ISSN 2466-2852

SCIENTIFIC JOURNAL

meat technology

59 Vol.

1 No.

2018 Belgrade

FOUNDER AND PUBLISHER
INSTITUTE OF MEAT HYGIENE AND TECHNOLOGY

"Meat Technology" is the scientific journal that publishes results of basic and applied research in the field of biotechnical sciences i.e. the following subcategories: veterinary sciences, food engineering and biotechnology.

Journal "Meat Technology" is abstracted in FSTA (Food Science and Technology Abstract). Full text is available in CABI Database, EBSCO publishing, AGRIS Database and www.inmes.rs. "Meat Technology" is an open access journal. All articles can be downloaded free and used in accordance with the Creative Commons Attribution 4.0 International (CC BY 4.0).

Editorial board of Scientific Journal "Meat Technology"

SSN 2466-2852

meat technology
scientific journal

Meat Technology Vol. 59 No. 1 P. 1–74 Belgrade 2018

CONTENT

INSTITUTE OF MEAT HYGIENE AND TECHNOLOGY • Belgrade 1955 •
INMES
www.inmes.rs

FOUNDER AND PUBLISHER

Institute of Meat Hygiene and Technology

11000 Belgrade, Kacanskog 13
P.O. Box 33-49
Phone 381 11 2650-655
Fax 381 11 2651-825
e-mail: institut@inmes.rs
www.inmes.rs

DIRECTOR
Vesna Z. Djordjevic, PhD

EDITOR IN CHIEF
Vesna Z. Djordjevic, PhD

PROOFREADER FOR ENGLISH LANGUAGE
Sheryl Avery, PhD

TEHNICAL EDITION
Danijela Sarcevic, PhD
Slavisa Sobot

Based on the opinion issued by Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 413-00-00416/2000-01), this publication is of special interest for the science.

Subscription

Annual subscription rate for aboard is: 100 EUR. Orders should be sent to Institute for Meat Hygiene and Technology, P.O. Box 33-49, Kacanskog 13, 11000 Belgrade, R. Serbia.

Annual subscription rate for Republic of Serbia is 5000.00 RSD. Payments can be made to an account of Institute no 205-7803-56 at Commercial Bank with a note subscription to the journal.

www.journalmeattechnology.com

Computer processing and printing
„Naučna KMD“, Beograd
www.naucnakmd.com
Circulation 150 copies

PUBLICATION OF THIS JOURNAL IS FINNANCIALLY SUPPORTED BY:
Ministry of Education, Science and Technological Development of the Republic of Serbia

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.5'64(497.11)"2017"
ID: 265895180
https://doi.org/10.18485/meattech.2018.59.1.1

Original scientific paper

A hygiene report regarding slaughter process of pig and cattle carcasses for 2017 in Serbia

Lazar Milojevic[1], Brankica Lakicevic[1], Vesna Jankovic[1], Radmila Mitrovic[1], Aleksandra Nikolic[1], Milijana Babic[2], Branko Velebit[1]

A b s t r a c t: For the successful validation and verification of the HACCP system, a food business operator is obliged, among other duties, to have continuous microbiological data of carcasses which are followed by a certain dynamic that the subject himself prescribed. To obtain these data, it is necessary to perform systematic monitoring of indicator microorganisms. The most important meat hygiene indicators are Total Viable Counts (TVC) and Enterobacteriaceae (EC). TVC are defined as indicators of overall slaughter hygiene (equipment, environment, tools, workers), while EC are indicators of faecal contamination on carcasses. The aim of this study was to determine the microbial contamination of cattle and pig carcasses, level of hygiene of the slaughter process as well as whether variations hygiene levels were related to seasonality in Serbia during 2017. The year was divided into four quarters of three months each, while the microbiological results were classified into three levels of hygiene status (unsatisfactory, satisfactory or acceptable). The highest percentage of the results surveyed during the entire study was at a satisfactory hygiene level. Furthermore, we found there were differences in results between the quarters, which could be associated with seasonality. The best microbiological results, and so the best hygiene of carcasses, was recorded in the period April, May, June, while the worst microbiological results were observed in the period of July, August and September.

Keywords: *slaughterhouse, process hygiene, Total Viable Count, Enterobacteriaceae, seasonality.*

Introduction

Meat consumption is increasing worldwide due to rapid population growth and urbanization. This has resulted in increased concerns and challenges when it comes to meat safety and hygiene. Therefore, the meat safety regulations should be implemented from farm, slaughterhouse, processing, storage, distribution, retail outlets up until the products reach the consumers (*Sofos and Geornaras*, 2010). During the food production process, each step of production is controlled and has a set of specific rules. The process of hazard analysis and critical control point (HACCP) is a systematic, science-based approach process control, designed to prevent, reduce or eliminate identified hazards in food products (*Kukay et al.* 1996). Therefore, a food business operator (FBO) is obligated to achieve compliance with these regulations. FBOs must apply compulsory self-checking programs following the HACCP approach.

The slaughterhouse is an indispensable part of the meat processing chain, being the place where animals are humanely killed to produce meat and meat products, and the entire process is under the supervision of an official veterinarian. Codex Alimentarius guidelines deal with hygiene of animals presented for slaughter: "the degree of contamination of the external surfaces of the animal is likely to compromise hygienic slaughter and dressing, and suitable interventions such as washing, or shearing are not available" (*Codex Alimentarius,* 2005). Regulation EU 853/2004 lists requirements that FBOs operating slaughterhouses must comply with, and one of them is that animals must be clean. Strict maintenance of good abattoir hygiene practices is of central importance to ensure both public health protection and meat quality. Implementation of good manufacturing practices (GMP) as well as HACCP for slaughterhouses and meat processing facilities plays a major role in enhancing the safety of meat products (*Bohaychuk et al*., 2011). Generally, it is accepted that the HACCP approach is the most effective way of reducing or eliminating contamination during food processing. For successful implementation of a HACCP system, it is necessary to have data on microbiological contamination, since the carcasses

[1]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia;*
[2]*University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11000 Belgrade, Republic of Serbia.*

Corresponding author: Lazar Milojevic, lazar.milojevic@inmes.rs

can be contaminated despite the absence of visible dirt (*Tergney and Bolton*, 2006). Scientific research has shown that with regard to long-term hygienic conditions in abattoirs, regular microbiological examination of the carcasses provides reliable conclusions (*Zweifel and Stephan*, 2003). This microbiological data can also be used for compliance with abattoir-specific performance criteria (*Zweifel et al.*, 2005). In order to obtain these data, it is necessary to perform systemic monitoring of indicator microorganisms (*Brown et al.*, 2000).

Regulation (EC) No. 2073/2005 and Serbian Regulation (*Official Gazette of the Republic of Serbia*, *No. 72/10*, 2011) require the obligatory control of hygiene indicators in slaughter processes. The most important meat hygiene indicators are Total Viable Counts (TVC) and *Enterobacteriaceae* (EC). TVC are defined as indicators of overall slaughter hygiene (equipment, environment, tools, workers), while EC are indicators of faecal contamination on carcasses (*Martelli et al*. 2017). Following the indicator microorganisms, allows indirect monitoring of the presence of pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni/coli*, *Escherichia coli* and *Yersinia enterocolitica* (*Nørrung et al*., 2009). These food-borne pathogens are still considered as important sources of human infections (*Nørrung et al*., 2009).

Analysis of data which were collected from slaughterhouses in Serbia in 2017 was conducted to understand the microbiological contamination of cattle and pigs carcasses, as well as establish the level of hygiene of the slaughter process for each animal. Further analysis of the obtained data should establish whether there are any variations in the hygiene results and if they exist, any relationship with seasonality.

Materials and methods

Abattoirs and carcass sampling

The study encompassed abattoirs with different production capacities in Serbia, and which were regularly subjected to veterinary inspection. A total of 554 wetdry swabs were collected during one year for cattle carcasses and 769 swabs were collected for pig carcasses. Following national regulations on the general and specific food hygiene requirements, we took samples in the production and processing phases. The number of samples by month was in line with the selfcontrol plans developed by HACCP teams in every abattoir. Samples were obtained by the wet-dry swab technique from four different sites (4×100 cm^2; cattle: neck, brisket, flank, and rump; pig: back, cheek, ham, and belly) of one half of each chosen carcass, as stipulated by SRPS ISO 17604 (*ISO*, 2016). Sampling was conducted by an authorized and trained laboratory technician and swabs were taken from five cattle and/or five pig carcasses randomly selected at the end of slaughter line, after the final wash but before chilling. Sample testing was performed by an ISO 17025 (*ISO*, 2005) accredited laboratory in accordance with respective ISO methods for TVC and EC enumeration.

TVC and Enterobacteriaceae enumeration

Each sample, consisting of four swabs, was added to 100 mL BPW (Oxoid, UK) and homogenized for 1 min in a stomacher (Laboratory Blender, Stomacher 400). TVC was obtained by inclusion of 1 mL of the suspension (M) and a series of 10-fold dilutions (10^{-1}, 10^{-2}, 10^{-3}) in duplicate into Plate Count Agar plates ([PCA] Oxoid, UK). EC counts were obtained by spread plating 1 mL of the suspension (M) and a series of 10-fold dilutions (10^{-1}, 10^{-2}, 10^{-3}) in duplicate onto Violet Red Bile Glucose Agar plates ([VRBGA] Oxoid, UK). Five characteristic colonies per plate were confirmed following SRPS ISO 21528−2:2009 (oxidase test and glucose fermentation test). The media was incubated as described above, according to SRPS EN ISO 4833−1:2014 and SRPS ISO 21528−2:2009. TVC and EC were calculated according to SRPS EN ISO 7218:2008/A1:2014. Bacterial recovery was expressed as $\log_{10}$ colony-forming units (cfu)/cm^2 and calculated as described in SRPS ISO 18593:2010, using the following formula:

$$\text{cfu cm}^{-2} = N \times F / A \times d$$

N = the number of cfu in 1 mL of dilution liquid (or neutralizing liquid);

F = the amount, in millilitres, of dilution fluid (or neutralizing liquid) in the tube or homogenizer bag;

A = is the area swabbed, in square centimetres;

d = is the dilution corresponding

Analysis of results

The year was divided into four quarters of three months. The first quarter (Q1) was January, February and March, the second quarter (Q2) comprised April, May and June. The third (Q3) comprised July, August and September, while October, November and December made up the fourth quarter (Q4).

All carcasses were classified into the following hygiene categories: unsatisfactory, satisfactory and acceptable, according to *Anonymous* (2017). For cattle carcasses, unsatisfactory results were those counts above 4.3 log cfu cm^{-2} for TVC and above 1.8 log cfu cm^{-2} for EC. Acceptable results were those counts below 4.3 log cfu cm^{-2} for TVC and below 1.8 log cfu cm^{-2} for EC. Satisfactory results were those equal to or below 2.8 log cfu cm^{-2} for TVC and equal to or below 0.8 log cfu cm^{-2} for EC. Pig carcasses were deemed unsatisfactory if they had more than 4.3 log cfu cm^{-2} TVC and more than 2.3 log cfu cm^{-2} for EC; acceptable pig carcasses had less than 4.3 log cfu cm^{-2} for TVC and less than 2.3 log cfu cm^{-2} for EC. Microbial levels on satisfactory pig carcasses were equal to or below 3.3 log cfu cm^{-2} for TVC and equal to or below 1.3 log cfu cm^{-2} for EC.

The obtained microbial results/data were analysed using Microsoft Office Excel 2016.

Results and Discussion

In Serbia in 2017, cattle carcass contamination levels ranged from 0.0 log cfu cm^{-2} to 3.7 log cfu cm^{-2} for TVC and from 0.0 log cfu cm^{-2} to 2.4 log cfu cm^{-2} for EC, while pig carcass contamination levels ranged from 0.0 log cfu cm^{-2} to 3.88 log cfu cm^{-2} for TVC and 0.0 log cfu cm^{-2} to 2.7 log cfu cm^{-2} for EC.

Cattle carcasses

TVC values for cattle carcasses

In Q1, TVC levels on cattle carcasses were within the satisfactory range (equal to or less than 2.8 log cfu cm^{-2}) in 85.5% of tested cattle carcasses, while in 14.5 % of tested carcasses, TVC levels were within the acceptable range, between 2.8−4.3 log cfu cm^{-2}. In Q2, 92.86% of tested carcasses were classified in the satisfactory hygiene group, and the remaining 7.14% were in the acceptable group. Altogether in Q3, 85.62% of carcasses belonged to the satisfactory group, while the rest (14.38%) were within the acceptable group. In Q4, we established that 82.71% of carcasses belonged in the satisfactory group, while the remaining 17.29% were classified in the acceptable group (Figure 1).

EC values for cattle carcasses

EC levels in Q1 were lower than 0.8 log cfu cm^{-2} in 95.42% of tested carcasses, which therefore, belonged to the satisfactory group, while 3.05% had a level between 0.8 log cfu cm^{-2} and 1.8 log cfu cm^{-2}, which put them in the acceptable group. Some cattle carcasses (1.53%) had an EC level higher than 1.8 log cfu cm^{-2}, and therefore, they belonged to the unsatisfactory group. In Q2, 97.14% of pig carcasses belonged to the satisfactory group and the remainder (2.86%) belonged to the acceptable group. In the third quarter, EC levels on cattle carcasses were within the satisfactory range in 86.93% of tested cattle carcasses, while in 7.84% of tested carcasses, EC levels were within the acceptable range and the remaining 5.23% of carcasses were within the unsatisfactory group. In Q4, 88.72% of tested carcasses belonged to satisfactory group, 7.52% of carcasses were within the acceptable range and 3.76% were classified as unacceptable (Figure 2).

Pig carcasses

TVC values for pig carcasses

The results of tested pig carcasses in Q1 showed that for TVC, 97% fell into the satisfactory group (equal to or less than 3.3 log cfu cm^{-2}), while 3% belonged to the acceptable group (4.3−3.3 log cfu cm^{-2}).

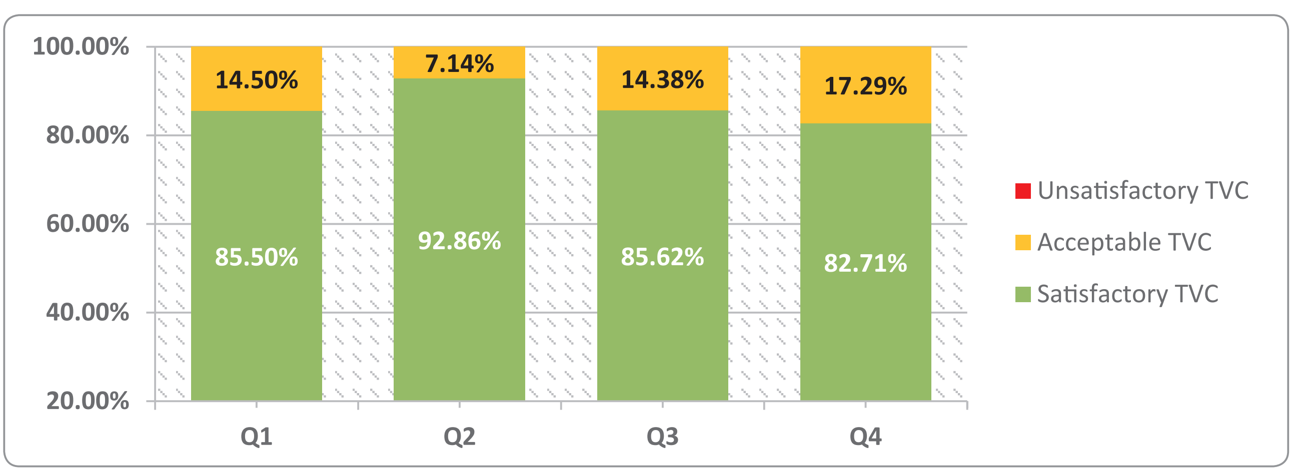


Figure 1. Trend analysis of Total Viable Count for cattle carcasses in 2017

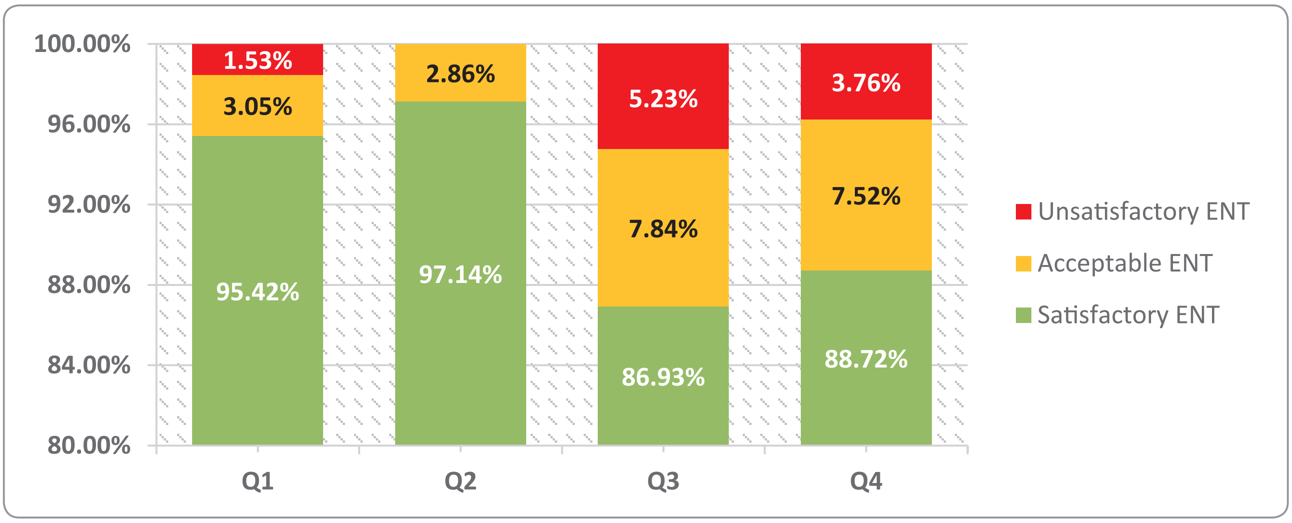


Figure 2. Trend analysis of *Enterobacteriaceae* for cattle carcasses in 2017

In Q2, 99% of tested carcasses were within the satisfactory group, and 1% were within the acceptable group. In Q3, 98% of tested carcasses belonged to the satisfactory group and 2% of pig carcasses belonged to the acceptable group. In Q4, 96% of pig carcasses belonged to the satisfactory group, while 4% of tested carcasses were within the acceptable group (Figure 3).

EC values for pig carcasses

EC values for pig carcasses in Q1 were such that 99% of tested pig carcasses belonged to the satisfactory group (equal to or less than 1.3 log cfu cm^{-2}) and 1% of carcasses belonged to the acceptable group (1.3−2.3 log cfu cm^{-2}). Similar results were observed in remaining quarters, where 99% of tested carcasses in Q2, 98% in Q3 and 96% in Q4 were within the satisfactory group, while 1% of pig carcasses in Q2, 2% in Q3 and 4% in Q4 belonged in the acceptable group. In Q3, 1% of tested carcasses were classified in the unsatisfactory group (Figure 4).

TVC and EC are indicators for hygiene process in slaughterhouses (*Delhalle et al*., 2008). Following these two parameters indicates the overall hygiene (TVC) in the slaughterhouses, as well as faecal contamination (EC) of the carcasses. In 2017 (divided into four separate periods), we observed that over 82% of the studied cattle carcasses had satisfactory TVC levels (≤ 2.8 log cfu cm^{-2}). However, the results were different for each quarter. Among the four annual quarters, Q2 had the highest percentage of carcasses with satisfactory hygiene status according to TVC levels. After that time, the percentage of carcasses with satisfactory hygiene status decreased

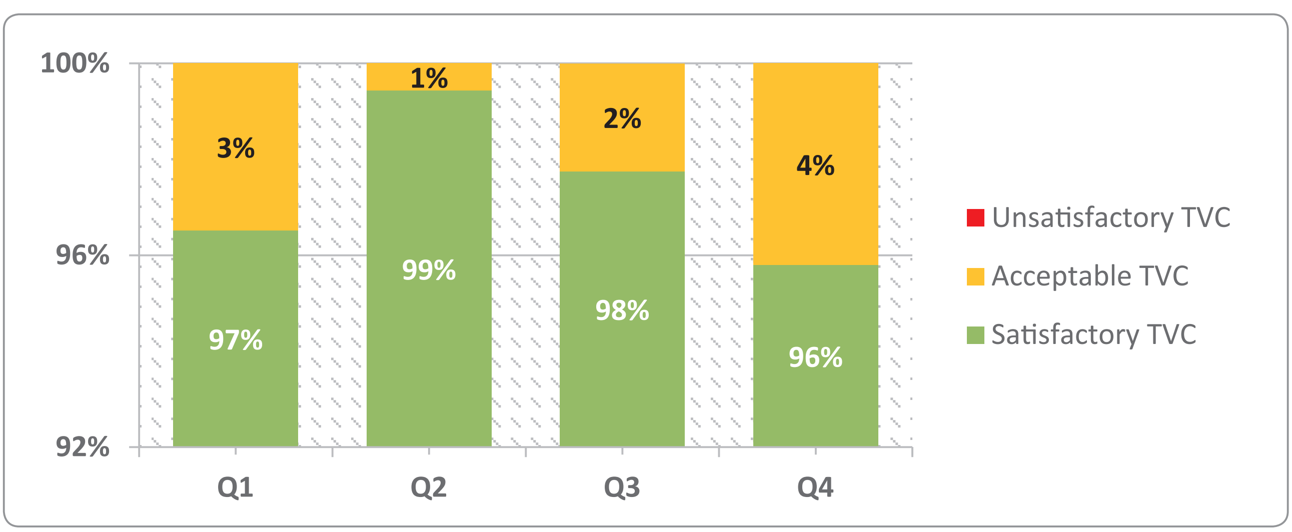


Figure 3. Trend analysis of Total Viable Count for pig carcasses in 2017

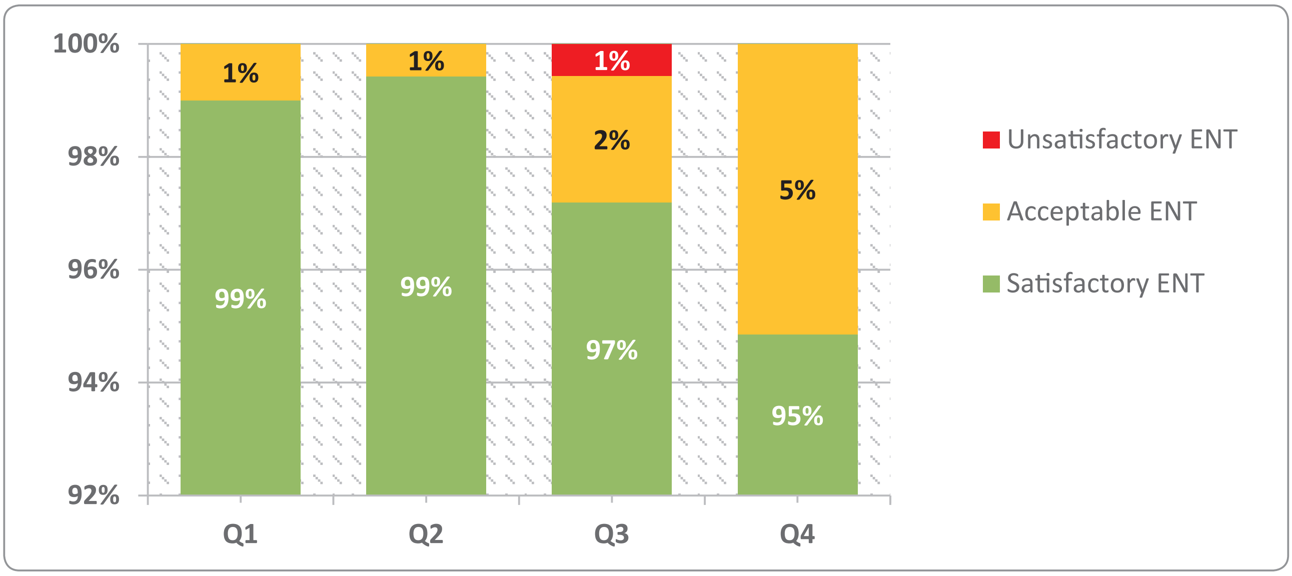


Figure 4. Trend analysis of *Enterobacteriaceae* for pig carcasses in 2017

during Q3 and Q4. Q4 had the highest percentage of carcasses with acceptable hygiene status according to TVC levels. Similar results were obtained for EC levels, where the highest percentage of carcasses with a satisfactory hygiene status was observed in Q2. Q1, Q3 and Q4 were periods when carcasses had unsatisfactory EC levels (> 4.3 log cfu cm^{-2}), and this was especially true for Q3, when over 5% of carcasses had unsatisfactory EC levels. This difference between quarters can probably be related to the colder and warmer parts of the year. *Rony et al.* (2007) came to a similar conclusion in their research, in which they confirmed the differences in the microbial levels on carcasses between these two periods of the year. *Barco et al.* (2014) highlighted that the season appears to have a direct impact on EC counts on beef carcasses, associating low counts with the dry season and cold months. Every step from the farm, through transport, storage, general hygienic conditions in the slaughterhouse and activities before and during slaughter can have an impact, which can be seen by microbiological analysis. If we observe the farm, during the warmer months, animals use a higher amount of water; they also try to cool down in different ways. All of this results in a higher percentage of dirty animals, and therefore, a special focus on animals with dirt of faecal origin is required during the hotter, summer months. The transport of animals to slaughterhouses in the summer months should be given special attention, since any failure made during transport will have consequences on the microbiological results of the carcasses. The hide of dirty animals is the most important source of contamination for carcasses, in particular during skinning operations. Bacteria including important foodborne pathogens can be transferred from hides onto carcasses via direct or indirect contact (*Brichta-Harhay et al.*, 2008). By the fact that the animals are dirtier, the possibility of contamination of the carcasses is greater. *Nastasijevic et al.* (2008) stated that in cattle carcass processing, one of the main concerns is related to observed soil and faecal contamination of carcasses and cross contamination during storage. The percentage of cattle carcasses which belonged to the unsatisfactory group in our study is probably because these carcasses had a greater potential for contamination with EC (*Zweifel et al.*, 2004). It should also be noted that slaughterhouses of different sizes were included in the current study.

Treatment of pig carcasses is different from cattle carcasses, because during pig processing, the carcasses pass through various characteristic and specific technological procedures. Due to this, microbiological contamination of pig carcasses is specific and one should expect different microbiological results and hygiene status compared to cattle carcasses. *Raseta et al.* (2015) obtained medium levels of 2.87 log cfu cm^{-2} for TVC and 1.05 log cfu cm^{-2} for EC levels on pig carcasses in a single slaughterhouse located in Banat, Serbia, for a period of one year. Out of all pig carcasses examined in our study, over 95% of them belonged to the satisfactory group. Observing TVC, the yearly period with the highest percentage of carcasses belonging to the satisfactory hygiene group was Q2, while the worst results occurred during Q4. Similarly poor results were obtained for EC in Q3, during which some carcasses were classified in the unsatisfactory group.

These results differ from the results of *Mrdovic et al.* (2017) who carried out research in one district in Serbia and found that TVC levels of pig carcasses were within the satisfactory range in 74.33% of tested carcasses and EC levels of carcasses were within satisfactory range in 100%, for a period of six years (2011−2016). These variations of results could reflect the improvement of hygienic conditions in slaughterhouses, which naturally, is an objective to strive for.

Conclusion

The hygienic conditions of slaughter are a focal point in the food chain. Despite the challenge of analysing results obtained from slaughterhouses of different sizes, the results obtained in this study indicate that the highest percentage of carcasses have satisfactory/acceptable levels of TVC and EC contamination. These results showed that most of the examined slaughterhouses keep managing and maintaining high quality standards. However, the percentage of carcasses with higher levels of TVC and/or EC contamination, and so classified as unsatisfactory, shows there are still opportunities for improvement. Attention must be paid to every link in the food chain from the farm, through the transport, residence of the animals in lairage and on the slaughter processing line in order to achieve the best performance. Hygiene in a slaughterhouse must be at a high level, as must the education of workers who should have regular training in accordance with new scientific knowledge. A high degree of hygiene and quality should be maintained throughout the year. Special attention should be paid during the summer months, during which a higher percentage of carcasses classified as unsatisfactory from the point of view of microbial contamination was observed in this survey.

Disclosure statement: No potential conflict of interest was reported by authors.

References

Anonymous (2017). Meat Industry Guide – Chapter 13 Microbiological Criteria, Food Standards Agency, London, UK.

Barco, L., Bellico, S., Roccato, A. & Ricci, A. (2014). *Escherichia coli* and *Enterobacteriaceae* counts on pig and ruminant carcasses along the slaughter line, factors influencing the counts and relationship between visual fecal contamination of carcasses and counts: A review. *EFSA supporting publication* 2014: EN-634, 111.

Bohaychuk, V. M., Gensler, G. E. & Barrios, P. R. (2011). Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. *The Canadian Veterinary Journal, 52 (10)*, 1095−1100.

Brown, M. H., Gill, C. O., Hollingsworth, J., Nickelson, I. R., Seward, S., Sheridan, J. J., Stevenson, T., Sumner, J. L., Theno, D. M., Usborne, W. R. & Zink, D. (2000). The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology, 62* (7), 16.

Brichta-Harhay, D. M., Guerini, M. N., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L. & Koohmaraie, M. (2008). *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology*, 74, 6289−6297.

Codex Alimentarius (2005). Code of hygienic practice for meat[1] CAC/RCP 58–2005: 1−51

Delhalle, L., De Sadeleer, L., Bollaerts, K., Farnir, F., Saegerman, C., Korsak N. *et al*. (2008). Risk factors for *Salmonella* and hygiene indicators in the 10 largest Belgian pig slaughterhouses. *Journal of Food Protection, 71* (7), 1320−1329.

EC (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (vol. L 338). Brussels, Belgium: European Commission. *Official Journal of the European Union*.

EFSA (2004) Clean Beef Cattle for slaughter. A guide for producers. Published by the Food Standard Agency November 2004, Crown Copyright 2004, Printed in England 5k FSA/0951/1104.

International Organization for Standardization (ISO) (2009). Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony count method. ISO 21528−2:2009, Geneva, Switzerland.

International Organization for Standardization (ISO) (2014). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony count technique at 30 degrees C. ISO 4833−1:2014, Geneva, Switzerland.

International Organization for Standardization (ISO) (2007). Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. ISO 7218:2007. Geneva, Switzerland.

International Organization for Standardization (ISO) (2005). General requirements for the competence of testing and calibration laboratories. ISO 17025:2005. Geneva, Switzerland.

International Organization for Standardization (ISO) (2016). Microbiology of the food chain – Carcass sampling for microbiological analysis SRPS EN ISO 17604:2016.

Kukay, C. C., Holcomb, L. H., Sofos, J. N., Morgan, J. B., Tatum, J. D., Clayton, P. P. & Smith, G. C. (1996). Applications of HACCP by small-scale and medium-scale meat processors. *Dairy, Food and Environmental Sanitation 16* (2), 74−80.

Martelli, F., M. Lambert, P. Butt, T. Cheney, F. A. Tatone, R. Callaby & R. P. Smith. (2017). Evaluation of an enhanced cleaning and disinfection protocol in *Salmonella* contaminated pig holdings in the United Kingdom. *PLoS ONE*, *12*(*6*), e0178897. http://doi.org/10.1371/journal. pone.0178897.

Ministry of Agriculture, Forestry and Water Management Serbia. (2010). Ordinance on general and specific food hygiene requirements at any stage of production, processing and trade. Republic of Serbia. *Official Gazette of the Republic of Serbia*. No. 72/10.

Mrdovic, B., Raseta, M., BrankovicLazic, I., Milijasevic, M., Baltic, B., & Nastasijevic, I. (2017). Pigs and cattle slaughter process hygiene in a largescale and a smallscale abattoir: A report from one county in Serbia. *Meat Technology, 58* (2), 65−72.

Nastasijevic, I., Mitrovic, R., & Buncic, S. (2008). Occurrence of *Escherichia coli* O157 on hides of slaughtered cattle. *Letters in Applied Microbiology, 46* (1), 126−131.

Nørrung, B., J. K. Andersen & S. Buncic. (2009). Main concerns of pathogenic microorganisms in meat. In: Todra F. (Ed) Safety of Meat and Processed Meat (Food Microbiology and Food Safety). Springer, New York, USA, pp. 3−30 (ISBN 978–0–387 89025–8).

Raseta, M., Teodorovic, V., Jovanovic, J., Lakicevic, B., LazicBrankovic, I. & Vidanovic, D. (2015). The hygiene of the process of slaughtering and processing of pigs for one year on one slaughterhouse in the North Banat District in Serbia. *Meat Technology, 56* (1), 26−3.

Ruby, J., Zhu, J., Ingham, S. (*2007*). Using indicator bacteria and *Salmonella* **test results from three large-scale beef abattoirs over an 18-month period to evaluate intervention system efficacy and plan carcass testing** for *Salmonella*. *Journal of Food Protection*, *70* (12), 2732−2740.

Sofos, J. N. & Geornaras. I. (2010). Overview of current meat hygiene and safety risks and summary of recent studies on bio-films and control of *Escherichia coli* O157:H7 in non-intact and *Listeria monocytogenes* in ready to eat meat products. *Meat Science*, *86*, 2−14.

Tergney, A. & Bolton, D. J. (2006). Validation studies on an online monitoring system for reducing faecal and microbial contamination on beef carcasses. *Food Control, 17*, 378−382.

Zweifel, C., Baltzer, D. & Stephan, R. (2005). Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU decision 2001/471/EC. *Meat Science,* 69, 559−566.

Zweifel C. & Stephan R. (2003). Microbiological monitoring of sheep carcass contamination in three Swiss abattoirs. *Journal of Food Protection*, *66*, 946−952.

Paper received: 23.4.2018.
Paper accepted: 27.06.2018.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 614.97(497.11) ; 636.4.083
ID: 265901580
https://doi.org/10.18485/meattech.2018.59.1.2

Original scientific paper

Evaluation of pig welfare in lairage and process hygiene in a single abattoir

Ivan Nastasijevic[1], Brankica Lakicevic[1], Mladen Raseta[1], Vesna Djordjevic[1], Vesna Jankovic[1], Boris Mrdovic[1], Ivana Brankovic-Lazic[1]*

A b s t r a c t: Food safety is indirectly affected by the welfare of food animals, due to close links between animal welfare, animal health and food borne diseases. Stress factors and poor welfare can lead to increased susceptibility to disease among animals and may intensify the fecal shedding of food borne pathogens, e.g. Salmonella, Campylobacter, Yersinia, and human pathogenic STEC in the pre-slaughter phase: on-farm, in transport and in lairage. This study evaluated two aspects: a) assessment of pig welfare in abattoir lairage founded on animal-based categories, and b) the relationship between pig welfare and microbial process hygiene at slaughter. The results revealed that the animal-based category 'manure on the body' assessed in abattoir lairage corresponded with microbial process hygiene at slaughter.

