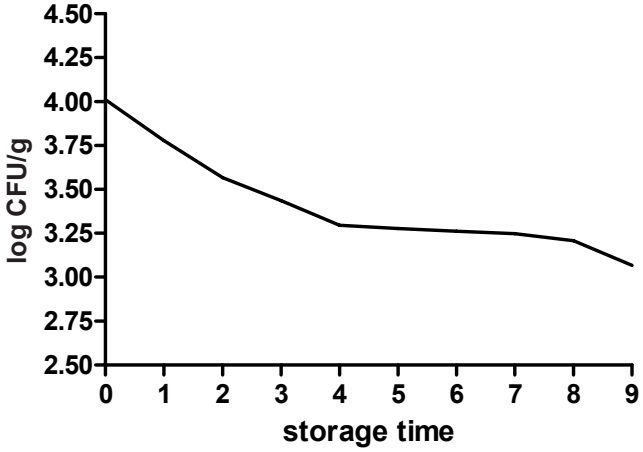
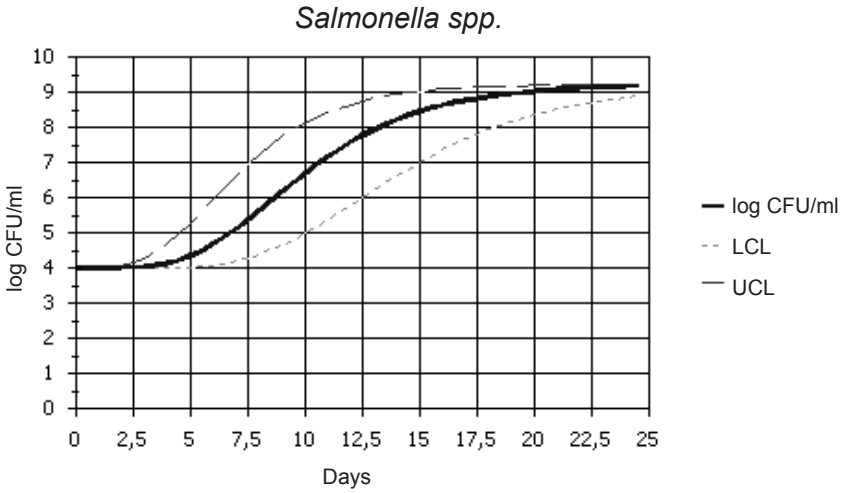


Fig. 3. *Salmonella enteritidis* survival curve in probiotic maturing blue cheese stored at 10°C



LCL – Lower Confidence limit
UCL – Upper Confidence Limit

Fig. 4. *Salmonella* spp. growth at 10°C estimated using the Pathogen Modelling Program ver. 6.0

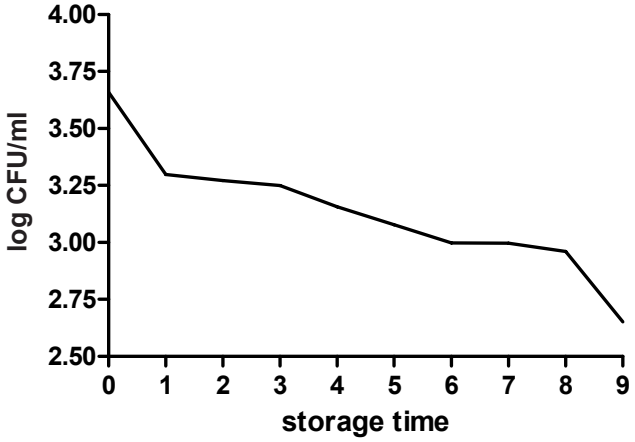
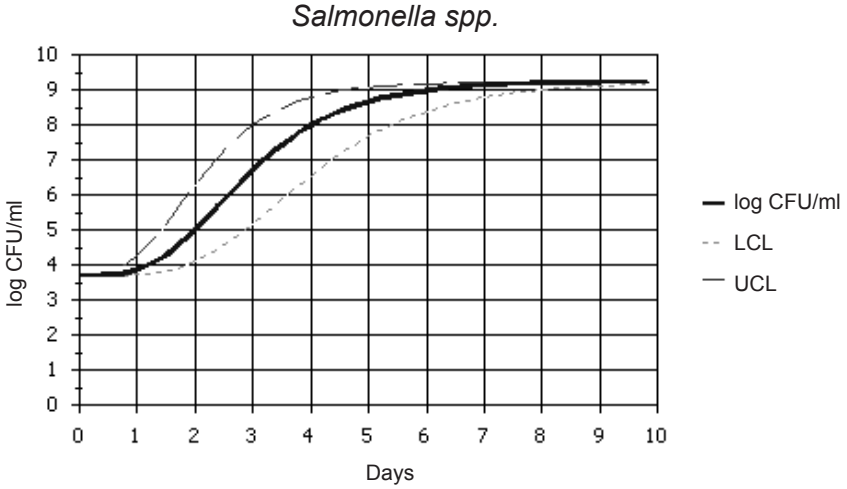


