Monitoring of microbiological quality in the process of production of steak tartare

IVANA REGECOVÁ – PETER TUREK – PAVLINA JEVINOVÁ – MONIKA PIPOVÁ – JÁN MAČANGA

Summary

The aim of this study was to determine the level of microbial contamination (aerobic plate counts, counts of *Escherichia coli,* presence of *Listeria monocytogenes* and *Salmonella enterica*) during the production of a steak tartare. The results showed that total viable counts (*TVC*) ranged from 1.20 ± 0.00 log CFU·cm^{-2} to 2.90 ± 0.00 log CFU·cm^{-2} on the surface of tenderloin, and from 1.30 ± 0.01 log CFU·g^{-1} to 1.90 ± 0.02 log CFU·g^{-1} in its centre. After grinding and adding ingredients, a significant increase ($p < 0.001$) was observed in both the temperature (from 9.7 °C to 14.3 °C) and *TVC* of the final products (from 3.49 ± 0.01 log CFU·g^{-1} to 3.80 ± 0.02 log CFU·g^{-1}). In steaks tartare stored at 4 °C, a significant decrease ($p < 0.001$) in *TVC* was determined. The counts of *E. coli* were below the detection limit (< 2.00 log CFU·g^{-1}). No presence of *S. enterica* or *L. monocytogenes* was determined in any inspected sample. In terms of bacterial contamination, the results of this study demonstrate that the health risk of steak tartare to the consumer is minimal provided that all principles of good hygienic practice are kept to during its production.

Keywords

steak tartare; total viable counts; *Escherichia coli*; *Salmonella*; *Listeria monocytogenes*

In member states of the European Union (EU), steak tartare is a very popular meat delicacy. It is prepared from raw minced beef and from numerous ingredients such as onion, eggs, spices and sauces. The presence of raw meat makes this product very susceptible to bacterial spoilage and, therefore, a potential source of food-borne infections such as campylobacteriosis, salmonellosis, listeriosis, cysticercosis or toxoplasmosis [1]. Salmonellosis is an acute bacterial diarrhea disease. Eggs, in particular, can serve as the source of this infection in the steak tartare. Currently, both salmonellosis and campylobacteriosis show the highest incidence in all European countries (23.7 and 45.6 cases per 100 000 inhabitants, respectively). Despite its low incidence (0.4 cases per 100 000 inhabitants), listeriosis is also ranked among serious zoonotic diseases characterized by a high mortality. Most frequently, the consumption of insufficiently heat-treated meat is the cause of this disease [2]. Based on the reported mortality and the total number of confirmed cases, it is estimated that in 2015 approximately 270 deaths in humans were caused by listeriosis [3]. In recent years, the usage of contaminated raw materials in the preparation of food, incorrect technology in the preparation of food and the shortcomings in the storage of raw materials are the most common causes of epidemics of salmonellosis and other food-borne illnesses in EU countries.

To avoid the risk of alimentary diseases after consumption of ready-to-eat food products, including the steak tartare, it is necessary to ensure health safety of such products made of raw meat and eggs by applying EU legal acts [4–8]. The correct and qualified post-mortem carcass inspection is one of the basic steps that will ensure health safety of the final product and minimize the risk of food-borne diseases of parasitic origin at the same time [9]. Moreover, the preparation of thermally untreated meals (e.g. steak tartare) puts high demands on the application of good hygienic practice rules.

The need for enhancing microbial food safety and quality, without compromising the nutritional, functional and sensory characteristics of

Ivana Regecová, Peter Turek, Pavlina Jevinová, Monika Pipová, Ján Mačanga, Department of Food Hygiene and Technology, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovakia.

Correspondence author
Ivana Regecová, e-mail: ivana.regecova@uvlf.sk

foods, has created an increasing interest in innovative technologies in food industry. Various post-processing technologies (e.g. high-pressure processing – HPP) were recently introduced into meat industry to extend the shelf-life and to improve the safety of ready-to-eat meat products. As reported, a company in Netherlands already uses HPP to produce steak tartare [10, 11]. The novel green plasma technology has also been developed to improve the quality of food while ensuring the microbial safety of tartare steak and other non-thermally treated meat products [12].

However, no technological step in the preparation of such meals is able to ensure their full safety. Therefore, it is necessary to draw attention to specific categories of consumers for which the consumption is not recommended, in particular for children under 5 years of age, pregnant and breastfeeding women, seniors and consumers with impaired immunity [13].

For the above reasons, the aim of this study was to analyse and specify health risks based on results of microbiological investigation of the basic raw materials, all ingredients used for preparation of the steak tartare, as well as the finished ready-to-eat products. The study was also intended to demonstrate that the risk of eating the steak tartare may be reduced significantly by the compliance of all technological procedures with the basic rules of hygiene.