Keywords: *abattoir lairage, pig welfare, slaughter, process hygiene.*

Introduction

Animal welfare is considered an important factor of an overall 'food quality' concept. Recently, there has been growing awareness and interest by the major stakeholders, e.g. industry, scientific community and consumers, in how animal welfare could also significantly impact food safety (*EFSA*, 2012). The safety of the food chain is indirectly affected by welfare of animals farmed for food production, due to the close links between animal welfare, animal health and food borne diseases. Namely, the gastrointestinal tract of farm animals can be colonized by enteric, food borne pathogens, e.g. *Salmonella*, *Campylobacter*, human pathogenic Shiga toxin-producing *E. coli* (STEC), and *Yersinia*, and their subsequent dissemination into the human food chain is a major public health and economic concern for the food (meat) industries (*Rostagno*, 2009). Stress factors and poor welfare can lead to increased susceptibility to disease among animals and can intensify fecal shedding of food borne pathogens in the pre-slaughter phase: on-farm, in transport/livestock markets and in abattoir lairage (*Buncic et al.*, 2013). Increased fecal shedding subsequently can increase the pre-slaughter cross-contamination of animals' skin with soil and fecal material during the transport and lay-over in lairage via contacts: animal-litter-animal, animal-floor-animal and animal-animal. In a study carried out by Berends et al. (1997), it was reported that the initial source of pig carcass contamination was the carrier pig itself (70%), while the remaining 30% of the carcass contamination was related to the hygiene of slaughter and dressing.

Furthermore, poor hygienic practices during slaughter/dressing (e.g. evisceration) can additionally contribute to cross-contamination of pig carcasses and increase the probability for transfer of pathogens onto meat (*Petruzzelli et al.*, 2016). These hazards can pose risks to consumers through meat consumption and provoke common food borne infections such as salmonellosis, yersiniosis, campylobacteriosis, or STEC infections.

Over recent years, several scientific opinions and guidelines on pig and cattle welfare at slaughter were developed. These documents were mainly focused on specific monitoring indicators to evaluate the effectiveness of stunning methods (*EFSA*, 2013a; 2013b). For instance, the current European Union legislation defines requirements for protection of animals at the time of killing, which is supported by the statement that "improving the protection of animals at the time of slaughter contributes to higher meat quality and indirectly has a positive

[1]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia.*

***Corresponding author:** Ivan Nastasijevic, *ivan.nastasijevic@inmes.rs*

impact on occupational safety in slaughterhouses" (*EC*, 2009). The US legislation does not define regulatory requirement for a written systematic approach to humane handling, but provides a guideline with current thinking on the systematic approach to humane handling of livestock (*USDA FSIS*, 2013). With a systematic approach, establishments should focus on treating livestock in such a manner as to minimize excitement, discomfort, and accidental injury the entire time they hold livestock in connection with slaughter. Therefore, an establishment may choose to develop and implement a written animal handling program that effectively addresses the most important aspects of animal welfare. Evidently, although official guidelines in the EU and US on specific, pre-slaughter animal welfare indicators exist, they do not necessarily reflect specific welfare issues associated with abattoir lairage. Animal welfare implications for microbial process hygiene at slaughter/dressing are not considered in available literature.

Increased fecal shedding of food borne pathogens by animals intended for slaughter, due to higher levels of stress, as well as poor maintenance of abattoir lairage, e.g. slick floors (causing animals to slip and fall), poorly designed holding pens (insufficient space allowance) associated with management of lay-over time in lairage, inadequate environmental conditions (high or low temperature, high humidity, poor ventilation) could subsequently introduce higher levels of microbial load (including pathogens) to the slaughter line. This could affect the process hygiene at slaughter/dressing and increase the public health risk for consumers (*Grandin*, 1996) due to increased exposure to foodborne pathogens. Therefore, this study aimed to cover two aspects: a) assessment of pig welfare in abattoir lairage based on specific animal-based categories, and b) impact of pig welfare in abattoir lairage on microbial process hygiene at slaughter.

Materials and methods

Company's profile

The study was conducted in one meat processing company. The company had two separated slaughter lines (pig and cattle) with registered capacity of 240 pigs/h and 30 cattle/h. The total space of lairage where pigs were kept prior to slaughter was composed of four holding pens (HP) and occupied in total: 342 m^2 (HP1: 99 m^2; HP2: 85 m^2; HP3: 59 m^2 and HP4: 99 m^2).

Pre-slaughter phase

The company had two lairage units, one for pigs and another for cattle. Each lairage unit had its own unloading ramp. In this study, we evaluated lairage and farm/transport factors that could contribute to pig welfare prior to slaughter (Table 1).

Lairage

Hygienic-technical conditions. A team of three assessors evaluated the empty lairage in order to assess the position of premises versus the loading ramp and the corridor leading to the slaughter line, as well as to assess the general hygienic-technical conditions of holding pens.

Environmental conditions. The assessors measured: the level of lairage illumination, using the light intensity measuring instrument, a pocket-sized Lux meter (Testo 540, Germany); the ventilation, using an anemometer for quick and precise measurements of air flow speed, direction and volumetric flow rate (Testo 417, Germany), and; the ambient temperature, using a digital thermometer (Testo 905i, Germany). The ambient temperature was recorded once per week in the morning hours (between 6–7 am), corresponding with the start of operations in the abattoir, during February, March and April; this included the temperature measurements during the three scheduled visits to the meat company. Overall, 12 measurements of the ambient temperature were conducted during the study and the average monthly temperature was calculated for each of those three months.

Pre-slaughter sampling. A total of 3286 pigs were monitored over three scheduled visits in February (visit 1), March (visit 2) and April (visit 3), through deliveries to the abattoir in 13 consignments. The variables that were observed or measured are presented in Table 1. Delivery time, breed/age, lay-over time in lairage, weight of pigs and space allowance were recorded and measured on the day of arrival by the lairage supervisor appointed by company's general manager, while other data (farm of origin, farm location, housing system), including animal identification and health status report issued by the official veterinarian at the farm of origin, were collected by assessors from the company's lairage reception recordkeeping during each of three scheduled visits.

Assessment of animal-based categories. Criteria intended for assessment of animal welfare in abattoir lairage are scarce in available literature. Therefore, the protocol recommended by the European Food Safety Authority (EFSA) intended for assessment

Table 1. The variables assessed or measured in lairage related to abattoir premises, farms and traceability information

Hygienic-technical conditions	general hygienic-technical conditions of holding pens
	position of lairage
	loading ramp
	corridor to slaughter line
Environmental conditions	lairage illumination
	ventilation (air flow speed, direction, volumetric flow rate)
	ambient temperature
Pre-slaughter sampling	delivery time
	farm of origin
	farm location
	housing system
	breed/age
	weight of pigs
	lay-over time
	space allowance

of on-farm pig welfare (*EFSA*, 2012) was adapted in this study and used in the abattoir lairage and at the slaughter line to evaluate the factors that could contribute to pigs' welfare scores (Table 2). This approach is based on a group level assessment of animal-based categories, e.g. the factors that could contribute to pigs' welfare scores, in the lairage (*ante-mortem*) and at the slaughter line (*post-mortem*). In this study, the assessment protocol was applied in two stages: 1) assessment of welfare-related factors of pigs in the abattoir lairage (pre-slaughter), and 2) assessment of internal organs and carcasses at slaughter line (post-slaughter). The EFSA protocol uses the presence/absence of some of the animal-based categories (Table 2) and also uses given scores based on the availability of preventive measures.

Delphi method (scoring). The scoring of welfare of the fattening pigs in abattoir lairage was conducted using the Delphi method (*Yousuf*, 2007). A numerical scoring system ranging from 0 (impossible to prevent hazard through short-term management) to 5 (full prevention possibility, the hazard can be prevented/eliminated almost instantly, on-the-spot) was used. Three experienced assessors participated in this survey; each assessor had more than seven years of professional experience working within the meat industry extension program. For each hazard in Table 2, the assessor visually observed the pigs in the abattoir lairage. The assessment was carried out in the abattoir lairage, and afterwards, the three assessors compared their scores while still on the abattoir premises. Mean values were calculated from the three assessor scores for each animal-based category. The highest score (5) corresponded to a 'management definition' as follows: a) management is anything that the responsible persons (be it animal owner or stockperson) can easily do themselves (e.g. moving barriers/gates) almost instantly, on-the-spot, but should exclude major activities, such as new buildings or replacing structural features of existing stables; b) management encompasses changes that can be made in a short term (to be implemented and consequences seen within a short period, but excluding long term management plans); c) management includes changes within the long term, without consideration of potential financial constraints (i.e. assuming that the manager can always take decision to change, if wanted). The lowest score (0) corresponded to the lowest management potential, e.g. construction-related housing, which also requires significant financial input and is time consuming.

In this study (Table 3), we also used the terminology 'space allowance' (*FAWC*, 2013) which denotes the space per animal (m^2/head) or 'capacity' (the maximum number of animals in a specific holding pen).

Table 2. Summary of animal-based categories for fattening pigs (adapted from *EFSA*, 2012)

[1]**Animal-based category**		**Pig category (fattening pigs)**	**Score (0–5)**
Lesions	Skin lesions		
	Vulva lesions		
	Tail lesions		
	Ear lesions		
	Shoulder sores		
	Foot lesions		
Total score			
Mortality (dead on arrival and dead in lairage)	Mortality rate		
Total score			
Disease signs (in lairage: ante-mortem & at slaughter: post-mortem)	Coughing		
	Sneezing		
	Laboured breathing		
	Twisted snout		
	Rectal prolapse		
	Scouring		
	Constipation sign		
	Skin inflammation or discolouration		
	Ruptures and hernias		
	Local infection sign		
	Neurological disorder sign (tremor)		
	Tear staining (indicating eye irritation, e.g. by ammonia)		
	Swollen bursae (resulting from excessive pressure on bony areas)		
	[2]Lung and respiratory tract pathologies in slaughtered pigs		
	Gut pathologies in slaughtered pigs		
	Heart pathologies in slaughtered pigs		
	Liver pathologies in slaughtered pigs		
	Joint pathologies in slaughtered pigs		
	Other pathologies in slaughtered pigs (e.g. lymph node infection, abscesses)		
Total score			
Injurious behaviour	Tail-biting		
	Ear-biting		
	Flank-biting		
	Vulva-biting		
	Aggression resulting in injury		
Total score			

[1]Animal-based category		Pig category (fattening pigs)	Score (0–5)
Other behaviours	Persistent investigatory behaviour (directed at pen-mates or pen-fittings)		
	Exploratory behaviour (involving diverse behavioural elements, e.g. directed towards manipulable materials, not pen mates)		
	Activity level (increased in specific circumstances as predictor of tail-biting)		
	Mounting behavior score		
	Play behaviour score		
	Social isolation (self-separation from the group as indicator of illness or pain)		
	Feeding and drinking behaviour – abnormal or presence or absence – (from automated records)		
	Qualitative behavior assessment score		
Total score			
Thermoregulation	Panting		
	Huddling and shivering		
	Body temperature		
	Lying location (lying in dunging or other inappropriate area due to spatial or thermal inadequacy)		
	Lying posture (sterna lying due to spatial or thermal inadequacy)		
Total score			
Mutilations	Tail intact or docked		
Total score			
Other measures	Approach to humans score (fear of humans or positive reaction to humans)		
	Manure on the body score		
	[3]Acute phase proteins (at present only pigMAP in blood or meat juice is practicable)		
	Locomotion score		
	Slipping and falling		
	Body condition score (under nutrition or sickness or incorrect feeding leading to a low score – bad welfare conditions)		
	Tail posture (as predictor of tail-biting or indicator of disease)		

[1]Animal-based category		Pig category (fattening pigs)	Score (0–5)
Total score			
Insufficient space or too high stocking density	Rest and sleep disruption		
	Stress and lesions		
	Behavioural restriction		
	Damaging behavior from pen mates (biting, massaging, belly nosing, etc.).		
	Pain due to leg problems.		
	Being tail bitten.		
Total score			
Rest and sleep disruption	No comfortable lying place, insufficient solid floor or no bedding material like straw		
	Inappropriate pen design: Inadequate separation of dunging and lying area and other inadequacy (feeders, drinkers, etc.)		
	Inappropriate pen lay out: open sides to pens		
Total score			
TOTAL			

Legend: [1]Animal-based category: "a response of an animal or an effect on an animal" (*EFSA*, 2012); [2]Post-mortem animal-based categories; [3]Acute phase proteins in blood or meat juice were not considered in this study

Human approach test. The human approach test took place in abattoir lairage. A total of 120 pigs were observed (one batch included 10 pigs from each of four holding pens; in total, 40 pigs per visit), over the three visits. The test was carried out in the four holding pens where fattening pigs intended for slaughter were placed. Assessors wore jumpsuits and boots which had been freshly cleaned before every test. One assessor approached carefully and stood still in front of each of the four holding pens, for one minute. The pigs could generally use their snouts to make contact with the boots or the legs of the assessor. During that time, the other two assessors recorded which pigs made physical contact with the person and noted the latency of the pigs (LC) to touch the first assessor. Pigs which did not contact the assessor in the test time of 60 s were scored as having the maximum latency time (latency to the first escape attempt/LEA = 60 s). Each pig had an ear tag with an individual number and the latency was recorded for each individual pig.

Physiological measurements. The rectal temperatures of selected fattening pigs were recorded. Six pigs that approached the assessor in the human approach test and had no visible signs of disease were randomly selected from each of the four holding pens. The selected pigs were restrained in the lairage pen and rectal temperature was measured with the digital thermometer (Nasco C28178N, US); the selected pigs did not necessarily originate from the same farm.

Post-slaughter phase

In the post-slaughter phase, meat pH/temperature of carcasses was measured; process hygiene levels at slaughter were also determined.

After slaughter (60 min post-slaughter), initial pH and temperature (pH/Temperature meter Testo 205, Germany) were measured in 15 selected pig carcasses on each of three visits, to reveal the potential distress of pigs prior to slaughter; pH and temperature measurements were taken at the middle region of the LTL muscle (muscularis longissimuss thoracis et lumborum). In total, pH and temperature was measured in 45 selected pig carcasses.

Process hygiene sampling. The sampling was carried out in the abattoir lairage and on the slaughter line. In the lairage, six samples were taken in a systematic way (i.e. three samples from the floor of the holding pen and three samples from the corners between concrete walls of the holding pen). The samples were taken with sponge covering 1000 cm^2

for each swabbing. The sponge samples were wetted with 10 mL of maximum recovery diluent (MRD, Oxoid UK), packed in a stomacher bags (19 x 30 cm, Nasco, Whirl-pak, USA) and transported within 3–4 h in a cool bin at <4°C to laboratory and processed on a same day; only the presence/absence of *Salmonella* spp. was examined in these lairage environmental samples (*ISO* 6579:2002).

Five pigs were individually identified in the abattoir lairage (i.e. black color mark on the back) for subsequent sampling on the slaughter line. Sponge swab samples were taken from four sites on the carcasses of the five pigs (rump, back, flank and jowl) (*ISO* 17604:2015) using a 100 cm^2 sterile template and one sponge per site, at four locations along the slaughter line: lairage/after stunning; after scalding/singeing/polishing (pre-evisceration); after evisceration; and after the final wash. Swabs were packed in a stomacher bags (19 x 30 cm, Nasco, Whirl-pak, USA), transported within 3–4 h in a cool bin at <4°C to the laboratory, and processed (see below) on the same day.

For each sponge swab sample of pig carcasses, the levels of process hygiene indicators, e.g. Total Viable Counts (TVC) (*ISO* 4833:2003), *Enterobacteriaceae* (EC) (*ISO* 21528–2:2004), generic *E. coli* (GEC) (*ISO* 16649–1:2001) and Coliforms (*ISO* 4832:2006) were determined to verify the process hygiene level at slaughter/dressing. Further, the presence/absence of *Salmonella* spp. was also determined in these pig carcass swab samples (*ISO* 6579:2002).

The four counts for TVC/EC/GEC/Coliforms per carcass were first converted into log CFU/cm^2, those log values for each of the four sampling sites/carcass were summed, and then the mean log CFU/cm^2 per carcass was calculated. As the EU legislation (*EC*, 2005) set limits for daily log mean counts, this was calculated from the five sampled carcasses. However, it has to be taken into account that EU process hygiene assessment legislation relates only to the final carcasses, and not to carcasses at earlier stages of the slaughter line that were sampled in this study.

Statistical analysis. Statistical analysis of the results was conducted using the software GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California USA). The group of pigs examined on one visit was considered as the experimental unit. The parameters obtained at ante-mortem and post-mortem assessment (animal-based measure categories) were described by descriptive statistics (mean, standard deviation, range). Relationships between ante-mortem animal-based categories' scores, obtained by the Delphi scoring method and microbiological status of carcasses (TVC, EC, GEC, Coliforms) were determined by Fisher's exact test. Values of $p<0.05$ were considered significant.

Results and discussion

The hygienic-technical and environmental conditions in the abattoir lairage, status of pigs prior to slaughter, as well as observation of animal-based categories, human approach test, pH and temperature of dressed carcasses and microbiological process hygiene at slaughter/dressing were addressed in this study.

Observation in pre-slaughter phase

Hygienic-technical conditions in lairage

The unloading ramp from truck to lairage was sloped at a 15° uphill angle. The isolation room for pigs potentially unfit for slaughter and requiring additional veterinary examination was in accordance with regulations.

Loading facilities and holding pens for pigs were made of a solid concrete non-slipping floor; flooring surfaces were uniform in appearance and mostly free from puddles and excessive cracks. Slats in holding pens were positioned in the proper direction so that pigs could walk across the slats instead of parallel with them. No intensive light and/or water reflections were observed under the slats, which facilitates the movement of animals. Drains were properly located outside the areas where animals walk. Watering (round pipe posts) and feeding facilities were installed so as to allow easy and smooth access by animals. However, some surfaces which came into contact with animals, including sharp corners, were not smooth and rounded. The majority of holding pen gates were equipped with tie-backs to prevent them from swinging out into the alley, except one. On the other hand, guillotine gates were adequate, counter-weighted and padded on the bottom. No differences regarding hygienic-technical conditions in the lairage across our three visits were observed.

Environmental conditions in lairage

The lairage was dimly illuminated, which supported the tendency of animals to move more easily in comparison to brightly illuminated space, as recommended by *Grandin* (1996). The lairage was well-ventilated and also had equipment available for water spraying and cooling of pigs. However, the steel gate strike posts did not have rubber stops to reduce noise and were operated manually. Major differences in ambient temperature (Table 3) were measured in our three separate visits, but other environmental conditions in the lairage were similar across our three visits.

Status of pigs prior to slaughter

Deliveries of fattening pigs intended for slaughter usually occurred in the early afternoon hours (between 1pm and 6pm). The pigs originated mostly from farms with controlled housing systems (e.g. biosecurity and herd health surveillance programs), belonging to the company's own supply chain (>90%). Most of the farms were located in the region with an average transportation time to the abattoir of 7–8 h, while only a few farms were located in relative proximity, with an average transportation time of 3–4 h. Fattening pigs were of Landrace breed (80%), as well as Yorkshire or Berkshire (20%). The live weights of animals arriving in the abattoir lairage ranged from 95–110 kg. All fattening pigs intended for slaughter were accompanied with a valid veterinary health certificate, issued by the local veterinary authority, stating the health status of each animal, including the traceability. Upon arrival in abattoir lairage, the pigs' access to feed was restricted for up to 12 h prior to slaughter in order to prevent diarrhea and possible cross-contamination of carcasses during slaughter (i.e. scalding, evisceration); drinking water was available at all times from an appropriate watering system-ound pipe posts.

Observation of animal-based categories

During our three visits to the abattoir, the space allowance ranged from 0.39–1.00 m^2/pig (Table 3). These findings were not in line with recommendations for space allowance in abattoir lairage of 0.66 m^2/pig, as suggested by Weeks (2008), but were in accordance with the Royal Society for the Prevention of Cruelty to Animals 'Freedom Foods scheme' (*RSPCA*, 2014); this scheme applies the minimum total area for pigs (m^2 per animal) of 0.15 when <100 pigs, and 0.225 when <101–250 in a holding pen. In available literature, space allowances for groups of animals of a uniform weight (*Spoolder et al.,* 2008; *Weeks*, 2000; *Faucitano*, 2010; *FAWC*, 2013, *RSPCA*, 2014) or space allowances during transport (*Sutherland et al.* 2010) are usually described. In this study, the live weight of pigs intended for slaughter ranged from 95–110 kg and this created some difficulty in specifying the area allowance when animals within one group were of relatively different weights. The management of space allowance can be effectively performed by the company itself, with the responsible person moving flexible barriers/gates and so adjusting the available space within the holding pen based on the dynamic of the incoming animals. However, space management will also require careful planning of the slaughter logistics which should include scheduling the truck deliveries to reduce waiting times for unloading, as well as lay-over in abattoir lairage prior to slaughter. Such adjustment could be a cost-effective approach to fulfilling animal welfare requirements, because it should not require expensive reconstruction/adaptation of the lairage and it can be implemented and/or performed in a short period of time.

The relatively long pre-slaughter lay-over times in abattoir lairage observed in this study (>14 h; Table 3) were not associated with fighting, aggressive behavior or excessive skin damage as reported in a study carried out in a Dutch abattoir (*Geverink et al.,*1996), as well as in other studies conducted in Spain (*Guàrdia et al.*, 2009) and the UK (*Weeks*, 2008). On the other hand, this was in line with a study in which it was observed that most fighting and aggressive behavior among pigs in abattoir lairage occurs within the first 30–60 minutes, and it is usually not significantly increased with up to 18 h of lay-over time (*Fraqueza et al.,* 1998). *Guàrdia et al*. (2010) carried out a study in Spain and also discussed high stocking density and lairage time related to increased risk of dark, firm, dry (DFD) meat, associated with pre-slaughter/on-farm fasting times longer than 22 h; the results from their study revealed that lowering the stocking density from 0.37 to 0.50 m^2 per 100 kg pig during transport would reduce the risk of DFD pork by 11%, but no observations regarding the lay-over time in abattoir lairage and implications on process hygiene at slaughter were provided in that study. Also, *Candiani et al.* (2008) evaluated physiological and behavioral indicators to provide useful information on pig welfare on farm, but without taking into consideration the status of pigs in abattoir lairage. Nonetheless, it should always be taken into account that a longer lairage lay-over time allows for rest but sometimes may increase the risk of aggression and thereby excessive skin damage (*Faucitano*, 2010).

The five ante-mortem animal based-categories with the lowest scores, in increasing order, (Table 4) were 'thermoregulation', 'manure on the body', 'insufficient space', 'injurious behavior', and 'lesions'. Although data on animal based-categories for fattening pigs in abattoir lairage in the available literature are scarce, these findings are similar to observations reported by *Gispert et al.* (2000) and *Spoolder et al.* (2000), who observed that major concerns in

Table 3. Pre-slaughter conditions in abattoir lairage (three visits; n=3286)

Holding Pen (HP)	Space allowance m^2/pig X±SD, range	Lairage lay-over time X±SD	[3]Rectal temperature (°C) X±SD, range	In-lairage ambient temperature (°C) X±SD, range
Visit I				
HP1	0.66±0.41, 0.24–0.75	>14h (14.5±0.24)	38.5±0.25, 38.1–39.0	
HP2	0.70±0.56, 0.27–0.81	>14h (14.8±0.35)	38.1±0.23, 38.0–38.8	
HP3	0.39±0.18, 0.22–0.48	>14h (14.9±0.42)	38.3±0.21, 38.1–38.9	9±0.17, 8.7–9.3
HP4	0.49±0.37, 0.29–0.57	>18h (18.6±0.51)	38.5±0.27, 38.0–39.3	
Visit II				
HP1	0.58±0.23, 0.23–0.71	>18h (18.9±0.18)	38.3±0.18, 37.9–39.1	
[1]HP2	–	–	–	
HP3	0.39±0.21, 0.22–0.45	>16h (17.2±0.37)	38.2±0.22, 38.0–38.8	14±0.21, 13.8–14.2
HP4/1	0.44±0.32, 0.33–0.58	>20h (20.4±0.28)	39.3±0.28, 38.4–39.6	
HP4/2	0.42±0.19, 0.34–0.51	>19h (19.6±0.21)	38.1±0.23, 38.0–38.7	
Visit III				
[2]HP1/1	0.40±0.31, 0.26–0.52	>19h (19.2±0.29)	37.8±0.35, 37.7–38.8	
HP1/2	1.00±0.37, 0.29–0.1,4	>14h (14.3±0.31)	38.2±0.23, 38.0–38.9	
[1]HP2	–	–	–	
HP3	0.44±0.23, 0.31–0.56	>14h (14.8±0.36)	38.3±0.27, 38.1–39.2	12±0.19, 11.9–12.1
HP4/1	0.46±0.34, 0.25–0.57	>16h (16.4±0.17)	38.1±0.20, 37.9–38.8	
HP4/2	0.83±0.42, 0.34–0.94	>14h (14.1±0.19)	39.0±0.29, 38.2–39.5	

Legend: HP1: $99m^2$(HP1/1: $49m^2$, HP1/2: $50m^2$); HP2:$85m^2$; HP3:$59m^2$, HP4:$99m^2$ (HP4/1: $49m^2$, HP4/2: $50m^2$)
[1]The holding pen no. 2 (HP2) was not used in third visit, since no fattening pigs were kept in this pen during this time;
[2]HP1/1, HP1/2, HP4/1 and HP4/2 denotes holding pens where there is a possibility to move/change the position of internal barrier/gate to adjust the space allowance within the respective holding pen; [3]Normal temperature range for pigs: 38.7–39.8°C

fattening pigs in abattoir lairage were associated with the skin damage and injurious behavior. The findings regarding post-mortem animal-based categories revealed that the three categories with the lowest scores (Table 4), which could be deserving of being regularly monitored on the slaughter line, were 'gut pathologies', 'lung and respiratory tract pathologies' and 'liver pathologies'. Such findings could be very helpful to identify animal health and welfare issues related to zoo-technical (e.g. ventilation) or biosecurity (e.g. feed disposal, manure management, vaccination) conditions at the farm of origin, as well as to provide the valuable feedback to farmers (*Horchner and Pointon,* 2011). The correlation analyses between five ante-mortem animal-based categories and microbiological status of carcasses showed that 'manure on the body' was associated with all defined process hygiene indicators ($p<0.05$), e.g. TVC, EC, GEC and Coliforms, while none of these five animal based-categories correlated with post-mortem findings (i.e. the visually observed pathologies observed in internal organs).

Table 4. Scores for hazard-pig category combinations for fattening pigs in abattoir lairage

<table>
<tr><th rowspan="2">No.</th><th rowspan="2">Animal-based measure category</th><th colspan="3">Overall score (0–5)
X ± SD</th></tr>
<tr><th>Session I</th><th>Session II</th><th>Session III</th></tr>
<tr><td colspan="5">Ante-mortem (n=3286)</td></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Lesions</td><td colspan="3">3.83 ± 1.05</td></tr>
<tr><td>Session I
3.80</td><td>Session II
3.50</td><td>Session III
4.20</td></tr>
<tr><td rowspan="2">2</td><td rowspan="2">Mortality</td><td colspan="3">5.00 ± 0.00</td></tr>
<tr><td>Session I
5.00</td><td>Session II
5.00</td><td>Session III
5.00</td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">Disease signs
(in lairage: ante-mortem & at slaughter: post-mortem)</td><td colspan="3">4.05 ± 1.83</td></tr>
<tr><td>Session I
3.90</td><td>Session II
4.10</td><td>Session III
4.15</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Injurious behaviour</td><td colspan="3">3.80 ± 1.67</td></tr>
<tr><td>Session I
3.90</td><td>Session II
3.4</td><td>Session III
4.1</td></tr>
<tr><td rowspan="2">5</td><td rowspan="2">Other behaviours
(persistent investigatory behavior, exploratory behavior, mounting behavior, social isolation, feeding and drinking behavior)</td><td colspan="3">3.90 ± 1.78</td></tr>
<tr><td>Session I
4.10</td><td>Session II
3.70</td><td>Session III
3.90</td></tr>
<tr><td rowspan="2">6</td><td rowspan="2">Thermoregulation</td><td colspan="3">3.50 ± 1.42</td></tr>
<tr><td>Session I
3.2</td><td>Session II
3.8</td><td>Session III
3.5</td></tr>
<tr><td rowspan="2">7</td><td rowspan="2">Mutilations</td><td colspan="3">4.12 ± 2.54</td></tr>
<tr><td>Session I
3.9</td><td>Session II
3.4</td><td>Session III
4.2</td></tr>
<tr><td rowspan="2">8</td><td rowspan="2">Manure on the body</td><td colspan="3">3.65 ± 1.91</td></tr>
<tr><td>Session I
4.1</td><td>Session II
3.3</td><td>Session III
3.6</td></tr>
<tr><td rowspan="2">9</td><td rowspan="2">Insufficient space</td><td colspan="3">3.73 ± 1.21</td></tr>
<tr><td>Session I
4.0</td><td>Session II
3.4</td><td>Session III
3.8</td></tr>
<tr><td rowspan="2">10</td><td rowspan="2">Rest and sleep disruption</td><td colspan="3">4,10 ± 2.15</td></tr>
<tr><td>Session I
4.1</td><td>Session II
4.0</td><td>Session III
4.2</td></tr>
<tr><td colspan="5">Post-mortem (n=3286)</td></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Lung and respiratory tract pathologies in slaughtered pigs</td><td colspan="3">1.42 ± 0.53</td></tr>
<tr><td>Session I
1.85</td><td>Session II
0.85</td><td>Session III
1.55</td></tr>
<tr><td rowspan="2">2</td><td rowspan="2">Gut pathologies in slaughtered pigs</td><td colspan="3">1.05 ± 0.21</td></tr>
<tr><td>Session I
1.25</td><td>Session II
0.70</td><td>Session III
1.20</td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">Heart pathologies in slaughtered pigs</td><td colspan="3">3,27 ± 1.15</td></tr>
<tr><td>Session I
3.65</td><td>Session II
2.80</td><td>Session III
3.35</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Liver pathologies in slaughtered pigs</td><td colspan="3">2.90 ± 0.94</td></tr>
<tr><td>Session I
3.25</td><td>Session II
2.50</td><td>Session III
2.95</td></tr>
<tr><td rowspan="2">5</td><td rowspan="2">Joint pathologies in slaughtered pigs</td><td colspan="3">4.05 ± 0.15</td></tr>
<tr><td>Session I
4.20</td><td>Session II
3.85</td><td>Session III
4.10</td></tr>
<tr><td rowspan="2">6</td><td rowspan="2">Other pathologies in slaughtered pigs
(e.g. lymph node infection, abscesses)</td><td colspan="3">3.38 ± 0.23</td></tr>
<tr><td>Session I
3.90</td><td>Session II
2.85</td><td>Session III
3.40</td></tr>
</table>

Human approach test

The test showed that, out of 120 pigs observed over the three visits, the response of animals was very good, so all pigs from each batch, and from each holding pen, wanted to have physical contact with the assessor. The latency of the pigs (LC) to touch the assessor was always less than 60 s (Table 5).

pH and temperature of dressed carcasses (post-slaughter)

pH values and temperatures of dressed carcasses, measured 60 min post-slaughter, ranged between batches/three visits from pH 5.81 to 6.60 and 39.5–42.0°C; after 12 h, from pH 5.62 to 5.83 and 3.0–3.9°C, and; after 24 h, from pH 5.60 to 5.79 and 2.20–3.50°C. These values are similar to those reported in a study carried out by *Dokmanovic et al.* (2014). In our study, recorded pH/temperature values of carcasses were in line with usual values for the post-slaughter period and did not indicate the animals had undergone excessive stress. A significant relationship between pre-slaughter stress and meat quality has been documented (*Ferguson et al.,* 2001; *del Campo et al.,* 2014). On the other hand, there is a lack of valid scientific data on stress-inducing factors in abattoir lairage. In a study conducted in Spain, *Gispert et al.* (2000) collected a random blood samples at exsanguination to determine cortisol, creatine phospho-kinase (CPK), lactate, and the halothane genotype; it was concluded that the most relevant stress indicators that may influence the carcass and meat quality in abattoir are associated with environmental aspects at pre-slaughter phase, e.g. high stocking density, long lay-over time in lairage and on-line skin damage.

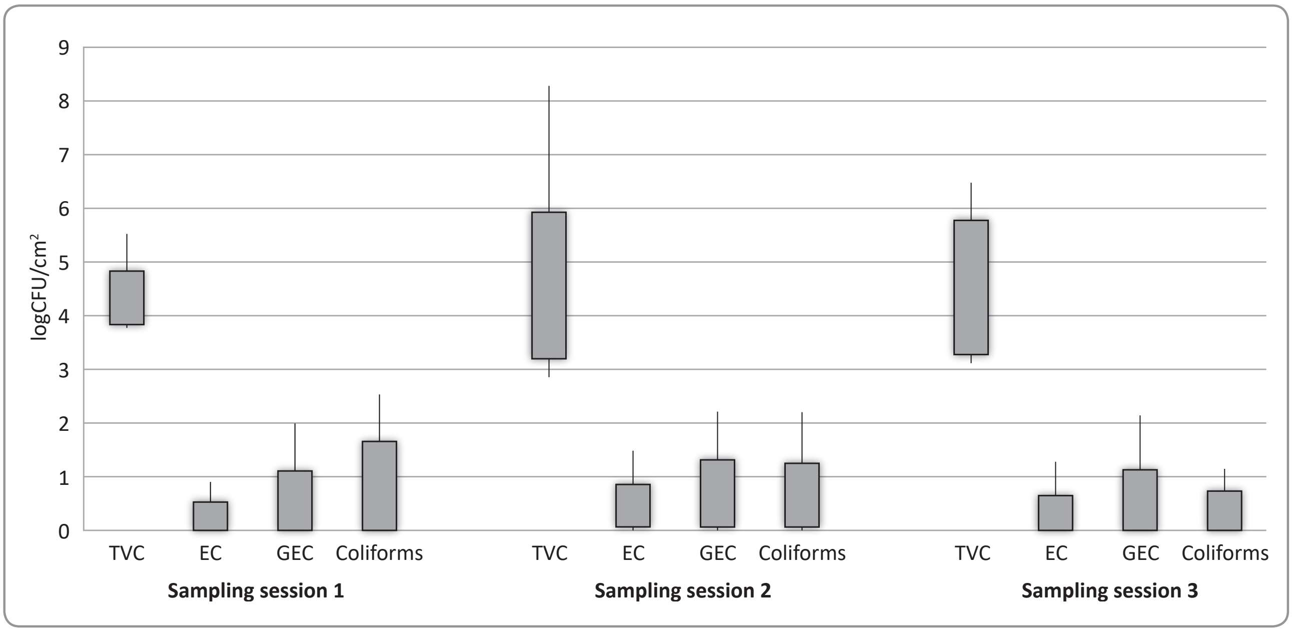
Microbiological process hygiene at slaughter/ dressing

Microbiological process hygiene indicators monitored on pig carcasses (TVC, *EC*, GEC and Coliforms) differed on the three visits (Figure 1). In the three visits, the carcasses were sampled at two stages along the slaughter line (at stunning/before bleeding and after scalding/singeing/polishing).

