Update of concepts in food microbiology education in Slovakia: from food microbiology to predictive microbiology and microbiological risk assessment in foods

ĽUBOMÍR VALÍK – ALŽBETA MEDVEĎOVÁ – PAVEL AČAI

Summary

To implement food-oriented theoretical knowledge and skills in practice, mutual knowledge exchanges, communications and cooperation among professionals are needed for the sake of final product quality and safety. Identical principles should be applied generally in university education regardless of the type of school. However, the specifics of food studies exist at technical universities, especially those with a chemical and technological background. These are achieved by controlling the chemical, physical, biochemical processes as well as the skills needed in the production as well as in the evaluation of food quality and safety. Therefore, their approaches have to be process-oriented, preventive and, in critical cases, they must provide solutions by applying reverse knowledge from scientific literature, past microbiological observations, trends of microbial loads and from actual situations in the food chain. Thus in food microbiology, a shift from descriptive qualitative toward quantitative approaches enables graduates to predict the behaviour of microorganisms and assess the potential risks resulting from growth, survival and contamination.

Keywords

education concept; predictive microbiology; microbiological risk assessment

No matter from which angle we look at the complex interactions between microorganisms, foods, environment and human beings, and how deeply we specify associated factors existing among these individual points in an imaginary quadrangle, microbiologists play a significant role in society. Together with university colleagues and in accordance with their professional teaching orientation, microbiologists follow a noble goal to work beneficially for the human population. In providing the knowledge and skills to students and the general public, they protect humans directly as medical microbiologists, indirectly and preventively through food chain protection as food microbiologists, or by addressing challenges in the microbiology of soil, water and air as environmental microbiologists. Recognizing the life cycle of microorganisms and their progressive development in a nutritionally rich nutrient environment on the one hand, and on other hand, destructive decay in a period of deficiency, we assume that the entire scientific community of microbiologists is very sensitive and understanding of global sustainability. By this we mean respect for the sustainability of quality of life and the health of human and animal populations, for agricultural crops and food production, as well as for environmental diversity. In particular, microbiologists are united in their understanding of microorganisms, certainly the most numerous population among the living organisms on earth. According to Doyle and Buchanan [1], food microbiologists have long been in the forefront of many microbiological concepts, and new research topics have arisen as a results of the unique challenges in areas such as biofilms, probiotics, naturally occurring antimicrobials, microbial diversity, predictive microbiology and microbiological risk assessment.

Ľubomír Valík, Alžbeta Medveďová, Department of Nutrition and Food Quality Assessment, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovakia.
Pavel Ačai, Department of Chemical and Biochemical Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovakia.

Correspondence author:
Ľubomír Valík, e-mail: lubomir.valik@stuba.sk

Food microbiology as a challenge in a transition country

Food microbiology, as one of the microbiological scientific branches, focuses on a wide variety of current research areas involving microbes that have both beneficial and deleterious effects on the safety as well as quality of foods, and are thus a concern of public health. As scientists in the country that has passed from socialism to the market economy, we have had many opportunities to consider which of the previous knowledge, experiences or approaches used in food microbiology have been useful. We were aware of the new challenges we would face in the future in a democratic open society. However, we still kept in our minds two quotes by our supervisor: "Do not speak of what you were working on, tell me what you have done" as well as a sort of encouragement for graduated food scientists and technologists, including microbiologists: "A graduate can become a good food chemist after 5 years, but a good food microbiologist after 10 to 15 years".

From the past to the present

It should be noted at this point that in the previous socialist order, the food production enterprises belonged to the state, with their most important task being to ensure food self-sufficiency for the country. Due to the state ownership of food companies as well as governmental supervisory authorities, it appeared that the system of control was not independent. In view of the current level of knowledge and opportunities for technological innovation, food quality assessment was based primarily on the control parameters of final products as detailed in the technical standards. Although at the end of this period newer concepts too were discussed among food professionals, their implementation was ideologically and bureaucratically managed and lacked a sufficient space.

So the concept, defined as preventive, and now referred to as the classic, incorporating the prerequisites of the Good Manufacture Practice (GMP), Good Hygiene Practice (GHP) and Hazard Analysis and Critical Control Points (HACCP) system, could only be implemented after the transformation of businesses operators and official control public authorities. Only after these conceptual changes, the activity of state authorities was considered independent, as food business operators were not linked by an ownership relationship.

