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Food safety and microbiological criteria*

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A b s t r a c t: The major cause of food borne diseases according to epidemiological data and risk analysis, are microorganisms and toxins of microorganisms. Uniform approach to ensure food safety is essential to achieve the same level of protection of human health and facilitate food trade between countries. Therefore with the new approach to food safety it was necessary to set up adequate microbiological criteria for foods. Microbiological criteria may be applied at various points in the food chain. They may be used during production and/or to asses the final product. Microbiological criteria may, however, play a supplementary role in the verification of HACCP.
Key words: *Microbiological criteria, limits, food safety, HACCP, FSOs, POS.*

Introduction

Economic importance and ubiquity of food in everyday life suggests that most attention in this area must be devoted to food safety by the society as a whole, public authorities, especially from direct producers (*Bunčić* et al., 2006). In recent decades there has been an enormous development in food manufacturing, processes and procedures necessary to ensure the achievement of acceptable standards of food safety. The situation in the field of food safety in the last decades of the twentieth century and the major issues that were being reported (BSE, emerging and re-emerging pathogens, polychlorinated biphenyls, dioxins, GM food ...) moved step closer to general conclusion that there were serious deficiencies in food safety, which has caused the need to change the policy of food safety. Uniform approach to ensuring food safety is essential to achieve the same level of protection of human health and facilitate food trade between countries. Today, the approach to food safety is based on an objective, rational scientific approach, on hazard analysis and risk assessment (*Katić*, 1997; *Bunčić i dr.,* 1996). The major cause of food borne disease according to epidemiological data and risk analysis, are microorganisms and toxins of microorganisms. Therefore with the new approach to food safety it was necessary to set up adequate microbiological criteria for foods. Principles for the development of risk assessment of microbiological hazards have been developed by the Codex Alimentarius (CAC) and EU Scientific Committee for Food. Codex Alimentarius has published principles for the development of microbiological criteria (*CAC*, 1997). In its development CAC used opinions of the International Commission on Microbiological Specification for Foods (*ICMSF,* 1986). Taking in account the principles of the CAC, and opinions of the Scientific Committee on Veterinary Measures relating to Public Health, the Commission of the European Union laid down the microbiological criteria for food in Regulation (EC) No 2073/2005. The main objective of this Regulation was to harmonize the microbiological criteria in the European Union countries. Harmonization of regulations in the field of food safety in Serbia with the European Union includes also the harmonization of legislation on microbiological criteria for foods. Ministry of Agriculture, Forestry and Water Management is October 2010, adopted „Regulation on general and special conditions of food hygiene at any stage of production, processing and transport (72/10)", which is in correspond dance in line with Regulation (EC) No. 2073/2005. Food safety is ensured through the application of food safety systems (HACCP), which starts from raw materials through the manufacturing

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process with the GHP, processing (including labeling), handling, distribution, storage, sale and consumption of food. Microbiological criteria used for verification of the applied control system. The limit values for certain microorganisms and their toxins were selected based on their importance to food safety, as well as for evaluation of hygiene in the production.

Regulation on microbiological criteria for food

The main causes of foodborne illnesses are microorganisms or their toxins.

Determination of microbiological criteria for foods should contribute to a certain degree of confidence that food is safe and appropriate quality throughout shelf-life, if it is handled properly with (*Regulation (EC) No.* 178/2002). Application of microbiological criteria should ensure that products are obtained under good hygienic conditions and that they are from a microbiological standpoint, safe for consumption, and to separate acceptable from unacceptable manufacturing products, to separate acceptable from unacceptable manufacturing practices and should be an integral part of the procedure for ensuring food safety, applied by food business operators. Also they should help in the validation and verification procedures that were established on the principles of HACCP (*Montville et al*., 2005).

Microorganisms in food are distributed heterogeneously, so testing a single unit sample is not sufficient to detect pathogenic microorganisms in the batch of food. Microbiological testing of final products can never ensure food safety even when examining a large number of samples (eg n = 60), and that when it does not get any positive result.