MATERIALS AND METHODS

Sample collection and preparation

Tenderloins were removed from farmed cattle (Michalovce, Slovakia) not older than 24 months of age after a qualified *post-mortem* inspection, then stored in the slaughterhouse–chilling room for 3 days (sample S) and used for preparing the steak tartare. In the chilling room, surface swabs were taken from the visceral side of the tenderloin, as well as from the area where the tenderloin was attached to lumbar vertebra. After chilling, the meat was transported in cooling boxes to the Department of Food Hygiene and Technology of University of Veterinary Medicine and Pharmacy in Košice, Slovakia (about 20 min).

The tenderloins were further divided into the following four samples:

- sample I (meat processed immediately after the delivery),
- sample II (vacuum-packed meat stored at 4 °C for 5 days before processing),
- sample III (vacuum-packed meat stored at –18 °C for 14 days before processing),
- sample IV (vacuum-packed meat stored at –18 °C for 21 days before processing).

Samples of tenderloin (I–IV) were used for preparation of the steak tartare according to the following procedure. Each sample of meat was washed, dried and fibrous connective tissues (tendons and fasciae) were removed. Minced beef was prepared with the help of commercial meat grinder. Fresh eggs, onion and other ingredients (black pepper powder, sweet red pepper powder, caraway powder, ketchup, Worcestershire sauce and mustard; all these purchased in the retail sale in Slovakia) were then mixed with the minced beef and shaped into small disks (diameter 9.0 cm, height 3.0 cm). The final products were steaks tartare, raw ready-to-eat non-thermally treated meals. Steaks were packaged individually into sterile containers covered with a foil and stored in the dark for 4 h at 4 °C or at room temperature.

Microbiological examination

During manufacturing of steak tartare, pH value, temperature and microbiological quality of the individual samples were determined.

Swabs for microbiological examination were taken from the visceral surface of the tenderloin and from the area adjacent to the lumbar vertebrae by a non-destructive method, using a sterile cotton swab and a sterile template of the total area of 100 cm^2, according to STN EN ISO 17604 [14]. Samples for microbiological examination were taken subsequently from the depth of tenderloin, minced beef and the final product immediately after its manufacturing, as well as after the storage at different temperatures (4 h at 4 °C or at room temperature). Sampling and preparation of samples for microbiological testing, as well as preparation of the initial suspension and further decimal dilutions, were performed in accordance with the legislative requirements [15].

Total viable counts (*TVC*) and counts of *E. coli* (*ECC*) were determined by the quantitative microbiological examination according to STN EN ISO standards [16, 17] using the plate count agar (Oxoid, Basingstoke, United Kingdom) for *TVC* and tryptone bile X-glucuronide medium (Oxoid) for *ECC*. Inoculated media were incubated at 30 °C for 72 h (*TVC*) or at 44 °C for 24 h (*ECC*). The presence of *L. monocytogenes* and *S. enterica* was determined by the culture-dependent qualitative examination according to STN EN ISO standards [18, 19].

Ingredients added to minced beef were also submitted to microbiological testing. In samples of black pepper powder, sweet red pepper powder,

caraway powder, ketchup, Worcestershire sauce and mustard, *TVC*, *ECC* and the presence of both *L. monocytogenes* and *S. enterica* were determined. Raw eggs, the most frequent source of food-borne salmonellosis in the steak tartare, were checked for the presence of *S. enterica*. After sampling for microbiological investigation, the pH values of those ingredients which were able to affect pH of the final product significantly (i. e. ketchup and mustard), were also measured.

Statistical analysis

Microbial counts were determined in six independent experiments with replicate samples. The results were statistically evaluated using GraphPad Prism 6.01 software (GraphPad Software, La Jolla, California, USA) with one-way ANOVA and Tukey's test for multiple comparison of means with a confidence interval set at 95 %.

RESULTS AND DISCUSSION

From the viewpoint of human health, parasites and bacteria found in raw meat of warm-blooded animals are different and more dangerous than those occuring in raw fish, such as sushi. In raw beef, the risk is mostly related to the presence of bacteria (e.g. *S. enterica*, *Campylobacter* spp., *L. monocytogenes*, *E. coli*), parasites (e.g. *Toxoplasma gondii*, *Cysticercus bovis*) and viruses (e.g. hepatitis E virus). Parasites and bacteria originate mainly from the animal intestines and could contaminate the meat at the slaughterhouse during slaughtering and meat processing (slicing, cutting, milling, transporting, packing, preparation etc.) [20]. As reported, bacteria of the families *Pseudomonadaceae* and *Enterobacteriaceae* are the predominant microflora on the meat surface [1].