During the transition period, everybody dreamed about new conditions, instruments and methodologies, without any information or knowledge barriers. Naturally, some of the scientific dreams became reality.

Changes in food microbiology education

At the present as in the past, the study of food sciences at the Faculty of Chemical and Food Technology (Slovak University of Technology, Bratislava, Slovakia) has been influenced by the requirements of food industry, since most graduates find their jobs there. The education of future food engineers consisted of passing the compulsory general subjects in chemistry, physics, mathematics, biology, general microbiology, toxicology and possibly other subjects. Further training of the students at the Faculty of Chemical and Food Technology was oriented to the needs of the individual branches of food industry only within the second half of the study. Contrary to the present situation, the Bachelor study did not exist, which, I consider personally as an advantage. Subjects such as 'Chemical Engineering', 'Food Technology' and 'Food Processing' have become of key importance. The food science courses were conceptually focused according to food commodities, in terms of animal origin (milk and dairy products, meat and meat products) and plant origin (carbohydrates and cereals, beverages). Food quality and safety assessment has also been addressed through a commodity approach and divided among the various departments.

The changes in the transforming society, the opening of the food market, the obsolescence of technology, the shabbiness of equipment and reduced capacities resulting in low production efficiency, as well as the specific problems of the emerging private companies, caused a drop in demand for educated food specialists. We had to transform our food study. Paradoxically, the new situation and industrial conditions, as well as new concepts and advances in food microbiology, helped us considerably in the transformation of the study program. In our case, this primarily concerned clear responsibility for food quality and safety, addressed to compliance with GMP and GHP requirements, as well as HACCP implementation. These circumstances led to a need to adapt the lessons for students. As far as the microbiological progression of the university study was concerned, this in particular meant introducing new subjects. Subjects such as 'Biology', 'Food and Nutritional Toxicology' and 'General Microbiology' currently represent the basic package needed to direct students into the food and biotechnology fields. Secondary microbiological-hygienic package has been created from two subjects, 'Food Microbiology' and 'Food Hygiene and Safety', including the appropriate laboratory exercises and seminars. Besides these subjects, teaching in 'Chemical and Food Engineering' is provided to the students. 'Food Technology', understood in general as well as specifically according to food industry sectors, has followed along. Due to tradition, the range and scope of the 'Microbiology of Milk and Milk Products' subject remained in the system. Finally, since 2014, we have managed to introduce the subject of 'Predictive Microbiology and Microbial Risk Assessment' for all food-oriented students as the assessors in their last year of study.

Nowadays, the Department of Nutrition and Food Quality Assessment is primarily responsible for the following subjects: 'Food Microbiology', 'Food Hygiene and Safety', 'Food Quality and Safety Assessment' and 'Predictive Microbiology and Microbiological Risk Assessment'. With regard to the character of the Faculty of Chemical and Food Technology in the concept of 'Food Microbiology', the descriptive properties of food-relevant microorganisms are taken as closely related to the technological processes used in the specific food productions.

Food microbiology

Education in food microbiology should enable our food science graduates to become familiar with microorganisms and their importance in the production and control of foodstuffs. They should also know the principles and procedures of microbiological control with indicator microorganisms as well as the evaluation of the results obtained. They have to be aware of the hazardous microorganism transmission routes from their niches to humans. To prevent the foods acting as carriers, heat-treatment, pasteurization, sterilization, ultra-pasteurization and other technological processes responsible for safety and the shelf-life extension of foods are applied. That is why food microbiologists should not only understand the microbial load coming from raw materials and the principles of food processing but, based on their knowledge and experience, they are obliged to bring high professionality and sound solutions not only to processing control, but process efficacy as well.

Essential for our subject is that the students link the properties of the microorganisms responsible for diseases and food spoilage or used in fermentations with their behaviour (growth, devitalization, survival) in the specific environment of a food matrix created by the technological processes and characterized by intrinsic environmental factors (Fig. 1).

In light of these main conceptual ideas and based on our pedagogical and consultation experience, following the previous monographs by Adams and Moss [2], Robinson [3], Marth and Steele [4], the textbook Applied Food Microbiology by Görner and Valík [5] was written for the students. Later, the educational concepts in the subject 'Food Microbiology' were periodically complemented by the work of Doyle and Buchanan [1] or Matthews, Kniel and Montwille [6], as well as by current scientific literature.