Microbiological criteria defined in the Regulation (72/10) are based on a scientific risk assessment. Regulation introduced some new criteria which apply to certain categories of food, and microorganisms that have not previously been an integral part of the Regulation on microbiological safety of food (e.g. criteria for cut fruits and vegetables, *Salmonella* species in carcasses of slaughtered animals, and *Listeria monocytogenes* in certain foods ready for consumption). Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and Regulation (72/10) introduced two types of criteria: the criterion for food safety and process hygiene criteria. Food safety criterion is the criterion by which the acceptability of a product or series of products applicable to products placed on the market. These criteria are not applicable to products intended for further processing by the food business operator. Food safety criteria include pathogenic microorganisms, their toxins and metabolites. The advantage of establishing criteria for the safety of food pathogens is that the harmonized standards on eligibility for food authorities and food industry. The process hygiene criterion indicating the acceptable functioning of the production process and does not apply to products that are put on the market. It sets an indicative contamination values above corrective actions which are required to maintain the hygiene of the process in compliance with the food law Offical Journal of Republic of Serbia no (41/09).

Microbiological criteria in food safety system

Microbiological criteria in the Regulation (72/10) intend to be used by food business operator at various points in the food chain. They may be used during production and / or to asses the final product. However, microbiological criteria may be involved in primary production, the customer requires that the primary product meets certain microbiological conditions that are considered to affect the safety and quality of its final product (e.g. improve process hygiene and selection of raw materials). Food business operator must ensure that food is fully compliant with the requirements of the Regulations (72/10) also has to take necessary actions if it determines that the food does not meet any of the criteria.

The Regulations (72/10) doen not specify the minimum requirements for testing (except for the carcasses of cattle, pigs, sheep's, goats, horses and poultry and also for minced meat, meat and semi-mechanically separated meat. If microbiological testing are required, with the exception of the above mentioned cases, Regulation (72/10) allows food business operators to use their own food safety management procedures to establish an appropriate sampling regime. The number of required samplings will depend on the applied procedures of food safety risks associated with the product, as well as the nature and extent of production.

Food business operator has to define in what circumstances it is appropriate to use microbiological testing to prove compliance with the criteria, however, the microbiological examination (with the exceptions mentioned above) is not always necessary. For example, when the established food safety management procedures based on the principles of HACCP and good hygiene practice, routine monitoring of physical parameters (such as monitoring the temperature and time, pH, levels of preservatives and water activity) can provide adequate security to the criteria met (*Bunčić et al.,* 2009).

Sampling and microbiological testing are not the only measures that are applied to ensure food safety and do not replace the application of measures to prevent the occurrence of hazards and risk

management by the food business operator shall carry out. Mandatory microbiological criterion is defined only for food and / or stages in the production process for which there is no other effective mean of monitoring measures applied to prove the usefulness of such criteria to ensure greater consumer protection.

Sampling plan and limit values for microorganisms are selected on the basis of the risk to human health, the conditions in which certain foods are stored and how it is consumed. The sustainability and growing of microorganisms in food depend on the characteristics of microorganisms, factors related to food (nutrients, aw, pH, redox potential, antimicrobial compounds) and environmental factors (humidity, concentration of gases, temperature) (*Mossel et al*., 1991, *Katić*, 1995, *Katić et al.,* 1998). In determining the limits all the factors are taken into account that determine microbial growth and/or formation of toxins.

For microbiological testing of food, used 2--class or plan of 3-class plan are most commonly plan. Pathogenic microorganisms may not be present in a given quantity of food. To prove it the "2-class plan" used. The results obtained by such sampling are classified as "presence" and "absence".

When a firm 2-class plan is not necessary, 3--class plan frequently used to examine for hygiene indicators where enumeration of microbes units--volume or mass is possible. 3-class plan allows the proportion of the units of the sample between acceptance and non-acceptance.