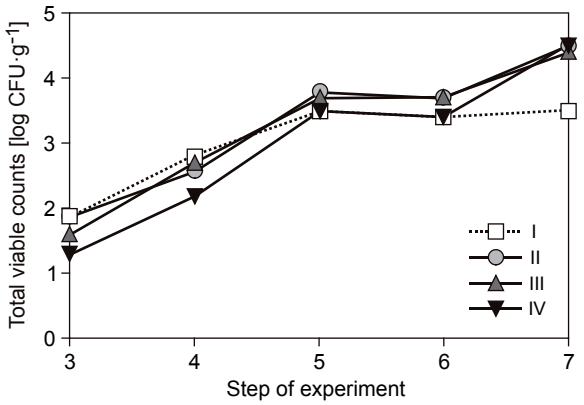

Fig. 1. Comparison of the average total viable counts among tenderloin samples during preparation of the steak tartare.

Step of experiment: 3 – sample from center of tenderloin; 4 – sample of minced beef; 5 – sample of fresh steak tartare; 6 – sample of steak tartare after storage at 4 °C; 7 – sample of steak tartare after storage at room temperature.
Samples of tenderloin: I – fresh, II – stored at 4 °C for 5 days, III – stored at –18 °C for 14 days, IV – stored at –18 °C for 21 days.

In addition to the above mentioned potential and obligatory pathogens, the presence of commensal flora can also be detected in/on fresh meat, this including *Brochothrix thermophacta*, *Lactobacillus agendas*, *Lactococcus piscium*, *Photobacterium kishitani*, *Xanthomonas oryzae* and *Leuconostoc gelidum* [1]. In general, microbial contamination of surfaces of raw meat usually exceeds 1.0 log CFU·cm^{-2} but is lower than 7.0 log CFU·cm^{-2} [21], as also demonstrated in this study. In swabs from the surface of fresh tenderloin, *TVC* ranged from 1.40 ± 0.01 log CFU·cm^{-2} to 2.90 ± 0.00 log CFU·cm^{-2}. The average *TVC* in samples taken from the center of tenderloin was 1.90 ± 0.02 log CFU·g^{-1} (Tab. 1).

After cold storage of the tenderloin at 4 °C and also its long-term frozen storage at –18 °C, a significant decrease in *TVC* ($p < 0.001$) was observed in both the surface swabs and the samples taken from the depth of the tenderloins (Tab. 1; Fig. 1). A significant decrease ($p < 0.01$) in *TVC* of raw beef during cold storage at 4 °C was also confirmed by Koutsoumanis et al. [21]. As reported by Kröckel and Hechelmann [22], the storage of fresh meat at 2 °C immediately after slaughtering did not result in microbial growth within the first 24 h. Due to conversion of glycogen to lactic acid, pH of the meat decreased to 5.7–6.0 at the same time. Similarly, the results of this study also confirmed an average pH value of 5.8 in all tenderloin samples before mincing (samples I–IV). This value indicates a correct ripening process of fresh meat.

After mincing, the pH value of meat remained unchanged. However, both the temperature of the minced meat as well as *TVC* increased significantly, the average temperature in sample III reached a value of 13.2 °C (Fig. 1). It is a well known fact that the grinding process contributes to an increase in the total viable counts in minced beef [23]. Increased levels of *TVC* (4.11 log CFU·g^{1}) in raw minced beef were also reported by Hayes et al. [24] and other authors [25, 26]. On the basis of these results, it is strongly recommended to mince the meat used for preparation of the steak tartare at refrigeration temperatures, thereby reducing the temperature of the raw material as well as the microbial load at this step.

As seen in Fig. 1, the temperatures have risen again after addition of ingredients to the minced

Tab. 1. Microbial contamination of steak tartare.