Table 5. Human approach test carried out in abattoir lairage (three visits; n=3286)

Holding Pen (HP) (the batch – 10 pigs)	**[1]LC X±SD, range**	**[2]LEA X±SD, range**
Visit I		
HP1	35±0.42, 10–52	7±0.12, 3–12
HP2	24±0.53, 7–46	3±0.15, 1–8
HP3	49±0.17, 17–51	12±1.32, 9–15
HP4	53±0.34, 32–57	4±0.14, 2–7
Visit II		
HP1	23±0.37, 13–41	3±0.17, 1–5
HP2	–	–
HP3	44±0.27, 27–51	9±0.11, 3–14
HP4/1	48±0.31, 31–54	6±1.32, 1–12
HP4/2	52±0.23, 38–58	7±1.23, 2–15
Visit III		
HP1/1	25±0.19, 12–34	11±1.47, 4–17
HP1/2	37±0.27, 23–46	4±0.25, 1–6
HP2	–	–
HP3	42±0.31, 35–49	8±0.17, 4–12
HP4/1	32±0.24, 19–43	9±1.34, 2–11
HP4/2	30±0.41, 17–40	10±0.17, 8–11

Legend: [1]LC – Latency of the pigs to touch the experimenter (expressed in seconds); [2]LEA – Latency to the first escape attempt (expressed in seconds; the maximum time = 60s



Legend: [1]rump, back, belly, jowl

Figure 1. The levels of Total Viable Counts (TVC), *Enterobacteriaceae* (*EC*), generic *E. coli* (GEC) and Coliforms expressed in log CFU/cm^2 at four sampling sites on pig carcasses[1], obtained in three sampling sessions; sampling points along the slaughter line: stunning/before bleeding and scalding/singeing/polishing.

Total viable counts (TVC)

The mean log TVC levels, encompassing all levels determined at the four stages along the slaughter line, ranged between 3.80 and 5.51 log CFU/cm^2, 2.86 and 8.26 log CFU/cm^2 and between 3.12 and 6.46 log CFU/cm^2, respectively, on visits 1, 2, and 3. The highest TVC levels were measured after stunning/before bleeding (8.20 log CFU/cm^2), while the lowest TVC levels were obtained after the final wash/before chilling (2.80 log CFU/cm^2), which was in accordance with the EU legislation (*EC*, 2005). The TVC levels obtained after the final wash were slightly higher than reported in a Canadian study (*Gill et al.,* 2000), but within the range reported in a study carried out in Swiss abattoirs (*Zweifel et al.* 2008). Furthermore, similar TVC levels were reported in a four-year monitoring study conducted in Italy (*Petruzzelli et al.,* 2016).

Enterobacteriaceae (EC), Generic E. coli (GEC) and Coliform counts

The overall *EC* levels observed in our three visits varied considerably, ranging from lower than the limit of detection to 1.48 log CFU/cm^2. The highest *EC* levels were observed after stunning/before bleeding (1.48 log CFU/cm^2), while the lowest *EC* levels were obtained after scalding/singeing/polishing (lower than the limit of detection). The *EC* levels after polishing were significantly lower than those reported by *Zweifel et al.* (2008) and *Blagojevic et al.* (2011).

The GEC levels varied appreciably during our three visits and ranged from lower than the limit of detection up to 2.22 log CFU/cm^2. The highest GEC levels were observed after stunning/before bleeding (2.22 log CFU/cm^2), while the lowest GEC levels were obtained after scalding/singeing/polishing (lower than the limit of detection). The results were similar to GEC levels reported by Gill et al. (2000) in a study in Canada, where 8 abattoirs with medium-to-high throughput were assessed for the level of process hygiene. Using the *E. coli* performance criteria (*FSIS*, 1996), the pig carcasses in the current study were categorised as within the acceptable range.

The Coliform levels determined during our three visits were similar to *EC* and GEC levels. Coliform levels ranged from lower than the limit of detection up to 2.53 log CFU/cm^2. Similarly, as with *EC* and GEC levels, the highest Coliform levels were observed after stunning/before bleeding (2.53 log CFU/cm^2), while the lowest values were obtained after scalding/singeing/polishing (lower than the limit of detection). Overall, these results indicated slightly lower level of hygiene than in a study carried out by Gill et al. (2000), where Coliform levels ranged from values lower than the limit of detection to 2.09 log CFU/cm^2.

The levels of EC, GEC and Coliforms can be a useful indicator of abattoir-specific hygienic level (*Zweifel et al.* 2008). However, none of the aforementioned studies reflected on the interface between animal welfare in abattoir lairage and process hygiene at slaughter.

Salmonella spp.

No *Salmonella* was detected on pig carcasses or in environment surface samples taken from the abattoir lairage, which was in line with the EU criteria for this hazard (≤5 positive carcasses out of 50, respectively; *EC*, 2005). Another study conducted in the same region in Serbia also found low *Salmonella* occurrence on carcasses (*Nastasijevic et al.,* 2016).

Interface between animal-based categories and slaughter process hygiene

Relationships between pig welfare variables (transportation time, animal-based categories, space allowance, lay-over time in lairage, ambient temperature), and microbial load values at slaughter were determined. The transportation time (3–8h from farm of origin to abattoir) observed in our three visits was not reflected in bacterial loads on carcasses (TVC, EC, GEC, Coliforms). Space allowance coupled with the lay-over time in lairage and ambient temperature tended to be related to process hygiene, as the lowest levels of hygiene indicators were observed in the first visit, where in-lairage space allowance was adequate, the lay-over time was shorter than in visits 2 and 3, and the ambient temperature was also lower than in visits 2 and 3 (Table 3, Figure 1). However, the trends were non-significant. Nonetheless, after all these circumstances, we measured lower microbial loads on carcasses, e.g. in visit 1, the TVC, EC, and *GEC* levels reached a peak of 5.5 log CFU/cm^2, 0.8 log CFU/cm^2 and 2.0 log CFU/cm^2, respectively compared to higher levels in visits 2 and 3, where the highest confirmed TVC levels were 8.1 and 6.4 log CFU/cm^2, respectively, EC levels were 1.4 and 1.2 log CFU/cm^2, respectively and *E. coli* levels were 2.2 and 2.1 log CFU/cm^2, respectively (Figure 1). Among the ten selected ante-mortem animal-based categories of importance for animal welfare, it was evident that the 'manure on the body' correlated with microbial loads on carcasses. 'Insufficient space', 'lesions', 'injurious behaviour', 'feeding and drinking behaviour' and 'rest and sleep disruption' tended to correlate to microbial load levels to some extent; for instance, the higher scores given for those animal-based categories meant better process hygiene levels were measured, although no statistical correlation was found.

Other ante-mortem animal-based categories, 'mortality', 'disease signs', 'thermoregulation', and 'mutilation' were not correlated with levels of process hygiene. The post-mortem animal-based categories for fattening pigs with the poorest scores were 'gut pathologies', 'lung and respiratory tract pathologies' and 'liver pathologies'. The categories 'gut pathologies' and 'liver pathologies were associated with microbial loads on carcasses but not significantly, in terms that higher scores for those categories were associated better process hygiene levels, while 'lung and respiratory tract pathologies' did not correspond with microbial levels on carcasses. This finding also revealed that these three post-mortem categories should be regularly monitored at the slaughter line.

These findings highlight the importance of the animal-based category 'manure on the body' as important for both aspects – pig welfare in abattoir lairage, as well as for the level of process hygiene at slaughter. Improved status of pig welfare in abattoir lairage, associated with pre-slaughter assessment of animal-based categories, e.g. visual evaluation of animal cleanliness, can be a useful tool for maintaining control of the overall slaughter process hygiene and may be effective in reducing microbial contamination of carcasses (*Delhalle et al.*, 2008).

Conclusion

The safety of the food chain is indirectly affected by welfare of animals farmed for food production, due to the close links between animal welfare, animal health and food borne diseases. Stress factors and poor welfare can lead to increased susceptibility to disease among animals and can intensify fecal shedding of food borne pathogens in the pre-slaughter phase: on-farm, in transport/livestock markets and in abattoir lairage. This could affect the process hygiene at slaughter/dressing and increase the public health risk for consumers. In this study, the European Food Safety Authority protocol intended for assessment of on-farm pig welfare was adapted to be used for assessment of welfare of fattening pigs in abattoir lairage. This approach is based on a group level assessment of animal-based categories. The results indicated that transportation time was not correlated with bacterial loads on carcasses (TVC, EC, GEC, Coliforms), while space allowance coupled with the lay-over time in lairage and ambient temperature tended to correlate with process hygiene; better space allowances, shorter lay-over times and higher temperatures were associated (but not statistically significantly) with lower microbial loads on carcasses. The ante-mortem animal-based categories for fattening pigs in abattoir lairage with the lowest scores were 'thermoregulation', 'manure on the body', 'insufficient space', 'injurious behavior' and 'lesions'. However, only manure on the body had any correlation or impact on process hygiene levels. Therefore, focus should be put, in particular, on 'manure on the body' category. The post-mortem animal-based categories for fattening pigs with the lowest scores were 'gut pathologies', 'lung and respiratory tract pathologies' and 'liver pathologies'. This finding revealed that these three categories should be regularly monitored on the slaughter line, as they could provide valuable feedback to the farm of origin, and reflect on the farm biosecurity level. Further and deeper research is needed to understand better the interface between animal welfare variables in abattoir lairage and slaughter process hygiene outcome.

Acknowledgment: We would like to thank to the meat industry "Yuhor", Jagodina, Serbia for its support in providing the full access to the production facilities needed to carry out this study.

Disclosure statement: No pontential conflict of interest was reported by authors.

References

Berends, B. R., VanKnapen, F., Snijders, J. M., & Mossel, D. A. (1997). Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. *International Journal of Food Microbiology*, *36*, 199–206.

Blagojevic, B., Antic, D., Ducic, M., & Buncic, S. (2011). Ratio between carcass-and skin-microflora as an abattoir process hygiene indicator. *Food Control*, *22*, 186–190.

Buncic, S., Steinhauserova, I., Smulders, F., Paulsen, P., Braun, P., Albert, T., & Steinhauser, L. (2013). The Food Research, Safety and Training Network (FRST-NET) initiative, step one: Issues relevant for meat safety assurance at abattoir level. http://www.maso-international.cz/wp-content/uploads/2013/04/maso-international-2012-2-page-083-093.pdf (accessed on 9 September 2016).

Candiani, D., Salamano, G., Mellia, E., Doglione, L., Bruno, R., Toussaint, M. & Gruys, E. (2008). A Combination of Behavioral and Physiological Indicators for Assessing Pig Welfare on the Farm. *Journal of Applied Animal Welfare Science*, *11*, 1–13.

Del Campo, M., Brito, G., Montossi, F., de Lima, J. M. S., & San Julián, R. (2014). Animal welfare and meat quality: The perspective of Uruguay, a "small" exporter country. *Meat Science*, *98*, 470–476.

Delhalle, L., De Sadeleer, L., Bollaerts, K., Farnir, F., Saegerman, C., Korsak, N., Dewulf, J., De Zutter, L., & Daube, G. (2008). Risk factors for *Salmonella* and hygiene indicators in the 10 largest Belgian pig slaughterhouses. *Journal of Food Protection*, *71*, 1320–1329.

Dokmanovic, M., Velarde, A., Tomovic, V., Glamoclija, N., Markovic, R., Janjic, J., & Baltic, M. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. *Meat Science*, *98*, 220–226.

EC. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

EC. (2009). Council Regulation 1099/2009 on the protection of animals at the time of killing.

EFSA. (2012). Scientific Opinion on the use of animal-based measures to assess welfare in pigs. *EFSA Journal*, *10*, 2512.

EFSA. (2013a). Scientific Opinion on monitoring procedures at slaughterhouses for pigs.

EFSA Journal, *11*, 3523.

EFSA. (2013b). Scientific Opinion on monitoring procedures at slaughterhouses for bovines.

EFSA Journal, *11*, 3460.

Faucitano, L. (2010). Effects of lairage and slaughter conditions on animal welfare and pork quality. *Canadian Journal of Animal Science*, *90*, 461–469.

FAWC. (2013). Space allowances in slaughterhouse lairage (Farm Animal Welfare Committee. http://www.defra.gov.uk/fawc (accessed on 2 September 2016).

Ferguson, D. M., Bruce, H. L., Thompson, J. M., Egan, A. F., Perry, D., & Shorthose, W. R. (2001). Factors affecting beef palatability – Farmgate to chilled carcass. *Australian Journal of Experimental Agriculture*, *41*, 879–892.

Fraqueza, M. J., Roseiro, L. C., Almeida, J., Matias, E., Santos, C., & Randall, J. M. (1998). Effects of lairage temperature and holding time on pig behaviour and on carcass and meat quality. *Applied Animal Behaviour Science*, *60*, 317–330.

Geverink, N. A., Engel, B., Lambooij, E., & Wiegant, V. M. (1996). Observations on behavior and skin damage of slaughter pigs and treatment during lairage. *Applied Animal Behaviour Science*, *50*, 1–13.

Gill, C. O., Dussault, F., Holley, R. A., Houde, A., Jones, T., Rheault, N., Rosales, A., & Quessy, S. (2000). Evaluation of the hygienic performances of the processes for cleaning, dressing and cooling pig carcasses at eight packing plants. *International Journal of Food Microbiology*, *58*, 65–72.

Gispert, M., Faucitano, L., Oliver, M. A., Guardia, M. D., Coli, C., Siggens, K., Harvey, K., & Diestre, A. (2000). A survey of pre-slaughter conditions, halothane gene frequency, and carcass and meat quality in five Spanish pig commercial abattoirs. *Meat Science*, *55*, 97–106.

Grandin, T. (1996). Animal Welfare in Slaughter Plants. 29th Annual Conference of American Association of Bovine Practitioners. *Proceedings*, 22–26. http://www.grandin.com/welfare/general.session.html (accessed on 29 August 2016).

Guàrdia, M. D., Estany, J., Balasch, S., Oliver, M. A., Gispert, M., & Diestre, A. (2009). Risk assessment of skin damage due to pre-slaughter conditions and RYR1 gene in pigs. *Meat Science*, *81*, 745–751.

Guàrdia, M. D., Estany, J., Balasch, S., Oliver, M. A., Gispert, M., & Diestre, A. (2010). Risk assessment of DFD meat due to pre-slaughter conditions in pigs. *Meat Science, 70*, 709–716.

Horchner, P. M. & Pointon, A. M. **(2011).** HACCP-based program for on-farm food safety for pig production in Australia. *Food Control*, *22*, 1674–1688.

ISO 16649–1. (2001). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

ISO 6579. (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

ISO 4833. (2003). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C.

ISO 21528–2. (2004). Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method.

ISO 4832. (2006). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique.

ISO 17604. (2015). Microbiology of the food chain – Carcass sampling for microbiological analysis.

Nastasijevic, I., Tomasevic, I., Smigic, N., Milicevic, D., Petrovic, Z., & Djekic, I. (2016). Hygiene assessment of Serbian meat establishments using different scoring systems. *Food Control*, *62*, 193–200.

Petruzzelli, A., Osimani, A., Pasquini, M., Clementi, F., Vetrano, V., Paolini, F., Foglini, M., Micci, E., Paoloni, A., & Tonucci, F. (2016). Trends in the microbial contamination of bovine, ovine and swine carcasses in three small-scale abattoirs in central Italy: A four-year monitoring. *Meat Science*, *111*, 53–59.

Rostagno, M. H. (2009). Can stress in farm animals increase food safety risk? *Foodborne Pathogens and Disease*, 6(7), 767–776.

RSPCA (2014). RSCPA welfare standards for pigs. http://science.rspca.org.uk/sciencegroup/farmanimals/standards/pigs (accessed on 29 August 2016).

Spoolder, H. A. M., Edwards, S. A., & Corning, S. (2000). Legislative methods for specifying stocking density and consequences for the welfare of finishing pigs. *Livestock Production Science*, *64*, 167–173.

Sutherland, M. A., Bryer, P. J., Davis, B. L. & McGlone, J. J. (2010). A Multidisciplinary Approach to Assess the Welfare of Weaned Pigs During Transport at Three Space Allowances. *Journal of Applied Animal Welfare Science*, *13*, 237–249.

USDA FSIS. (1996). Guidelines for *Escherichia coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments. http://www.fsis.usda.gov/wps/wcm/connect/cebac8d0–954b-4cc2-abcf-205ad4f4ddcd/Guideline_for_Ecoli_Testing_Cattle_Swine_Estab.pdf?MOD=AJPERES (accessed on 7 September 2016).

USDA FSIS. (2013). FSIS Compliance Guide for a Systematic Approach to the Humane Handling of Livestock. http://www.fsis.usda.gov/wps/wcm/connect/da6cb63d-5818–4999–84f1–72e6dabb9501/Comp-Guide-Systematic-Approach-Humane-Handling-Livestock.pdf?MOD=AJPERES (accessed on 7 September 2016).

van Erp-van der Kooij, E., Kuijpers, A. H., Schrama, J. W., van Eerden-burg, F. J. C. M., Schouten, W. G. P., & Tielen, M. J. M. (2002). Can we predict behaviour in pigs? Searching for consistency in behaviour over time and across situations. *Applied Animal Behaviour Science*, *75*, 293–305.

Weeks, C. A. (2008). A review of welfare in cattle, sheep and pig lairages, with emphasis on stocking rates, ventilation and noise. *Animal Welfare*, *17*, 275–284.

Yousuf, M. I. (2007). Using experts' opinions through delphi technique. *Practical Assessment, Research and Evaluation*, *12*, 8.

Zweifel, C., Fischer, R., & Stephan, R. (2008). Microbiological contamination of pig and cattle carcasses in different small-scale Swiss abattoirs. *Meat Science*, *78*, 225–231.

Paper received: 19.01.2018
Paper corrected: 13.03.2018.
Paper accepted: 8.03.2018.

UDK: 637.5'62.05(497.11)
ID: 265914380
meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade
https://doi.org/10.18485/meattech.2018.59.1.3

Original scientific paper

Analysis of beef meat quality in a slaughterhouse in Raska district

Milos Z. Petrovic[1], Radojica Djokovic[1], Dragan Vasilev[2], Vesna Djordjevic[3], Mirjana Dimitrijevic[2], Silvana Stajkovic[2], Nedjeljko Karabasil[2]*

A b s t r a c t: The quality of slaughtered animals is a subject of interest, for both primary production and the meat industry. Classification of the carcasses is performed in slaughterhouses immediately after a veterinary examination and measurement of the carcasses weight. The present study examined the quality of young cattle carcasses in a slaughterhouse in the Raska district, according to the standard applied in the EU but not in Serbia. In total, 100 cattle carcasses (young bulls) were examined. For meat quality evaluation and grading, the following parameters according to the European standard for the classification of cattle carcasses SEUROP were used: slaughter weight, carcass weight and carcass yield, i.e. the carcass conformation, development of the muscles of the carcass as well as the development of basic parts (round, back and shoulder) and the degree of carcass fat tissue coverage.

***Key words:** beef meat quality, carcass classification, young bulls.*

Introduction

The meat production process has several steps and each of them is significant for production of safe and quality product. Consumption of beef meat ranks in third place in Serbia, after pork and poultry (*Ostojic et al.,* 2006). Improving carcass performance and meat quality traits are the main objectives of most research carried out in the beef production area. Meat quality is an important criterion that influences consumer decisions to purchase beef (*Baltic and Boskovic*, 2015; *Djordjevic*, 2016). Local demand is partially covered by imported beef because domestic production can not fulfill the requirements of the local market. In order to improve the current local situation, it is necessary to enhance and maintain agro-economic policies and strengthen the primary production. Some of the possible solutions for better production and quality are improving the qualities of breeding stock, nutrition and animal breeding technology (*Aleksic et al.*, 2011; *Sefer et al.,* 2015). Development of greater beef production volumes, improved beef meat quality and placement of higher-value meat on the market require improvement to the quality of meat from carcasses. Understandably, this refers to the edible parts of the carcass, carcass conformation and the carcass fat coverage, plus processing quality and sensory properties (*Sretenovic et al.,* 2011; *Ostojic-Andric et al.,* 2012).

The quality of the slaughtered animals is a subject of interest for both primary production and the meat industry. Based on the estimated value and classification of carcasses, it is possible to appropriately compensate producers i.e. the owners of animals, but also to assess the market value and industry profit. In order to assess carcass quality (meatiness) more thoroughly, parameters such as: slaughter weight, age of animal, carcass weight, carcass yield, carcass conformation, fat coverage, musculature length (m. *longissimus dorsi*) etc. should be considered. Animals are classified based on age, sex, physiological status and meatiness (*Stamenkovic and Radovanovic,* 2004). Carcass classification should be performed in slaughterhouses immediately after the veterinary examination and measurement of the carcasses weight.

In developed countries, quality is taken into account through a balanced approach of carcass meat quality assessment. The SEUROP classification system, used in the EU, enables prediction of the amount of meat in the carcass (*EC No. 1249*, 2008), which is the basis for determining the selling price of each animal. Given that monetary compensation

[1]University of Kragujevac, Faculty of Agronomy Cacak, Ljubicska 30, 32000 Cacak, Republic of Serbia;
[2]University of Belgrade, Faculty of Veterinary Medicine, Bulevar osobodjenja 18, 11000 Belgrade, Republic of Serbia;
[3]Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia.

***Corresponding author:** Petrovic Z. Milos, *petrovic.milos87@kg.ac.rs.*

depends on the achieved quality, it is also an incentive for the improvement of cattle breeding, zootechnical conditions including hygiene, and welfare and health of animals in primary production. Slaughterhouses in which over 75 animals are slaughtered weekly (annual average) are obliged to apply the SEUROP classification system. The selection of personnel involved in this activity is of great importance and they require adequate education and training. The uniformity of the carcass quality assessment is achieved by precisely defined rules that include parameters and criteria in the corresponding regulations that concern: the category of carcass according to age and physiological status (calves, elderly calves, heifers, young bulls, castrated male animals and cows), carcass processing at slaughter for classification and categorization, criteria for scoring the carcass parameters of conformation and the fat tissue degree coverage. As already emphasized, carcass meat quality assessment has material significance since it enables payment for meat on the basis of quality achieved, and therefore, the SEUROP system has been further improved with subclasses within each class in order to determine the quantity of meat in the most complete manner.

The aim of the present study is to examine young bull carcass quality in a Serbian slaughterhouse according to slaughter weight, carcass yield, carcass conformation, and fat coverage degree, the criteria used in EU countries.

Materials and methods

The study was conducted from July 10 2014 to August 4 2014 in a slaughterhouse in Raska district, Serbia. The examination included carcasses of 100 slaughtered young bulls of the domestic Simmental breed, aged about one year from purchase.

Slaughter weight was measured after unloading at the slaughterhouse, while carcass weight was determined 45 minutes after slaughter, both on scales with accuracy of ±0.5 kg.

Carcass weight included the processed carcass without the following: internal organs (with the exception of the kidneys, which were included), skin, head, lower parts of legs (separated at the lower part of the carpal, tarsal joints were included), large blood vessels, spinal cord and the genital organs.

Carcass conformation and fat coverage were determined 45 minutes after slaughter, according to SEUROP classification (*EC No. 1249*, 2008). Based on the carcass conformation, carcasses were classified into six classes: S (superior): all profiles extremely convex; exceptional muscle development, double-muscled carcass type; E (excellent): all profiles convex to super-convex; exceptional muscle development; U (very good): profiles on the whole convex; very good muscle development; R (good): profiles on the whole straight; good muscle development; O (fair): profiles straight to concave; average muscle development; and P (poor): all profiles concave to very concave; poor muscle development.

Carcass fat coverage was estimated by numerical grades, from: 1 (low): none up to low fat cover; 2 (slight): slight fat cover, flesh visible almost everywhere; 3 (average): flesh, with the exception of the round and shoulder, almost everywhere covered with fat, slight deposits of fat in the thoracic cavity; 4 (high): flesh covered with fat, but on the round and shoulder still partly visible, some distinctive fat deposits in the thoracic cavity; to 5 (very high): entire carcass covered with fat; heavy fat deposits in the thoracic cavity.

Statistical analysis was performed using the statistical package Stats Soft INC (Statistica For Windows, version 6.0 computer program manual Tulsa, Stat Soft Inc., 1995). Descriptive statistical parameters (mean, standard deviation, standard error of the mean, minimum, maximum, and coefficient of variation) are presented in Table 1.

Results and discussion

Table 1 shows the average mean slaughter weight, carcass weight and carcass yield of all 100 cattle carcasses.

Results from Table 1 show that average cattle slaughter weight was 518.77 kg with a coefficient of variation of 10.74%. The mean carcass weight was 275.21 kg with a coefficient of variation of 10.4%. The mean carcass yield was 52.61% and ranged from 48.00 to 63.00%.

According to statistical data in Serbia, the average weight of adult animals before slaughter during 1995 to 2000 was 478 kg and from 2006 to 2011 was 504 kg (*Dokmanovic et al*., 2014). Lower weights compared to our results could be due to the fact that in those data, cattle were not separated by age and sex. *Aleksic et al. (*2002) showed the average animal weight before slaughter was 592.7 kg, hot carcass weight with the lard was 329.9 kg, while average carcass yield was 55.66%. Similar results were found by *Ostojic et al. (*2007*),* who reported Simmental bulls weighed 579 kg after 477 days, while the average carcass yield was 57.1%. In the same study, crossbreeds of Charolais and Limousine of younger age (446 and 443 days) achieved higher body weight at the end of fattening (621 kg and 590 kg, respectively).

Table 1. Average slaughter weight, carcass weight and carcass yield of young domestic Simmental bulls (n=100) in a slaughterhouse in Serbia

Parameter	Slaughter weight (kg)	Carcass weight (kg)	Carcass yield (%)
Mean±standard deviation	518.77±55.74	275.21±28.6	52.61±2.14
Standard error	5.6	2.8	–
Minimum	376.0	210.0	48.00
Maximum	652.0	342.0	63.00
Coefficient of variation (%)	10.74	10.4	–

Drca (2009) reported that male domestic Simmental type cattle from three different producers in Serbia had carcass yields between 54.20% and 55.40%. Similar results were reported by *Lukic et al.* (2016), who showed the average slaughter weight of male Simmental beef cattle was 586.9 kg. The average carcass yield of male Simmental beef cattle was 56.56% (*Lukic et al*., 2016), which is higher than the Simmental carcass yield in our study (52.61%). Among 54 young Simmental bull carcasses, the average weights of two groups of cattle were 478.40 kg and 569.42 kg (*Petrovic et al*., 2016). Average carcass yield was 51.87% (total for both groups), and ranged from 42.00 to 57.00% (*Petrovic et al*., 2016). *Ciric et al*. (2017) found mean slaughter weight of domestic Simmental bulls was 583.9 kg, while average carcass yield was 56.32%. Similar results were determined by *Petrovic et al.* (2017), who showed the average slaughter weight of male Simmental beef cattle was 516.23 kg with coefficient of variation of 10.47%, while average carcass yield was 52.37% and ranged from 39.00 to 63.00%. Finally, *Petrovic et al.* (2017) analyzed 80 young bull carcasses and found the average live weight was 497.74 kg with a coefficient of variation of 5.83%, while average carcass yield was 52.74%, ranging from 42.00 to 59.00%.

The following parameters are used for cattle carcass classification in the EU (*EC No. 1249*, 2008): carcass weight, conformation, meatiness, as well as the development of the prime cuts (round, the back and the shoulders), fat coverage degree, etc. A favorable conformation (excellent) of carcasses implies that all profiles are extremely well developed and convex. The round in excellent carcasses has a convex profile, the back is well developed and wide and the shoulders are filled and well-formed. Poor or unfavorable carcass conformation is characterized by concave, poorly developed leg, narrow back, straight shoulders and convex bones. Fat coverage refers to the amount and arrangement of subcutaneous, kidney and pelvic fat and residues on the inside surfaces of the chest and abdominal cavity. Fat protects the meat from oxidation, slows down the surface drying of meat, reduces the toughness and contributes to good juiciness and aroma *(Vukovic,* 2012*)*. From a quality point of view, it is

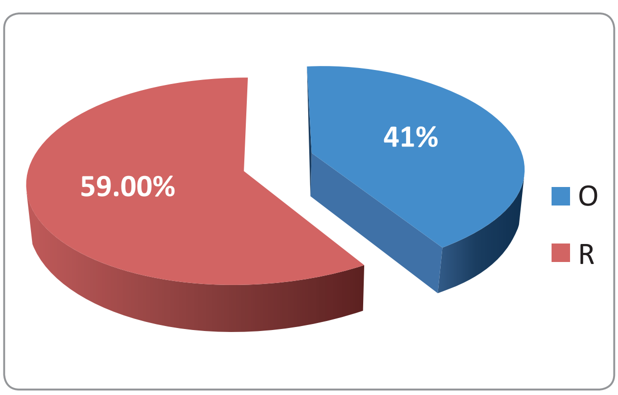


Figure 1. Percentage of carcass classes O and R among the studied cattle

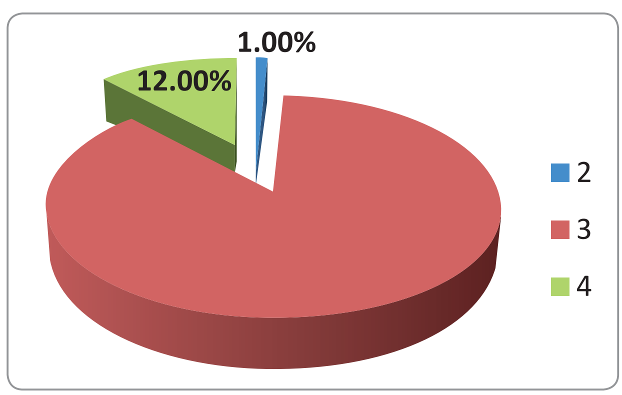


Figure 2. Percentage of studied cattle with degrees of carcass fat tissue coverage

considered as favorable that a beef carcass has a uniform and well-distributed, continuous, but not too thick, layer of fat.

Figure 1 shows the results of carcass classification according to SEUROP classification, while Figure 2 shows the results of carcass classification according to fat coverage degree (n=100). In terms of SEUROP classification, only two categories were observed among our studied cattle: O (fair) (n=41) and R (good) (n=59). Regarding degree of fat coverage, three categories were determined (2, 3 and 4). One carcass was labeled as category 2. More carcasses were labeled as category 3 (n=71) than category 4 (n=12).

Similar results were obtained by *Petrovic et al.* (2016) who evaluated class of carcass based on the conformation as O for 77.5% of carcasses and as R for 22.5% of carcasses. The carcass fat tissue coverage degree was rated as 3 for 88.75% and 4 for 11.25% of carcasses (*Petrovic et al*., 2016). *Petrovic et al.* (2017) analyzed fat tissue coverage in 123 young bull carcasses. Their results showed three categories of fat cover (2, 3, 4), similar to our current study. More (n=107) carcasses were classified as category 3 than category 2 (1 carcass) or category 4 (15 carcasses) (*Petrovic et al*., 2017). In research by *Petrovic et al.* (2017) for all 80 young bull carcasses, only two categories, in terms of class were seen: O (fair) (n=62, 77.5%) and R (good) (n=18, 22.5%). Regarding fat tissue coverage, two categories were determined: 3 (mid coverage) and 4 (high coverage). More carcasses were labeled as category 3 (n=71, 88.7%) compared to number of carcasses labeled as category 4 (n=9, 11.2%).

Meat quality can be affected by pre-slaughter factors and post slaughter factors of animals including gender, age, feeding, animal handling, animal welfare, slaughter of animal, genotype of animals. In the EU, beef carcass classification for conformation and fatness plays an important role in international meat trade marketing. This is why meat price in the market depends on carcass conformation.

Conclusion

Based on the results and their critical considerations the following can be concluded:

- The mean carcass weight was 518.77±55.74 kg;
- The mean carcass yield was 52.61±2.14% and it ranged from 48.00 to 63.00%;
- Carcasses were evaluated as having conformation O in 41% of cases and as R in 59% of cases;
- The carcass fat tissue coverage degree was rated as 2 for 1%, 3 for 12% and 4 for 87% of carcasses.

Acknowledgments: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Projects No. TR 31034 and TR 31001.

Disclosure Statement: No potential conflict of interest was reported by the authors.

References

Aleksic, S., Petrovic, M. M., Pantelic, V., Novakovic, Z., Ostojic-Andric, D., Stanisic, N. & Niksic, D. (2011). Chemical, technological and sensory properties of meat from different cattle genotypes. *Biotechnology in Animal Husbandry*, *27 (3)*, Book 2, 913–918.

Aleksic, S., Miscevic, B., Petrovic, M. M., Pavlovski, Z., Josipovic, S. & Tomasevic, D. (2002). Investigation on factors affecting the results regarding the dressing percentage value of male young cattle of domestic Simmental breed and crossbreeds of domestic Simmental and Limousine breed (in Serbian). *Biotechnology in Animal Husbandry*, *18 (3–4)*, 1–14.

Baltic, Z. M. & Boskovic, M. (2015). When man met meat: meat in human nutrition from ancient times till today. *Procedia Food Science*, *5*, 6–9.

Ciric, J., Lukic, M., Radulovic, S., Janjic, J., Glamoclija, N., Markovic, R. & Baltic, Z. M. (2017). The relationship between the carcass characteristics and meat composition of young Simmental beef cattle. IOP Conference Series: Earth and Environmental Science, 85, conference 1.

Dokmanovic, M., Lukic, M., Baltic, M. Z., Ivanovic, J., Markovic, R., Grbic, S. & Glamoclija, N. (2014). Analysis of beef production volume in Serbia from 1985 to 2011. *Tehnologija Mesa*, *55 (1)*, 73–80.

Djordjevic, M. (2016). Comparative analysis of the lean meat of carcasses and selected quality parameters of meat of cattle in fattening. Doctoral dissertation. Faculty of Veterinary Medicine, University of Belgrade.

Drca, D. (2009). Examination of lean meat in beef cattle carcasses in slaughterhouse in Cajetina. University of Belgrade, Serbia.

Lukic, M., Ivanovic, J., Starcevic, M., Djordjevic, J., Markovic, R. & Baltic, Z. M. (2016). Carcass performance of Simmental and Holstein Friesian beef cattle in Serbia *Meat Technology, 57 (2)*, 95–101.

Ostojic, D., Bogdanovic, V., Petrovic, M. M., Aleksic, S., Miscevic, B. & Pantelic, V. (2006). Criteria of consumers when purchasing beef in retail stores, Part B: Position of beef compared to other meat types, reasons and frequency of its use in everyday nutrition and preparation methods. *Biotechnology in Animal Husbandry*, *22 (3–4)*, 45–53.