Fig. 5. *Salmonella enteritidis* survival curve in probiotic maturing blue cheese stored at 15°C

The results presented confirmed how bacterial growth on selected microbiological media differed from the growth of the same bacteria on a medium consisting of a food product alone. This was also confirmed by the studies by Jeppesen and Huss [1993] concerning the growth of *L. monocytogenes* and *Y. enterocolitica* in fish products. Many factors connected with food. such as e.g. availability of food components. antimicrobial agents. effect of accompanying microflora. are not taken into account in models constructed on the basis of experiments performed on microbiological media. Therefore. many such models cannot be adjusted to the results of studies carried out on a food product. The growth rate coefficient and lag-phase duration differ significantly from those obtained in studies with liquid microbiological media, even considering the same environmental conditions [Muermans et al. 1993].



LCL – Lower Confidence limit
UCL – Upper Confidence Limit

Fig. 6. *Salmonella* spp. growth at 15°C estimated using the Pathogen Modeling Program ver. 6.0

Avery et al. [1996] demonstrated in their comparative studies and in the construction of the computer programme that the growth obtained on a microbiological medium was always more rapid than in an actual food product. Similar differences were observed also by other authors using the computer Pathogen Modelling Program (PMP). Szczawiński et al. [1996] compared the behaviour of *L. monocytogenes* in several selected food products (kefir. yoghurt. raw milk. heat-processed cottage cheese and corned ham) with the results estimated by means of the mentioned programme. A more rapid inactivation of *Listeria* organisms than expected in the programme was found in dairy products (possibly caused by lactic acid bacteria effects) and a slower than expected growth was seen in corned ham. Jałosińska-Pieńkowska [1999] studied the growth of *S. enteritidis* and *L. monocytogenes* in "meat-ball" – type products kept at various temperatures. including 10ºC and 15ºC. The results of the survival rate of the studied pathogens were compared with the growth estimated by the PMP. In all cases the predicted growth was more rapid and more intense than that in the food product. Dalgaard and Jorgensen [1998] also demonstrated that the growth of *Listeria monocytogenes* estimated based on the PMP was significantly more rapid than that observed on the basis of studies in the food product. namely smoked salmon. The prediction obtained using Food Micro Model in the same paper also differed from the results of microbiological tests performed with the mentioned fish product. In both cases. on the basis of the computer programme. higher growth rate coefficients and longer lag-phase duration were obtained. compared with the results of tests carried out with the food product. The prediction most similar to the microbiological tests in the discussed paper was that obtained using Murphy's model [1996]. the construction of which included the experiments carried out on dairy products. It was. however. not identical. Besides that. the Murphy's programme failed to consider the temperatures (0–3ºC) used for salmon storage.

In the case of the studies presented. the differences in the predicted and actual growth are so great undoubtedly due to the food matrix used. i.e. probiotic blue ripening cheese.