Sample	Step of experiment	Temperature of samples [°C]	Total viable counts [log CFU·g^{-1}] or [log CFU·cm^{-2}]*	*E. coli* counts [log CFU·g^{-1}] or [log CFU·cm^{-2}]*
S Tenderloin at the slaughterhouse	A	3.2	2.90 ± 0.00 *	< 2.00 *
	B	3.2	1.40 ± 0.00 *	< 2.00 *
I Fresh tenderloin	1	3.0	2.90 ± 0.00 *	< 2.00 *
	2	3.0	1.40 ± 0.01 *	< 2.00 *
	3	3.0	1.90 ± 0.02	< 2.00
	4	12.8	2.80 ± 0.01	< 2.00
	5	13.5	3.50 ± 0.00	< 2.00
	6	3.0	3.39 ± 0.00	< 2.00
	7	15.6	3.51 ± 0.01	< 2.00
II Tenderloin stored at 4 °C, 5 days	1	2.9	2.60 ± 0.00 *	< 2.00 *
	2	2.9	1.80 ± 0.01 *	< 2.00 *
	3	2.9	1.90 ± 0.00	< 2.00
	4	10.6	2.60 ± 0.00	< 2.00
	5	12.3	3.80 ± 0.02	< 2.00
	6	3.5	3.70 ± 0.00	< 2.00
	7	15.6	4.50 ± 0.01	< 2.00
III Tenderloin stored at –18 °C, 14 days	1	2.9	2.60 ± 0.01 *	< 2.00 *
	2	2.9	1.59 ± 0.01 *	< 2.00 *
	3	2.9	1.60 ± 0.00	< 2.00
	4	13.2	2.70 ± 0.01	< 2.00
	5	14.3	3.70 ± 0.01	< 2.00
	6	3.9	3.70 ± 0.00	< 2.00
	7	15.5	4.40 ± 0.00	< 2.00
IV Tenderloin stored at –18 °C, 21 days	1	2.1	2.08 ± 0.02 *	< 2.00 *
	2	2.1	1.20 ± 0.00 *	< 2.00 *
	3	2.1	1,30 ± 0.01	< 2.00
	4	9.7	2.19 ± 0.01	< 2.00
	5	12.2	3.49 ± 0.01	< 2.00
	6	3.6	3.40 ± 0.00	< 2.00
	7	14.9	4.50 ± 0.02	< 2.00

The results are expressed as the (mean ± standard deviation) of six independent measurements.
* – values expressed as logarithm of colony forming units per square centimeter.
Step of experiment: A – swab sample from visceral surface; B – swab sample from surface of connection to the backbone; 1 – swab sample from visceral surface after rinsing; 2 – swab sample from surface of connection to the backbone after rinsing; 3 – sample from the center of tenderloin after rinsing; 4 – sample of minced beef; 5 – sample of the final product; 6 – sample of the final product after storage at 4 °C; 7 – sample of the final product after storage at room temperature.

meat, which was also accompanied by a significant increase in *TVC* ($p < 0.001$) of the final products. The increase in temperature after addition of ingredients in this study was most probably caused by the temperature of ingredients themselves, as they had been stored at 20 °C during the experiment. *TVC* in final products ranged from 3.49 ± 0.01 log CFU·g^{-1} to 3.80 ± 0.02 log CFU·g^{-1}. The most significant increase in *TVC* ($p < 0.001$) was observed in sample IV between the stages of minced meat (2.19 ± 0.01 log CFU·g^{-1}) and the final product (3.49 ± 0.01 log CFU·g^{-1}).

The increase in *TVC* could also be caused by the ingredients added. In this study, *TVC* of spices ranged from 1.00 ± 0.00 log CFU·g^{-1} to 1.80 ± 0.01 log CFU·g^{-1}, the *ECC* being below the limit of the detection method (< 2.00 log CFU·g^{-1}). The presence of *S. enterica* was not determined in any sample. Despite of results of microbiological testing presented in this study, food ingredients may be contaminated with pathogenic microorganisms involved in foodborne infections. This also applies to spices with low water activity. Although the low water activity

contributes to long-term sustainability, pathogenic microorganisms still survive in a dry environment. To proof that spices are a cause of food-borne disease is difficult, because the detection is often limited to the main food components. In the European legislation, microbiological limits specific for dried herbs and spices are not yet established. To meet valid EU criteria, in particular for beef steak and steak tartare, it is therefore recommended to use ingredients decontaminated by various methods [27].

Pathogenic bacteria are routinely detected or quantified in spices using standard horizontal microbiological methods. However, various spices contain compounds that may interfere with plating on nutrient agar media and/or proliferation of bacteria during culture-based enrichment. Such interference may lead to false negativity or compromised quantitative results [28–30]. A way to overcome the described methodological problems is to utilize a culture-independent approach, i.e. to extract DNA from spices and then analyse it by pathogen-specific real-time polymerase chain reaction [31].

In the case of steak tartare, additional raw ingredients, such as chicken egg yolks, are the main source of health risk because of their possible microbial contamination (*S. enterica*, *E. coli*). *Salmonella* egg contamination is a complex issue affected by each stage of the food production process. In view of the current shift in consumer preferences and the growing demand for raw food products, it is necessry to strengthen *Salmonella* control measures after egg collection by washing, pasteurization or irradiation [32]. Chicken eggs used in this study were checked for the presence of *S. enterica* with a negative result.