Ostojic-Andric, D., Aleksic, S., Hristov, S., Novakovic, Z., Petrovic, M. M., Niksic, D. & Stanisic, N. (2012). Serbia in the implementation of SEUROP standard for beef carcass classification legislation, parameters and evaluation criteria. *Biotechnology and Animal Husbandry*, *28 (1)*, 47–58.

Ostojic-Andric, D., Bogdanovic, V., Aleksic, S., Petrovic, M. M., Miscevic, B., Pantelic, V. & Josipovic, S. (2007). The effect of genotype on dressing percentage and tissue content of beef carcasses. *Biotechnology in Animal Husbandry*, *23 (3–4)*, 31–39.

Petrovic, M., Djokovic, R., Petrovic, D. M, Cincovic, M, Ilic, Z. Z, Cobanovic, N. & Karabasil, N. (2017). Analysis of the degree covering of young bull carcass with fat tissue according to EU standard. 6th International Symposium on Agricultural Sciences AgroReS 2017, Abstracts, Banja Luka, Bosnia and Herzegovina, 46.

Petrovic, M., Karabasil, N., Djokovic, R., Cincovic, M., Petrovic, D. M, Ilic Z. Z. & Stanimirovic Z. (2016). Influence of the final mass of young bulls of the Simmental breed on dressing percentage. XXI Conference on Biotechnology, Faculty of Agronomy in Cacak, Proceedings, *21 (24)*, 577–580.

Petrovic, M., Djokovic, R., Cincovic, M., Ilic Z. Z, Petrovic, D. M, Cobanovic, N. & Karabasil, N. (2017). Effect of age of young Simmental bulls on dressing percentage. *Journal Acta Agriculturae Serbica, (XXII (43)*, 11–21.

Petrovic, M., Djokovic, R., Cincovic, M., Ilic, Z. Z, Petrovic, D. M, Doskovic, V. & Karabasil, N. (2017). Analysis of the quality of carcasses of young Simmental bulls according to EU standard. *Journal Acta Agriculturae Serbica (XXII) 44,* 147–156.

Regulation (EC) No. 1249. 2008. Laying down detailed rules on the implementation of the Community scales for the classification of the beef, pig and sheep carcases and the reporting of prices thereof. *Official Journal of the European Parliament.*

Sretenovic, Lj., Pantelic, V., Novakovic, M., Novakovic, Z., & Ostojic-Andric, D. (2011). Production of beef meat with functional food properties. *Biotechnology in Animal Husbandry, 27 (3)*, Book 1, 373–385.

Stamenkovic, T. & Radovanovic P. (2004). A contribution to study on carcasses of slaughtered cattle, pigs, sheep and fowls. *Tehnologija Mesa, 45 (5–6)*, 219–224.

Vukovic, K. I. 2012. Basics of Meat Technology. Veterinary Chamber of Serbia, Belgrade.

Sefer, D., Markovic, R., Nedeljkovic-Trailovic, J., Petrujkic, B., Radulovic, S. & Grdovic, S., 2015. The application of biotechnology in animal nutrition. *Veterinarski Glasnik, 9 (1–2)*, 127–137.

Paper received: 11.12.2017.

Paper corrected: 26.01.2018.

Paper accepted: 18.12.2017.

UDK: 639.371:597.551.2
ID: 265903116
https://doi.org/10.18485/meattech.2018.59.1.4

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

Original scientific paper

Evaluation of the diet supplementation strategy on the flesh quality and fatty acid profiles in common carp (*Cyprinus carpio* L)

Dejana Trbovic[1], Vesna Djordjevic[1], Aurelija Spiric[1], Radivoj Petronijevic[1], Vlado Teodorovic[2], Nenad Parunovic[1], Zoran Markovic[3]*

A b s t r a c t: The objective of this study was to evaluate the influence of three supplementary diets on the nutritional quality of marketable common carp. Supplementary diets (Carp1–maize, Carp2–extruded and Carp3–pelleted feed) exhibited significantly different ($p \leq 0.05$) influences on the protein, lipids, moisture and ash content in market-sized carp. The most abundant saturated FA (SFA) was palmitic acid (C16:0), the most predominant monounsaturated FA (MUFA) was oleic acid (C18:1n-9), and the most abundant polyunsaturated FA (PUFA) was linoleic acid (C18:2n-6). The highest content of SFAs was established in Carp1 (24.57% and 1888.72 mg/100 g wet fillet). MUFAs were presented in the highest quantities (61.77%, i.e. 4854.91 mg/100 g wet fillet) in Carp1, and PUFA accounted the highest quantities (33.48%, i.e. 1763.01 mg/100 g wet fillet) in Carp3. The most nutritionally beneficial n−6/n−3 ratio was obtained in Carp2 (5.83). The results obtained in this study indicate that introducing supplementary diets containing extruded or pelleted feed instead of maize improved carp nutritional quality. PCA (principal component analysis) and LDA (linear discriminant analysis) of the FAs demonstrated that carp could be reliably classified based on their supplementary feed.

Key words: *common carp, supplementary diets, proximate composition, fatty acid profiles.*

Introduction

The lipid content and fatty acid (FA) composition differ greatly within fish species depending on various conditions, including the type and availability of food and/or feed, the state of the ecosystem inhabited by the fish, the environmental conditions, season, and age (Kaçar et al., 2016; Ljubojevic et al., 2013a; Ivanovic et al., 2015). However, fish feed is the most responsible of these factors for the FA profile of fish, and evidence suggests that under similar farming conditions fish feed rich in n–3 FAs decreases the n–6/n–3 PUFA ratio in the fish tissue (Tesic et al., 2014). The FA composition of farmed fish differs from the FA composition of fish from open waters (Ljubojevic et al., 2013b). The growing demand for food in densely populated or less developed countries is partially satisfied through aquaculture with cyprinid fish species (Jeney & Jian, 2009). The farming of cyprinid fish species is more developed in Asia and in central and eastern European countries (Váradi et al., 2012). The dominant form of cyprinid production is the semi-intensive farming of common carp (Cyprinus carpio L.) where the diet of the fish is based primarily on a combination of natural food and supplementary feed (cereals, such as wheat, maize and barley) (Mráz et al., 2012). To improve the production and nutritional value of the carp over the last decade, cereals have been replaced by pelleted and extruded feed (Tkaczewska et al., 2014). Research into carp aquaculture became more focused on the evaluation of the supplemental feed type that is essential for increasing carp production (*Ciric et al.,* 2015) and on nutritional quality of the fish under the influence of different food/feed type (*Böhm* et al., 2014).

Although carp production is widespread in the world, data on the nutritional quality of marketable carp are limited in the literature. In Serbia, carp is also the most cultivated fish species, and is considered as a valuable fishery product and a traditional dish. Therefore, the aim of this study was: (a) to evaluate the influence of three supplementary diets (maize, extruded and pelleted feed) on the proximate composition and FA profile of marketable common carp collected

[1]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia;*
[2]*University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11080 Belgrade, Republic of Serbia;*
[3]*University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade – Zemun, Republic of Serbia.*

***Corresponding author:** Vesna Djordjevic, *vesnazdjordjevic@gmail.com*

from three commercial fish farms using semi-intensive farming conditions, and (b) based on the obtained data to derive conclusions about the nutritional quality of the carp offered to commercial market.

Materials and methods

According to the opinion of the Ethics Commission of the Institute of meat hygiene and technology (Opinion No. 3294) all experimental procedures conducted in this study have been performed in accordance with the national Animal Welfare Act (2009).

Samples

For the study, two and a half years old market-sized carp (n=8) were obtained in October 2015 from three commercial carp farms from batches of fish harvested for the market. The fish farms were

Figure. 1 The geographical positions (GPS data) of the fish farms. (Zivaca: Carp supplementary fed maize) latitude 44.73, longitude 20.18; Ecka: (Carp supplementary fed extruded feed) latitude 45.24, longitude 20.36; Despotovo: (Carp supplementary fed pelleted feed) latitude 45.45, longitude 19.54)

located in Vojvodina, the lowland part of Serbia (Fig. 1), and all of them practiced semi-intensive aquaculture.

From April until they were harvested for the market, in October 2015, the carp were fed commercial diets supplemented with maize (Carp1), extruded feed (Carp2), which consisted of 61% maize, 30% soybean meal, 3% fish meal, 3% yeast, 0.8% chalk, 0.2% lysine, 1% vitamin premix and 1% other ingredients, and pelleted feed (Carp3), which consisted of 28% soybeans, 5% fish meal, 6% yeast, 22% wheat, 30% maize, 6.4% soybean meal, 0.8% chalk, 0.20% lysine, 1% vitamin premix, 0.8% monocalcium phosphate and 0.1% salt. The mean body weights of the live fish at harvest were 1,834±443 g for Carp1 fed maize, 1,984±322 g for Carp2 fed extruded feed, and 2,235±423 g for Carp3 fed pelleted feed. The proximate and FA composition of the supplementary diets are shown in Table 1.

After the fish were caught, the percussive stunning method has been carried out (EFSA, 2009), and afterwards the slaughtered fish were transported to the laboratory in coolers with ice. After removal of the skin, heads, tails, fins, and intestines, the fish were deep-frozen and maintained at −25 °C until analysis, but no longer than two weeks. Before the analysis, the frozen fish were allowed to equilibrate at room temperature for an hour. The obtained fish fillets were individually disintegrated in a CombiMax 600 blender (Braun GmbH, Kronberg, Germany).

Proximate composition

The protein content (N × 6.25) in the fish was determined using the Kjeldahl procedure on a Kjeltec Auto 1030 Analyser (Manual book, Tecator, Höganäs, Sweden). The moisture content was determined by drying the sample at 103±2°C to a constant mass based on the ISO 1442:1997 method. The total fat content was determined by extraction with petroleum ether in a Soxhlet apparatus, after the sample was hydrolysed with acid, based on the ISO 1443:1973 method. The ash content was determined gravimetrically through dry ashing in a muffle furnace at 550±25°C based on the ISO 936:1998 method.

Fish feed was analysed for its lipid (ISO 6492:1999), moisture (ISO 6496:1999), ash (ISO 5984:2002) and crude protein contents (Kjeldahl method using a conversion factor of 6.25 for the total nitrogen). The crude fibre content was determined using a standard method with intermediate filtration (ISO 6865:2000). The nitrogen-free extract (NFE) was calculated by adding the values obtained for moisture, crude protein, lipid, crude fibre and ash, and subtracting the sum from 100. All proximate composition analyses were performed in triplicate.

Table 1. Proximate and FA composition of the diets

Proximate composition (%)	**Maize**	**Extruded feed**	**Pelleted feed**
Crude protein	8.58	24.96	22.22
Lipids	4.47	8.17	5.43
Moisture	11.64	9.66	8.27
Ash	1.10	6.81	6.61
Crude fibre	1.71	3.12	3.17
Nitrogen-free extract (NFE)	72.50	47.28	54.30
Fatty acids (% of total FAs)			
C18:2n-6	56.15	54.02	54.96
C18:3n-3	0.98	1.62	3.74
ΣSFA	13.84	14.02	15.47
ΣMUFA	28.15	27.73	25.62
ΣPUFA	58.00	58.18	58.91
n-6	57.03	54.69	55.13
n-3	0.98	3.49	3.78
n-6/n-3	58.19	15.67	14.59

FA fatty acid, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

Extraction of total lipids

The total lipids used to determination the FA contents were extracted from fish fillets through accelerated solvent extraction (ASE), (ASE 200, Dionex, Sunnyvale, CA, USA). The operating conditions were the same as previously reported (*Spiric et al.,* 2010). Briefly, after consecutively loading the Dionex extraction cell with diatomaceous earth and a homogenised fish fillet sample, the cells were extracted with a mixture of n-hexane and isopropanol (60:40, v/v) at 100°C over two static cycles under nitrogen at 10.3 MPa. The solvent from the collected extracts was removed under a stream of nitrogen (Dionex Solvent evaporator 500) at 50°C until dry. The ASE method for extracting lipids from fish fillets has been previously validated and accredited.

Fatty acids

FAMEs were prepared by transesterifying the lipids extracted through ASE using 0.25 M TMSH based on the ISO 5509:2000 method. The FAMEs were analysed on a Shimadzu 2010 gas chromatograph (Kyoto, Japan) equipped with a split/splitless injector, a fused silica cyanopropyl HP-88 column (J&W Scientific, Santa Clara, USA) (length=100 m, i.d.=0.25 mm, film thickness=0.20 µm), a flame ionisation detector, and a workstation. The injector and detector temperatures were 250°C and 280°C, respectively. To achieve complete separation of the tested compounds, a programmed column oven temperature, starting at 125ºC and ending at 230ºC, was applied.

The chromatographic peaks in the samples were identified by comparing their relative retention times to the FAME retention times in the Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA). The relative quantities of FAs, which were expressed as the weight percentages of the total FAs, were converted to mg of FA per 100 g of wet fillet (wf) based on the method described by *Exler et al.* (1975).

Statistical analysis

The obtained data are reported as the mean values±the standard deviations. ANOVA and the Tukey–Kramer test were used to analyse the data at the level of significance of 0.05 ($p \leq 0.05$). PCA and LDA were performed using JMP 10 software (SAS Institute Inc. NC, USA https://www.jmp.com).

Results and discussion

Proximate composition

The proximate compositions (protein, total lipids, moisture and ash) of the market-sized carp supplemented with different diets are presented in Table 2.

The data obtained show that different diets led to differences in the chemical composition of the fish, as reported by *Shearer* (1994). Significant differences ($p \leq 0.05$) in protein content were observed between Carp1, which were fed supplementary maize (16.12%), Carp2, which were fed supplementary extruded feed (17.26%) and Carp3, which were fed supplementary pelleted feed (18.42%). *Cirkovic et al.* (2011) reported similar results for proteins in carp farmed in polyculture in carp ponds in Serbia. Carp fed supplementary maize contained 8.59% lipids ($p \leq 0.05$), a higher content than in carp fed supplementary extruded feed (4.72%) and in carp fed supplementary pelleted feed (5.42%). However, from the data presented in Table 1, the NFE content, which is considered as a measure of soluble carbohydrates such as starch and sugar in the feed, accounted for 72.50%, 47.28% and 54.30% of the weight in maize, extruded and pelleted diets, respectively. Literature data (*Henderson*, 1996) demonstrates that fish diet rich in carbohydrates, such as maize, increased the synthesis of FAs and led to higher lipid content in the carp. Taking into consideration the geographical distribution of the fish farms, variation in amounts of natural and/or supplementary food/feed, environmental

Table 2. Proximate composition (% wf) of market-sized carp (Cyprinus carpio L.) (n=8)

Parameters	Carp1	Carp2	Carp3
Protein	16.12 ± 0.92^{a}	17.26 ± 0.30^{b}	18.42 ± 0.62^{c}
Total lipids	8.59 ± 0.34^{a}	4.72 ± 0.71^{b}	5.42 ± 1.04^{b}
Moisture	73.01 ± 2.63^{a}	75.72 ± 0.93^{b}	73.41 ± 1.55^{a}
Ash	0.96 ± 0.04^{a}	1.12 ± 0.06^{b}	1.33 ± 0.10^{c}

Legend: Values are means ± SD; ^{a,b,c} Means with different letters within the same row are significantly different ($p \leq 0.05$); wf wet fillet; n number of samples; Carp1 supplementary fed maize; Carp2 supplementary fed extruded feed; Carp3 supplementary fed pelleted feed conditions, etc, it can be considered that the lipid contents obtained in this study are similar to other reported data for the farmed carp (*Cirkovic et al.*, 2011; *Trenovszki et al.*, 2011). Regarding fat content, according to literature data (*Ackman*, 1989), carp fed supplementary maize can be considered as a fatty fish (>8%), while carp fed supplementary extruded and pelleted feed are considered as a medium fatty fish (4−8%). Due to differences in the mineral content of the diets, the ash content among the studied carp differed significantly (p≤0.05), ranging from 0.96% to 1.33%. However, other endogenous (genetic, like size, sex and life cycle stage) and exogenous (environment, like temperature, salinity, etc.) factors might also affect the proximate composition of the fish, including ash content (*Shearer*, 1994).

Fatty acids expressed as relative percentage of total fatty acids

The FA compositions of the carp supplemented with maize (Carp1), extruded (Carp2) and pelleted feed (Carp3), expressed as percentage of the total FAs and mg/100 g wf are presented in table 3.

The proportions of SFAs in the carps did not reflect their proportions in the diets. These results are similar to other reported data in carp supplied with supplementary feed diets (*Mráz et al.*, 2012).

The MUFA contents were 61.77% in the carp supplemented with maize, 43.45% in the carp supplemented with extruded feed and 43.46% in the carp supplemented with pelleted feed, which reflect their contents in the applied supplementary diets. The content of MUFAs in the carp fed supplementary maize was significantly higher (p≤0.05) than in the carp fed supplementary extruded or pelleted feed. No significant difference was observed in the MUFA contents in the carp supplemented with extruded and pelleted feed (p≥0.05). Among the individual MUFAs, oleic acid (C18:1n-9) was the most abundant FA in the carp, and it was significantly higher (p≤0.05) in carp fed supplementary maize (50.43%) than in carp fed supplementary extruded (34.11%) or pelleted (37.83%) feed. Evidence in the literature (*Farkas et al.*, 1978; *Henderson*, 1996) suggests that supplementary diets rich in carbohydrates (maize, wheat) lead to increased *de novo* synthesis of oleic acid in the carp, which led to around 1.5 times higher content of MUFAs in the carp fed supplementary maize compared to carp fed supplementary extruded or pelleted feed.

The total PUFA content was the highest in carp supplemented with pelleted feed (33.48%), and was followed by carp supplemented with extruded feed (31.53%). The lowest PUFA content was observed in the carp supplemented with maize (12.89%). Differences in the PUFA contents between carps supplemented with extruded and pelleted feed were not statistically significant (p≥0.05), while carp supplemented with maize contained significantly lower amounts of PUFAs (p≤0.05). However, the contents of PUFAs in the carp were not correlated with the quantities of PUFAs in the supplementary feeds (58.00%, 58.18% and 58.91% in maize, extruded and pelleted feed, respectively). Linoleic acid (C18:2n-6) was the most abundant PUFA in carp, comprising 10.19%, 25.01% and 28.01% of total FAs in carp supplemented with maize, extruded and pelleted feed, respectively. The linoleic acid (C18:3n-3) contents in the carps fed supplementary extruded and pelleted feed were 2.45 and 3.75 fold higher than in the carp fed supplementary maize, even though the content of this FA in the diets was similar (56.15%, 54.02% and 54.96% in the maize, extruded and pelleted feed, respectively). Literature data suggests that carp preferentially retain PUFAs from diets (*Böhm et al.*, 2014). This indicates that other effects (e.g. different metabolic pathways, zooplankton levels in fishponds, etc.) apart from diets were involved (*Henderson*, 1996).

Carp supplemented with pelleted feed contained the highest proportion of *n*–6 PUFAs (30.14%), followed by carp supplemented with extruded feed (26.91%) and carp supplemented with maize (11.89%). The differences in the *n*-6 PUFA contents were statistically significant (p≤0.05). The content of *n*–3 PUFAs was the highest in the carp supplemented with extruded feed (4.62%), followed by the carp supplemented with pelleted feed (3.33%), and the lowest was in the carp supplemented with maize (1.00%). The differences were statistically significant (p≤0.05). The proportion of linolenic acid, which was the most abundant n–3 PUFA in the carp, was the highest in the carp supplemented with pelleted feed (2.53%), followed by the carp supplemented with the extruded feed (2.26%), and the lowest was in the carp fed supplementary maize (0.63%), which is a consequence of the linolenic acid content in the carp feed (*Buchtová et al.*, 2007). The EPA (C20:5*n*-3) and DHA (C22:6*n*-3) contents were lower in the carp supplemented with maize (0.11% and 0.13%, respectively) and higher in the carp supplemented with extruded (0.58% and 1.01%, respectively) and pelleted (0.24% for both FAs) feed. The reason for higher quantities of EPA and DHA in the carp supplemented with extruded and pelleted feeds might be higher quantities of linolenic acid in the diets and the increased digestibility of these diets, which affected their utilisation by the fish (*Takeuchi*, 1996). Some literature data (*Böhm et al.*, 2014) indicate that different diets led to significant variations in the MUFA, n–6 and n–3 PUFA

Table 3. FA composition of carp (*Cyprinus carpio* L.) (n=8) supplementary fed different diets (% of total FAs and mg/100 g wf)

Fatty acids	Carp1		Carp2		Carp3	
	(% of total FA)	(mg/100 g wf)	(% of total FA)	(mg/100 g wf)	(% of total FA)	(mg/100 g wf)
C14:0	0.71 ± 0.07[b]	58.30[x]	0.84 ± 0.07[a]	38.09[y]	0.46 ± 0.06[c]	25.55[y]
C15:0	0.09 ± 0.02[b]	7.20[x]	0.19 ± 0.04[a]	8.17[x]	0.13 ± 0.06[b]	7.26[x]
C16:0	18.73 ± 1.27[a]	1423.42[x]	17.81 ± 0.76[ab]	806.54[y]	16.76 ± 0.70[b]	886.76[y]
C16:1	6.95 ± 0.76[a]	541.68[x]	5.01 ± 0.83[b]	229.68[y]	3.47 ± 0.96[c]	189.85[y]
C17:0	0.12 ± 0.03[b]	10.37[x]	0.34 ± 0.08[a]	14.99[x]	0.18 ± 0.09[b]	9.85[x]
C18:0	5.16 ± 0.66[a]	390.99[x]	4.49 ± 0.28[ab]	203.02[y]	4.38 ± 0.50[b]	228.09[y]
C18:1n-9	50.43 ± 3.46[a]	3968.34[x]	34.11 ± 2.37[c]	1560.15[y]	37.83 ± 2.89[b]	2019.36[y]
C18:1n-7	2.67 ± 0.20[a]	211.93[x]	2.57 ± 0.18[a]	115.37[y]	ND	ND
C18:2n-6	10.19 ± 2.18[c]	737.25[z]	25.01 ± 1.73[b]	1125.34[y]	28.01 ± 1.56[a]	1476.88[x]
C18:3n-6	0.24 ± 0.05[a]	19.07[x]	0.34 ± 0.05[a]	15.64[x]	0.33 ± 0.16[a]	15.88[x]
C18:3n-3	0.63 ± 0.23[b]	54.62[z]	2.26 ± 0.23[a]	101.30[y]	2.53 ± 0.24[a]	133.42[x]
C20:1n-9	1.76 ± 0.26[b]	132.95[x]	1.76 ± 0.24[b]	80.27[y]	2.16 ± 0.17[a]	114.32[x,y]
C20:2n-6	0.31 ± 0.06[c]	23.76[y]	0.68 ± 0.09[b]	30.35[y]	0.98 ± 0.20[a]	52.76[x]
C20:3n-6	1.09 ± 0.17[a]	82.78[x]	0.88 ± 0.10[b]	39.61[y]	0.83 ± 0.09[b]	43.33[y]
C20:3n-3	0.04 ± 0.08[b]	2.82[y]	0.48 ± 0.11[a]	21.03[x]	0.07 ± 0.06[b]	6.36[y]
C22:1n-9+20:4n-6	0.55 ± 0.12[b]	45.35[x]	1.35 ± 0.28[a]	59.34[x]	1.14 ± 0.51[a]	59.17[x]
C20:5n-3	0.11 ± 0.08[b]	8.62[y]	0.58 ± 0.14[a]	25.66[x]	0.24 ± 0.17[b]	12.09[y]
C22:5n-3	0.01 ± 0.02[b]	3.17[y]	0.28 ± 0.08[a]	12.47[x]	0.19 ± 0.12[a]	9.50[x]
C22:6n-3	0.13 ± 0.05[b]	11.04[y]	1.01 ± 0.27[a]	45.25[x]	0.24 ± 0.26[b]	12.79[y]
ΣSFA	24.57 ± 1.29[a]	1888.72[x]	23.66 ± 0.80[a]	1070.82[y]	21.91 ± 0.98[b]	1157.55[y]
ΣMUFA	61.77 ± 3.99[a]	4854.91[x]	43.45 ± 2.78[b]	1985.41[y]	43.46 ± 3.04[b]	2323.52[y]
ΣPUFA	12.89 ± 2.46[b]	944.94[y]	31.53 ± 2.41[a]	1416.65[x]	33.48 ± 1.96[a]	1763.01[x]
n-6	11.89 ± 2.29[c]	862.87[z]	26.91 ± 1.86[b]	1210.95[y]	30.14 ± 1.62[a]	1588.95[x]
n-3	1.00 ± 0.27[c]	72.57[y]	4.62 ± 0.64[a]	207.71[x]	3.33 ± 0.43[b]	175.57[x]
n-6/n-3*	11.89±2.53[a]		5.83±0.56[c]		9.05±0.81[b]	

Legend: Values are means ± SD; [a,b,c;] Means (% of total FA) with different letters within the same row are significantly different ($p \leq 0.05$); [x,y,z] Means (mg/100 g) with different letters within the same row are significantly different ($p \leq 0.05$); wf wet fillet; n number of samples; Carp1 supplementary fed maize; Carp2 supplementary fed extruded feed; Carp3 supplementary fed pelleted feed; ND not detected; FA fatty acid; SFA saturated fatty acid; MUFA monounsaturated fatty acid; *PUFA* polyunsaturated fatty acid; * n-6/n-3 ratio

contents in the carp, as well as in our study. The lowest n–6/n–3 ratio was established in the carp fed extruded feed (5.83). *Li et al.* (2011) reported that low n3 PUFAs and high n6 PUFAs in the diets provided unfavourable n–6/n–3 ratios in the fish.

Data we obtained in this study indicate that nutritional quality of common carp cultivated in commercial farms was, to a great extent, influenced by the composition of the applied supplementary diets. Aside from selective retention of the FAs from the applied diets in carp tissue, the state of the ecosystem, the availability of the natural food in the fish farms, the genetic differences between carp, water temperature (which is closely related to regional climate), pond management system, etc. probably had additional influences on the nutritional quality of the carp (*Mráz et al.*, 2012; *Tkaczewska et al.*, 2014). However, *Trbovic et al.* (2016) reported that the applied pelleted feed in a semi-intensive farming system of carp manifested dominant influence compared to natural food.

Fatty acids expressed as fatty acid amounts (mg/100 g wf)

When FAs in the fish are expressed as mg of FAs per 100 g of wf (Table 3), the obtained data are more important for consumers and demonstrate the nutritional value of the fish consumed per specific fish serving (*Karakatsouli,* 2012).

The carp that were supplemented with maize cwontained significantly lower ($p \leq 0.05$) amounts of n–6 PUFAs than carp that were supplemented with extruded or pelleted feed. The amounts of n–3 PUFAs were the highest in the carp supplemented with extruded and pelleted feed ($p \leq 0.05$). The quantities of EPA) and DHA were higher in the carp that were supplemented with the extruded feed than in the carp supplemented with the pelleted feed. Due to the benefits of n-3 PUFAs on human health and particularly the suppression of many diseases, the requirements for fish production and intake by humans is increasing worldwide (*FAO*, 2014). However, clinical studies indicated that high amounts of n-6 PUFAs and high n-6/n-3 ratios promote the pathogenesis of many diseases, and an optimal n-6/n-3 ratio, between 1:1 and 4:1, is more desirable for reducing the risk of disease (*Simopoulos*, 2002). The n–6/n–3 ratio we obtained was more nutritionally beneficial in the carp that were supplemented with the extruded feed (5.83) and this ratio is closer to the ratio which was suggested by *Simopoulos* (2002) as optimal one.

Statistical evaluation of data

PCA performed on the FAs expressed as a percentage of the total FAs in the 24 carp samples provided better insight into the data structure. A biplot of the PCA score values for the first two principal

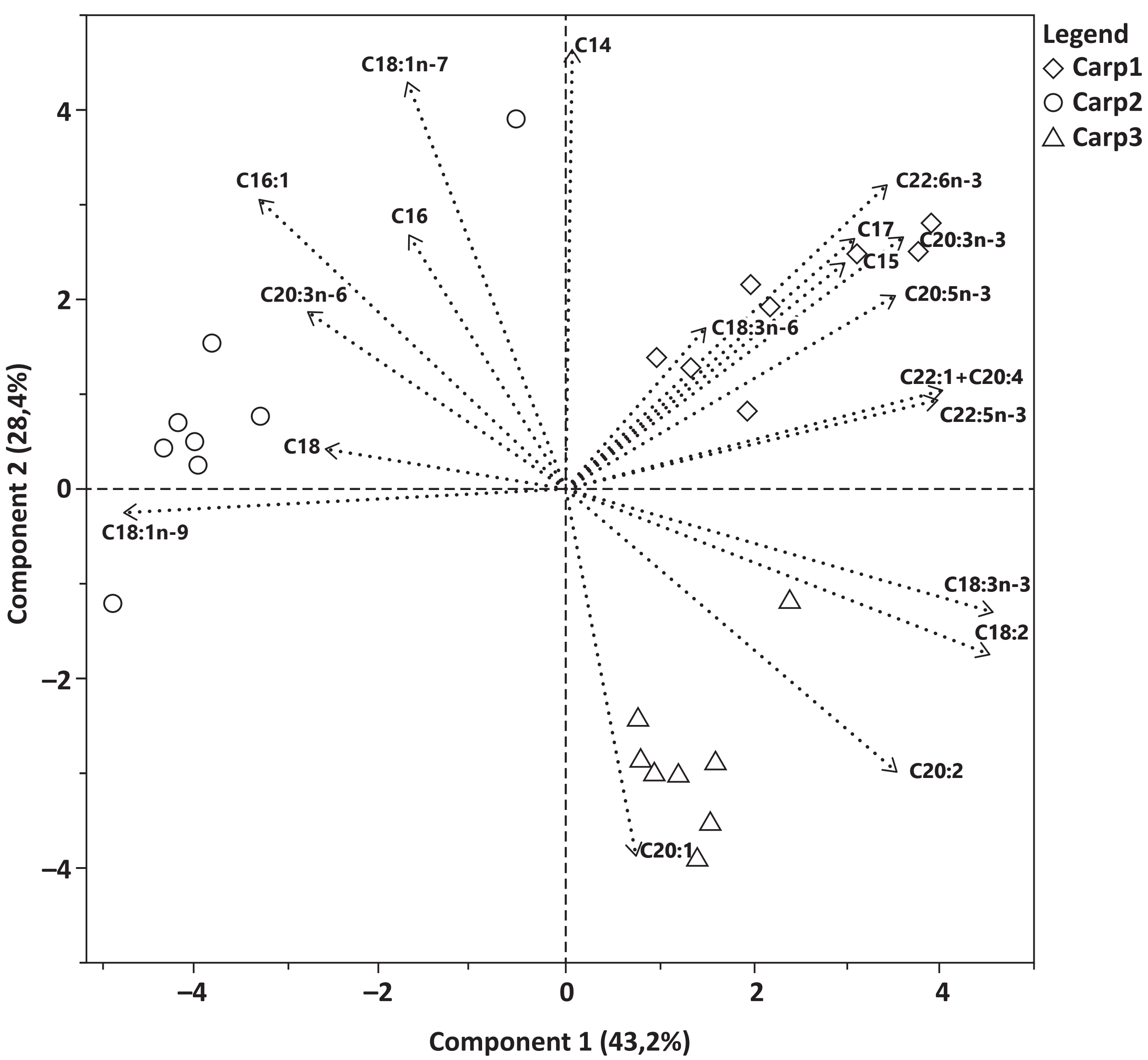


Figure 2. Biplot of PCA score values for the first two principal components of FAs expressed as percentage of the total FAs (Carp1 supplementary fed maize, Carp2 supplementary fed extruded feed, Carp3 supplementary fed pelleted feed)

components of FAs expressed as a percentage of the total FAs is shown in Figure 2.

The analysis resulted in a two-principal-component model that explained 71.6% of the total variance. The first principal component (PC1) accounted for 43.2%, and the second (PC2) accounted for 28.4%.

The score values for the first PC1 and PC2 of the FAs show that carp supplemented with maize (Carp1) were characterised by increased oleic, stearic (C18:0), dihomo-γ-linolenic (C20:3n–6) and palmitoleic (C16:1) FA contents. The carp supplemented with extruded feed (Carp2) were characterised by higher contents of the DHA, eicosatrienoic (C20:3n–3) FA, EPA and DPA (docosapentaenoic acid, C22:5*n*–3). Higher contents of eicosenoic (C20:1) and eicosadienoic (C20:2) FAs were observed in the carp supplemented with the pelleted feed (Carp3). The PCA showed that the carp supplemented with the extruded or pelleted feed had greater linolenic, linoleic, and γ-linoleic (C18:3n–6) FA contents compared to the carp supplemented with maize.

Preliminary statistical evaluation showed that PCA was unsuitable to evaluate the FAs expressed as mg/100 g wf. Instead, LDA was applied. The first and the second discriminant function (Canonical1 and Canonical2) were used to obtain the scoring coefficients. Canonical1 comprised 84.8%, while Canonical2 comprised 15.2% of the data variance. The canonical correlation, which involved Canonical1 and Canonical2, totalled 0.997 and

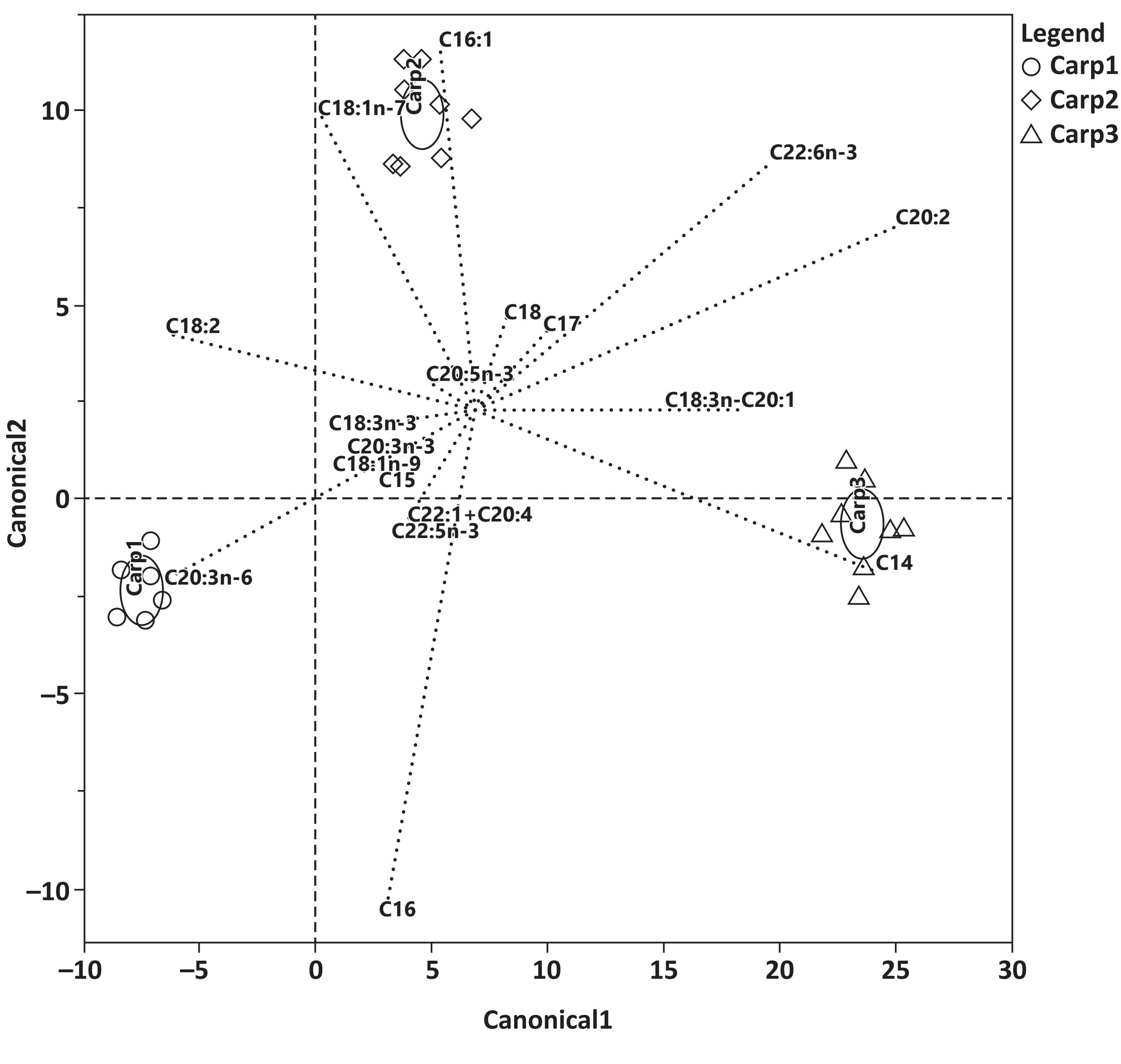


Figure 3. Canonical plot of the FAs expressed as FA amounts (mg/100 g wf) (Carp1 supplementary fed maize, Carp2 supplementary fed extruded feed, Carp3 supplementary fed pelleted feed

0.985, respectively. For discriminant analysis a total of 24 samples were used: eight samples from each group of fish. The number of misclassified samples was zero.