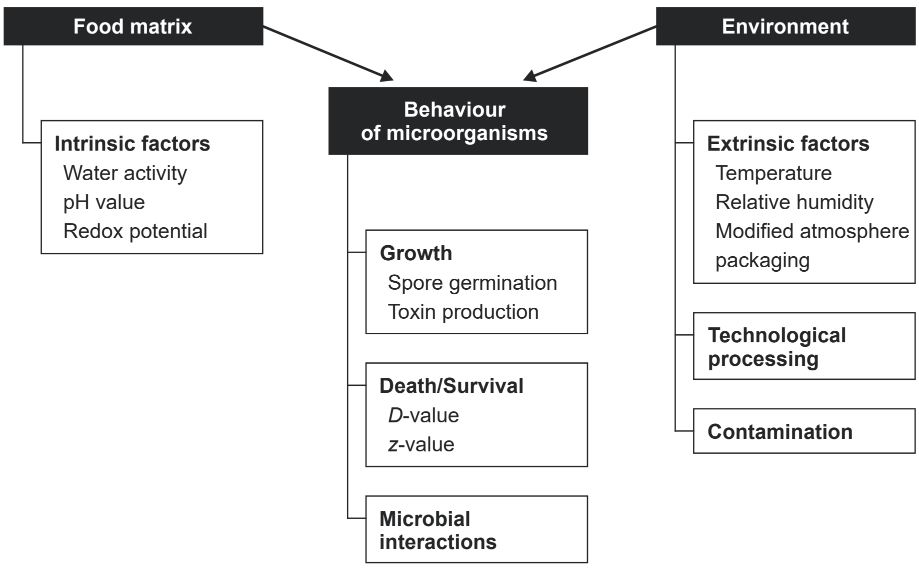

Fig. 1. Factors influencing behaviour of microorganisms in foods.

D-value – the time required, at a given condition (e.g. temperature), to achieve 1 log reduction, that is, to kill 90 % of relevant microorganisms, *z*-value – the increase of the temperature to achieve a ten-fold reduction in the *D*-value.

Food hygiene and safety

In the subject 'Food Hygiene and Safety', the students are provided in detail with a description of the microorganisms considered as food hazards and the symptoms of the diseases they cause through the consumption of contaminated food [7]. Emphasis is placed on knowledge of the cardinal values of the intrinsic and extrinsic factors determining behaviour of pathogenic microorganisms. As was mentioned above, this includes their growth, inactivation, survival as well as toxin or biofilm production. In the remaining two-thirds of the semester, our attention is devoted to the prerequisite requirements of GMP, GHP and HACCP, including the appropriate documentation (within the seminar part). An explanation of new concepts in food safety management, as proposed by International Commission for the Microbiological Specification of Foods (ICMSF) [8] based on Food Safety Objective (FSO) and optimally derived from the so-called Appropriate Level of Protection (ALOP), is also provided to the students within the tutorial [9].

Towards predictive microbiology and microbiological risk assessment

According to Matthews, Kniel and Montwille [6], food microbiologists must understand microbiology, molecular biology as well as food systems, and be able to integrate them to solve problems in complex food ecosystems. In full agreement with this statement, all the subjects mentioned above gave us the opportunity to incorporate the experience of each of the teachers involved in the education, including the laboratory exercise assistants. The main outcome of their work should be a student or graduate able to orientate and work independently in the laboratory, get the result, evaluate it, formulate his own opinion and, if his work is a part of a larger issue, to assess presumed causes and bring about a solution. This means that in the near future, without knowledge of predictive microbiology, it will become impossible to formulate practical microbiological solutions for the food industry.

In the effort to improve our skills in the field of predictive microbiology and mathematical modelling, a breakthrough moment was our participation in growth modelling courses within the COST projects in 1997. Dr. Baranyi kindly enabled me to take part in the courses and provided the DMFit tool for Excel [10].

In order to be able to evaluate the impact of microbial behaviour on microbiological safety or food quality, we need in particular cases to describe at least the behaviour of undesirable microbial populations in the foods. This includes the growth, metabolism, death or survival of pathogenic and spoilage microorganisms, determining the rate and extent of the detrimental effect on foods and finally on consumers. Both the microbial populations as well as the food characteristics are determined by specific intrinsic and extrinsic factors, and the knowledge in context can be used to improve quality and safety of the newly developed foods. Applying mathematical models for a description of the relationships between the behaviour of microorganisms and food environmental factors, a microbiologist can predict the rate and extent of their impact on food microbiological quality and safety. Not to put it too simply, also microbial interactions, whether antagonistic, competitive or other, should, in specific cases be included in the modelling (Fig. 1).