Ingredients added to the final products, such as spices, can also change pH and osmotic state of the product, which can ultimately affect the microbial load of the steak tartare. The change in pH was confirmed in this study. The pH value of the final product imediatelly after its preparation decreased from 5.8 to 5.3 after a four-hour storage period. This significant decrease was probably caused by the addition of acidic ingredients, such as mustard (pH 3.9) or ketchup (pH 3.7). Low pH values correlated well with the results of microbiological examination of the final products, where both the pH values and *TVC* decreased after the four-hour-storage (Fig. 1).

In the final products (steaks tartare) stored at 4 °C, a significant decrease ($p < 0.001$) in *TVC* was determined. This result could be attributed not only to the reduced pH, but also to the low storage temperature (4 °C), which suppressed the levels of some bacteria. On the other hand, in products that were stored at room temperature, all *TVC* were significantly higher (by 0.10 log CFU·g^{-1} on average; $p < 0.001$), in particular in samples II, III, and IV, where the average *TVC* increased within 4 h from 4.40 ± 0.00 log CFU·g^{-1} to 4.51 ± 0.01 log CFU·g^{-1}. These results confirm the fact that, at an interruption of cold chain, an increase in the counts of microorganisms, including food-borne pathogens, can take place. Therefore, in the case of delayed serving, the steak tartare must be cooled to 0–4 °C as soon as possible. Gram-negative bacteria (*E. coli*, *S. enterica*) are generally more sensitive to temperatures of 0–7 °C. Under cold conditions, they lose their ability to use inorganic nitrogen to synthesize cellular proteins and have to rely solely on peptides because they cannot directly use aminoacids [33].

In all the samples inspected, *ECC* were below the limit (< 2.00 log CFU·g^{-1}). Similar results were reported by Bohaychuk et al. [34]. The authors did not determine the presence of *E. coli* neither in raw beef nor in chicken eggs. The only *E. coli* isolate obtained from the sample of minced beef was not confirmed by serotyping as E. *coli* O157: H7, the serotype most frequently associated with large outbreaks of epidemics in Canada, United States and United Kingdom [35]. However, other *E. coli* serotypes (e.g. STEC-O26, O91, O103, O111, O118, O145 and O166) were isolated from whole beef throughout the world. Those serotypes are known to cause human diseases ranging from bloody diarrhea and hemorrhagic colitis to life-threatening hemolytic uremic syndrome [36]. Recent studies also showed a level of prevalence of *E. coli* in raw beef and ground beef samples: 7 % in Turkey, 4.7 %, in the Netherlands and 9 % in Spain [37–39].

On the contrary, a relatively high prevalence of *L. monocytogenes* (52 %) in raw minced beef and products from it (steak tartare) was confirmed by Bohaychuk et al. [34]. However, the determined rate was comparable to 34.9 % for raw beef in Spain [40]. Other researchers found *L. monocytogenes* in raw sausage products in Denmark, Switzerland, Mexico, Ireland and Italy [41–45]. In 2013, most reported cases of gastrointestinal disease caused by *L. monocytogenes* in EU were, with a mortality rate of 15.6 %, after consumption of raw meat products [46]. No presence of *L. monocytogenes* was determined in this study.

The qualitative microbiological examination did not determine the presence of *S. enterica* in any sample of minced beef or any ingredient used in this study. Our results are similar to those of Bohaychuk et al. [34], who did not detect any

S. enterica in raw minced beef or in the products made thereof, including the steak tartare.

CONCLUSION

The results of this study demonstrate that the microbiological risks associated with the consumption of steak tartare can be significantly reduced by keeping to basic rules of good manufacturing practice, strict control of product temperature and the use of very fresh meat. Moreover, storage conditions of the finished products are also of great importance. Despite the appropriate manipulation, mincing and temperature control during storage of meat, there is always a risk of contamination of the steak tartare as a raw ready-to-eat product. Therefore, every consumer should be properly instructed that the consumption of products intended to be eaten raw may lead to a serious foodborne illness.

Acknowledgements

This work was supported by Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0705/16)