LDA of the FAs expressed as FA amounts (mg/100 g wf) is presented in Figure 3.

All three groups of carps are clearly separated with no overlaps. Carp1 (supplemented with maize) are grouped in the lower left part of the figure, and have negative values for Canonical1 and Canonical2. The Canonical1 and Canonical2 values for Carp2 (carp supplemented with pelleted feed) are positive, while Carp3 (carp supplemented with extruded feed) have positive values for Canonical1 and negative values for Canonical2.

The differences in the FA contents among the groups strongly influenced the LDA grouping of the carp fed different diets. Carp1 differed from the other two groups (Carp2 and Carp3) based on dihomo-γ-linolenic FA contents. Carp2 differed from the other two groups based on palmitoleic FA content, and Carp3 differed from the other two groups based on the miristic FA content. The LDA of the FAs in the carp supplemented with different diets expressed as amounts of FAs (mg/100 g wf) shows that carp might be classified reliably based on the type of supplemental feed.

Conclusions

The results obtained in this study indicate that introducing supplementary diets containing extruded or pelleted feed instead of maize improved carp nutritional quality. The obtained n–6/n–3 ratio is more nutritionally beneficial in the carp that were supplemented with the extruded feed, and this ratio is close to the optimal one. The PCA showed that the carp supplemented with the extruded or pelleted feed had greater linolenic, linoleic, and γ-linoleic FA contents compared to the carp supplemented with maize. The LDA of the FAs in the carp supplemented with different diets shows that carp might be classified reliably based on the type of supplemental feed. In general, great potential remains for improving feed quality and feeding strategy to achieve FA compositions of carp such that higher levels of n-3 PUFAs and more desirable n-6/n-3 ratios are obtained.

Acknowledgements: This research was supported by grants from the Ministry of Education and Science of the Republic of Serbia (Projects No. TR31075 and TR31011).

Disclosure Statement: No pontential conflict of interest was reported by the authors.

References

Ackman, R. G. (1989). Seafood lipids and fatty acids. *Food Reviews International, 6*, 617–646.

Böhm, M., Schultz, S., Koussoroplis, A-M. & Kainz, M.J. (2014). Tissue-specific fatty acids response to different diets in common carp (*Cyprinus carpio* L.). *PLoS ONE, 9*, 1–8.

Buchtová, H, Svobodová, Z, Křízek, M, Vácha, F, Kocour, M & Velísek, J. (2007). Fatty Acid Composition in Intramuscular Lipids of Experimental Scaly Crossbreds in 3-Year-Old Common Carp (*Cyprinus carpio* L.). *Acta Veterinaria Brno, 76*, 73–81.

Cahu, C., Salen, P. & de Lorgeril, M. (2004). Farmed and wild fish in the prevention of cardiovasular diseases: Assessing possible differences in lipid nutritional values. *Nutrition, Metabolism and Cardiovascular Diseases, 14*, 34–41.

Ciric, M., Subakov-Simic, G., Dulic, Z., Bjelanovic, K., Cicovacki, S. & Markovic, Z. (2015). Effect of supplemental feed type on water quality, plankton and benthos availability and carp (*Cyprinus carpio* L.) growth in semi-intensive monoculture ponds. *Aquaculture Research, 46*, 777–788.

Cirkovic, M., Trbovic, D., Ljubojevic, D. & Djordjevic, V. (2011). Meat quality of fish farmed in polyculture in carp ponds in Republic of Serbia. *Tehnologija mesa, 52*, 106–121.

EFSA – European Food Safety Authority (2009). Scientific Opinion of the Panel on Animal Health and Welfare. Species-specific welfare aspects of the main systems of stunning and killing of farmed carp. *EFSA Journal, 1013*, 1–37.

Exler, J., Kinsella, J. E. & Watt, B. K. (1975). Lipids and fatty acids of important finfish. New data for nutrient tables. *Journal of the American Oil Chemists' Society, 52*, 154–159.

FAO – Food and Drug Administration (2014). The State of World Fisheries and Aquaculture: Opportunities and challenges. Rome, ISBN 978−92−5−108275−1 (print).

Farkas, T., Csengeri, I., Majoros, F. & Olah, J. (1978). Metabolism of fatty acids in fish. II. Biosynthesis of fatty acids in relation to diet of the carp, (*Cyprinus carpio* Linnaeus 1758). *Aquaculture, 14*, 57–65.

Ivanovic, J., Baltic, M. Z., Janjic, J., Markovic, R., Boskovic, M., Djordjevic, V., Dokmanovic, M., 2015. The scope and structure of the production and catch of the fish in Serbia in the period from 2006–2012. *Veterinarski glasnik, 69 (1–2)*, 139–154.

Henderson, R. J. (1996). Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition, 49*, 5–22.

Jeney, Z. & Jian, Z. (2009). Use and exchange of aquatic resources relevant for food and aquaculture: common carp (*Cyprinus carpio* L.). *Reviews in Aquaculture, 1*, 163–173.

Kaçar, S., Başhan, M. & Oymak, S. A. (2016). Effect of seasonal variation on lipid and fatty acid profile in muscle tissue of male and female Silurus triostegus. *Journal of Food Science and Technology – Mysore, 53*, 2913–2922.

Karakatsouli, N. (2012). An Overview of the Use of Fatty Acids in Fish Farming Research during the Last Decade, with Particular Emphasis on Fish Quality. *Journal of the World Aquaculture Society, 43*, 291–320.

Li, G., Sinclair, A.J. & Li, D. (2011). Comparison of Lipid Content and Fatty Acid Composition in the Edible Meat of Wild and Cultured Freshwater and Marine Fish and Shrimps from China. *Journal of Agricultural and Food Chemistry, 59*, 1871–1881.

Ljubojevic, D., Cirkovic, M., Djordjevic, V., Puvaca, N., Trbovic, D., Vukadinov, J. & Plavsa, N. (2013a). Fat quality of marketable fresh water fish species in the Republic of Serbia. *Czech Journal of Food Science, 31*, 445−450

Ljubojevic, D., Cirkovic, M., Djordjevic, V., Trbovic, D., Vranic, D., Novakov, N. & Masic, Z. (2013b). Chemical composition, cholesterol content and fatty acid profiles of common carp (*Cyprinus carpio*) from free-catch, semi-intensive and cage system. *Tehnologija Mesa, 54*, 48–56.

Mráz, J., Máchová, J., Kozák, P. & Pickova, J. (2012). Lipid content and composition in common carp – optimization of n-3 fatty acids in different pond production systems. *Journal of Applied Ichthyology, 28*, 238–244.

Shearer, K. D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture, 119*, 63–88.

Simopoulos, A.P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*, 365–379.

Spiric, A., Trbovic, D., Vranic, D., Djinovic, J., Petronijevic, R. & Matekalo-Sverak, V. (2010). Statistical evaluation of fatty acid profile and cholesterol content in fish (*Common carp*) lipids obtained by different sample preparation procedures. *Analytica Chimica Acta, 672*, 66–71.

Takeuchi, T. (1996). Essential fatty acid requirements in carp. *Archiv für Tierernaehrung (Berlin) 49*, 23–32.

Tesic, M., Baltic, M., Teodorovic, V., Nedic, D., Mirilovic, M., Markovic, R. & Aleksic-Agelidis, A. (2014). Effects of various meal compositions on production results, economic performance and fish meat quality. *Acta Veterinaria Beograd, 64*, 338–348.

Tkaczewska, J., Migdał, W. & Kulawik, P. (2014). The quality of carp (Cyprinus carpio L.) cultured in various Polish regions. *Journal of the Science of Food and Agriculture, 94*, 3061–3067.

Trbovic, D., Zivic, I., Stankovic, M., Zivic, M., Dulic, Z., Petronijevic, R. & Markovic, Z. (2017). Dependence of the common carp (*Cyprinus carpio* L.) fatty acid profile on diet composition in a semi-intensive farming system: tissue and time variability. *Aquaculture Research, 48*, 3121−3133.

Trenovszki, M.M., Lebovics, V. K., Müller, T., Szabo, T., Hegyi, Á., Urbányi, B., Horváth, L. & Lugasi, A. (2011). Survey of fatty acid profile and lipid peroxidation characteristics in common carp (*Cyprinus carpio* L.) meat taken from five Hungarian fish farms. *Acta Alimentaria, 40*, 153–164.

Váradi, L., Lane, A., Harache, Y., Gyalog, G., Békefi, E. & Lengyel, P. (2012). Regional Review on Status and Trends in Aquaculture Development in Europe – 2010. In: FAO Fisheries and Aquaculture Circular. 1061/1, Rome, FAO 2010, pp 257.

Paper received:20.03.2018.
Paper corrected: 17.05.2018.
Paper accepted: 29.05.2018.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 613.261
ID: 265916428
https://doi.org/10.18485/meattech.2018.59.1.5

Review paper

The safety and quality of sous vide food

Natasa Kilibarda[1], Ivana Brdar[1], Branislav Baltic[2], Vladan Markovic[1], Hava Mahmutovic[3], Nedjeljko Karabasil[4], Svetlana Stanisic[1]

A b s t r a c t: The demands of contemporary health conscious consumers are increasingly focused on minimally processed, convenient and affordable food that retains its natural sensory qualities along with nutritional value. In order to fully respond to these expectations, the use of sous vide processing technology, which refers to cooking vacuum-sealed food in heat-stable plastic pouches under precisely monitored conditions, has been widely adopted. Due to the low temperatures of sous vide processing, most research on this processing method is associated with biological hazards such as Listeria monocytogenes, Bacillus cereus and Clostridium botulinum. Not only has sous vide long been accepted by the food industry, in recent years, it has also been applied in households and in restaurants which are searching for innovative ways to attract more health conscious consumers. In this review, the authors present basic techniques, benefits and disadvantages of sous vide cooking and consider the great efforts the modern food industry is undertaking to extend shelf-life, ensure microbiological safety and maintain nutritional and organoleptic quality of sous vide food products.
Keywords: *sous vide, food, microbiological safety, nutritional quality, gastronomy.*

Introduction

Different food choices in human history have resulted from complex interactions of nutritional requirements, ecology, historical events and human logic or lack thereof (*Anderson,* 2005). Greenland Inuits consume more than 400 g of fish per day, vegans avoid animal-based food products while Maasai of Kenya and Tanzania mostly rely on meat and milk, but all of them have adequate nutrient consumption (*Oniang'o,* 2016; *Jacob, 2016; Odusanya and Atanda,* 2018). While our human ancestors ate to survive and maintain body temperature, for some of their modern descendants, food requirements are far beyond the primary instinct for self-sustainment. For the contemporary human, gustatory, olfactory and visual attributes of food are undoubtedly important drivers of food choice and intake. Hence, the tendency for sensation-seeking and the desire to taste and explore the unknown and unfamiliar is deeply rooted in the long story of human evolution. In addition to this, food serves as a communication tool for defining one's individuality and is an indicator of religion, ethnicity and culture, lifestyle affiliation, class and other social groupings, and therefore, the maintenance of food habits can serve as a cohesive force in an unfamiliar environment (*Kalenjuk et al.,* 2015; *Henderikx*, 2017). In the modern world of hedonism, food shows off wealth, personal power and authority (*Baltic et al*., 2010). Moreover, consumption of special-status foods is utilised by elite groups and by people aspiring to higher social ranking (*Baltic et al*., 2010). When it comes to health aspects of dietary habits, a vast number of literature sources over the last few decades have been focused on exploring the adverse effects of recent shifts towards indulgent, high-fat and high-sugar diets (*Caplan,* 2013). Nowadays, the limited time available for cooking at home and lack of cooking skills are the main barriers to the adoption of healthy dietary choices. Therefore, modern consumers often search for pre-prepared food commodities that are nutritionally valuable, safe for use, free of additives and preservatives, affordable and feature similar sensory qualities to those of home-prepared food (*García-Linares et al.,* 2004; *Kilibarda,* 2010; *Kalenjuk et al.,* 2015). Finally, apart from food's contribution to human existence, longevity and physical sensations, food is intimately bound with one's emotional state. The memories of personal events evoked by eating experience can provide strong and complex relationships to early

[1]Singidunum University, 32 Danijelova st., 11010 Belgrade, Republic of Serbia;
[2]Institute of Meat Hygiene and Technology, 13 Kacanskog st, 11000 Belgrade, Republic of Serbia;
[3]University of Tuzla, Faculty of Technology, 8 Univrzitetska st., 75000 Tuzla, Bosnia and Herzegovina;
[4]University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Republic of Serbia.

Corresponding author: Natasa Kilibarda, nkilibarda@singidunum.ac.rs

childhood experiences, or certain occasions in later life. From a psychological point of view, consumer memory is a significant predictor of a food product's success on the market. In this context, consumer memory encompasses the emotions experienced during eating and the after-effects of the meal all weighted and integrated by the person in an individual way. This emotional content from past eating experiences forms the basis for every consumer's future expectations, as well as for their perception and appreciation of food. In spite of their importance, emotional responses to food triggered by events such as memories of a grandmother's cuisine, dinner with friends or fine dining on exotic trips have been often neglected in food-related consumer research (*Drpic and Vukman,* 2014; *Köster and Mojet,* 2015).

The aim of this paper is to consider the potential benefits and risks associated with consumption of sous vide food. Sous vide is a modern food preparation technique that addresses the expectations of demanding consumers for minimally processed, safe, nutritionally valuable food which has visual appeal, smell, taste and texture comparable with those of fresh food (*Schellekens,* 1996).

Brief history

The French term sous vide means "under vacuum". In contrast to conventional food, sous vide food is vacuum-sealed in heat-stable, food-grade plastic pouches, and subjected to precisely monitored temperatures over a pre-determined period of time (*Schellekens,* 1996; *Baldwin,* 2012). In recent decades, this sophisticated technique has been applied to plant foods, such as green bean pods, red cabbage, carrots and other root vegetables (*Iborra-Bernad et al*., 2013; *Iborra-Bernad et al*., 2014a; *Trejo Araya et al*., 2009), as well as meat (*Vaudagna et al*., 2008; *Sánchez del Pulgar et al.,* 2012; *Roldán et al.,* 2015; *Botinestean et al*., 2016) and fish (*Gonzalez-Fandos et al.,* 2004; *Picouet et al*., 2011; *García-Linares et al.,* 2014; *Espinosa et al*., 2015; *Dimitrijevic et al.* 2015). While sous vide cooking has been a part of the food industry for years, primarily because of its price and efficiency, in recent times, it has become more affordable for application in households and restaurants.

The modern era of sous vide cooking began in the 1970s when French biochemist and microbiologist Bruno Goussault observed that by using low temperatures (about 60°C) to cook vacuum packed beef, tender cuts with a juicy texture could be prepared, and according to him, that was exactly what consumers expected. The low-temperature food processing resulted in a final product that had better sensory attributes compared to conventionally prepared food. In contrast to Goussault, who has been researching the application of sous vide techniques in the food industry, European chefs have questioned the possibility of using sous vide techniques in restaurant kitchens *(Keller,* 2008). These directions of research (scientific and culinary) have resulted in two different applications of the sous vide cooking: one is in the food industry and serves for production of thousands of ready-prepared frozen meals in factory conditions, which are intended for home use after reheating; the other approach employs sous vide in restaurants and hotels for preparation of meals intended for uninterrupted in-house usage (*Nyati,* 2000). Nowadays, the expansion of the sous vide food market is driven by emerging circumstances – fewer family meals are prepared at home because employed parents face everyday challenges, struggling to integrate inflexible working hours and family lives (*Devine et al.*, 2006), while at the same time, the numbers of single and senior households in developing countries are growing, with people who are determined and have time to improve their dietary habits by adopting new cooking techniques. Apart from home consumers, the potential users of sous vide cooking techniques include commercial catering, food retailers, hotels and restaurants, airlines, trains, cruise ships, defence forces, hospital kitchens, the health-food market and schools (*Nyati,* 2000).

Sous vide equipment

The preparation of sous vide food necessitates higher training and monitoring costs as well as specialised equipment including: a) water bath or steam oven, b) vacuum packaging machine, c) pouches/vacuum bags, and d) needle thermometer. The vacuum packaging machine eliminates the air from the food packaging and draws different degrees of vacuum depending on the type of foodstuff, then seals the package so it remains impermeable to air. As regards vacuum degree, high pressure is not recommended for sous vide processing of fish fillets, since the texture of fish is very gentle, and strong pressure would damage the tissue. This type of food cannot be completely vacuumed, and the residual pressure inside the package typically amounts to 100–120 mbar. On the other hand, sous vide processing of root vegetables utilises high pressure in order to achieve the most efficient thermal treatment. For firm and physically resistant food ingredients, the residual pressure inside the package can be as low as 10–15 mbar. Plastic polymers for sous vide vacuum bags must have certain features: they must be temperature- and pressure-resistant, must have low permeability to gases and moisture, and must have the chemical composition to provide limited migration of plastic residues to food *(De Baerdemaeker and Nicola,* 1995*; Schellekens,* 1996*)*.

After sealing, the plastic pouches are completely immersed in a water bath or placed into a hot steam oven, in which the temperature is constantly monitored and adjusted (*Carlin*, 2014). The needle thermometer, which is used to monitor the temperature of cooking, should be regularly calibrated by an accredited laboratory.

Processing of sous vide foods

Sous vide cooking encompasses two types of products that differ in their production patterns – cook-hold or cook-serve and cook-chill or cook-freeze food *(Stringer and Metris,* 2018). Cook-hold or cook-serve sous vide techniques are intended for uninterrupted food processing in restaurants and households and include the following steps: food preparation, vacuum packaging, heating or pasteurising, finishing and serving. On the other hand, cook-chill or cook-freeze sous vide techniques include food preparation, vacuum packaging, heating or pasteurising, and finally refrigerating or rapid freezing in industrial conditions. This ready-to-take type of sous vide product is intended for use at home after reheating or re-thermalising and serving (*Baldwin,* 2012).

In contrast to conventional cooking, the temperatures of sous vide processing are typically in the range from 65 to 95°C, with temperatures commonly exceeding 70°C, but not 100°C (*Schellekens, 1996; Carlin*, 2014). There are four general time-temperature regimes for sous vide processing: i) 90°C for 10 minutes; ii) 70°C for 2 minutes (this process results in a large log reduction of vegetative cells, without affecting spores, and the products obtained by application of this temperature-time regime are usually frozen after processing); iii) minimum heating process with optional pasteurisation; and iv) light processing, which refers to neither cooking nor pasteurisation (*Steward and Onyeaka*, 2015).

The lowest sous vide temperatures are applied to fish, seafood and meat processing (50–75°C, with an average temperature of 55°C) and maintained for several hours or even days, while the highest temperatures of 90–100°C (with an average temperature of 85°C) are applied to vegetable processing, which typically takes only a few minutes. For red meat which is cooked for less than 4 hours, the average temperature is 56°C, while for red meat which is cooked for 4 hours or more, the average temperature is up to 60°C. The average sous vide temperature for poultry is 63.5°C, egg products are prepared at 64.5°C and dairy products at 82°C *(Stringer and Metris,* 2018).

The shelf-life of sous vide products depends on both the temperature-time treatment and the storage temperature, and typically ranges from 6 to 42 days (*Schellekens, 1996; García-Linares et al.,* 2004).

The advantages of sous vide food

The current evidence from relevant literature suggests that sous vide food has a number of advantages, and the most important benefits of sous vide food are associated with, but not limited to: prevention of aerobic bacterial growth, low-risk of contamination after the packaging step, efficient heat transfer from oven or water to the food inside the package, minimal loss of volatile flavour compounds and moisture during thermal processing, superior sensory quality of product and inhibition of oxidation and related off-flavours *(Carlin,* 2014). Continuously monitored conditions contribute significantly to the success of sous vide techniques, to the extent that some authorities consider the name “precise cooking” more appropriate for this type of thermal processing than the current designation (*Keller,* 2008). Furthermore, following a specific processing regime results in food product of consistent, reliable and reproducible sensory qualities that strongly affects consumers’ loyalty.

Plastic foil prevents the loss of aromatic volatile compounds and water during sous vide cooking, which enhances sensory attributes and contributes to juiciness and tenderness of meat. Additionally, the meat, compressed during the sous vide packaging, retains many of its desirable qualities, palatability, natural colour and the shape into which it has been formed, and this fresh appearance of meat is pleasing and acceptable to consumers (*Roascio-Albistur and Gámbaro,* 2018). *Roldán et al.* (2015) reported the formation of volatile compounds in the amino acid-involved reactions during sous vide processing of lamb meat. As suggested, the aromatic volatiles were associated with a specific savoury flavour, due to which fewer spices and less salt was required.

In addition to this, mild sous vide thermal treatment has a superior capability to preserve nutritional value of food (*Baldwin,* 2012; *Iborra-Bernad et al.,* 2013, 2014a; *Kosewski et al.,* 2018) and to minimise the generation of chemical species known for their deleterious effects on human health, such as heterocyclic amines and polycyclic aromatic hydrocarbons. Mutagenic and carcinogenic polycyclic aromatic hydrocarbons and the main compound of concern in this group, benzopyrene, are formed during the incomplete combustion or pyrolysis of organic material and become concentrated in well-cooked meat during charcoal-broiling, grilling and traditional smoking *(Kilibarda et al.*, 2009), but do not form during sous vide processing. Due to the fact that sous vide's plastic pouches prevent direct contact between food and oxygen, the oxidation of plant pigments, chlorophyll and carotenoids is limited and the colour of vegetable tissue is preserved (*Rondanelli et al*., 2017). Food colour is known to be one of the most desirable sensory attributes associated with freshness (*Baltic et al.,* 2007). *Iborra-Bernad et al.* (2014a) found sous vide red cabbage had brighter colour, more intensive taste and aroma, and higher anthocyanin content than traditionally cooked cabbage.

Physicochemical characteristics of sous vide foods

Sous vide foods are commonly prepared without the additives widely used in traditional food production. The pH and water activity of sous vide foods are similar to those of the raw ingredients. In order to enhance the taste, salt is often added, but in concentrations that do not affect water activity, which is reported to be over 0.98, and even more often over 0.99 in final sous vide product *(Carlin,* 2014).

Because of the elimination of air/oxygen during vacuum packaging, sous vide foods provide either strictly anaerobic conditions or contain a small amount of residual air/oxygen that should theoretically inhibit the growth of strictly anaerobic bacteria. However, this is not the case in practice – it has been shown that growth and reproduction of strict anaerobes is possible despite the presence of low levels of oxygen (*Baldwin,* 2012; *Djordjevic et al.,* 2016*; Stringer and Metris,* 2018). Due to the fact that the physicochemical features of sous vide foods are suitable for the growth of a wide range of bacteria, which is further supported by low processing temperatures, much of the research dealing with sous vide processing has been focused on the microbiological safety of sous vide products.

The safety of sous vide products

The research dealing with sous vide food and related issues since the 1990s has mainly focused on exploring the effects of sous vide cooking on extending food sustainability. Apparently the research outcomes and contribution to knowledge in the field of food science have been substantial, since to date, no case of foodborne disease caused by sous vide food has been recorded in scientific literature (*Peck et al.,* 2006).

The majority of sous vide processing includes long-term (in the bacteriological sense) treatment in the temperature danger zone between 4 and 60°C. Such circumstances would normally allow pathogens to grow and reproduce to numbers that are sufficient to cause foodborne disease *(Stringer and Metris,* 2018). The safety of sous vide products mostly relies on: (i) temperature control during cooking, (ii) rapid cooling, and (iii) temperature control during storage (*Schellekens,* 1996; *Gonzalez-Fandos et al.,* 2005).

As shown by a number of studies, the presence of pathogens in final sous vide products most likely results from those microorganisms being in raw ingredients and surviving during processing. The pathogens of concern in sous vide food can be divided into four categories. The first category is that of the vegetative bacteria that are unable to grow at refrigeration temperatures, such as *Escherichia coli, Salmonella, Vibrio, Staphylococcus aureus* and *Campylobacter*. These bacteria are predominantly inactivated by pasteurisation. The second category includes vegetative bacteria that can grow and reproduce at refrigeration temperatures such as *Listeria monocytogenes, Yersinia* and *Aeromonas*. Most of these bacteria are sensitive to pasteurisation temperatures, but some cells can survive mild heat treatment. The third category of concern is the psychrotrophic spore-forming pathogenic bacteria that are able to survive pasteurisation treatments and then grow and reproduce at low storage temperatures (*Church and Parsons*, 1993). These include non-proteolytic *Clostridium botulinum*, enterotoxigenic *E. coli* and spore-forming, psychrotrophic *Bacillus cereus.* Finally, the fourth category is the mesophilic spore forming bacteria that are able to survive pasteurisation treatments, but are unable to grow and reproduce at refrigeration temperatures, such as proteolytic *Clostridium botulinum*, mesophilic *B. cereus* and *Clostridium perfringens (Stringer and Metris,* 2018).

Vacuum packed sous vide food could provide favourable conditions for the growth and reproduction of anaerobic bacteria, ideal for one of the major biological hazards cited for sous vide processing, *C. botulinum* (*Nyati,* 2000). *Clostridium botulinum* type E is able to grow and produce toxin at 3°C (*Briley,* 1992; *Jay*, 1992; *Gould*, 1999; *Kilibarda,* 2010). Furthermore, any leakages in the seal and/or packaging bags could result in contamination of sous vide product with other types of pathogens during production or storage (*Nyati*, 2000).

Cook-hold or cook-serve sous vide techniques are considered relatively safe due to the fact that the food is consumed immediately after preparation, usually with a delay of not longer than two hours, during which food is stored at temperatures above 54.4°C to prevent or slow down the reproduction of pathogenic bacteria *(Baldwin,* 2012*; Carlin,* 2014; *Stringer and Metris,* 2018). For these types of sous vide products, the pathogenic bacteria of concern are *Salmonella* and pathogenic *E. coli* because they are relatively heat resistant and a small number of vegetative bacteria can cause alimentary infection in immunocompromised people *(Baldwin,* 2012).

On the other hand, cook-chill or cook-freeze is more popular, involving food products that are consumed reheated after days or weeks of cold storage. In these foods, the pathogenic bacteria of concern are *L. monocytogenes*, *B. cereus* and *C. botulinum* (*Baldwin*, 2012, *Carlin,* 2014; *Stringer and Metris*, 2018). *L. monocytogenes* is generally considered to be the most heat-resistant vegetative pathogen, and in food with high water activity, it can grow anaerobically below 4°C (*Dimitrijevic et al.,* 2008). For this reason, pasteurisation is a critical control point for the prevention of *Listeria* growth in cook-chill or cook-freeze sous vide food. The European Union guidelines recommend the minimum heat treatment for sous vide pasteurisation is equivalent to heating at 70°C for two minutes *(ECFF, 2006; Stringer and Metris*, 2018*).*

B. cereus and *C. botulinum* are considered important pathogens in heat-treated foods, because of their ability to produce spores and toxins. Therefore, the recommended shelf-life of sous vide foods is limited to 10 days (*Peck et al.,* 2008), except if the storage temperature is below 2.5°C, when shelf-life should not exceed 90 days (*Baldwin*, 2012). According to *Baldwin* (2012), shelf-life and storage temperature of sous vide foods are critical factors for preventing spores of non-proteolytic *C. botulinum* from outgrowing and producing neurotoxin. Although sous vide vacuum packs contain some residual oxygen, it might not be sufficient to inhibit the growth and reproduction of strictly anaerobic species such as *Clostridium*, which could grow inside the product (*Kilibarda,* 2010; *Carlin,* 2014).

It should be noted that consumption of sous vide food could be also associated with non-bacterial infection or intoxication risks from Norwalk virus, Rotavirus and hepatitis viruses, and parasites such as *Trichinella, Taenia, Toxoplasma, Cryptosporidium* and *Giardia*. It is very important to design guidelines for the safe production of unheated foods where the presence of such pathogens is a risk (*Stringer and Metris*, 2018). Proper design and implementation of a hazard analysis and critical control point (HACCP) system with its prerequisite programes (good manufacturing practice/good hygienic practice) is considered the best preventive measure for foodborne illnesses generally. The food safety criteria related to biological hazards for sous vide processing are mainly based on HACCP, which is recommended at all stages of sous vide production, storage and distribution for control of microbiological and other hazards. This includes trained personnel using specialised time-temperature recorders to monitor potential temperature abuse at all stages of production and storage (*Roascio-Albistur and* Gámbaro, 2018). Furthermore, the use of high-quality, HACCP-controlled ingredients is critical for assuring high-quality and safety of final products, as is the uniform heating of the food that is achieved by complete soaking in a water bath.

The optimal growth temperature of most pathogenic bacteria is between 30 and 50°C, whereas inhibition of bacterial growth and reproduction starts at temperatures above 52.3°C. Thus, the core temperature of sous vide food during processing should not be below 54.4°C and it should be maintained for 6 hours. When the prepared sous vide food is served immediately after thermal processing, no risk of pathogen growth exists. However, if sous vide product is frozen for later use, freezing must be conducted rapidly and immediately following the thermal procedure to prevent/limit risks, *e.g*. toxin production by *C. perfrigens* during the formation of spores. Furthermore, storing sous vide food in vacuumed pouches is appropriate for preventing recontamination, while toxin production by *C. botulinum* and *B. cereus* is prevented by proper storing at correct temperatures in a refrigerator or freezer (*Baldwin,* 2012; *Stringer and Metris*, 2018).

The nutritional quality of sous vide food

The fact that sous vide food is processed in low oxygen environments, under mild and precisely controlled temperatures is important for preserving the nutritional content of final product without ignoring consumers' sensorial experience and satisfaction

(Iborra-Bernad et al., 2014b*)*. In contrast to conventional cooking, the plastic pouches used in sous vide minimise the loss of minerals and can enhance their bioavailability *(Ronadelli et al.,* 2017*)*. *Da Silva et al.* (2017) confirmed these findings by exploring the bioavailability of calcium, cooper, iron, potassium and magnesium from bovine liver. In addition to this, sous vide food processing is superior than widespread steaming and boiling in terms of preventing the loss of vitamins, particularly those most vulnerable to high temperatures and the presence of oxygen, including thiamine (vitamin B1), riboflavin (vitamin B2) and ascorbic acid (vitamin C) (*Petersen,* 1993; *Creed,* 1995). Furthermore, the content of anthocyanins and polyphenols in sous vide vegetables remains comparable to that of fresh plants (*Baardset et al.,* 2010; *Iborra-Bernad et al.,* 2015; *Renna et al*., 2014), and a number of studies have confirmed that sous vide red onion, shallot, broccoli, tomato, green beans, artichokes, carrots, parsley root and cauliflower retain their antioxidative potential (*Kosewskia et al., 2018*; *Guillén et al., 2017).*

Adding small amounts of fat to food ingredients prior to vacuum sealing prevents adhesion of proteins to the pouch cooking surfaces and improves texture, juiciness and flavour of final product. The plastic barrier significantly limits diffusion of oxygen into the food and subsequent oxidation of lipids, which is important for preserving the quality and health benefits of essential polyunsaturated fatty acids in human nutrition (*Pavlicevic et al.,* 2014). As confirmed by *Schellekens* (1996), the oxidation of unsaturated fatty acids was less pronounced in sous vide fish than in fish prepared at the higher temperatures during traditional cooking.

Conclusions

Sous vide food processing confers numerous advantages with regards to the quality of the final products, primarily the preservation of nutritional ingredients in foods, and the excellent sensory characteristics such as well-preserved colours, rich flavours, and intense tastes. Sous vide satisfies, to a significant extent, the demands of consumers who are searching for quality, nutritionally valuable food with sensory properties similar to those of raw food. As regards safety of sous vide food, special attention should be paid to choice of raw ingredients, continual control of processing (temperature/time regime), temperature control during storage and proper determination of shelf-life of the final product. While sous vide food products can be considered safe, this does not mean that foodborne illness outbreaks will never happen, particularly when having in mind the specific features of sous vide processing, *i.e.* mild heat treatment and avoidance of preservatives. Therefore, it is important to strictly adhere to the requirements of prerequisite programs (good manufacturing practice/good hygienic practice) and to safety criteria based on the HACCP approach. As a modern food preparation technique, sous vide provides opportunities for restaurants and food industries to expand their product base and, thus, achieve greater profits and become more competitive on the market. By applying well-designed marketing, sous vide processing should help food manufacturers gain consumers who are interested in healthy diets and new food experiences.

Disclosure Statement: No potential conflict of interest was reported by the authors.

References

Anderson, E. N. (2014). *Everyone eats: understanding food and culture*. NYU Press.

Baldwin, E. D. (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science 1,* 15–30

Baltic, Z. M., Andjelkovic, R., Stevanovic, J., Karabasil, N., Dimitrijevic, M. & Kilibarda, N. (2007). Importance of the Sense of Smell in the Sensory Analysis of Food, *Meat Technology*, *48* (1–2), 131–137.