Predictive microbiology and microbiological risk assessment

A conceptual definition by Ross and McMeekin [11] says that "Predictive microbiology is based upon the premise that the responses of populations of microorganisms to environmental factors are reproducible, and that by considering environments in terms of identifiable dominating constraints it is possible, from past observations, to predict the responses of those microorganisms" and is still valid today. Furthermore, predictive microbiology provides a rational framework for understanding the microbial ecology of food, and is "a large step toward making food microbiology more science than art". Predictive microbiology models can be useful to assess the growth, survival or death (or time to toxin production) of microorganisms as a function of the food and environmental conditions encountered throughout the food chain, and are particularly important when making quantitative estimates, e.g. within exposure assessment.

Microbiological risk assessment (MRA) is a comprehensive and systematic approach for addressing the risk of pathogens in specific foods and/or processes. MRA consisting of four elements (hazard identification, hazard characterization, exposure assessment and risk characterization) is a part of the framework of "risk analysis", provided by the Codex Alimentarius Commission (Codex), which also includes "risk management" and "risk communication" as interdependent concepts [12].

Respecting the conditions at the faculty, especially the timetable we were provided, the 'Predictive Microbiology' and 'Microbiological Risk Assessment' modules were combined in one subject for students of the Master's level (second year) in the field of food quality and safety assessment. Both are compulsory and include both lectures and tutorials. In the lectures, we deal with the rationale of the concept and the theoretical background of the most important predictive models within classifications according to phenomenon, approach and variables as included in the models [13]. Primary growth and inactivation models provide changes in numbers of the microbial population as a function of time ($N = f(t)$) in constant environmental conditions. Modelling experimental growth and inactivation data enable determination of relevant parameters such as the duration of the lag phase (lag), the maximal specific growth rate (μ_{max}) and rate of inactivation or D-value, respectively. Secondary models describe the relations between growth or inactivation parameters (e.g. rates) as a function of environmental conditions such as temperature, water activity or pH [14]. So-called "tertiary models" include databases, fitting utilities, growth, growth/no growth and inactivation prediction tools, probability models and risk assessment modules. This enables a wide range of applications such as HACCP system support, product and process design, shelf life assessment, microbiological criteria compliance, exposure assessment, sampling plans and experimental design and education. However, the most important benefit for the user is that the software can assist decision making so that appropriate action may be carried out as early as possible [15].

A structure similar to our classification one that we applied referring to the teaching of predictive food microbiology models (primary and secondary), fitting experimental growth or inactivation data, using the web-based tools and resources (tertiary) and comparing the outputs found by students or from free web resources was also presented in the work of Dubois-Brissonnet et al. [16]. However, we should also take into consideration advanced optional modules, which would use methodologically well prepared case studies to support and encourage the team work. We also appreciate an agreement with a representative of Sym'Previus Operational Unit (ADRIA développement, Quimper, France) that allows our students to have full approach to their application software and Sym'Previus microorganisms' database for future winter semesters.

In relation to the complexity and relevance of MRA, the tutorials for students provide basic information and knowledge from various scientific sources, reports and European Food Safety Authority (EFSA) scientific opinions [12, 17]. Also, due to time constraints and insufficient software equipment, students are provided with examples of some exposure assessments related to traditional Slovakian raw milk cheeses [18, 19] and pasteurized milk [20], or with quantitative microbiological risk assessment studies, e.g. Mataragas et al. [21] and Pérez-Rodríguez et al. [22].