REFERENCES

1. Delhalle, L. – Korsak, N. – Taminiau, B. – Nezer, C. – Burteau, S. – Delcenserie, V. J. – Poullet, B. – Daube, G.: Exploring the bacterial diversity of Belgian steak tartare using metagenetics and quantitative real-time PCR analysis. Journal of Food Protection, *79*, 2016, pp. 220–229. DOI: 10.4315/0362-028X.JFP-15-185.
2. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA Journal, *9*, 2011, Article 2090. DOI: 10.2903/j.efsa.2011.2090.
3. Zoonoses report: *Listeria* infections stable but frequently reported among the elderly. In: European Food Safety Authority (EFSA) [online]. Parma: European Food Safety Authority, 2016 [cit. 6 Juny 2017]. <http://www.efsa.europa.eu/en/press/news/161216>
4. 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. Official Journal of the European Communities, *45*, 2002, L 31, pp. 1–25. ISSN: 1725-2555. <http://eur-lex.europa.eu/eli/reg/2002/178/oj>
5. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Communities, *47*, 2004, L 139, pp. 1–54. ISSN: 1725-2555. <http://eur-lex.europa.eu/eli/reg/2004/852/oj>
6. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Communities, *47*, 2004, L 139, pp. 55–205. ISSN: 1725-2555. <http://data.europa.eu/eli/reg/2004/853/oj>
7. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. Official Journal of the European Communities, *47*, 2004, L 139, pp. 206–320. ISSN: 1725-2555. <http://data.europa.eu/eli/reg/2004/854/oj>
8. Commission regulation (EC) No 2073/2005 of 15 November 2005, on microbiological criteria for foodstuffs. Official Journal of the European Communities, *48*, 2005, L 338, pp. 1–26. ISSN: 1725-2555. <http://data.europa.eu/eli/reg/2005/2073/oj>
9. Steinhauserová, I. – Bořilová, G. – Hulánková, R.: Výskyt vybraných zoonóz spojených s konzumací masa a masných výrobků. (The occurrence of selected zoonoses associated with the consumption of meat and meat products.) Maso, *25*, 2014, No. 3, pp. 16–21. ISSN: 1210-4086. In Czech.
10. Marcos, B. – Kerry, J. P. – Mullen, A. M.: High pressure induced changes on sarcoplasmic protein fraction and quality indicators. Meat Science, *85*, 2010, pp. 115–120. DOI: 10.1016/j.meatsci.2009.12.014.
11. Bajovic, B. – Bolumar, T. – Heinz,V.: Quality considerations with high pressure processing of fresh and value added meat products. Meat Science, *92*, 2012, pp. 280–289. DOI: 10.1016/j.meatsci.2012.04.024.
12. Rivalain, N. – Roquain, J. – Demazeau, G.: Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in biotechnologies. Biotechnology Advances, *28*, 2010, pp. 659–672. DOI: 10.1016/j.biotechadv.2010.04.001.
13. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods – Technical report. Microbiologial Risk Assessment Series 5. Rome : Food And Agriculture Organization, 2004. ISBN: 9251051275. <http://www.fao.org/3/a-y5394e.pdf>
14. STN EN ISO 17604. Mikrobiológia potravín a krmív. Odber vzoriek z tiel jatočných zvierat na mikrobiologickú analýzu (ISO 17604: 2015). (Microbiology of the food chain – Carcass sampling for microbiological analysis (ISO 17604:2015).) Bratislava: Slovak Standards Institute, 2016.
15. STN EN ISO 6887-2/O1. Mikrobiológia potravín a krmív. Úprava analytických vzoriek, príprava základnej suspenzie a desaťnásobných riedení na mikrobiologické skúšanie. Časť 2: Špecifické pokyny na úpravu mäsa a mäsových výrobkov (ISO 6887-2:2003). (Microbiology of food and animal feeding stuffs. Preparation of test samples, initial