The presence of moulds and probiotic strain. as well as other lactic acid bacteria strains must have affected quite significantly the shape of *Salmonella enteritidis* survival rate curve. and the observed reduction of the studied bacterium count from the first day of the storage at both temperature values. One of the more important benefits produced by probiotic strains are their bactericidal and bacteriostatic effects against pathogens. Lactic acid plays the role of antimicrobial factor. Its effect includes neutralisation of the electrochemical potential of cell membranes. denaturation of intracellular proteins and pH decrease in the cytoplasm of pathogenic cells. The simultaneous occurrence of lactic and acetic acids in the environment causes an intensification of their antibacterial properties. Acetic aldehyde. an intermediate product for lactic acid bacteria. is also a strong antibacterial compound. It usually undergoes further metabolism to ethanol. with the participation of alcohol dehydrogenase. The situation is different in the case of *Lactobacillus acidophilus* strains (thus also the strain present in the product). These bacteria are characterised by low alcohol dehydrogenase activity what results in accumulation of acetic aldehyde. Some *Lactobacillus acidophilus* strains can also synthesize H_2O_2 in amounts that are toxic for pathogens – particularly for anaerobic bacteria having no enzymes protecting against oxidation of disulfide bridges in cell proteins [Motyl and Libudzisz 1996].

Other compounds produced by probiotics. inhibiting the growth and development of pathogenic bacteria. include specific peptides of antibiotic-like character. i.e. bacteriocins. Bacteriocins produced by probiotics form a large group of heterogeneous chemical compounds diversified in respect of molecular weight. chemical structure. biochemical properties and range of activity and mode of action against microorganisms. *Lactobacillus acidophilus* strains are well known producers of bacteriocins and they synthesize substances inhibiting the development of the following species of pathogens: *Staphylococcus aureus*, *Salmonella enteritidis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Mycobacterium* spp. [Motyl and Libudzisz 1996, Libudzisz 2006].

Conclusions

1. Survival curves for *Salmonella* rods estimated using Pathogen Modelling Program v. 6.0 are significantly different from those obtained for the foodstuff (maturing probiotic cheese). Presence of probiotic strains caused the number of bacteria to go down from the beginning of the storage period at both studied temperatures which was not reflected by curves obtained using the predictive model.
2. The obtained results confirm that microbiological studies to develop predictive models should be carried out using model foodstuffs rather than liquid microbiological mediums.

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References

Avery S.M., Hudson J.A., Phillips D.M., 1996. Use of response surface models to predict bacterial growth from time/temperature histories. Food Control, 7, 121.

Buchanan R.L., 1991. Using spreadsheet software for predictive microbiology applications. J. Food Safety, 11, 123.

Buchanan R.L., 1993. Predictive food microbiology. Trends in Food Sci. Technol., 4, 6.

Cole M.B., 1991. Databases in modern food microbiology. Trends in Food Sci. Technol., 2, 11, 293.

Czajkowska D., 2003. *Salmonella* w żywności jako przyczyna chorób ludzi. Materiały firmy Biomerieux. Warszawa.

Dalgaard P., Jorgensen L.V., 1998. Predicted and observed growth of *Listria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. Int. J. Food Microbiol., 40, 105.

Doleżko-Marciniak H., 1986. Pałeczki Gram-ujemne (duże), [in:] Jabłoński L. (Ed.). Podstawy mikrobiologii lekarskiej – podręcznik dla studentów. PZWL, Warszawa.

Gawęcki J., Libudzisz Z., 2006. Mikroorganizmy w żywności i żywieniu. Wydawnictwo Akademii Rolniczej im. A. Cieszkowskiego. Poznań.

Ilnicka-Olejniczak O., Hornecka D., 1995. Prognozowanie w mikrobiologii żywności, [in:] Food Product Development. Opracowanie nowych produktów żywnościowych. Ed. AR Poznań.

Jałosińska M., 2006. Mikrobiologia żywności. Wydawnictwo Format-AB. Warszawa.

Jałosińska-Pieńkowska M., 1999. Prognozowanie bezpiecznego okresu trwałości modelowych wyrobów mięsnych gotowych do spożycia. Warszawa (in Polish, doctoral thesis, manuscript).

Jeppesen V.F., Huss H.H., 1993. Antagonistic activity of lactic acid bacteria against *Listeria monocytogenes* and *Yersinia enterocolitica* in a model fish product at 5ºC. Int. J. Food Microbiol., 19, 179.

Jiang T., Savaiano D.A., 1995. Lactobacilli supplementation and short chain fatty acid production by human fecal bacteria grown in continuous culture. Gastroenterology, 108, 4. Suppl. 1, 293.

Kołożyn-Krajewska D., 1994. Ogólne zasady prognozowania w mikrobiologii żywności. Cz. I. Matematyczne modelowanie wzrostu mikroorganizmów w żywności. Przem. Spoż., 48, 11, 362.