Next generation predictive microbiology and microbiological risk assessment

At the present level of work experience with students, we are facing various challenges associated with the ability of students to absorb the type and amount of knowledge within the assigned time span. On the other hand, lecturers should master also information on progressive, novel scientific approaches and outputs. It is clear that predictive microbiology is becoming an increasingly multidisciplinary field. The next generation predictive models are based on dynamic models able to quantify the undesirable organisms, the concentration of critical metabolites as well as the population potential to adapt and proliferate or survive in a given environment at dynamically changing conditions. This category includes also the models able to describe interactions between bacterial populations in a co-culture, relation between behaviour of single cells and population, as well as to reveal the molecular influence that defines the bacterial response and population kinetics throughout the food chain [23]. To obtain quantitative information on the real activity of microorganisms in their ecosystem, transcriptomic and meta-transcriptomic approaches are necessary. As Cocolin et. al. [24] pointed out, the industry also identifies ways how to move forward and incorporate metagenomics into their classical Pasteurian-based microbiological food safety and quality systems. This interest as well as other drive forces as Next Generation Sequencing (NGS) aimed at RNA have already encouraged the scientist to discover new approaches even the fields. For example, Predictive Metagenomic Profiling (PMP) is predicting the abundance of functional genes in microbial populations in terms "What will the microbial community potentially do?" [25]. In this context, RNA-based approaches can provide important information on activity of microorganisms, e.g. information on actual production of secondary toxic metabolites by pathogenic microorganisms, relevant to MRA [24].

CONCLUSIONS

To implement food-oriented theoretical knowledge and skills in practice, mutual knowledge exchanges, communications and cooperation among professionals are needed for the sake of final product quality and safety. Identical principles should be applied generally in university education regardless of the type of school. However, the specifics of food studies exist at technical universities, especially those with a chemical and technological background. These are achieved by controlling the chemical, physical, biochemical processes as well as the skills needed in the production as well as in the evaluation of food quality and safety. Therefore, their approaches have to be process-oriented, preventive and, in critical cases, they must provide solutions, either immediately on site or later by applying reverse knowledge from scientific literature, past microbiological observations, trends of microbial loads in the important production steps or from trends and actual situations in the food chain. Thus, a shift from descriptive qualitative food microbiology toward quantitative approaches enables graduates to predict the behaviour of microorganisms and assess the potential risks resulting from growth, survival and contamination. Furthermore, the microbiologists will not only face the new challenges in application of new stochastic modelling in variable single-cell behaviour or in processing amounts of genetic data, but also incorporate them in holistic microbiological risk assessments of the next generation. Moreover, their outputs should be clear and applicable for risk managers.

Acknowledgements

This work was presented at 3nd symposium "Food microbiology education in practice" at 26th Food Micro 2018 organized by International Committee on Food Microbiology and Hygiene (Berlin, Germany) and supported by the Slovak Research and Development Agency under the contract No. APVV-15-0006 and VEGA Agency VEGA-1/0532/18.