suspension and decimal dilutions for microbiological examination. Part 2: Specific rules for the preparation of meat and meat products (ISO 6887-2:2003). Bratislava: Slovak Standards Institute, 2006.
16. STN EN ISO 4833-1. Mikrobiológia potravinárskeho reťazca. Horizontálna metóda na stanovenie počtu mikroorganizmov. Časť 1: Metóda počítania kolónií kultivovaných pri 30 °C zalievaním inokula (ISO 4833-1:2013). (Microbiology of food chain. Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 degrees C by the pour plate technique.) Bratislava: Slovak Standards Institute, 2014. In Slovak.
17. STN ISO 16649-2. Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu β-glukuronidázopozitívnych *Escherichia coli*. Časť 2: Metóda počítania kolónií pri 44 °C použitím 5-bromo-4-chloro-3-indolyl-β-D-glukuronidu. (Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of β-glucuronidasepositive *Escherichia coli*. Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide.) Bratislava: Slovak Standards Institute, 2007. In Slovak.
18. STN EN ISO 11290-1. Mikrobiológia potravín a krmív. Horizontálna metóda na dôkaz a stanovenie počtu baktérií *Listeria monocytogenes*. Časť 1: Metóda dôkazu (ISO 11290-1:1996). (Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method.) Bratislava: Slovak Standards Institute, 2000. In Slovak.
19. STN EN ISO 6579/AC. Mikrobiológia potravín a krmív. Horizontálna metóda na dôkaz baktérií rodu *Salmonella (*ISO 6579:2002). Oprava AC. (Microbiology of food and animal feedings stuffs. Horizontal method for the detection of *Salmonella* spp. (ISO 6579:2002/AC:2006).) Bratislava: Slovak Standards Institute, 2006. In Slovak.
20. Kopper, G. – Mirecki, S. – Kljujev, I. S. – Raicevic, V. B. – Lalevic, B. T. – Jovicic-Petrovic, J. – Stojanovski, S. – Dimovska-Blazekovic, D.: Chapter 23 – Hygiene in primary production. In: Motarjemi, Y. – Lelieveld, H. (Eds.): Food safety management – A practical guide for the food industry. Amsterdam : Elsevier, 2014, pp. 559–621. ISBN: 9780123815040. DOI: 10.1016/B978-0-12-381504-0.00023-8.
21. Koutsoumanis, K. P. – Geornaras, I. – Sofos, J. N.: Chapter 52. Microbiology of land muscle food. In: Hui, Y. H. (Ed.): Handbook of food science, technology and engineering. Vol. 4. New York : CRC Press, 2005, pp. 52.1–52.43. ISBN: 978146650787. DOI: 10.1201/b15995-57.
22. Kröckel, L. – Hechelmann, H.: Mikrobiologie der Kühlung, Kühllagerung und Fleischreifung. (Microbiology of chilled meats.) Fleischwirtschaft, *79*, 1999, No. 3, pp. 90–93. ISSN: 0015-363X. In German.
23. Zambuchini, B. – Fiorini, D. – Verdenelli, M. C. – Orpianesi, C. – Ballini, R.: Inhibition of microbiological activity during sole (*Solea solea* L.) chilled storage by applying ellagic and ascorbic acids. LWT – Food Science and Technology, *41*, 2008, pp. 1733–1738. DOI: 10.1016/j.lwt.2007.11.004.
24. Hayes, J. E. – Stepanyan, V. – Allen, P. – O'Grady, M. N. – O'Brien, N. M. – Kerry J. P.: The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. Meat Science, *83*, 2009, pp. 201–208. DOI: 10.1016/j.meatsci.2009.04.019.
25. Lambropoulou, K. A. – Drosinos, E. H. – Nychas G. J. E.: The effect of glucose supplementation on the spoilage microflora and chemical composition of minced beef stored aerobically or under a modified atmosphere at 4 °C. International Journal of Food Microbiology, *30,* 1996, pp. 281–291. DOI: 10.1016/0168-1605(96)00954-3.
26. Bañón, S. – Díaz, P. – Rodríguez, M. – Garrido, M. D. – Price, A.: Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. Meat Science, *77*, 2007, pp. 626–633. DOI: 10.1016/j.meatsci.2007.05.015.
27. Mader, A. – Schaarschmidt, S.: Bakteriologische Sicherheit von Gewürzen : Mikrobiologische Kontaminationen können zu lebensmittelbedingten Krankheitsausbrüchen führen. (Bacteriological safety of spices : Microbiological contamination can lead to outbreaks of foodborne disease.) Fleischwirtschaft, *95*, 2015, No. 11. pp. 42–47. ISSN: 0015-363X. In German.
28. Vitullo, M. – Ripabelli, G. – Fanelli, I. – Tamburro, M. – Delfine, S. – Sammarco M. L.: Microbiological and toxicological quality of dried herbs. Letters in Applied Microbiology, *52*, 2011, pp. 573–580. DOI: 10.1111/j.1472-765X.2011.03040.x.
29. Cabicarová, T. – Kaclíková, E. – Mader, A. – Minarovičová, J. – Koreňová, J. – Kuchta, T.: Improvement of the detection sensitivity for *Staphylococcus aureus* in spices and herbs. Food Analytical Methods, *9*, 2016, pp. 1980–1984. ISSN: 1936-9751 (print), 1936-976X (online).
30. Lins, R.: Detection of *Salmonella* spp. in spices and herbs. Food Control, in press, corrected proof, available online 29 May 2017 [cit. 24 July 2017]. DOI: 10.1016/j.foodcont.2017.05.040.
31. De Medici, M. – Kuchta, T. – Knutsson, R. – Angelov, A. – Auricchio, B. – Barbanera, M. – Diaz-Amigo, C. – Fiore, A. – Kudirkiene, E. – Hohl, A. – Horvatek Tomic, D. – Gotcheva, V. – Popping, B. – Prukner-Radovcic, E. – Scaramaglia, S. – Siekel, P. – To, K. A. – Wagner, M.: Rapid methods for quality assurance of foods: The next decade with polymerase chain reaction (PCR)-based food monitoring. Food Analytical Methods, *8*, 2015, pp. 255–271. ISSN: 1936-9751 (print), 1936-976X (online).
32. Whiley, H. – Ross, K.: *Salmonella* and eggs: From production to plate. International Journal of Environmental Research and Public Health, *12*, 2015, pp. 2543–2556. DOI: 10.3390/ijerph120302543.
33. Rosset, R.: Chilling, freezing and thawing. In: Brown, M. H. (Ed.): Meat microbiology. London: Applied Science Publishers, 1982, pp. 265–318. ISBN: 9789401198219.