Baltic, Z. M., Nedic, D., Djuric, J., Dimitrijevic, M., Karabasil, N. & Kilibarda, N. (2010). Food and everlasting concern about health. *Veterinary Journal of Republika Srpska, 10* (1), 5–10.

Botinestean, C., Keenan, D. F., Kerry, J. P. & Hamill, R. M. (2016). The effect of thermal treatments including sous vide, blast freezing and their combinations on beef tenderness of *M. Semitendinosus* steaks targeted at elderly consumers. *LWT – Food Science and Technology, 74,* 154–159.

Briley, M. E. (1992). Sous vide processed foods: are they safe for the elderly? *Journal of Nutrition for the Elderly, 11* (3)*,* 45–52.

Caplan, P. (2013). *Food, health and identity*. Routledge.

Carlin, F. (2014). Microbiology of Sous vide Products. In. Encyclopedia of Food Microbiology. Eds. C. A. Batt & M. L. Tortorello, Elsevier, London, UK, pp. 621–626.

Church, I. J. & Parsons, A. L. (1993). Review: sous vide cook-chill technology. *International Journal of Food Science and Technology, 28,* 563–574.

Creed, P. G. (1995). The sensory and nutritional quality of 'sous vide' foods. *Food Control, 6* (1), 45–52.

Da Silva, F. L. F., de Lima, J. P. S., Melo, L. S., da Silva, Y. S. M., Gouveia, S. T., Lopes, G. S. & Matos W. O. (2017). Comparison between boiling and vacuum cooking (sous vide) in the bioaccessibility of minerals in bovine liver samples. *Food Research International*, *100* (1), 566–571.

De Baerdemaeker, J. & Nicola B. M. (1995). Equipment considerations for sous vide cooking. *Food Control, 6* (4), 229–236.

Devine, C. M., Jastran, M., Jabs, J., Wethington, E., Farell, T. J. & Bisogni, C. A. (2006). "A lot of sacrifices:" Work–family spillover and the food choice coping strategies of low-wage employed parents. *Social Science & Medicine*, *63* (10), 2591–2603.

Dimitrijevic, M., Karabasil, N., Kilibarda, N., Teodorovic V. & Baltic, M. Z. (2008). Control of *Listeria monocytogenes* in food production plants**.** *Veterinarski Glasnik, 62* (5–6), 301–315.

Dimitrijevic, M., Boskovic, M., Baltic, M. Z., Karabasil, N., Teodorovic V., Vasilev, D., Katic, V., 2015. The importance and use of nanopacking in food industry. *Veterinarski Glasnik, 69* (1–2), 130–154.

Drpic, K. & Vukman, M. (2014). Gastronomija kao vazan dio turisticke ponude u Hrvatskoj. *Prakticni Menadzment, V* (1), 62–67.

Djordjevic, J., Pavlicevic, N., Boskovic, M., Baltic, T., Ivanovic, J., Glamoclija, N. & Baltic, M. Z. (2016). Sensory evaluation of cold-smoked trout packaged in vacuum and modified atmosphere. *Meat Technology,* 57 (2), 141–149.

Espinosa, M. C., Díaz, P., Linares, M. B., Teruel, M. R. & Garrido, M. D. (2015). Quality characteristics of sous vide ready to eat seabream processed by high pressure. *LWT - Food Science and. Technology, 64,* 657–662.

European Chilled Food Federation (ECFF). (2006). Recommendations for the production of prepackaged chilled foods. (http://www.ecff.net/images/ECFF_Recommendations_2nd_ed_18_12_06.pdf).

García-Linares, M. C., Gonzalez-Fandos, E., García-Fernández, M. C. & García-Arias, M. T. (2004). Microbiological and nutritional quality of sous vide or traditionally processed fish: influence of fat content. *Journal of Food Quality, 27*, 371–387.

Gonzalez-Fandos, E., Garcia-Linares, M. C., Villarino-Rodriguez, A., Garcia-Arias, M. T. & Garcia-Fernandez, M. C. (2004). Evaluation of the microbiological safety and sensory quality of rainbow trout (*Oncorhynchus mykiss*) processed by the sous vide method. *Food Microbiology, 21,* 193–201.

Gould, G. W. (1999). Sous vide foods: conclusions of an ECFF botulinum working party. *Food Control, 10,* 47–51.

Guillén, S., Mir-Bel, J., Oria, R. & Salvador, M. L. (2017). Influence of cooking conditions on organoleptic and health-related properties of artichokes, green beans, broccoli and carrots. *Food Chemistry, 217,* 209–216.

Henderikx, F., 2017. Labeling of food: A challenge for many. *Veterinarski Glasnik, 71* (1), 16–23.

Iborra-Bernad, C., Philippon, D., García Segovia, P. & Martínez Monzó, J. (2013). Optimizing the texture and color of sous vide and cook-vide green bean pods. *LWT - Food Science and Technology, 51* (2), 507–513.

Iborra-Bernad, C., Tárrega, A., García-Segovia, P. & Martínez-Monzó, J. (2014a). Advantages of sous vide cooked red cabbage: Structural, nutritional and sensory aspects. *LWT - Food Science and Technology, 56*, 451–460.

Iborra-Bernad, C., Tárrega, A., García-Segovia, P. & Martínez-Monzó, J. (2014b). Comparison of sous vide treatments and traditional cooking using instrumental and sensory analysis*. Food Analytical Methods, 7,* 400–408.

Iborra-Bernad, C., García, P. & Martínez-Monzó, J. (2015). Physico-chemical and structural characteristics of vegetables coked under sous vide, cook-vide, and conventional boiling. *Journal of Food Science, 8*, 1725–1734.

Jacob, J. J. (2016). Fish consumption and omega-3-fatty acids in prevention of diet-related noncommunicable diseases. *Journal of Social Health and Diabetes*, *4* (2), 115–120.

Jay, J. M. (1992). Microbiological food safety. *Critical Reviews in Food Science and Nutrition, 31,* 177–190.

Kalenjuk, B., Tesanovic, D., Gagic, S., Erdeji, I. & Banjac, M. (2015). Offer of authentic food as a condition for gastronomic tourism development. *The European Journal of Applied Economics, 12* (2), 27–34.

Keller, T. (2008). Under pressure: cooking sous vide. Artisan, New York, USA

Kilibarda, N., Baltic, Z. M., Dimitrijevic, M., Karabasil, N. &Teodorovic, V. (2009). The smoked fish - Producing and quality, 4. International Conference "Fishery" Faculty of Agriculture, Belgrade-Zemun, Proceedings, 296–306.

Kilibarda, N. (2010). Comparative study of selected quality parameters during storage of cold smoked trout packed in vacuum and modified atmosphere, PhD Thesis, Faculty of Veterinary Medicine, University of Belgrade, Serbia

Kosewski, G., Górnaa, I., Bolesławska, I., Kowalówka, M., Więckowska, B., Główka, A. K., Morawska, A., Jakubowski, K., Dobrzyńska, M., Miszczuk, P. & Przysławski, J. (2018). Comparison of antioxidative properties of raw vegetables and thermally processed ones using the conventional and sous vide methods. *Food Chemistry, 240,* 1092–1096.

Köster, E. P. & Mojet, J. (2015). From mood to food and from food to mood: A psychological perspective on the measurement of food-related emotions in consumer research. *Food Research International*, *76*, 180–191.

Nyati, H. (2000). An evaluation of the effect of storage and processing temperatures on the microbiological status of sous vide extended shelf-life products. *Food Control, 11,* 471–476.

Odusanya, I. A. & Atanda, A. A. (2018). Income-health nexus in sub-Saharan Africa: Evidence from heterogeneous panel models. *The European Journal of Applied Economics*, 15 (1), 94–109.

Oniang'o, R. (2016). "You are what you eat", indeed: Nutrition Matters. *African Journal of Food, Agriculture, Nutrition and Development*, *16* (2), Editorial.

Pavlicevic, N., Baltic, M. Z., Dimitrijevic, M., Karabasil, N., Djordjevic, V., Markovic, R. & Grbic, S. (2014). Polyunsaturated fatty acids in the fish meat and their significance for human health. *Meat Technology, 55* (1)*,* 1–7.

Peck, M. W., Goodburn, K. E., Betts, R. P. & Stringer, S. C. (2006). *Clostridium botulinum* in Vacuum Packed (VP) and Modified Atmosphere Packed (MAP) Chilled Foods. Final Project Report (b13006). Technical Report. Institute of Food Research.

Peck, M. W., Goodburn, K. E., Betts, R. P. & Stringer, S. C. (2008). Assessment of the potential for growth and neurotoxin formation by non-proteolytic *Clostridium botulinum* in short shelf-life commercial foods designed to be stored chilled. *Trends in Food Science and Technology, 19* (4), 207–216.

Petersen, M. A. (1993). Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli florets. *Zeitschrift fur Lebensmittel Untersuchung und Forschung,197* (4), 375–380.

Picouet, P. A., Cofan-Carbo, S., Vilaseca, H., Ballbè, L. C. & Castells, P. (2011). Stability of sous vide cooked salmon loins processed by high pressure. *Innovative Food Science and Emerging Technologies, 12,* 26–31.

Renna, M., Gonnella, M., Giannino, D. & Santamaria, P. (2014). Quality evaluation of cook-chilled chicory stems (*Cichorium intybus* L., Catalogna group) by conventional and sous vide cooking methods. *Journal of the Science of Food and Agriculture*, *94*, 656–665.

Roascio-Albistur A. & Gámbaro, A. (2018). Consumer perception of a non-traditional market on sous vide dishes. *International Journal of Gastronomy and Food Science*, *11*, 20–24

Roldán, M., Ruiz, J., del Pulgar, J. S., Pérez-Palacios, T. & Antequera, T. (2015). Volatile compound profile of sous vide cooked lamb loins at different temperature-time combinations. *Meat Science, 100,* 52–57.

Rondanelli, M., Daglia, M., Meneghini, S., Di Lorenzo, A., Peroni, G., Faliva, M. A. & Perna, S. (2017). Nutritional advantages of sous vide cooking compared to boiling on cereals and legumes: Determination of ashes and metals content in ready-to-eat Products. *Food Science and Nutrition*, *5* (3), 827–833.

Sánchez del Pulgar, J., Gázquez, A. & Ruiz-Carrascal, J. (2012). Physico-chemical, textural and structural characteristics of sous vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science, 90*, 828–835.

Schellekens, M., (1996). New research issues in sous vide cooking. *Trends in Food Science and Technology 7,* 256–262.

Steward, M. & Onyeaka, H. (2015). Sous vide and/or low temperature cooking or processing. Food Standards Agency (https://www.food.gov.uk/sites/default/files/sousvide.pptx)

Stringer, S. C & Metris, A. (2018). Predicting bacterial behavior in sous vide food, *International Journal of Gastronomy and Food Science,* article in press.

Trejo Araya, X. I., Smale, N., Zabaras, D., Winley, E., Forde, C., Stewart, C. M. & Mawson, A. J. (2009). Sensory perception and quality attributes of high pressure processed carrots in comparison to raw, sous vide and cooked carrots. *Innovative Food Science and Emerging Technologies, 10,* 420–433.

Vaudagna, S. R., Pazos, A. A., Guidi, S. M., Sanchez, G., Carp, D. J. & Gonzalez, C. B. (2008). Effect of salt addition on sous vide cooked whole beef muscles from Argentina. *Meat Science, 79,* 470–48.

Paper received: 3.05.2018.
Paper corrected: 1.06.2018.
Paper accepted: 18.05.2018.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 639.162(497.6)"2016/2017"
ID: 265892364
https://doi.org/10.18485/meattech.2018.59.1.6

Original scientific paper

Characterisation of Bosnia and Herzegovina honeys according to their physico-chemical properties during 2016−2017

Jelena Ciric[1], Drago Sando[2], Danka Spiric[3], Jelena Janjic[1], Marija Boskovic[1], Milica Glisic[1], Milan Z. Baltic[1]

A b s t r a c t: This study evaluated the quality of 78 honeys of six different floral types (Acacia, sage [Salvia officinalis L.], linden, chestnut, honeydew and blossom), mainly from Bosnia and Herzegovina. Reducing sugars, sucrose content, moisture, hydroxymethylfurfural (HMF), free acidity, water-insoluble content, diastase activity, electrical conductivity were analysed. The samples of honey, collected during 2016−2017, were analysed using recommended methods. Results show that in 2016 and 2017, a great number of individual honeys sampled were of insufficient quality to satisfy regulatory requirements. Among the overall determined parameters, hydroxymethylfurfural and diastase activities in some honeys were not acceptable according to national and international regulations. A correlation between free acidity and electrical conductivity was found in both acacia and blossom honeys. HMF content and diastase activity was strongly negatively correlated in both acacia and blossom honeys. The quality of the honeys was varied, based on botanical origins, and presumably, handling and storage conditions.
Keywords: *honey, quality, regulations, physico-chemical parameters.*

Introduction

Honey is a sweet and flavourful natural product produced by honey bees, *Apis mellifera*, and which is consumed for its high nutritive value and its contribution to consumer health (*Habib et al*., 2014). The composition of honey is variable and depends on its floral source or botanical origin and geographical region (*Ivanovic et al*., 2015; *Matovic et al*., 2018). The quality of honey is mainly determined by physico-chemical and microbiological characteristics. The physico-chemical quality criteria of honey are well specified by the EC Directive 2001/110 (*EU*, 2002) and national regulations (*Official Gazette*, 2009; *Official Gazette*, 2011). The major criteria of interest are moisture content, electrical conductivity, ash, reducing sugars, free acidity, diastase activity and hydroxymethylfurfural (HMF) content (*Alvarez-Suarez et al.*, 2010; *Lazarevic et al.*, 2012). The manipulation of honey (heating) and its possible adulteration is reflected by many of these parameters (*Celechovská and Vorlová*, 2001; *Karabagias et al*., 2017). Damage caused by heating can be evidenced by measuring quality control parameters, such as diastase activity and hydroxymethylfurfural (HMF) content (*Bodganov et al*., 1997; *Tosi et al*., 2008). HMF is usually absent in fresh and untreated honey (*Matovic et al*., 2018), but its concentration is reported to increase as a result of heating processes (*Fallico et al*., 2006) or due to long storage. For this reason, HMF is a recognized parameter related to the freshness and quality of honey. Also, the activity of diastase is related to its structure, which can be modified by denaturation and heating (*Tosi et al*., 2008).

Bosnia and Herzegovina produces different types of honey, since honey production is a traditional practice well-implemented in several regions. The detailed characterisation of the different honey types existent in Bosnia and Herzegovina is important, because it will allow the establishment of technical specifications, thus limiting the possibility of adulteration (*Grujic and Komic*, 2012). Honey quality must be analytically controlled with the aim of guaranteeing its declared specifications. Also, consumers have increasing interest in monofloral honeys (acacia, linden, sunflower etc.) to the detriment

[1]*University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11000 Belgrade, Republic of Serbia;*
[2]*Food Safety Agency of BiH, Kneza Viseslava, Mostar 88000, Bosnia and Herzegovina;*
[3]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11 000 Belgrade, Republic of Serbia.*

Corresponding author: Jelena Ciric, 1310jecko@gmail.com

of multifloral honeys (blossom honey). According to other research, honey has functional properties which promote human health (*Giorgi et al*., 2011).

The information available on the physico-chemical characteristics of honeys of differing botanical origins produced in Bosnia and Herzegovina is limited. Therefore, the current study was conducted to assess the physico-chemical properties composition of honeys (acacia, sage, linden, chestnut, honeydew and blossom honey) available in Bosnia and Herzegovina during 2016−2017.

Materials and methods

A total of 78 honeys (nineteen samples of acacia, three samples of sage, eight samples of linden, three samples of chestnut, three samples of honeydew and forty-two samples of blossom honey) collected from different locations in Bosnia and Herzegovina (BiH) (Figure 1) during 2016−2017 were provided by the Food Safety Agency of BiH (http://www.fsa.gov.ba/fsa/bs/). Among the 78 honeys, 26 were imported (from Serbia or Croatia), while 52 were produced by local beekeepers, but purchased on the retail market. The botanical origin of all honeys was established based on their declarations, sensory characteristics and geographical origins. All honeys were kept in their original packages at 20−21°C in the dark before laboratory analysis. Triplicate samples of each honey were then taken to establish physico-chemical parameters.

All physico-chemical parameters of honey were investigated using International Honey Commission (2009) recommended standard methods or official analysis methods from the Association of Official Analytical Chemists (*AOAC*, 1990). Physico-chemical parameters of honey were evaluated according to the BiH regulation (*Official Gazette*, 2009; *Official Gazette*, 2011) and European Council Directive 2001/110/EC (2002). The minimum quality requirements for honey's physico-chemical parameters according to these regulations are presented in Table 1.

The reducing sugar and sucrose contents were analysed following Codex Alimentarius Committee on Codex standard for honey (2001), using HPLC with refractometric detection, with results presented in percent (%). The moisture content was estimated from the refractive index of all honey samples, using an ABBE refrectometer (CETI, Medline, UK). All measurements were performed at 20°C, after waiting for 6 min for equilibrium and results were converted to percent (%). Electrical conductivity (EC) was determined in a 20% (w/v) honey solution using a

Figure 1. Map of the geographic origins of examined honeys from Bosnia and Herzegovina

Table 1. Values of physico-chemical parameters for honey, according national and international regulations (*Official Gazette*, 2009; *Official Gazette*, 2011; *Council Directive 2001/110/EC*, 2002)

Parameter	Criteria*
Glucose and fructose content	Blossom honey 60 ≤ Honeydew honey, blends of honeydew honey with blossom honey 45% ≤
Sucrose content	In general: Not more than 5%
Moisture	In general: Not more than 20%
Electrical Conductivity	In general: Not more than 0.8 mS/cm
HMF	In general: Not more than 40 mg/kg
Diastase activities	In general: Not less than 8
Water-insoluble content	In general: Not more than 0.1%
Free acidity	In general: Not more than 50 meq/kg

*The criteria for physico-chemical parameters of honeys of declared origin are presented in detail in regulations (*Official Gazette*, 2009; *Official Gazette*, 2011; *Council Directive 2001/110/EC,* 2002).

conductometer (CyberScan, Thermo Scientific). The method for the determination of EC is described in Bogdanov *et al.* (1997), and results are presented as mS/cm. Determination of HMF was by the Winkler method, according to the Harmonised Methods of the European Honey Commission (*Bogdanov et al*., 1997). A Shimadzu UV-1800 spectrophotometer was used, and results are presented as mg/kg. Diastase activity was measured according to the Harmonised Methods of the European Honey Commission (*Bogdanov et al*., 1997), expressed as the diastase number, Schade units. The free acidity was determined by the titrimetric method (using NaOH and HCl). The solution was titrated with 0.05N NaOH at a rate of 5 ml/minute until pH 8.50 was achieved, and results are presented as mEq/kg. Water-insoluble content was measured by the gravimetric method and is expressed as % of material insoluble in water. Twenty grams of honey were diluted with water and filtered and washed carefully. The presence of sugars was tested by the addition of 1% phloroglucinol in ethanol to some filtrate and then a few drops of concentrated sulphuric acid. The crucible was dried at 135°C for an hour. The following equation was used (where m = mass of dried insoluble matter and m_1 = mass of honey taken):

$$\text{Insoluble Matter (\%)} = m/m_1 \times 100$$

Analysis of the results was conducted using GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters are described by means±standard deviation. Pearson's correlation (*r*) was used to determine relationships among different physico-chemical parameters.

Results and discussion

Table 2 illustrates the sugar content (reducing sugar and sucrose contents) of different honeys. The mean reducing sugar content of the examined honeys ranged from 59.57±12.93 g/100g (honeydew) to

Table 2. Reducing sugars and sucrose in honeys (n=78) of differing botanical origins (mean ±standard deviation)

Botanical origin	Reducing Sugars (g/100g)	Sucrose (g/100g)
Acacia (n=19)	72.26±6.53	2.55±1.06
Sage (*Salvia officinalis L.)* (n=3)	72.80±2.80	2.63±1.00
Linden (n=8)	73.36±3.99	2.44±1.25
Chestnut (*Castanea sativa mill.* Fam. Fagacea) (n=3)	73.57±5.62	1.73±0.45
Honeydew (n=3)	59.57±12.93	2.47±0.89
Blossom (n=42)	71.11±6.20	2.95±1.51

Table 3. Moisture content and electrical conductivity of honeys (n=78) of differing botanical origins (mean ±standard deviation)

Botanical origin	Moisture (%)	Electrical Conductivity (mS/cm)
Acacia (n=19)	16.00±1.33	0.13±0.15
Sage (*Salvia officinalis L.)* (n=3)	17.49±0.28	0.17±0.10
Linden (n=8)	16.71±0.98	0.16±0.08
Chestnut (*Castanea sativa mill.* Fam. Fagacea) (n=3)	16.63±1.00	0.48±0.19
Honeydew (n=3)	15.54±0.75	0.27±0.19
Blossom (n=42)	16.86±1.38	0.31±0.28

73.36±3.99 g/100g (chestnut). Reducing sugar levels in all honeys was acceptable according to *Codex Alimentarius* (2001), except honeydew honey, which had a value (59.57±12.93 g/100g) lower than the Codex limit. *El Sohaimy et al.* (2015) showed that fructose and glucose are the dominant sugars in honey, and together, they make up the reducing sugars specified in the international standard for honey established by Codex Alimentarius Commission (with a level of not less than 60 g/100 g) (*Codex Alimentarius*, 2001).

The sucrose content of the examined honeys was from 1.73±0.45 g/100 g (chestnut) to 2.95±1.51 g/100 g (blossom). All honeys had acceptable sucrose content according to national and international regulations, which state sucrose should be not more than 5 g/100 g (*Codex Alimentarius*, 2001).

Several previous studies have been published on the use of physico-chemical parameters for characterisation of honeys from different production regions (*Piazza and Persano*, 2004, *Mărghitaş et al.*, 2009; *Grujic et al*., 2011; *Grujic and Komic*, 2012; *Lazarevic et al*., 2012; *Matovic et al*., 2018). As the result of comparing quality characteristics, water content, electrical conductivity and free acidity of honey were the most important parameters for classification of analysed honeys according to their geographical origin. The moisture contents and electrical conductivities of the honeys are presented in Table 3. The moisture content in all honeys indicated a proper degree of maturity, in compliance with the national and international regulations, which state moisture in honey should be not more than 20% (*Official Gazette*, 2009; *Official Gazette*, 2011; *Council Directive 2001/110/EC,* 2002). This parameter is very important for the shelf life of honey during storage. The moisture contents of Bosnia and Herzegovinian honeys were similar to previously reported moisture levels in other European honeys (*Oddo and Piro,* 2004; *Lazarevic et al*., 2012; *Grujic and Komic*, 2012; *Matovic et al*., 2018).

The electrical conductivity of the examined honeys is shown in Table 3. The lowest mean electrical conductivity was obtained for acacia honey (0.13±0.15 mS/cm) and the highest mean electrical conductivity was measured in chestnut

Table 4. HMF and diastase activity of honeys (n=78) of differing botanical origins (mean ±standard deviation)

Botanical origin	HMF (mg/kg)	Diastase activity (Schade number)
Acacia (n=19)	31.36±33.87	10.19±8.33
Sage (*Salvia officinalis L.)* (n=3)	44.67±53.61	11.07±10.27
Linden (n=8)	24.25±16.80	18.86±10.53
Chestnut (*Castanea sativa mill.* Fam. Fagacea) (n=3)	32.37±8.06	14.83±7.25
Honeydew (n=3)	18.37±8.41	12.73±11.03
Blossom (n=42)	30.79±50.68	23.37±14.35

Table 5. Water-insoluble content and free acidity of honeys (n=78) of differing botanical origins (mean ±standard deviation)

Botanical origin	Water-insoluble content (g/100g)	Free acidity (mEq/kg)
Acacia (n=19)	0.02±0.01	12.43±7.13
Sage (*Salvia officinalis L.)* (n=3)	0.01±0.009	15.87±6.17
Linden(n=8)	0.46±1.15	20.40±6.61
Chestnut (*Castanea sativa mill.* Fam. Fagacea) (n=3)	0.03±0.02	16.20±3.14
Honeydew (n=3)	0.03±0.02	19.03±12.75
Blossom (n=42)	0.04±0.07	23.56±10.49

honey (0.48±0.19 mS/ cm). Electrical conductivity is a good criterion for the botanical origin of honey (*Adenekan et al.*, 2010). The honeys from different botanical and geographical origins in Bosnia and Herzegovina were within the standard limit (not more than 0.8 mS/cm) (*Codex Alimentarius*, 2001).

The HMF and diastase activity of honey available in Bosnia and Herzegovina is reported in Table 4. HMF concentrations of honeys ranged from 18.37±8.41mg/kg (honeydew) to 44.67±53.61 mg/kg (sage honey). However, HMF concentrations of some samples of acacia, sage, linden, and blossom honey were above the maximum limit of 40 mg/kg as recommended by European Union (*EU Directive* 110/2001). The high concentration HMF is reported to have genotoxic effects and mutagenic potential on humans (*Janzowski et al.*, 2000). Several factors have been reported to influence the levels of HMF, such as temperature and time of heating, storage conditions and floral source (*Fallico et al.*, 2006; *Anacleto et al.*, 2009; *Vranic et al.*, 2017; *Matovic et al.*, 2018). Also, previous studies on HMF in honey have reported a considerable increase in its concentration when honey is stored at room temperature (*Khalil et al.*, 2010). *Turhan* (2009) demonstrated that honey should be consumed within six months of harvesting.

The mean diastase activities of the honeys were from 10.19±8.33 (acacia) to 23.37±14.35 Schade units (blossom). The current law stipulates a minimum value of 8.00 Schade units (*EU Directive 110/2001*). Same samples of all our honeys were less than than 8 Schade units. Similar to HMF content, the activity of diastase can be used as an indicator of heating, if the honey has been subjected to heating above 60°C (*Matovic et al.*, 2018). *Tosi et al*. (2008) showed that diastase activity loss occurred as temperature increases, and decrease in diastase activity was more evident at higher rates of temperature increase.

Table 5 shows the water-insoluble content and free acidity of our honeys. The honeys analysed had water-insoluble contents ranging from 0.01±0.009 g/100g (sage honey) to 0.46±1.15 g/100g (linden honey). Acacia, sage, chestnut, honeydew and blossom honeys had the greatest water-insoluble contents. Linden honey had values higher than the maximum limit of water-insoluble contents allowed by BiH legislation (0.1 g/100g) (*Official Gazette*, 2009; *Official Gazette*, 2011). This physico-chemical

Table 6. Correlations (Pearson's correlation coefficients – *r*) between physico-chemical parameters of acacia honey

Parameter	Free Acidity	Electrical Conductivity	Moisture	HMF	Diastase activity
Free Acidity	–	0.65**	−0.034	0.19	0.186
Electrical Conductivity	–	–	−0.08	−0.12	0.509*
Moisture	–	–	–	−0.33	0.226
HMF	–	–	–	–	−0.594**

* $P<0.05$; ** $P<0.01$

Table 7. Correlations (Pearson's correlation coefficients – *r*) between physico-chemical parameters of blossom honey

Parameter	Free Acidity	Electrical Conductivity	Moisture	HMF	Diastase activity
Free Acidity	–	0.50***	0.28	−0.06	0.373
Electrical Conductivity	–	–	−0.01	−0.25	0.564***
Moisture	–	–	–	−0.16	0.122
HMF	–	–	–	–	−0.384*

* P<0.05; ***P<0.001

parameter was used when a significant portion of the honey collected was harvested by pressing the combs. However, nowadays, almost all commercial honey is harvested by centrifugation, although wax is a major source of water-insoluble contamination (*Bogdanov et al.,* 1997; *Liberato et al*., 2013). Similar results for the water-insoluble content in honey were reported in a study of acacia and linden honeys in Serbia (*Vranic et al.,* 2017; *Matovic et al*., 2018).

The mean free acidity values of honey ranged from 12.43±7.13 mEq/kg (acacia) to 23.56±10.49 mEq/kg for blossom honey. Free acidity values of all examined honeys were below the legal limit (lower than 50 meq/kg), and concord with results presented in other studies (*Lazarevic et al*., 2012; *Vranic et al*., 2017; *Matovic et al*., 2018). Free acidity is an important parameter for honey. Higher values for free acidity can be indicative of fermentation of sugars into organic acids and is related to the deterioration of honey. The variation in free acidity among different honeys can be explained by their diverse botanical origins, the presence of different organic acids, and differing geographical origins or harvest season (*da Silva et al*., 2016).

Correlations between different physico-chemical parameters of acacia and blossom honeys are presented in Tables 6 and 7, respectively. The regression analysis clearly indicated that free acidity content in honeys significantly correlated with electrical conductivity. *Khalil et al*. (2010) showed that storage duration, free acid, total acidity, pH and lactone levels significantly correlated with HMF concentration in honey. The positive correlation between diastase activity and moisture content can be explained by the increase of the enzyme activity in water. Similar results were found by *Gomes et al*. (2010). HMF content was strongly negatively correlated with diastase activity in acacia honey (r = −0.594). Therefore, those two parameters are inversely proportionate to each other. This is in agreement with other studies (*Thrasyvoulou,* 1986; *Sahinler and Gul,* 2005; *Kędzierska-Matysek et al.,* 2016).

A positive correlation occurred between free acidity and electrical conductivity in blossom honey (r = 0.50; P<0.001). A weak correlation was also found between free acidity and moisture content (r = 0.28) in blossom honey (Table 7). Therefore data from both honeys (acacia and blossom) combined with statistical analysis indicate that free acidity and electrical conductivity could provide ways to assess honey quality. Similar results were presented by *Khalil et al*. (2010) and *Celechovská and Vorlová* (2001). The HMF was negatively correlated with moisture content (r = −0.16). This is in agreement with *Meda et al.* (2005). A significant negative correlation was found between HMF and diastase activity in blossom honey (r = −0.384; P<0.05).

Conclusion

Results show that in 2016 and 2017, a great number of honeys in Bosnia and Herzegovina were of insufficient quality to satisfy regulatory requirements. In the future, the quality of honey will need to be monitored in order to improve its quality over the whole territory of Bosnia and Herzegovina.

Acknowledgment: This paper was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project "Selected biological hazards to the safety/quality of food of animal origin and the control measures from farm to consumer" (31034).

Disclosure Statement: No potential conflict of interest was reported by the authors.

References

Adenekan, M. O., Amusa, N. A., Lawal, A. O. & Okpeze, V. E. (2010). Physico-chemical and microbiological properties of honey samples obtained from Ibadan. *Journal of Microbiology and Antimicrobials*, *2(8),* 100−104.

Alvarez-Suarez, J. M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F. & Battino, M. (2010). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology*, *48(8−9)*, 2490−2499.

Anacleto, D. A., Souza, B. A., Marchini, L. C. & Moreti, A. C. (2009). Composicao de amostras de mel de abelha Jatai (Tetragonisca angustula latreille, 1811). *Ciencia e Tecnologia de Alimentos, 29*, 535–541.

AOAC. (1990). Official Methods of Analysis.15th edn. Pp. 770–771. Virginia, USA: Association of Official Analytical Chemists, Inc.

Bogdanov, S., Martin, P., Lüllmann, C., Borneck, R., Flamini, C., Morlot, M., Heretier, J., Vorwohl, G., Russmann, H., Persano-Oddo, L., Sabatini, A. G., Marcazzan, G. L., Marioleas, P., Tsigouri, K., Kerkvliet, J., Ortiz, A. & Ivanov, T. (1997) Harmonised methods of the European honey commission. *Apidologie (extra issue)*, 1−59.

Celechovská, O. & Vorlova, L. (2001). Groups of honey-physicochemical properties and heavy metals. *Acta Veterinaria Brno, 70(1)*, 91−95.

Codex Alimentarius. (2001). Revised Codex standard for honey. Codex Stan 12−1982: Rev.1 (1987)., Rev. 2, 2001. www.fao.org/docrep/w0076e/w0076e30.htm

Council Directive 2001/110/EC. (2002). *Official Journal of the European Communities, 10*, 51–52.

da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O. & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry, 196*, 309−323.

El Sohaimy, S. A., Masry, S. H. D. & Shehata, M. G. (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences, 60(2)*, 279−287.

Fallico, B., Arena, E., Verzera, A. & Zappala, M. (2006). The European Food Legislation and its impact on honey sector. *Accreditation and Quality Assurance, 11*, 49–54.

Giorgi, A., Madeo, M., Baumgartner, J. & Lozzia, G. C. (2011). The relationships between phenolic content, pollen diversity, physicochemical information and radical scavenging activity in honey. *Molecules, 16(1)*, 336−347.

Gomes, S., Dias, L. G., Moreira, L. L., Rodrigues, P. & Estevinho, L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology, 48(2)*, 544−548.

Grujic, S. & Komic, J. (2012). Classification of honeys from three geographical regions based on their quality control data. *Quality of life, 5* (1−2).

Grujic, S., Grujic, R., Popov-Raljic J. & Komic, J. (2011). Characterization of Black Locust (*Robinia pseudoacacia*) honey from three geographical regions of North-West Bosnia and Herzegovina. Proceedings. 7th International Congress of Food Technologists, Biotechnologists and Nutritionists, (pp.). Opatija, Croatia, 274−278.

Habib, H. M., Al Meqbali, F. T., Kamal, H., Souka, U. D. & Ibrahim, W. H. (2014). Physicochemical and biochemical properties of honeys from arid regions. *Food Chemistry, 153*, 35−43.

International Honey Commission (2009) Harmonised Methods of the International Honey Commission www.bee-hexagon.net/en/network.htm.

Ivanovic, J., Baltic, M. Z., Jelic, D., Janjic, J., Boskovic, M., Markovic, R. & Starcevic-Dokmanovic, M. (2015). Research of production volume and market turnover of honey from 2004 to 2014. *Veterinarski Glasnik, 69(5−6)*, 467−478.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J. & Eisenbrand, G. (2000). 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food and Chemical Toxicology, 38(9),* 801−809.

Karabagias, V. K., Karabagias, I. K. & Gatzias, I. (2017). The impact of different heating temperatures on physicochemical, color attributes, and antioxidant activity parameters of Greek honeys. *Journal of Food Process Engineering*. DOI: 10.1111/jfpe.12668.

Kędzierska-Matysek, M., Florek, M., Wolanciuk, A., Skałecki, P. & Litwińczuk, A. (2016). Characterisation of viscosity, colour, 5-hydroxymethylfurfural content and diastase activity in raw rape honey (*Brassica napus*) at different temperatures. *Journal of Food Science and Technology, 53(4)*, 2092−2098.

Khalil, M. I., Sulaiman, S. A. & Gan, S. H. (2010). High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. *Food and Chemical Toxicology, 48(8−9),* 2388−2392.

Lazarevic, K. B., Andric, F., Trifkovic, J., Tesic, Z. & Milojkovic-Opsenica, D. (2012). Characterisation of Serbian unifloral honeys according to their physicochemical parameters. *Food Chemistry, 132(4)*, 2060−2064.