REFERENCES

1. Doyle, M. P. – Buchanan, R. L.: Food microbiology: Fundamentals and frontiers. 4th edition. Washington DC : ASM Press, 2013. ISBN: 9781555816261 (print), 9781555818463 (e-book). DOI: 10.1128/9781555818463.
2. Adams, M. R. – Moss, M. O.: Food microbiology. Cambridge : The Royal Society of Chemistry, 1995. ISBN: 9780854045099.
3. Robinson, R. K.: Dairy microbiology: Microbiology of milk. 2nd edition. London : Elsevier Applied Science, 1990. ISBN: 978-1851663910.
4. Marth, E. – Steele, J. L.: Applied dairy microbiology. 2nd edition. New York : Marcel Dekker, 2001. ISBN: 9780824705367.
5. Görner, F. – Valík, Ľ.: Aplikovaná mikrobiológia požívatín. (Applied food microbiology.) Bratislava : Malé Centrum, 2004. ISBN: 9788096706495. In Slovak.
6. Montville, T. J. – Matthews, K. R. – Kniel, K. E.: Food microbiology: An introduction. 4th edition. Washington, D. C. : ASM Press, 2017. ISBN: 978-1555819385.
7. Valík, Ľ. – Prachar, V.: Pôvodcovia ochorení z požívatín a minimalizácia ich rizika. (Food-borne disease agents and risk minimization.) Bratislava : Slovak University of Technology, 2009. ISBN: 978-80-227-3200-0. In Slovak.
8. Microorganisms in foods 7: Microbiological testing in food safety management. 2nd edition. New York : Kluwer Academic/Plenum Publishers, 2018. ISBN: 978-3-319-68458-1, 978-3-319-68460-4 (e-book). DOI: 10.1007/978-3-319-68460-4.
9. Gorris, L. G.: Food safety objective: An integral part of food chain management. Food Control, *16*, 2005, pp. 801–809. DOI: 10.1016/j.foodcont.2004.10.020.
10. Baranyi, J. – Roberts, T. A.: A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, *23*, 1994, pp. 277–294. DOI: 10.1016/0168-1605(94)90157-0.
11. Ross, T. – McMeekin, T. A.: Predictive microbiology. International Journal of Food Microbiology, *23*, 1994, pp. 241–264. DOI: 10.1016/0168-1605(94)90155-4.
12. Bassett, J. – Nauta, M. – Lindqvist, R. – Zwietering, M.: Tools for microbiological risk assessment. Brussels: International Life Sciences Institute (ILSI), 2012. ISBN: 9789078637349.
13. Valík, Ľ. – Ačai, P.: Prediktívna mikrobiológia a mikrobiologické hodnotenie rizika. (Predictive microbiology and microbiological risk assessment.) Bratislava : Slovak University of Technology, 2016. ISBN: 9788022746557. In Slovak.
14. McKellar, R. – Lu, X.: Modeling microbial responses in food. Boca Raton : CRC Press, 2004. ISBN: 9780849312373.
15. Koutsoumanis, K. P. – Lianou, A. – Gougouli, M.: Latest developments in foodborne pathogens modeling. Current Opinion in Food Science, *8*, 2016, pp. 89–98. DOI: 10.1016/j.cofs.2016.04.006.
16. Dubois-Brissonnet, F. – Guillier, L. – Naitali, M.: Teaching microbiological food safety through case studies. International Journal of Food Studies, *4*, 2015, pp. 134–140. DOI: 10.7455/ijfs/4.2.2015.a2.
17. EFSA Panel on Biological Hazards (BIOHAZ): Scientific opinion on the development of a risk ranking framework on biological hazards. EFSA Journal, *10*, 2012, article 2724. DOI: 10.2903/j.efsa.2012.2724.
18. Ačai, P. – Valík, Ľ. – Medveďová, A. – Studeničová, A.: *Staphylococcus aureus* in unripened ewes' lump cheese. Part 1: Exposure assessment after first 24 h of fermentation. Journal of Food Nutrition Research, *53*, 2014, pp. 143–151. ISSN 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=1608>
19. Ačai, P. – Valík, Ľ. – Medveďová, A. – Studeničová, A.: *Staphylococcus aureus* in unripened ewes' lump

cheese. Part 2: Exposure assessment at the time of consumption. Journal of Food Nutrition Research, *53*, 2014, pp. 271–277. ISSN 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=1622>

20. Ačai, P. – Valík, Ľ. – Liptáková, D.: Quantitative risk assessment of *Bacillus cereus* in pasteurised milk produced in the Slovak Republic. Czech Journal of Food Sciences, *32*, 2014, pp. 122–131. DOI: 10.17221/106/2013-CJFS.
21. Mataragas, M. – Dimitriou, V. – Skandamis, P. N. – Drosinos, E. H.: Quantifying the spoilage and shelf-life of yoghurt with fruits. Food Microbiology, *28*, 2011, pp. 611–616. DOI: 10.1016/j.fm.2010.11.009.
22. Pérez-Rodríguez, F. – Carrasco, E. – Bover-Cid, S. – Jofré, A. – Valero, A.: Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage. EFSA supporting publication, *14*, 2017, EN-1252. DOI: 10.2903/sp.efsa.2017.EN-1252.
23. Pin, C. – Metris, A. – Baranyi, J.: Next generation of predictive models. In: Sofos, J. (Ed.): Advances in Microbial Food Safety. Vol. 1. Cambridge : Woodhead Publishing, 2013, pp. 498–515. ISBN: 978-0-85709-874-0. DOI: 10.1533/9780857098740.6.498.
24. Cocolin, L. – Mataragas, M. – Bourdichon, F. – Doulgeraki, A. – Pilet, M. – Jagadeesan, B. – Rantsiou, K. – Phister, T.: Next generation microbiological risk assessment meta-omics: The next need for integration. International Journal of Food Microbiology, *287*, 2018, pp. 10–17. DOI: 10.1016/j.ijfoodmicro.2017.11.008.
25. Wood, J.: Predictive metagenomics profiling: why, what and how? Bioinformatics Review, *2*, 2016, pp. 15–17. ISSN: 2455-6645. <https://issuu.com/bioinformaticsreview/docs/mar_2016>

Received 11 December 2018; 1st revised 14 January 2019; accepted 15 January 2019; published online 15 May 2019.