The sample was composed of one or several units or portion of matter selected by different means in a population or in an important, quantity of matter which is intended to provide information on a given characteristic of the studied population or matter and to provide a basses for a decision concerning the population or matter in question or concerning the process which has produced it. An integral part of microbiological criteria, the number of sample units that represent the sample (n), the threshold number of bacteria (m), the maximum value of the number of bacteria (M) and number of samples (c) where the number of bacteria may exceed the limits (m) and less than the maximum value (M). In the food safety criterion m = M, and c = 0, except for histamine, the result is considered satisfactory if the number of bacteria in all units of the sample below the threshold value, the result is considered not acceptable if the bacteria in one or more of the same sample unit or greater than M.

Salmonella spp. most frequent causes alimentary toxic infections, and and for the emergence of the disease is required a small number of microorganisms, and for *Salmonella* spp. within the food safety criteria stipulated in the absence of 25 g in all five sample units.

Limit for staphylococcal enterotoxin is prescribed for cheese, milk powder and whey powder if the number of coagulase-positive staphylococci greater than 10^5 / g of cheese made from raw milk or 1000 / g of cheese made from pasteurized milk. In the process hygiene criteria provided for the enumeration of coagulase-positive staphylococci during cheese making process when it is expected that the number of coagulase-positive staphylococci to be the biggest. In determining the threshold values for coagulase-positive staphylococci the conditions for the growing and production of enterotoxins in cheese were taken into account.

When it comes to inspections there is no specified sampling and testing frequency. In cases where the inspection decides on the basis of results of applied measures of self-control in such conditions there is no guarantee to obtain safe products. For the purposes of the monitoring plan adopted by the competent national authority sampling frequency and types of tests depend on the aim.

The results of microbiological analysis of food depend on the method used to isolate and identify microorganisms or detection of their metabolites, so the test must use the reference methods laid down in the microbiological criteria. Using reference methods food testing results are obtained after several days. In order to obtain data on the microbiological safety of food in shorter time alternative rapid methods can be used to test. The application of alternative methods is acceptable if the validation of the method is given in relation to the reference method specified in the Regulations (72/10) or if the food business operator has applied a method that is certified by a third party in accordance with the protocol established EN / ISO standard 16140 or other internationally accepted protocol, and their usage approved by the Ministry in charge of agriculture. Application procedures of sampling and testing methods other than those defined in the regulations, are permitted provided that the food business operator can demonstrate that the application of those procedures and practices provided at least the same level of reliability and the criterion prescribed in the Regulations (72/10). Testing for the presence of other microorganisms, as compared to the corresponding microbiological limits that apply to them, and to examine other parameters, except for microbiological, can be performed only in terms of process hygiene criteria. If a food business operator in the food business examines other microorganisms, they must be included in a plan of self-control (e.g. at the request of the manufacturing specifications, the recommended parameters, etc..), as well as all the components that make up the microbiological criteria (food to which relations, a microorganism, the sampling plan, the limit values, meth-

ods, the phase in which the criterion applies, the corrective measures in case of unsatisfactory results).

Besides sampling the food, the Regulations (72/10) stipulates that food business operators must be required to take samples from the production areas and equipment (swabs) in facilities that manufacture ready-to-eat food able to support the growth and development of *L. monocytogenes*, in order to test the presence of these bacteria. Food business operator must determine the frequency of sampling with production areas and equipment (swabs) and is included in the plan of self-control. When determining the frequency of sampling and determining the production areas from which samples are taken, the determination of eligibility criteria and corrective measures, food business operators must take into account all information is available about the potential dangers and ways of managing, and that can occur during all phases of production.

In general, how should the results of microbiological tests could take into account when assessing the assurance provided by the food business operator, methods and frequency of sampling must be described and explained in detail in the plan of self-control food business operator.

In the Regulations (72/10) there are clearly defined phases in which the microbiological criteria appliy. Food safety criterion is applied only to food placed on the market during their shelf-life, a process hygiene criterion points to the proper functioning of the production process, is used in various stages of production and can not be applied to products on the market.

Application of microbiological criteria in HACCP system and the development of HACCP should run in parallel. For the successful management of food safety, the manufacturer shall: establish microbiological hazards significant for food safety; establishes the procedures to be applied to prevent, eliminate or reduced the hazard to an acceptable level; to have information on the degree of variability and the factors that influence on it; determine the parameters process taking into account the variability to ensure that critical limits are met; to establish procedures for monitoring applied measures; collects and interprets data obtained during the process.