34. Bohaychuk, V. M. – Gensler, G. E. – King, R. K. – Manninen, K. I. – Sorensen, O. – Wu, J. T. – Stiles, M. E. – McMullen, L. M.: Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. Journal of Food Protection, *69*, 2006, pp. 2176–2182. DOI: 10.4315/0362-028X-69.9.2176.
35. Pradel, N. – Livrelli, V. – De Champs, C. – Palcoux, J.-B. – Reynaud, A. – Scheutz, F. – Sirot, J. – Joly, B. – Forestier, C.: Prevalence and characterization of Shiga toxin–producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. Journal of Clinical Microbiology, *38*, 2000, pp. 1023–1031. ISSN: 0095-1137. <http://jcm.asm.org/content/38/3/1023.full.pdf+html>
36. Hussein, H. S.: Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. Journal of Animal Science, *85*, pp. 63–72. DOI: 10.2527/jas.2006-421.
37. Doi, Y. – Paterson D. L. – Egea, P. – Pascual, A. – López-Cerero, L. – Navarro, M. D. – Adams-Haduch, J. M. – Qureshi, Z. A. – Sidjabat, H. E. – Rodríguez-Baño, J.: Extended-spectrum and CMY-type β-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. Clinical Microbiology and Infection, *16*, 2009, pp. 33–38. DOI: 10.1111/j.1469-0691.2009.03001.x.
38. Overdevest, I. – Willemsen, I. – Rijnsburger, M. – Eustace, A. – Xu, L. – Hawkey, P. – Heck, M. – Savelkoul, P. – Vandenbroucke-Grauls, C. – Zwaluw, K. – Huijsdens, X. – Kluytmans, J.: Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerging Infectious Diseases, *17*, 2011, pp. 1216–1222. DOI: 10.3201/eid1707.110209.
39. Egea, P. – Lopez-Cerero, L. – Torres, E. – Gomez-Sanchez, M. C. – Serrano, L. – Navarro Sanchez-Ortiz, M. D. – Rodriguez-Bano, J. – Pascual, A.: Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing *Escherichia coli* in the south of Spain. International Journal of Food Microbiology, *159*, 2012, pp. 69–73. DOI: 10.1016/j.ijfoodmicro.2012.08.002.
40. Vitas, A. I. – Aguado, V. – Garcia-Jalon, I.: Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). International Journal of Food Microbiology, *90*, 2004, pp. 349–356. DOI: 10.1016/S0168-1605(03)00314-3.
41. Busani, L. – Cigliano, A. – Taioli, E. – Caligiuri, V. – Chiavacci, L. – Di Bella, C. – Battisti, A. – Duranti, A. – Gianfranceschi, M. – Nardella, M. C. – Ricci, A. – Rolesu, S. – Tamba, M. – Marabelli, R. – Caprioli, A.: Prevalence of *Salmonella enterica* and *Listeria monocytogenes* contamination in foods of animal origin in Italy. Journal of Food Protection, *68*, 2005, pp. 1729–1733. DOI: 10.4315/0362-028X-68.8.1729.
42. Fantelli, K. – Stephan, R.: Prevalence and characteristics of Shiga toxin–producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. International Journal of Food Microbiology, *70*, 2001, pp. 63–69. DOI: 10.1016/S0168-1605(01)00515-3.
43. Inoue, S. – Nakama, A. – Arai, Y. – Kokubo, Y. – Maruyama, T. – Saito, A. – Yoshida, T. – Terao, M. – Yamamoto, S. – Kumagai S.: Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. International Journal of Food Microbiology, *59*, 2000, pp. 73–77. DOI: 10.1016/S0168-1605(00)00284-1.
44. Norrung, B. – Andersen, J. K. – Schlundt. J.: Incidence and control of *Listeria monocytogenes* in foods in Denmark. International Journal of Food Microbiology, *53*, 1999, pp. 195–203. DOI: 10.1016/S0168-1605(99)00157-9.
45. Soultos, N. – Koidis, P. – Madden, R. H.: Presence of *Listeria* and *Salmonella* spp. in retail chicken in Northern Ireland. Letters Applied Microbiology, *37*, 2003, pp. 421–423. DOI: 10.1046/j.1472-765X.2003.01423.x.
46. The European Union Summary on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal *13*, 2015, Article 3991. DOI: 10.2903/j.efsa.2015.3991.

Received 29 June 2017; 1st revised 27 July 2017; accepted 22 August 2017; published online 16 October 2017.