Liberato, M. D. C. T. C., Morais, S. M. D., Magalhães, C. E. D. C., Magalhães, I. L., Cavalcanti, D. B. & Silva, M. M. D. O. (2013). Physicochemical properties and mineral and protein content of honey samples from Ceará State, Northeastern Brazil. *Food Science and Technology (Campinas), 33(1)*, 38−46.

Mărghitaş, L. A., Daniel, D., Moise, A., Bobis, O., Laslo, L. & Bogdanov, S. (2009). Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry, 112(4)*, 863−867.

Matovic, K., Ciric, J., Kaljevic, V., Nedic, N., Jevtic, G., Vaskovic, N. & Baltic Z. M. (2018). Physico-chemical parameters and microbiological status of honey produced in an urban environment in Serbia. *Environmental Science and Pollution Research*. DOI: 10.1007/s11356−018−1659−1.

Meda, A., Lamien, C. E., Millogo, J., Romito, M. & Nacoulma, O. G. (2005). Physiochemical analyses of Burkina Fasan honey. *Acta Veterinaria Brno, 74(1)*, 147−152.

Oddo, L. & Piro, P. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie, 35*, 38−81.

Official Gazette. (2009). Bosnia and Herzegovina. Pravilnik o medu i drugim pcelinjim proizvodima i Pravilnik o metodama za kontrolu meda i drugih pcelinjih proizvoda. No. 37, 222.

Official Gazette. (2011). Bosnia and Herzegovina. Pravilnik o izmjenama i dopunama pravilnika o medu i drugim pcelinjim proizvodima. No. 25, 90.

Piazza, M. G. & Persano Oddo, L. (2004). Bibliographical review of the main European unifloral Honeys. *Apidologie 35*, 94–111.

Sahinler, N. & Gul, A. (2005). Effect of heating and storage on honey hydroxy methylfurfural and diastase activity. *Journal of Food Technology, 3*, 152−157.

Thrasyvoulou, A. T. (1986). The use of HMF and diastase as criteria of quality of Greek honey. *Journal of Apicultural Research, 25(3)*, 186−195.

Tosi, E., Martinet, R., Ortega, M., Lucero, H. & Ré, E. (2008). Honey diastase activity modified by heating. *Food Chemistry, 106(3)*, 883−887.

Turhan, K. (2009). Effects of thermal treatment and storage on hydroxymethylfurfural (HMF) content and diastase activity of honeys collected from middle Anatolia in Turkey. *In Innovations in Chemical Biology*, Springer, Dordrecht, 233−239.

Vranic, D., Petronijevic, R., Stojanovic, J. Dj., Koricanac, V., Milijasevic, J. B. & Milijasevic, M. (2017). Physicochemical properties of honey from Serbia in the period 2014−2016. In IOP Conference Series: *Earth and Environmental Science,* IOP Publishing, 85, 1, 012058.

Paper received: 8.03.2018.
Paper corrected: 9.05.2018.
Paper accepted: 14.05.2018.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 613.281(497.11)(091) ; 641.56(497.11)(091)
ID: 265894924
https://doi.org/10.18485/meattech.2018.59.1.7

Review paper

Meat in traditional Serbian cuisine

*Milan Z. Baltic[1], Janjic Jelena[1], Milka Popovic[2], Tatjana Baltic[3], Marija Boskovic[1], Marija Starcevic[4], Danijela Sarcevic[3]**

A b s t r a c t: The use of meat in traditional Serbian cuisine can be divided into three periods. The first period is the time from the arrival of Slavs in the Balkans, through the Middle Ages to the early 19th century. The second period, the 19th and the first half of the 20th centuries, can be marked as special due to significant changes that impacted traditional Serbian cuisine, compared to the first period. It was a period during which the Europeanization of Serbia took place in all spheres of life, including cuisine. After World War II, Serbia and many surrounding countries eperienced a great surge in industrialization in all economic fields, including that of food production. The last sixty years has seen the globalization of our food supply, with food being transported globally from one part of the world to another, and which resulted in neglected traditional, local cuisines. Therefore, many countries, including Serbia, recognize a need for preserving traditional cuisine. This recognition has initiated several mechanisms for maintaining Europe's gastronomic heritage. Preservation of traditional cuisine in Serbia has special significance for tourism development.
Keywords: *tradition, nutrition, history, heritage.*

Introduction

At a geographic, historic crossroads between East and West, Serbia has always been, and still is, interesting economically, politically and militarily to other, larger states. Hence, there were frequent conflicts (in the twentieth century alone there were five), shifting borders and population migrations. All of this has had a significant impact on the overall life of the people of Serbia, as well as on their cuisine, food preparation, food habits and their whole culinary culture. Serbian cuisine, without exaggeration, can be described as a gastronomic cauldron, a mixture of different cuisines, being the legacy of the original homeland with influences from Greece and Rome plus Byzantium, the East and the West. That makes Serbia host to a heterogeneously contrasting variety of foods.

Serbian cuisine can be regarded as very tasty and moderately spicy, able to satisfying to all the senses. Meat, especially barbecued meat and different types of roast meat, has an important place, and most ordinary meals contain at least one meat serving (*Sarcevic et al.*, 2011) Traditional Serbian dishes are very generous, while ingredients are not expensive and preparation is not complex. Generally, Serbian people like to prepare food and enjoy consuming the resultant meals. The term "national cuisine" can be used only conditionally, because there is no truly homogenous national cuisine.

Culinary culture is an important and inseparable part of the cultural heritage of each nation. Foods that are consumed, customs and habits related to food preparation and behavior at the table are part of the spiritual riches and heritage of each nation that is transmitted from generation to generation.

Traditional Serbian cuisine in the Middle Ages

There are almost no data about the cuisine of Serbian people in the centuries after their arrival in the Balkans. These Slavic peoples certainly brought with them their cuisine traditions. For these massive, strong people, adept in boat making and skilled fishermen, the Balkans was an arid environment. Their Slavic homeland was rich with pastures and water. However, the Balkan Peninsula did have pastures and forests, which provided the means for animal

[1]*University in Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Republic of Serbia;*
[2]*University in Novi Sad, Faculty of Medical Science, Institute for public health Vojvodina, Futoski put 121, 21000 Novi Sad, Republic of Serbia;*
[3]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia;*
[4]*Army of Serbia.*

***Corresponding author:** Danijela Sarcevic, *danijela.sarcevic@inmes.rs*

husbandry, and hence, the use of milk and meat in local diets. It is believed that the early Slavs also brought the today almost forgotten and certainly lost, breed of pig, the siska pig (*Baltic et al*, 2013, *Hrasnic et al.,* 1964). Serbia still has traditional pig breeding and it is quite understandable that pork continues to be the most common type of meat. In medieval Serbia, pigs and sheep were the most common and cherished meat animal species. Meat was sold fresh, and professional butchers are mentioned in various Serbian cities of the time. Butchers had to comply with different regulations, particularly those regulating prices. Besides fresh meat, people traded in salted meat. Meat was usually dried and used in winter. Meat products (smoked meat, sausages) were also familiar, especially in coastal areas. Medieval communities utilized significant amounts of wild game meat (wild pigs, chamois, deer, roe deer, hares, wild ducks, geese, partridges, pigeons). Besides meat, the most important product of animal origin was cheese, and by medieval times, several kinds of cheese were produced. Fish (freshwater and marine) also played an important role in the local cuisine. Fresh fish was sold in retail markets in cities situated on the shores of the sea or rivers, but salted fish was also accessible to consumers inland. Besides fish, other seafood (octopus, crabs, clams) was available on the tables of the rulers, feudal lords and nobles, and during fasting periods in local monasteries (*Marjanovic-Dusanic and Popovic*, 2004).

Among foods of plant origin, bread prepared from cereals (wheat, barley, millet, oats or sorghum) took pride of place. Vegetables (cabbage, garlic, onion, radishes, turnips, pumpkins, melons, lentils, broad beans, peas, poppy seeds, rice) and fruits (walnuts, hazelnuts, pears, apples, cherries, cherry, rowan) were used in the local cuisine. Foods of plant or animal origin were prepared with the addition of fat, tallow or oil, particularly olive oil. Lard was extensively used in the daily cuisine because ordinary, poorer people mainly bred pigs (*Spremic,* 2004).

At that time in Serbia, people had two meals daily, so breakfast was introduced later from the West. By the mid-nineteenth century, people ate mainly stews, and the spoon was the main, and often only, cutlery item. However, while European peoples had, for six or seven centuries, eaten relatively fatty foods and with their fingers, from the time of the Nemanjic dynasty, Serbs at court and the nobility ate with golden forks. In fact, the German emperor Frederick Barbarossa, on his crusade, was hosted by Serbian Prince Stefan Nemanja in Nis during 1189, and experienced eating with a fork for the first time (*Baltic et al,* 2010c).

For the average Serbian host, the collapse of the Serbian state and the arrival of the Ottoman Empire was not much influence on the cuisine. They ate twice a day (lunch before midday, between 10.00 and 11.00 and an evening meal before dusk). For those who worked in the fields, an additional snack meal was prepared.

Traditional Serbian cuisine in the 19th up to the mid-20th century

In the 19th century, Europeanization engulfed all areas of public and private life in Serbia. It was quite understandable for the country to desire its place in Europe, after centuries spent under Ottoman occupation. The influx of foreigners (diplomats, merchants, governors) influenced life in Serbia, the changes at first visible among upper social classes, but which soon spread to the middle class. The most interesting changes occurred in the private lives of wealthy, urban families, and the greatest changes were around the position of women and their household duties. The housewife was now tasked with supervising domestic chores and taking care of guests and how they were served. Cooking was left to the maidservant and food purchases to the house boy. The changes were also reflected in food preparation and serving. In the best hotel in Belgrade at the time, Novo Zdanje, breakfast was served at eight o'clock, lunch at noon (daily four different meals, and on Thursdays and Sundays, six meals), while dining was a la carte (*Grujic,* 1992).

We cannot say with certainty which meals were on the menu of the mid-19th century, but it is known that the food was simple but plentiful. Soup, boiled beef (Rindfleisch) and stewed vegetables were consumed daily, but on Thursdays and Sundays, roast meat and on Sundays, cakes, were prepared. Cheese and fruit were always available. Remainders of stews and cheese were served for dinner, and during winter, this meal was supplemented with mutton or fried sausages (*Zujovic*, 1986). Great feasts were infrequent, but on Sundays, friends were invited to lunch. Guests would come every day for brunch (pork crackling, offal sausages – kavurma – and cheese) (*Zujovic*, 1986). Breakfast was served between seven and eight o'clock, lunch between midday and one o'clock, and dinner at seven in the evening. The eating times of urban families were maintained until the Second World War, and choice of meals changed as the country grew and contacts with other European nations expanded. In one cookbook from 1877, many recipes for dishes from different backgrounds and requiring various ingredients were recorded (*Popovic Midzina*, 1877). The hostess had to take into account the balance between different dishes, ensuring there were not more soups than solid foods, or more sweets than savoury foods. Mixed dishes were intentionally created to produce enticing menus. In the menu prepared by *Stojan Novakovic* (1913), lunch options were: pureed bean soup with fried bread, veal stew with dumplings and butter-taiga for desert; hamburger steak, roast potatoes, dumplings with cheese and fruit, or; green soup, beef with horseradish sauce, lentils with bacon, veal chops and vegetables. For dinner, according to the same source (*Novakovic,* 1913), choices were: fried chicken, potatoes boiled in salted water and fruit; veal chops, spinach and fruit, and; chicken with white sauce, donuts and fruits. Naturally, official lunches and dinners offered different fare from the daily diet. At the official dinners at the Obrenovic's court, meals were served in a certain order. Thus, for dinner on 13 December 1894, the following items were served: poultry soup; Rhine salmon in a crayfish sauce; garden stake; garnished lobster; quail with rice; goose liver pate à la Parisienne; pineapple; pheasant on a spit; peas with roasted bread pieces; chocolate candy; ice cream and fruit. In the United Kingdom in 1913 during the reign of King George V, the following were served for one dinner: Indian-style soup; crab; lamb fillet; poultry meat in aspic; quail with vegetables; cold meat jelly; asparagus with sauce; and various types of cakes, strawberries and wild berries. Comparing these official Serbian and English royal family dinners, it is clear the Serbian court did not lag behind the table of the King of England. Everyday court lunches and dinners were more simple: soup; fish as an appetizer; roast meat as the first main course poultry as a second main course; cheese and fruit for dessert (*Colak-Antic*, 2004).

After the First World War, urban society was so modernized that wealthy households sometimes ordered in food, despite the fact that they had their own servants. Complicated, savory specialties for a cold buffet were usually ordered in, such as goose liver in aspic or hot roll of puff pastry stuffed with a filling of brain, mushrooms and chicken meat. One respectable Belgrade family had 26 dinner guests who were served soup, spinach pie, meat pie, ham with horseradish, cauliflower salad with mayonnaise, celery with goose liver pate sandwiches, pastry with ham, sweets and fruit.

Food is just one segment of culture, but it is an excellent indicator of the real state of society. From the 1850s until the Second World War, middle class society in Serbia lived and ate according to European standards. The diet changed, especially in urban environments in which different cultures intersected. A new meal was accepted (breakfast was introduced into the daily menu), attitudes towards food changed and eating times during the day were modified. These changes occurred not only in Belgrade, but spread to other Serbian cities (*Colak-Antic*, 2004).

People in rural areas lived and ate differently to the urban population. Rural meal patterns depended on the seasons and cycles of agricultural production. From late spring to early autumn, food was balanced and diverse, because there was plenty of fresh vegetables and fruits. Despite the hard field work at this time of year, food consumption was moderate and meals were light but frequent (four meals a day). In winter, the diet was more uniform, and people ate a greater proportion of fatty foods in larger portions. From this it could be concluded that people were moderately behaved when the nature was generous, until at the time of shortages, people ate plenty of food. In rural areas, some customs were retained steadfastly, to become rituals. So, the holiday roast pig or lamb was always prepared by male members of the household. For feasts (the family patron saint feast, weddings, funerals), many different dishes were prepared, most of which included meat (or fish if the celebration was at a time of fasting), but all the dishes were just a prelude to the main course. This was a spit-roasted pig or lamb, which stood in the middle of the table, taking a central and important place in the table presentation (*Prosic-Dvornic,* 2004).

The beginnings of active culinary literacy in Serbia in the late 19th and early 20th century was related largely to the female population, best shown through a real explosion of cookbooks which started to be published. These cookbooks, beside recipes, contained advice and instructions for housewives. *Spasenija Pata Markovic* wrote one of the most famous cookbooks which was published in 1907 and has had 24 editions to date. In the Introduction, among other things, she wrote: "Food is what keeps us alive. And that is why is not irrelevant what a person eats, how much he eats and how the meal is prepared. The diversity of food and different ways of preparing food are of great importance for the human body. Only diversified food can provide all the ingredients we need: proteins, fat, starch, sugar, various salts, vitamins, etc., and the lack of these ingredients contributes to a variety of disorders in the body" (*Markovic*, 1907). This way of thinking about food is completely in line with the modern approach to nutrition. From 1907 until today, cookbooks, with various types of content, and with different scopes and purposes, became numerous and found their way into the hands of many satisfied customers. Serbs from the mid-19th century onwards generally had three meals a day (the newly-introduced breakfast, plus lunch and dinner) and the most abundant was, and is, lunch. Until the mid-19th century, the only two meals were lunch and dinner, as is practiced in monasteries today. It is interesting that Nikola Tesla wrote: "It is natural that a person eats twice a day" (*Janic,* 2010).

Traditional Serbian cuisine in the second half of the 20th century

The peoples living in Serbia during the second half of the 20th century have had exposure to a very a rich and interesting culinary culture, which is the result of the country's geographic location, and contacts and influence from other cultures, especially Oriental and Central European ones, but is also due to the hedonistic habits and material development of the populace in the country. In the 20th century, much has changed in the world and in Serbia, especially after the Second World War. Much of what for centuries has been our heritage, has, within a brief moment in history, become worthless, obsolete. Restaurants have contributed to the standardization of cuisine in Serbia, and eventually to making gastronomy's ordinary shop window that of fast food. Over a considerable period of time, sit-down, more traditional restaurants (as opposed to those that are largely fast food outlets) have been replaced by ethnic and fusion restaurants that often do not pay much attention to traditional cuisine (*Baltic et al.*, 2010c).

Today, the real symbol of Serbian national cuisine, above all, is grilled meat. Our meals can appear greasy and heavy to many foreigners, which is a result of the fact that in Serbia, pork is the most common type of meat consumed (it accounts for 70 to 80% of consumed meat). Pork and/or lard have been the subject of many debates, and very frequently have been accused of causing cardiovascular diseases in humans. However, recently, the opinion that lard has been groundlessly and unscientifically demonized as causing cardiovascular diseases has started to be heard (*Baltic et al.* 2010b; *Boskovic et al.,* 2015). Regardless, though, of warnings of the catastrophic consequences that consuming fatty foods has on the human body at any age, fatty but delicious dishes always have strong appeal to human desires and appetites. Fat is not only nutritious and a basic requirement in human nutrition, but is also a food supplement that enriches and intensifies the flavor of other ingredients. Man is has always attributed great and manifold significance to fat. Lard, regardless of the part of the world and culinary traditions, has long enriched meals premised heavily on grains or other forms of carbohydrate. Fat, whether vegetable, dairy or animal fat, and no matter what culinary tradition, improves meals prepared from vegetables and other plant ingredients. It is known that breaded and fried foods carry large amounts of fat, but these fats also contribute to the diversity and fullness of meal taste (*Baltic et al*., 2010c).

In the latter part of the 20th century and to date, globalization has induced large and significant, sometimes serious or even irreparable damage in the food culture of nations, including our own. Globalization has led to a loss of traditional cuisine, since large, multinational companies have interests that we all have the same diet and that everyone purchases food from the same manufacturers. Environmental changes, the race for economic profit and need to produce more food have contributed to the disappearance of traditional varieties of fruit, vegetables and animal breeds, plus a number of fish species. The basic human right to life and food should include the right to enjoy food. This implies that societies and communities have some responsibility for preserving their own food cultures and traditional methods of food preparation in order that food enjoyment is feasible in the future, all in accordance with the principles of sustainable development (*Pulen*, 2004a).

Industrial production in all areas, including that of food, has wrought a series of changes. In industrial food production, the distribution system is the weak link between food and those who consume it. The culinary sector has now moved outside the household, and the food distribution system allows a wide variety of plant and animal products to be at our disposal, thus significantly changing the system of decision-making about our cuisines. Industrialization encroaches on the social function of the kitchen and partially excludes the act of eating from those of food preparation and indeed, from the kitchen. Industrialization has, in fact, two sides: production and transformation. In the production of foods of animal origin, animal nutrition has become a thing apart from human consumption of the food, so that foods like meat, eggs and dairy products have become devoid of animal attributes. In small manufacturing households that keep animals for their own use, and where the animals live in "a state of nature", animals are treated and cared for similarly to people. The animal is man's companion, enjoys a very special status and becomes the subject of enormous attention (*Pulen*, 2004b). This is confirmed by customs from our region, including that of women getting up at the moment oxen (working cattle) passed by them. Also, dairy cows or working livestock were practically never slaughtered for consumption in their own household (*Baltic et al.*, 2010a). Transformation in the food industry implies the efforts of industry to offer consumers something that has an identity, which is not anonymous, which is not without spirit, and which is acceptable socially. This is confirmed by the data showing modern food producers want to ameliorate the connotations of industrial food production by using advertising to draw consumers closer. This is usually achieved by pictures that are linked to traditional cuisine or food production, or product names such as "grandma's cake".

Industrialized food is produced in vast quantities, and it may be, overall, less recognizable and famous than traditional food, but more worrisome. Food, however, is not an ordinary consumer product; it enters the body, taking part in the maintenance of its integrity, but also in the construction of personal identity. Food binds man to nature, cuisine and behavior at the table in each society, and harbors a unique relationship between nature and culture. Industrialized food disturbs that arrangement and can cause issues that provoke fear, so that consumers now question where their food comes from, what changes it has suffered and who has handled it?

Food preservation, transport speed and temperature control have enabled food to be transferred from one to the other end of the world. Food in all its forms, from the seeds of plants to live animals to ready-to-eat meals, is now shipped from country to country by major road, sea, rail and air services. At the same time, it is not rare that traditional meals are found in exotic places, to which they were never previously linked. However, relocation and moving food – globalization of the food industry – have also contributed to the fact that man today, as never before, has a variety of affordable food (*Pulen*, 2004a).

This millennium is an era when we have increasingly less time for long feasts and when everyone lives faster. This acceleration of life dictates insipidity. People in the modern world have come to the fact that they cannot afford to lose a single moment of time. In an effort to save that precious time, by consuming fast food, we have chewed over our futures and our own souls. "I keep time," said the Romanian poet Jon Karajon. As a result, we have come to the point that we have lost part of our souls and may now be trying to turn back, if this is at all possible (*Domazet*, 2004).

Fast food is not a novelty in modern diets. In fact, fast food has existed since the time when people began to gather in public places to trade, socialize or participate in any joint activities. Markets, fairs, streets and squares and all other places of public assembly, are all traditional social places where special diets were developed, intended primarily for passengers or strangers passing through. When talking about modern fast food, speed and convenience are the most important characteristics, all in order to meet the requirements arising out of a specific type of demand. At the same time, it is important to preserve many elements of the traditional meal. When fast food is consumed, there is no cutlery, no dish washing, no tables, no chairs surrounding it. Fast food has long been accepted throughout the world, so the main aim of modern producers is not to completely satisfy our hunger, but to make their products enjoyable, practical and regularly consumed (*Janjic et al.,* 2015). Previously, prior to globalization, fast food was in accordance and harmony with the culture and environment in which it was created and in which it put down roots. However, only after the Second World War was it accepted all over the planet, so it is now a kind of global culinary tradition and habit that knows no cultural barriers, and, thereby, must meet a huge number of different culinary traditions and flavors (*Rozen*, 2004) .

This type of nutrition probably originally appeared among travelers and farmers. The main desirability of fast food is the fact that the humans simply love to hold food in their hands, because that enables the experience of unparalleled closeness and intimacy with the food that is consumed. This is one of the reasons why, and to what extent, the humble but popular sandwich can be almost a complete meal, since a mixture of ingredients can go together between two slices of bread. The variety and mix of different tastes, aromas and textures from the food from which a sandwich is prepared are an enjoyable mixture. Sandwiches can be open, that is, prepared so that various foods and spices, alone or in appealing combinations, are applied to one slice of bread. Among many people, including those in Serbia, this slice of bread was, until recently, but now more rarely, usually smeared with lard, lightly salted and spiced with sweet pepper.

The expansion of fast food and all phenomena related to it appears to have occurred because of what fast food offers to consumers – direct and efficient food access. This primarily refers to the satisfaction felt by consumers because of their complete sensory stimulus due to the presence of favorite ingredients, the fullness of taste, texture and aroma. Fast food feels good to most of the people who consume it; it offers meals and tastes to consumers which people have always sought after and craved, and which they have not always managed to afford.

In contrast, globalization and industrialization of food has also led to the fact that the products are standardized and uniform. This is often the reason for the loss of specific sensory characteristics. Despite strong pressure from international companies to place their products, national and regional efforts to preserve the cuisine specifics of any one country and one people have not vanished. Thus, in Serbia, cabbage with pork and rustic bread appears on city restaurant tables and take-out outlets, as do baked potatoes, various kinds of meat grilled over charcoal, local meat products and products from local fields, opposing industrial products from multinationals. In some countries, the movements described as new cuisine from local soil, which have many activities including, for example, publication of ethnic cuisine recipes, have become guardians of traditional gastronomy. This, however, often isn't just a book of old, traditional recipes, but can also include local habits and dining customs throughout the history of the region or country, anecdotal records of evening gatherings and older carnivals, and old photographs of related scenes. International companies such as McDonald's are largely aware of this type of movement and adapt the fast food they offer to local habits. Thus, while in the US, chips are served with ketchup, in France, the accompaniment is mayonnaise (*Rozen*, 2004; *Janjic et al.*, 2015)**.**

However, we cannot say that fast food has no opponents. The Slow Food movement is registered to date in about 150 countries, including in Serbia. Proponents of this movement have revolted against the quick and unhealthy eating of food and fast life of the modern world, the disappearance of people's interests in the food they eat and the disappearance of local culinary traditions. The Slow Food movement encourages slow and prolonged enjoyment of food from harvest to consumption, but also awareness of our permanent responsibility towards food and its origins (*Baltic et al*., 2010).

The gastronomic map of Serbia today

The gastronomic map of Serbia is based on geographically and historically defined areas, as well as similarity of gastronomic culture, and so is divided into four regions (Vojvodina, Central and Western Serbia, Eastern Serbia and Southwest Serbia). The cuisine of Vojvodina is significantly different to the cuisine of other parts of Serbia. This is understandable considering the influence from Austrian, German, Hungarian and Slovakian cuisines. This cuisine is characterized by significant use of pasta. Some municipalities within the region have a specific population composition. This is because settlers from different parts of former Yugoslavia after the Second World War brought with them their own habits in eating and preparing meals, and which have now been maintained for over 60 years. This means that their third generation descendants are now preparing those dishes in traditional ways (*Zagorac et. al*, 2010). Meat is often cooked in sauces, like a stew, or eaten as roast. A characteristic stew is prepared with mutton, chicken, fish, beef, venison or rooster. Another traditional dish is smoked fish, such as perch or bream. Famous meat products include sausages like kulen and other types of fermented sausages, Petrovska klobasa, sausages for roasting, dry ham and bacon. In Vojvodina there is long tradition of production of fermented sausages of various diameters, ham and bacon. Fermented sausage are produced maily from pork meat (Sremski kulen, Lemesko sausage, Sremska sausage, Petrovska klobasa) (*Baltic et al.,* 2011). These sausage types have protected geographical indication (PGI). Almost forgotten meat dishes include wild-style quail, minced meat with cabbage (galupci) and fried pork brains. Vrsac ham, produced in the middle of Banat in Vojvodina, also has PGI status. Lemesko sausage, Petrovska klobasa and Vrsac ham are produced exclusively in artisanal conditions, typically smallholder households. Sremska sausages, Sremski kulen and Sremska salami are produced in industrial and also in artisanal conditions. Traditional meat products such as fermented sausages, ham and bacon are produced in the period from November until April because of favorable climatic conditions (colder seasons) (*Vukovic et al.,* 2011a; *Vukovic et al.,* 2011b; *Ikonic et al.,* 2010; *Ivanovic et al.,* 2016; *Stevanovic et al.,* 2016).

Central and Western Serbia, regions between the Morava and Drina Rivers and to the south to Kraljevo, are regions of traditional livestock production. Here, people consume significant amounts of meat and milk products. Among them, some of the finest prosciutto and kajmak (a clotted, fermented heavy cream) are produced on Zlatibor Mountain. This area is characterized by chicken, lamb and veal soups, boiled and roast meats (particularly pork), fried and roasted dishes such as janija (a shallow-fried and then braised beef dish), mutton with potatoes, coarsely chopped fermented cabbage slow-boiled with several types of meat, podvarak (sauerkraut with ribs and/or other meats smothered with a sour cream-based custard and baked), sarma (here, minced meat and rice wrapped in sauerkraut leaves and boiled), kopacki pasulj (beans with meat, ribs and bacon), moussaka (here, baked, layered sliced potato interspersed with pre-fried minced meat), spit-roast lamb, furnance-burnt lamb, pork and veal, lamb or chicken baked under charcoal ash, jellied calf`s head with tripe – an icon of national cuisine from Loznica town – boiled lamb, tobacco crackling (finely shaven pork crackling) and pork hams.

In western Serbia, especially in the area of Zlatibor, beef and pork ham (made with pieces of meat from the back and leg) and Uzice bacon (made with pork bone side without the neck and shoulder musculature without forelegs, back and leg without shank) are traditionally produced. These products are protected by geographical indications and are produced from November to April in artisanal household and industrial conditions throughout the year (*Troeger et al.,* 2007; *Stevanovic et al.,* 2016). In Western Serbia, pork (tobacco) crackling from Valjevo (produced by thermal treatment of adipose tissue and oil-producing parts of the meat, neck and shoulder of pigs) has protected designation of origin. This pork crackling is produced both under household and industrial conditions (*Stevanovic et al.,* 2016) .

Eastern Serbia, with its natural beauty and ecologically preserved areas with ethnic and ethnological variety, is full of culinary surprises. Authentic Danube fish dishes (gap, perch or carp) are characteristic of Smederevo town. Sarma here is prepared with vine leaves and minced meat, while chicken stew comes from the Vlaska region.

In eastern Serbia, Pozeravac sausage has geographical protection. This is fermented sausage with prepared from pork meat and hard fat tissue with 20% beef. The products are produced in industrial and household conditions (*Stevanovic et al.,* 2016).

South Serbian cuisine can be regarded as typical of the Balkan Peninsula, with its influences from Bulgaria, Greece and Turkey. Barbecued (here, meaning charcoal-grilled) minced meat patties from the area around Leskovac are one of the most popular meat products with protected geographical name and origin (*Stevanovic et al.,* 2016). In southern Serbia, dishes such as janija (here, a soup of sheep meat and vegetables), sprza (a kind of young lamb, roasted and with crispy skin) and roasted turkey on banica (a type of turkey pie) are popular.

In the gastronomy of Southwest Serbia, a strong oriental influence in noticeable, which is in accordance with the harsh climatic conditions (cold winters). Beef and sheep meat and sausages named sudzuk are commonly found in local diets.

Gastronomic events in Serbia

Food is a very important part of tourism, and according to the World Trade Organization, is a key factor in the development of the world economy. It provides structured jobs, infrastructure and development. Tourism is currently ranked fourth place in the world economy. It is of particular importance for developing countries, and hence, for Serbia, since it can significantly contribute to economic recovery. An interesting sector of tourism includes specific, sometimes targeted events such as festivals, traditional ceremonies, parades, expositions etc. Such events are global phenomena, and they equalize the attendees (regardless of their status), allow them to indulge, and perhaps even carry them away, even if briefly, from the humdrum and stress of everyday life. One of the basic characteristics of such events is the desire that they remain as they are, providing unique experiences for all visitors. In Serbia, events include some with a long tradition such as Vukov Sabor, going since 1933, and the Guca Trumpet Festival, held annually since 1961. Among the most popular events in 2016 were the Belgrade Beer Fest (900,000 visitors), Guca Trumpet Festival (900,000 visitors) and the Barbecue Festival in Leskovac (500,000 visitors). Clearly, Serbian gastrofestivals can attract great attention from visitors (http://www.turistickisvet.com). Besides the Barbecue Festival, significant events related to meat are the Bacon Festival in Kacarevo, a Smoked Ham event in Mackat, Sausage Festival in Turija and the Valjevo Pork Crackling Festival, as well as many other local and less well-known showcase events (*Baltic et al.*, 2010a).

Food, in the context of the agricultural and food industries is, without doubt, an important resource in Serbia, although its position in the overall economy of Serbia is not high. The local cuisine is one of the most interesting aspects to tourists, often leaving the strongest impression and is also the reason for the re-arrival of many visitors. In Serbia, about one thousand different events are held annually, and in 2016, the Trumpet Festival in Guca and Exit in Novi Sad (fundamentally a music festival) produced income of 2.5 million euros (http://www.turistickisvet.com). Numerous events in Serbia, in which various rural associations participate, have become an interesting resource for the development of rural tourism. In Serbian villages, people still largely consume traditional foods and foster, in simple and harmonious ways, the culture of dining. This leaves a very good impression on tourists. Besides, knowledge and tastes are intimately intertwined. Today's interest in eating locally should enhance a general desire for living in a time and place with no fear, with a feeling of being protected by the known, and consuming foods that have kept their identity. The identity crisis in some European countries is partially linked to the food crises. For example, France, since 1990, has conducted an inventory of all that is being threatened by oblivion, including the country's gastronomic heritage (*Pulen*, 2004 b).

It is a fact that food is not only a theme in private life, but also in media. Television shows promote culinary artristy and world-famous chefs as well as foodie destinations and food production. Culinary stories are of interest to many viewers, regradless of their occupation, social status and gender.

Conclusion

From the time Slavs arrived in the Balkan Peninsula, meat has played an important role in Serbian cuisine. This especially applies to pork meat, which remains the most common type of meat in the diet of Serbian people. Serbian cuisine and and our use of meat has changed under different influences, whether it be from the impact of other national cuisines, or whether these changes resulted from industrialization, especially in the second half of the 20th century. Such changes have affected cuisines worldwide, not just those in Serbia. Globalization and industrialization in the food sector tend to suppress traditional national cuisines. Therefore, practically all countries of the world are making enormous efforts to preserve local culinary traditions as a part of the cultural heritage of each nation. Preserving traditional cuisine has special significance for tourism. In Serbia too, in many different ways, people are actively trying to preserve local culinary traditions, including those around meat production and consumption. This is, among other things, being achieved through the long-time promotion of various festivals dedicated to local culinary traditions for meat and meat products

Disclosure Statement: No pontential conflict of interest was reported by the authors.

References

Baltic, Z. M., Djuric, J., Karabasil, N., Dimitrijevic, M., Markovic, R. & Kilibarda, N., (2010a). Tradicionalni proizvodi od mesa u duhu dobre proizvodjacke prakse, Simpozijum tradicija i buducnost stocarstva u brdsko-planinskom podrucju sa posebnim osvrtom na Sjenicko-pestersku visoravan. *Zbornik radova Sjenica,* 86–107.

Baltic, Z. M., Djuric, J., Karabasil, N., Dimitrijevic, M., Markovic, R., Mirilovic, M. & Pavlicevic, N., (2010c). Istorijski osvrt na proizvodnju mesa u Srbiji, 21. savetovanje veterinara Srbije. *Zbornik referata i kratkih sadrzaja,* 249–259.

Baltic, Z. M., Nedic, D., Djuric, J., Dimitrijevic, M., Karabasil, N. & Kilibarda, N., (2010b). Hrana i vecna briga za zdravlje. *Veterinarski zurnal Republike Srpske X,* (1), 5–9.

Baltic, Z. M., Pecanac, B., Saric, M., Mandic, S., Filipovic, I., Djuric, J. & Dojcinovic, S. (2011). Fermented sausages – products with a tradition. *Veterinary Journal of Republic of Srpska*, *11*, (1), 5–11.

Baltic, Z. M., Djuric, J., Mandic, S., Pecanac, B. & Loncina J., (2013). Consumers and Food Safety. *Glasnik Hemicara, tehnologa i ekologa Republike Srpske,* 11–16.

Boskovic, M., Baltic, Z. M., Ivanovic, J., Dokmanovic, M., Markovic, R., Sarcevic, D., Baltic T. (2015). Uticaj svinjskog mesa na zdravlje ljudi. *Tehnologija mesa, 56,* (1), 8–15.

Colak-Antic, T. (2004). O posluzenju i ponasanju za stolom u Beogradu. Kultura, Beograd, 109/111, 340–350.

Domazet, S. (2004). Knjizevnost i hrana. Kultura, Beograd,109/111, 295–305.