Kołożyn-Krajewska D. 1995. Gwarantowana jakość mikrobiologiczna żywności a metody predyktywne. Żywność. Technologia. Jakość, 2(3), 53.

Kun Lee Y. Salminen S., 2009. Handbook of probiotics and prebiotics. A Jonh Wiley & Sons. Inc.

Libudzisz Z. 2006. Żywność probiotyczna, [in:] Gawęcki J.. Libudzisz Z. (Ed.). Mikroorganizmy w żywności i żywieniu. Ed. Akademii Rolnicza im. Augusta Cieszkowskiego w Poznaniu. Poznań.

Libudzisz Z., 2008. Bakterie fermentacji mlekowej, [in:] Libudzisz Z.. Kowal K.. Żakowska Z. (Ed.). Mikrobiologia techniczna. Mikroorganizmy w biotechnologii ochronie środowiska i produkcji żywności. Wydawnictwo Naukowe PWN. Warszawa.

McClure P.J., Blackburn C.W., Cole M.B., Curtis P.S., Jones J.E., Legan J.D., Ogden I.D., Peck M.W., Roberts T.A., Sutherland J.P., Walker S.J., 1994. Modeling the growth. survival and death of microorganisms in foods: the UK Food Micromodel approach. Int. J. Food Microbiol., 23, 265.

Medellin-Peña M., Griffiths M., 2009. Effect of molecules secreted by *Lactobacillus acidophilus* strain LA-5 on *Escherichia coli* O157:H7 colonization. Appl. Environ. Microb., 75, 4, 1165–1172.

Motyl I., Libudzisz Z., 1996. Właściwości probiotyczne bakterii mlekowych i ich wykorzystanie w przetwórstwie mleczarskim. Przegl. Mlecz., 3, 72–76.

Muermans M.L.T., Stekelenburg F.K., Zwietering M.H., Huis I., Veld J.H.J., 1993. Modeling of the microbiological quality of meat. Food Control, 4, 216.

Murphy P.M., Rea M.C., Harrington D., 1996. Development of a predictive model for growth of *Listeria monocytogenes* in skim medium and validation studies in a range of dairy products. J. Appl. Bacteriol., 80, 557.
Ozga-Michalski E., 2006. Choroby układu pokarmowego. Portal Farmaceutyczno-Medyczny. http://www.pfm.pl/u235/navi/199944. Internet. 22.10.2006.
Pijanowski E., Dłużewski M., Dłużewska A., Jarczyk A., 2004. Zdrowotność produktów spożywczych, [in:] Ogólna technologia żywności. WNT, Warszawa.
PN-EN ISO 6579:2003/A1, 2007. Mikrobiologia żywności i pasz. Horyzontalna metoda wykrywania Salmonella spp.
Ross T., McMeekin T.A., 1994. Predictive microbiology. Int. J. Food Microbiol., 23, 3–4. 241.
Saarela M., Maukonen J., von Wright A., Vilpponen-Salmela T., Patterson AJ., Scott K., Hämynen H., Mätto J., 2007. Tetracycline susceptibility of the ingested *Lactobacillus acidophilus* LaCH-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 strains during antibiotic/probiotic intervention. Int. J. Antimicrob. Ag., 29, 3, 271–280.
Szczawiński J., Szczawińska M., Stańczak B., 1996. Przewidywanie zachowania się *Listeria monocytogenes* w żywności na podstawie programu komputerowego Pathogen Modeling Program v.4.0 oraz badań własnych, [in:] Proceed. X Congress PTNW, Wrocław, 527.
Szewczyk E.M., 2001. Rodzina *Enterobacteriaceae* – jelitowe względnie beztlenowe pałeczki Gram-ujemne, [in:] Mikucki J. Szewczyk E.M. (Eed.). Mikrobiologia szczegółowa i diagnostyka mikrobiologiczna. Wydawnictwo Akademii Medycznej w Łodzi, Łódź,
de Vrese M., Schrezenmeir J., 2008. Probiotics. Prebiotics. and Synbiotics. Springer-Verlag. Berlin.
Walker S.J., Jones J.E., 1993. Protocols for data generation for predictive modeling. J. Ind. Microbiol., 12, 273.
Whiting R.C., Buchanan R.L., 1997. Predictive Modeling, [in:] Doyle M.P., Beuchat L.R. Montville-.T.J. (Ed.). Food Microbiology. fundamentals and frontiers. ASM Press. Washington D.C.
Zaręba D., 2008. Przeżywalność probiotycznego szczepu *Lactobacillus acidophilus* w mleku niefermentowanym i fermentowanym. Żywność. Nauka. Technologia. Jakość, 60, 5, 189–196.