Based on data collected through the time of the finding of micro-organisms, the producers sets the baseline process for the level of self-control that is achievable when the procedure GHP and HACCP in control. Data from the microbiological tests are used to obtain the baseline process, and are collected from at least five sources that vary by location or time of sampling within the process (e.g. raw materials, samples during production, samples from equipment/manufacturing area, and samples of end of shelf life). Once they establish a baseline of the process the next test results that differ from this line indicate deviations from normal due to changes in production conditions. Also, the baseline can be used for the establishment of microbiological specifications.

Control chart is made from data collected over time from one lot or from multiple lots (Ryan, 1989). On the X axis control chart is applied to the time at which the samples were taken during the process and the Y axis values obtained during testing. The control chart has three parallel lines: lower control limit, central line and upper control limit. In some instances where the lower control limit is below the lower limit detection (e.g. absence) assumed to be zero or predesignated value below lower limit of detection (*Jay et al*., 2005).

Each step in the production process has a certain degree of variability. When combined variability of each step in the system, we get the overall variability of the system. In well-controlled systems, the data show a tendency to claster around the baseline. In the case of propagation of bacteria in an environment where growth has been uneven, as in food, increase population density is exponential. Declines under adverse conditions are also roughly exponentially. It has long been observed that the concentration of microorganisms has a log - normal distribution. Control chart generally assumes that the distribution of data collected during the process is log-normal or approximately normal. Based on the normal distribution, approximately 68% of the value will fall between plus or minus one standard deviation of the mean, approximately 95% within 2 standard deviations and 99.7% within 3 standard deviations from the mean. Control limits are most commonly set at plus or minus 3 standard deviations. When the limit of 3 standard deviations set the probability that any data will be beyond the control is 0.3% if the process is truly under control. If the frequency at which the values fall above or below three standard deviations is greater than 0.3% then it is considered that the process is not under control (ICMSF, 2002).

Based on the collecting data on the control chart producer can evaluate any future results obtained and to determine whether the obtained result is within one, two or three standard deviations.

With improvement of food safety policy change and microbiological criteria for food makes use Food safety objectives (FSOs) and Performance objectives (POS). Food safety objectives (FSOs) and Performance objectives (POS) industry provide quantitative targets to be achieved in terms of species, in production, taking into account the hazard at the time of consumption (FSOs). As defined by Codex Alimentarius FSO is the maximum frequency and

/ or concentration of hazards in food, which at the time of consumption provides or contributes to a satisfactory level of health protection, and PO is the maximum frequency and / or concentration of hazards in food at the certain step of food production that contributes to a defined FSO or Appropriate Lovell off Protection (ALOP-in) in the case.

Microbiological criteria are not immutable, and future changes can be expected in relation to emergence of new microbial hazards in food, changes in industrial processes which consequently lead to changes in microbial population, and other factors important for food safety.

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Bezbednost hrane i mikrobiološki kriterijumi

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R e z i m e: Ujednačen prilaz osiguranju bezbednosti hrane je neophodan da bi se postigao isti nivo zaštite zdravlja ljudi i olakšao promet hrane između različitih zemalja. Danas se prilaz bezbednosti hrane zasniva na objektivnoj, racionalnoj i na naučnim osnovama zasnovanoj analizi hazarda i proceni rizika. Glavni uzrok bolesti prenosivih hrane, prema epidemiološkim podacima i analizi rizika, su mikroorganizmi i njihovi toksini. S toga je bilo potrebno da se, u skladu sa novim prilazom bezbednosti hrane, izvrše adekvatne promene mikrobioloških kriterijuma za hranu. Mikrobiološki kriterijumi se mogu primeniti na različitim mestima u lancu ishrane. Mogu se koristiti tokom proizvodnje hrane i / ili da se oceni gotov proizvod. Pored toga, mikrobiološki kriterijumi mogu imati ulogu u verifikaciji sistema HACCP.

Ključne reči: *mikrobiološki kriterijumi, limiti, bezbednosti hrane, HACCP, FSOs, POS.*

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