AUTHORS

Árvay Július – Slovak Agricultural University in Nitra, Slovakia
Bajzík Pavol – Slovak Agricultural University in Nitra, Slovakia
Bakker Evert J. – Wageningen University, Wageningen, Netherlands
Belej Lubomir – Slovak Agricultural University in Nitra, Slovakia
van Boekel M.A.J.S. – Wageningen University, Wageningen, Netherlands
Bojňanská Tatiana – Slovak Agricultural University in Nitra, Slovakia
Bulla Jozef – Slovak Agricultural University in Nitra, Slovakia
Bystrická Judita – Slovak Agricultural University in Nitra, Slovakia
Čičová Iveta – Slovak Agricultural University in Nitra, Slovakia
Czarniecka-Skubina Ewa – Warsaw University of Life Sciences, Warszawa, Poland
Godlewska Katarzyna – Warsaw University of Life Sciences, Warszawa, Poland
Goldsztejn Magdalena – National Veterinary Research Institute, Puławy, Poland
Golian Jozef – Slovak Agricultural University in Nitra, Slovakia
Górecka Justyna – Wrocław University of Environmental and Life Science, Wrocław, Poland
Górecki Jerzy – University of Science and Technology, Kraków, Poland
Górna Justyna – Poznań University of Economics, Poznań, Poland.
Grenda Tomasz – National Veterinary Research Institute, Puławy, Poland
Grodzicki Tomasz – University of Life Sciences, Lublin, Poland
Harangozo Ľuboš – Slovak Agricultural University in Nitra, Slovakia
Jakubczak Antoni – The State College of Computer Science and Business Administration, Łomża, Poland
Jałosińska Małgorzata – Warsaw University of Life Sciences, Warszawa, Poland
Ježo Peter – Slovak Agricultural University in Nitra, Slovakia
Jinap Selamat – University Putra Malaysia, Serdang, Selangor, Malaysia
Jónášová Diana – Slovak Agricultural University in Nitra, Slovakia
Kęsy Zbigniew – Wrocław University of Environmental and Life Sciences, Wrocław, Poland
Kołożyn-Krajewska Danuta – Warsaw University of Life Sciences, Warszawa, Poland
Korzeniowska Małgorzata – Wrocław University of Environmental and Life Sciences, Wrocław, Poland
Król Jolanta – University of Life Sciences, Lublin, Poland
Kukier Elżbieta – National Veterinary Research Institute, Puławy, Poland
Kukułowicz Anita – Maritime University in Gdynia, Poland
Kwiatek Krzysztof – National Veterinary Research Institute, Puławy, Poland
Lewandowski Włodzimierz – The State College of Computer Science and Business Administration, Łomża, Poland, Białystok University of Technology, Białystok, Poland
Litwińczuk Anna – University of Life Sciences, Lublin, Poland
Luning Pieternel A. – Wageningen University, Wageningen, Netherlands
Macherzyński Mariusz – University of Science and Technology, Kraków, Poland
Malicki Adam – Wrocław University of Environmental and Life Sciences, Wrocław, Poland
Marczewska Sylwia – University of Life Sciences, Lublin, Poland
Mazur Robert – Poznań University of Life Sciences, Poznań, Poland
Musilová Janette – Slovak University of Agriculture in Nitra, Slovakia
Nowicki Paweł – Cracow University of Economics, Kraków, Poland
Ostrowska Monika – National Veterinary Research Institute, Puławy, Poland
Revák Ondrej – Slovak Agricultural University in Nitra, Slovakia
Rola Jolanta G. – National Veterinary Research Institute, Puławy, Poland
Rzeźnik Radosław – Warsaw University of Life Sciences, Warszawa, Poland