Grujic, J. (1992). Etnografski zapisi Jevrema Grujica. Srpski etnografski zbornik, XCVI, Beograd, 123.

Hrasnica, F., Ivancic, D., Milosavljevic, S., Pavlovic, S., Rako, A. & Smacelj, I. (1964). Specijalno stocarstvo. Zavod za izdavanje udzbenika SR Srbije, Beograd, 229–340.

Ikonic, P., Petrovic, Lj. Tasic, T., Dzinic, N., Jokanovic, M. & Tomovic, V. (2010). Physicochemical, biochemical and sensory properties for the characterisation of Petrovska Klobasa (traditional fermented sausage). *Acta Periodica Technologica*, *41*,19–31.

Ivanovic, J., Baltic, Z. M., Janjic, J., Markovic, R., Baltic, T., Boskovic, M., Djordjevic, J., Mrdovic, B. & Jovanovic, D. (2016). Health aspects of dry-cured ham. *Meat Technology*, *57*, (1), 39–46.

Janjic, J. (2010). Srpska kuhinja – gastronomija Srbije. Panacomp, Beograd, 1–8.

Janjic, J., Lovrenovic, M., Grujic, R., Ivanovic, J., Boskovic, M., Sarcevic, D., Glisic, M., Baltic, Z. M. (2015). Fast food in the diet of adolescent. *Tehnologija mesa,* 56, *(2)*, 154–162.

Marjanovic-Dusanic, S. & Popovic, D. (2004). Privatni zivot u srpskim zemljama srednjeg veka, Klio, Beograd, 139–163.

Markovic, S. (1907). Moj kuvar i savetnik, Beograd.

Novakovic, S. (1913). Meni za novembar-decembar 1913. Arhiv Srbije.

Popovic-Midzina, K. (1877). Veliki srpski kuvar, Novi Sad, 9.

Prosic-Dvornic, M. (2004). Kulturni i drustveni znacaj hrane tradicionalne srpske kulture. Kultura, Beograd, 109/111, 315–339.

Pulen, Z. P. (2004a). Mondijalizacija, izmestanje i premestanje hrane. Kultura, Beograd, 109/111, 121–138.

Pulen, Z. P. (2004b). Izmedju domaceg i ekonomskog: plima i oseka kulinarstva. Kultura, Beograd, 109/111, 150–163.

Rozen, E. (2004). Njegovo velicanstvo, kralj hamburger. Kultura, Beograd, 109/111, 139–149.

Sarcevic, D., Lilic S., Djordjevic, V., Milicevic, D., Vranic D., Lakicevic B., Milijasevic, M. (2011). The role of consumer's perception and attitude in purchasing of meat and meat products. *Tehnologija mesa, 52,* (2), 283–290.

Spremic, M., 2004. Privatni zivot u srpskim zemljama srednjeg veka, Klio, Beograd, 139–163.

Stevanovic, J., Okanovic, Dj., Stevanetic, S., Mirilovic, M., Karabasil, N. & Pupovac, S. (2016). Tradicionalni proizvodi – osnove za odrzivi razvoj proizvoda zivotinjskog porekla u Srbiji. *Food and Feed Research*, *43*, (2), 127–134.

Troeger, K., Dederer, I., Ristic, M., Turubatovic, L., Beric, M. & Stojanovic, A. (2007). Raw cured meat products and raw sausage from Serbia – Quality of traditionally produced products. *Mitteilungsblatt der Fleischforschung Kulumbach, 46,* (175), 11–20.

Vukovic, I., Petrovic, Lj., Vasilev, D. & Saicic, S. (2011b). Mikroflora und Qualitat von nach traditinellem Verfahren herrgestllen Rowursten aus Nordserbien. *Flischwirtschaft, 91*, (11), 118–122.

Vukovic, I., Saicic, S. & Vasilev, D. (2011a). Contribution to knowledge of major quality parameters of traditional (domestic) kulen. *Tehnologija mesa, 52,* (1), 134–139.

Zagorac, D., Bjegovic, J., Majstorovic, M. (2010). Gastronomska mapa Srbije. Zavod za proucavanje kulturnog razvitka Srbije, 1–23.

Zujovic, J. (1986). Dnevnik I. Arhiv Srbije, Beograd, 32.

Paper received: 25.01.2018.
Paper accepted: 23.04.2018.

meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 613.261
ID: 265898252
https://doi.org/10.18485/meattech.2018.59.1.8

Review paper

Being a vegetarian: health benefits and hazards

Svetlana Stanisic[1], Vladan Markovic[1], Sarcevic Danijela[2], Milan Z. Baltic[3], Marija Boskovic[3], Milka Popovic[4], Natasa Kilibarda[1]*

A b s t r a c t: Although their anatomical features disclose the herbivorous nature of humans, an omnivorous diet can be considered an evolutionary advantage that has supported human survival. Over recent decades, vegetarianism has significantly increased in developed countries due to the support of scientific research, emerging supermarkets and restaurants, and easy-to-access soy products and healthy foods. According to current knowledge, vegetarian diets are associated with significantly lower prevalence of non-infectious chronic diseases. However, while the overall risk of cancer is slightly lower in vegetarians, the findings regarding the location and type of cancer that can be prevented by vegetarian diets are inconclusive. In addition to this, it remains unclear whether health benefits of vegetarian diets can be attributed to the avoidance of meat, or to the increased intake of dietary fibre, n-6 fatty acids, vitamins C, B9 and E, potassium, magnesium and phyto-chemicals, or to both of these factors. As a vegetarian diet becomes more restrictive, intake of adequate daily energy and of n-3 fatty acids, essential amino acids, vitamin B12, zinc, calcium and iron becomes more difficult to achieve, which is particularly challenging in children who have higher nutrient requirements relative to body weight than adults.
Keywords: vegetarianism, vegan, pescovegetarian, meat-based diet.

Vegetarianism through time and space

The anatomical features that allow predators to effectively stab and kill their prey include a wide mouth opening, blade-shaped molars for flesh ripping, a massive temporalis muscle and a jaw joint located in the same plane as the teeth. According to evolutionary theory, these anatomical features of carnivores are more primitive than herbivore adaptations, which suggest that herbivores are basically carnivores that adopted significant anatomical modifications consistent with a plant-based diet (*Provenza et al*., 2015). These modifications are reflected in a small oral cavity opening, flat and spade-like incisors for peeling and biting, an herbivore-style jaw joint that is more efficient for crushing and grinding plant tissue, and carbohydrate-digesting enzymes in saliva, all of which are features of modern human anatomy (*Mills*, 1996). However, the most striking anatomical differences between carnivores and herbivores are related to the stomach and intestines (*Danowitz and Solounias*, 2016). A single-chambered stomach, lengthening small intestine and a shrinking distensible colon in humans can be considered as appropriately designed for digestion of soft and pulpy plant food (*Armelagos*, 2014).

Although observations of comparative anatomy disclosing the herbivorous nature of humans have often been often cited as core arguments for vegetarian diets, the fact that some herbivores exhibit anatomical features consistent with carnivorous diets and vice versa clearly suggests that the anatomy of the gastrointestinal tract is not the only feature that predicts food-related behaviour of an animal species (*Ramalanjaona et al*., 2016). Another factor that has had a significant bearing on human food selection throughout history was the struggle for survival in new climatic conditions. In this terms, an omnivorous diet was one of the evolutionary advantages (*Spencer*, 1996).

The term 'vegetarianism' refers to a wide spectrum of dietary patterns characterised by an emphasis on plant foods and avoidance of animal foods. Ovovegetarians consume eggs, lactovegetarian's diet consists of legumes, grains, fruits, nuts and vegetables, together with milk and dairy products, while semivegetarians restrict the type of meat to only fish (pescovegetarian), poultry (pollovegetarian), or both fish and poultry (pesco-pollovegetarians).

[1]*Singidunum University, 32 Danijelova St., 11010 Belgrade, Republic of Serbia;*
[2]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia;*
[3]*University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11000 Belgrade, Republic of Serbia.*
[4]*Institute for Public Health of Vojvodina, Futoska 121, 21000 Novi Sad, Republic of Serbia.*

***Corresponding author:** Svetlana Stanisic, sstanisic@singidunum.ac.rs

Finally, the strictest form of vegetarianism is veganism, and it involves avoidance of all foods of animal origin, including eggs and milk (*Lanham-New*, 2009). The main goal of this review is to briefly summarise current knowledge on health advantages and disadvantages associated with vegetarian diets in order to provide a better understanding of the effects such a specific food choice exerts on human health.

Throughout history, different people have chosen vegetarian dietary patterns for a variety of reasons, including to demarcate important common cultural grounds. The first philosophical arguments for animal food avoidance dating back to ancient Egypt were related to the belief in transmigration i.e. reincarnation of souls. In the centuries that followed, avoidance of animal foods was adopted among ancient Greeks and Indians, while ethical principles supporting a plant-based diet were instilled in the teaching of Pythagoras and Buddha. Nowadays, among a variety of non-religious motivations for adopting a meat-free diet, the most highly ranked are weight loss, potential health benefits, disgust of meat smell and consistency, and moral imperatives to preserve the environment and avoid sacrificing animals (*Fox and Ward*, 2008b). In modern societies, vegetarianism can also be perceived as a cue to individual identity, with certain psychological factors or life events having been shown to provoke sudden rejection of meat (*Fox and Ward*, 2008a). In contrast to multiple ethical justifications for vegetarianism that have their roots in religion and philosophy of ancient civilisations, the scientific evidence for the health effects of vegetarian diets is relatively recent, emerging in the 19th century. The last but not least reason for adopting a plant-based diet is the environmental impact of food production, which remains a matter of scientific debate. In terms of biological diversity and habitat loss, human consumption of animal-based foods/products is estimated to be among the most negative factors affecting the preservation of terrestrial ecosystems (*Machovina et al*., 2015). Some researchers estimate that switching from an animal-based diet to vegetarianism is a feasible tool for climate change mitigation that would contribute to a three-fold reduction of greenhouse gas emissions (*Marlow et al*., 2009), while more recent research suggests that increased intake of fruits, vegetables, dairy and seafood might pose a higher risk for the environment due to relatively high greenhouse gas emissions per calorie produced (*Tom et al.*, 2015). Probably the most feasible explanation is offered in the recent study of *Rosi et al*. (2017), which showed that some vegan diets had greater negative environmental impacts than non-vegetarian diets. This suggests that the individual variability of dietary habits is more important than the type of diet itself.

Vegetarianism is not the only food phenomenon on the rise over recent decades. Namely, the two-fold increase in global meat consumption over the last 50 years has placed a significant burden on the environment, leading to unbalanced diets, particularly in industrialised areas and emerging countries, mainly Brazil and China (*Baltic et al*., 2010a; *Sans and Combris*, 2015; *Odusanya and Atanda, 2018*). Among developing countries, India is a notable exception because a significant proportion of the population (up to 35–40%) follows a traditional vegetarian diet and has done so for many generations (*Ponzio et al*., 2015). The study of *Vranken et al*. (2014) based on data from 120 countries reported an inverted U-shaped relationship between meat consumption and income level, complemented by the remark that at a turning point between income levels of US$32,000 to US$55,000, average meat consumption will stagnate or even decline. In compliance with this, the number of vegetarians is significantly increasing in some of the most affluent countries, although it still comprises a relatively small proportion of the population. Nowadays, approximately 9% of Germans, 8% of Canadians, and 3% of United States, United Kingdom and Australian citizens declare themselves as vegetarians. Apart from income, culture plays an important role in dietary preferences – in traditional societies, milk and meat are rated as more masculine foods than vegetables, and male meat-eaters are perceived as being more masculine than vegetarians. *Rozin et al*. (2012) analysed 23 world languages that use gendered pronouns, and confirmed that meat was associated with the male gender across most of them.

As regards personal characteristics, a typical vegetarian is described as a female of higher social status and academic or vocational qualifications, although these attainments need not be reflected in income. Interestingly, *Gale et al*. (2007) showed that higher IQ at a young age remains a statistically significant predictor of vegetarianism, even after accounting for social class, sex and academic or professional qualifications. On the other hand, although vegetarianism is supported by scientific research, emerging supermarkets and trendy restaurants that offer access to soy products and healthy foods, consumers of restrictive plant-based diets are often confronted with various challenges and social alienation because of their choices and beliefs. As noted by *Menzies and Sheeshka* (2012), five central food values, *i.e.* taste, health, time, cost, and social relationships, have the potential to significantly undermine people's commitment to a vegetarian diet chosen largely for health or moral reasons.

Health benefits of vegetarianism

Vegetarian diets contain high amounts of dietary fibre, n-6 fatty acids, vitamins C, B9 and E, magnesium, potassium, carotenoids, plant sterols and many other phyto-chemicals, which are commonly associated with numerous health benefits. The wide range of antioxidants in a plant-based diet prevents oxidative stress that plays an important role in carcinogenesis and development of endothelial dysfunction, and in the initial steps of pathogenesis in atherosclerosis (*Chauveau et al*., 2013). Unlike other types of vegetarianism, a vegan diet implies lower intake of saturated fatty acids, cholesterol, calcium, vitamin B12 and D, as well as a higher intake of dietary fibre (*Fields et al*., 2016).

To date, a large body of evidence has shown that a vegetarian diet is associated with significantly lower prevalence of overweight and obesity, as well as with a lower risk of cardiovascular hospital admission and 32% less mortality. As concluded by *Crowe et al*. (2013), even after accounting for body mass index, vegetarians remain 28% less likely to develop ischemic heart disease. A recent meta-analysis (*Yokoyama et al*., 2014) examining the relationship between vegetarian diet and blood pressure has shown that a diet excluding meat, but involving regular consumption of dairy products, eggs and fish was associated with 4.8–6.9 mm Hg lower systolic blood pressure, compared to an omnivorous diet. The estimated reduction in blood pressure was associated with 9% decreased risk of death from coronary heart disease and can be equated to the health benefits of a 5 kg weight reduction or a low-sodium diet.

In addition to this, the vast number of studies that have explored the link between plant-based diets and malignant diseases reported the overall risk of cancer is somewhat lower in vegetarians compared to omnivores. However, when it comes to the location and type of cancer that can be prevented by plant-based diets, findings are rather scarce and inconclusive (*Key*, 2017). The prospective cohort study of *Bradbury et al*. (2014) aimed at exploring the associations between fruit, vegetable and/or fibre intake and cancer risk and included more than 500,000 participants from 10 European countries. According to the results, the risk of gastrointestinal tract cancer and liver cancer was inversely associated with excessive consumption of plant-based foods, while for lymphoma, as well as for stomach, cervix, biliary tract, pancreas, prostate, kidney, endometrium and bladder cancer, no significant association was reported between incidence and total intake of fruit, vegetables or fibre. Similarly, *Gilsing et al*. (2016) reported that after accounting for confounding factors, vegetarians, pescovegetarians and 1 day-per-week meat-eaters did not have a reduced risk of postmenopausal breast, lung or prostate cancer compared to those consuming meat on a daily basis.

The largest body of epidemiological data relates to the risk of colorectal cancer and excessive consumption of red and processed meat (*Chauveau et al*., 2013), but the results also turned out to be divergent (*Boskovic and Baltic*, 2016). A recent study (*Michelle et al*., 2015) with 77,659 participants showed that vegetarians have a 22% lower risk of developing all colorectal cancers compared to non-vegetarians with a similar background. Furthermore, the authors emphasised that eating a pescovegetarian diet was associated with the lowest risk of colorectal cancer (a 43% risk reduction compared with omnivores), while the risk of colorectal cancer in semivegetarians (risk reduction of 8%) was closest to the risk that meat consumers face (*Michelle et al*., 2015). A similar conclusion was reported in a meta-analysis and systematic review of prospective cohort studies by *Godos et al*. (2017). According to their findings, the risk of colorectal cancer was lower in the population that consumed a semivegetarian diet (relative risk 0.86) and pescovegetarian diet (relative risk 0.67) when compared to non-vegetarians (*Godos et al*., 2017). However, in contrast to this, *Koushik et al*. (2007) followed 756,217 men and women for 6 to 20 years and showed that excessive intake of fruit and vegetables was not strongly associated with colon cancer risk reduction.

The controversial findings in the current literature are driven by the fact that studies exploring the relationships between diet and health face two main challenges. First, it is difficult to discriminate the specific effects of vegetarian diets from those lifestyle factors that are often associated with vegetarianism, such as lighter body mass index, higher levels of physical activity, and lower prevalences of smoking and alcohol consumption. In addition to this, it remains unclear whether the established health benefits of vegetarian diets can be attributed to the avoidance of red meat, avoidance of processed meat, limited intake of saturated fatty acids and cholesterol, increased intake of fruit, legumes, vegetables, grains nuts, and soya protein-foods, or to all or combinations of these.

Although nutritionally dense, red meat with high fat content and processed meat are often mentioned as risk factors for cancer development. According to the results of a study including 450,000 participants, the overall risk of cardiovascular and malignant mortality increases by 18% for every 50 grams of processed meat per day, due to the presence of carcinogenic nitrosamines, as well as the high content of cholesterol and saturated fats (*Rohrmann et al*., 2013). The core results of the study remained the same after taking into account the level of physical activity, smoking, alcohol consumption and other factors that can confound the relationship between nutrition and morbidity (*Rohrmann et al*., 2013). Furthermore, a positive association between excessive red meat consumption and colorectal cancer was found in a large number of cohort studies (*Bouvard et al*., 2015). In addition to this, high intake of red meat was associated with pancreatic and prostate cancers, while consumption of processed meat was strongly related to stomach cancer (*Bouvard et al*., 2015). In a review of meta-analyses, *Yip et al*. (2018) concluded that 21 morbidity burdens were significantly associated with meat intake, with the highest dose-response for a 50 g increase in processed meat daily intake for oesophageal, stomach and colon cancer, as well as for coronary heart disease and cardiovascular mortality. Furthermore, the highest dose-response for each 65 g increase in red meat daily intake was detected for endometrial, oesophageal and lung cancer.

The abundance of fruits and vegetables in vegetarian diets overlaps with conventionally recommended healthy dietary patterns, which leads us to the conclusion that a significant share of health benefits associated with vegetarianism comes from increased intake of plant-based foods, even though these benefits seem to remain rather restricted to cardiovascular health. The study of *Aune et al*. (2017) showed increasing fruit and vegetable daily intake by 200 g decreased the relative risk of stroke to 0.84, of coronary heart disease to 0.92 and of cancer morbidity to only 0.97. Furthermore, by reviewing the epidemiological evidence on diet and cancer, *Key et al*. (2004) concluded that consumption of fruits and vegetables probably reduces the risk of gastrointestinal cancers located in the oral cavity, oesophagus, stomach and colorectum.

Apart from cancer, high fruit and vegetable intake as well as vegetarian diets have been associated with lower risk of diabetes type II (*Cooper et al*., 2015; *Appleby and Key*, 2016). Furthermore, some authors mention auxiliary impacts of restrictive vegetarian diet on gut health. Namely, *Bauer and Yeh* (2014) showed that vegans displayed a gut microbiota that was most distinct from that of omnivores, but was not always significantly different from that of vegetarians, with a reduced concentration of pathological species and abundance of protective species. The unique gut microbial profile might be a key feature linking veganism with protective health effects.

Health hazards associated with plant-based diets

While higher intake of plant foods and moderate amounts of saturated fatty acids, cholesterol and processed meat can be considered beneficial for health, the existing evidence from cohort studies suggests that the complete elimination of animal foods might not be associated with additional benefits for human health (*Godos et al*., 2017). Meat is a source of biologically valuable proteins, long chain n-3 fatty acids, essential trace elements (iron, copper, manganese, iodine, zinc, selenium), vitamin D and several B vitamins (*Baltic et al*., 2010; *Ivanovic et al*., 2016; *De Smet and Vossen*, 2016). Therefore, the potential drawbacks of vegetarian diets mostly refer to the reduced supply of essential amino acids, n-3 fatty acids, vitamin B12, zinc, iron and calcium (*Petti et al*., 2017).

Vegetarian diets are abundant in n-6 fatty acids (linoleic acid), while lower serum levels of n-3 fatty acids, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are thought to be important for immune, cognitive and cardiac functions, have been reported in vegans (*Pavlicevic et al*., 2014). Plant-derived linolenic acid can be converted to EPA and DHA *in vivo*, but the conversion rate is rather slow and vegan sources of n-3 fatty acids are limited to canola oil, flaxseed and flaxseed oil, and olive oil (*McEvoy et al*., 2012).

Iron deficiency is a cause of anaemia in approximately 30% of the population in wealthy countries, particularly in urban residents and young females. The vegan population exhibits an even higher tendency for anaemia, not because their iron intake is below recommended levels, but because non-haeme iron from plants is less bioavailable and because plant-abundant diets contain substances such as phytic acid and polyphenols/tannin, which can impair mineral absorption.

A vast number of studies aimed at exploring vegan health with respect to vitamin B12 deficiency, since B12 requirements cannot be met without animal-based food intake or supplementation, and in affected people, B12 deficiency and the accompanying haematological symptoms can be mimicked by folic acid intake, which is high in vegan diets (*Baltic et al*., 2010; *McEvoy et al*., 2012). Although plasma levels of vitamin B12 are lower in the entire vegetarian population than in meat-eaters, cases of pronounced vitamin B12 deficiency with subsequent haematological and neurological damage, such as central nervous system demyelination, have been reported only in vegans (*Kapoor et al.*, 2017). This is because followers of less strict vegetarian diets, such as ovolactovegetarians, lactovegetarians and semivegetarians, obtain B12 through consumption of cheese, eggs, milk, and artificially fortified products. Apart from haematological and neurological effects, vitamin B12 deficiency is shown to be associated with atherosclerosis. As reported by *Woo et al*. (2014), low intake of meat, egg or dairy products in poor residents of northern Chinese rural communities and consequent vitamin B12 deficiency have been associated with impaired arterial endothelial function and increased thickness of carotid intima-media.

In addition to vitamin B12 deficiency, plant-eaters who avoid animal-based protein might be lacking several key nutrients, including sulphur amino acids, iron, zinc and omega-3 fatty acids, which can be associated with the elevated blood levels of homocysteine and decreased high-density lipoprotein levels often reported in vegans (*Ingenbleek and McCully*, 2012). In order to meet the daily requirements and decrease vulnerability to atherosclerosis, vegans should be encouraged to take vitamin B12 supplements and consume walnuts as a source of n-3 fatty acids (*Li*, 2011).

The relationship between vegetarian diets and skeletal integrity was a matter of scientific debate due to the fact that it is challenging to distinguish between the effects of diet and certain lifestyle factors (e.g. physical activity, smoking and caffeine intake) on bone health. The EPIC-Oxford study (European Prospective Investigation into Cancer and Nutrition – University in Oxford) performed between 1993 and 1999 showed veganism poses a risk of calcium and vitamin D deficiency, particularly for people living in northern latitudes with low sunlight exposure (*Crowe et al*., 2011). Nevertheless, more recent findings have shown the daily average vitamin D intake of vegans has increased noticeably by almost 12-fold in the last 20 years due to newer dairy replacement products that are typically fortified (*Dagbasi et al*., 2015). Moreover, the lower bone density in people consuming plant-based diets was confirmed, but it cannot be considered as clinically relevant as no significant differences in osteoporotic fracture rates between vegetarians and non-vegetarians were registered (*Chauveau et al*., 2013).

Vegetarianism and vulnerable population categories

One of the conclusions of the EPIC-Oxford study, that recruited more than 65,000 subjects of which approximately 50% were meat-eaters, was that mean energy intake was 14% lower in vegans than in non-vegetarians (*Davey et al*., 2003). While the mean fat intake was similar, the contribution of saturated fats to total energy intake was significantly lower in vegetarians. Moreover, vegetarianism has been shown to precede different eating disorders associated with low energy consumption and increase the risk of developing anorexia nervosa (*Aloufy and Latzer*, 2016).

Despite the aforementioned assumptions and facts, it has been convincingly highlighted that a vegetarian diet can be nutritionally adequate for all stages of the life cycle from infancy to old age (*Chauveau et al*., 2013). While experts claim that vegetarian diets can be adequate and while the majority of vegetarians interpret their nutrition as a transition to a new and healthier nutrition, some studies have shown that some vegetarians attribute their perceived decline in health or well-being to meat avoidance (*Menzies and Sheeshka*, 2012). A healthy diet is designed to provide the body with all essential nutrients and sufficient energy, but as a vegetarian diet becomes more restrictive, adequate daily energy intake becomes more difficult to achieve. Unlike proteins in animal foods, those in plant-based foods are less digestible and are often deficient in one or more essential amino acids, and thus, human requirements for a well-balanced intake of amino acids in vegetarians can be met only if a variety of plant foods is consumed. Furthermore, monotonous vegetarian diets are nutritionally inadequate and without the appropriate monitoring and supplementation can result in severe nutrient deficiencies with detrimental health effects. The study of *Satija et al*. (2017) based on a sample of 166,030 women and 43,259 men found an unhealthful plant-based diet which emphasised consumption of refined grains was linked to an equal risk of coronary heart disease as that of regular animal-based food intake.

As regards infants, study showed that the majority of vegan children grew and developed normally, although they tended to be smaller in stature and lighter in weight than the general population of their age, and their intake of energy, calcium, vitamin D, B2 and B12 were usually below the recommendations (*Di Genova and Guyda*, 2007; *Rogne et al.,* 2017). Ten years ago, an Internet search of the terms 'vegan' and 'children' produced 1,380,000 hits (*Di Genova and Guyda*, 2007), suggesting numerous parents were considering limiting their child/children to the most restrictive type of plant-based diet.

Healthy eating habits in childhood are of vital importance for prevention of under nutrition, growth retardation, and a number of other nutrition-related issues (*Djordjevic et al*., 2016). Due to higher nutrient requirements relative to body weight, vegan children are more likely than adult vegans to face nutritional deficiencies that adversely affect their bone mineral content, growth, as well as their motor and cognitive development. Bulky plant food with a high content of dietary fibre can restrict energy intake in children and lead to lower mean weight (*Katz and Meller*, 2014). Furthermore, vitamin B12 deficiency in toddlers results in severe, long-term megaloblastic anaemia and neurological disorders, including impaired cognitive performance and intelligence, as well as poor spatial ability and short-term memory (*Rogne et al*., 2017).

Conclusions

The current evidence from long-term studies based on large population samples and using methodology that precludes bias and confounding factors is too scarce to resolve the issue of a single best diet, but the weight of evidence strongly supports healthy dietary patterns while allowing for variations in food choice. While the positive effects of a less restrictive vegetarian diet on cardiovascular health are beyond doubt, the data show relatively small differences in overall cancer risk between vegetarians and non-vegetarians. In the absence of direct evidence, claims for the superiority of vegetarianism, and particularly its restrictive forms, are probably exaggerated. However, every diet that contains limited amounts of processed foods, moderate amounts of animal foods, and an abundance of fruit, vegetables and whole cereals will likely be nutritionally adequate, consistent with the current nutritional recommendations, and associated with health promotion and disease prevention.

Disclosure Statement: No potential conflict of interest was reported by the authors.

References

Aloufy, A. & Latzer, Y. (2006). Diet or health – the linkage between vegetarianism and anorexia nervosa. *Harefuah*, *145* (7), 526–31.

Appleby, P. N. & Key, T. J. (2016). The long-term health of vegetarians and vegans. *Proceedings of the Nutrition Society*, *75* (3), 287–293.

Armelagos, G. J. (2014). Brain evolution, the determinates of food choice, and the omnivore's dilemma. *Critical Reviews in Food Science and Nutrition*, *54* (10),1330–1341.

Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N., Norat, T., Greenwood, D. C., Riboli, E., Vatten, L. J. & Tonstad, S. (2017). Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. *International Journal of Epidemiology*, *46* (3), 1029–1056.

Baltic, Z. M., Djuric, J., Karabasil, N., Dimitrijevic, M., Markovic, R., Mirilovic, M., Pavlicevic, N. (2010a). Istorijski osvrt na proizvodnju mesa u Srbiji, 21. Savetovanje veterinara Srbije (sa medjunarodnim ucescem), Zlatibor 15–18 septembar 2010, Zbornik referata i kratkih sadrzaja, 249–259.

Baltic, Z. M., Nedic, D., Djuric, J., Dimitrijevic, M., Karabasil, N., Kilibarda, N. (2010b). Food and everlasting concern about health. *Veterinary Journal of Republika Srpska, 10* (1), 5–10.

Boskovic, M. & Baltic, M. (2016). Association between red meat consumption and cancer risk. *Meat Technology, 57* (2), 81–88.

Bouvard, V., Loomis, D., Guyton, K. Z., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Mattock, H. & Straif, K. (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet Oncology*, *16* (16), 1599–1600.

Bradbury, K. E., Appleby, P. N. & Key, T. J. (2014). Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *The American Journal of Clinical Nutrition*, *100* (suppl_1), 394S-398S.

Chauveau, P., Combe, C., Fouque, D. & Aparicio, M. (2013). Vegetarianism: advantages and drawbacks in patients with chronic kidney diseases. *Journal of Renal Nutrition*, *23* (6), 399–405.

Cooper, A. J., Sharp, S. J., Luben, R. N., Khaw, K. T., Wareham, N. J. & Forouhi, N. G. (2015). The association between a biomarker score for fruit and vegetable intake and incident type 2 diabetes: the EPIC-Norfolk study. *European Journal of Clinical Nutrition*, *69* (4), 449–454.

Crowe, F. L., Appleby, P. N., Travis, R. C. & Key, T. J. (2013). Risk of hospitalization or death from ischemic heart disease among British vegetarians and nonvegetarians: results from the EPIC-Oxford cohort study. *The American Journal of Clinical Nutrition*. *97* (3), 597–603.

Crowe, F. L., Steur, M., Allen, N. E., Appleby, P. N., Travis, R. C. & Key, T. J. (2011). Plasma concentrations of 25-hydroxyvitamin D in meat eaters, fish eaters, vegetarians and vegans: results from the EPIC-Oxford study. *Public Health Nutrition*, *14* (2), 340–346.

Dagbasi, A., Parisi, M., Robertson, M.D. & Tripkovic, L. (2015). An analysis of vitamin D and calcium intakes of 21st century vegans. *The Proceedings of the Nutrition Society*, *74(OCE5)*.

Danowitz, M. & Solounias, N. (2016). Embryology, comparative anatomy, and congenital malformations of the gastrointestinal tract. *Edorium Journal of Anatomy and Embryology*, *3*, 39–50.

Davey, G. K., Spencer, E. A., Appleby, P. N., Allen, N. E., Knox, K. H. & Key, T. J. (2003). EPIC–Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutrition*, *6* (3), 259–268.

De Smet, S. & Vossen, E. (2016). Meat: The balance between nutrition and health. A review. *Meat Science*, *120*, 145–156.

Di Genova, T. & Guyda, H. (2007). Infants and children consuming atypical diets: Vegetarianism and macrobiotics. *Paediatrics and Child Health*, *12* (3), 185–188.

Djordjevic, V., Petronijevic, R., Sarcevic, D., Jankovic, V., Lakicevic, B., Velebit, B., & Ljubojevic, D. (2017). The attitudes and habits of Serbian preschool children in consumption of meat and fish. *Meat Technology, 57* (1), 72–77.

Fields, H., Ruddy, B., Wallace, M. R., Shah, A., Millstine, D. & Marks, L. (2016). How to Monitor and Advise Vegans to Ensure Adequate Nutrient Intake. *The Journal of the American Osteopathic Association*, *116* (2), 96–99.

Fox, N. & Ward, K., (2008a). You are what you eat? Vegetarianism, health and identity. *Social Science & Medicine, 66,* 2585–2595.

Fox, N. & Ward, K., (2008b). Health, ethics and environment: a qualitative study of vegetarian motivations. *Appetite*, *50* (2–3), 422–429.

Gale, C. R., Deary, I. J., Schoon, I. & Batty, G. D. (2007). IQ in childhood and vegetarianism in adulthood: 1970 British cohort study. *British Medical Journal*, *334* (75–87), 245.

Gilsing, A. M. J., Weijenberg, M. P., Goldbohm, R. A., Dagnelie, P. C., Van Den Brandt, P. A. & Schouten, L. J. (2016). Vegetarianism, low meat consumption and the risk of lung, postmenopausal breast and prostate cancer in a population-based cohort study. *European Journal of Clinical Nutrition*, *70* (6), 723–729.

Godos, J., Bella, F., Sciacca, S., Galvano, F. & Grosso, G. (2017). Vegetarianism and breast, colorectal and prostate cancer risk: an overview and meta-analysis of cohort studies. *Journal of Human Nutrition and Dietetics*, *30* (3), 349–359.

Ingenbleek, Y. & McCully, K. S. (2012). Vegetarianism produces subclinical malnutrition, hyperhomocysteinemia and atherogenesis. *Nutrition*, *28* (2), 148–153.

Ivanovic, J., Baltic, M.Z., Janjic, J., Markovic, R., Baltic, T., Boskovic, M., Djordjevic, J., Mrdovic, B. & Jovanovic, D. (2016). Health aspects of dry-cured ham. *Meat Technology*, *57* (1), 39–46.

Kapoor, A., Baig, M., Tunio, S.A., Memon, A.S. & Karmani, H. (2017). Neuropsychiatric and neurological problems among Vitamin B12 deficient young vegetarians. *Neurosciences*, *22*(3), 228–232.

Katz, D. L. & Meller, S. (2014). Can we say what diet is best for health? *Annual Review of Public Health*, *35*, 83–103.

Key, T. J. (2017). Cancer Risk and Vegetarian Diets. In: Vegetarian and Plant-Based Diets in Health and Disease Prevention. Ed. François Mariotti, Elsevier, New York, 345–354.

Key, T. J., Schatzkin, A., Willett, W. C., Allen, N. E., Spencer, E. A. & Travis, R. C. (2004). Diet, nutrition and the prevention of cancer. *Public Health Nutrition*, *7* (1a), 187–200.

Koushik, A., Hunter, D. J., Spiegelman, D., Beeson, W. L., Van Den Brandt, P. A., Buring, J. E., Calle, E. E., Cho, E., Fraser, G. E., Freudenheim, J. L. & Fuchs, C. S. (2007). Fruits, vegetables, and colon cancer risk in a pooled analysis of 14 cohort studies. *Journal of the National Cancer Institute*, *99* (19), 1471–1483.

Lanham-New, S. A. (2009). Is "vegetarianism" a serious risk factor for osteoporotic fracture? *The American Journal of Clinical Nutrition*, *90* (4), 910–911.

Li, D. (2011). Chemistry behind vegetarianism. *Journal of Agricultural and Food Chemistry*, *59* (3), 777–784.

Machovina, B., Feeley, K. J. & Ripple, W. J. (2015). Biodiversity conservation: The key is reducing meat consumption. *Science of the Total Environment*, *536*, 419–431.

Marlow, H. J., Hayes, W. K., Soret, S., Carter, R. L., Schwab, E. R. & Sabate, J. (2009). Diet and the environment: does what you eat matter?. *The American Journal of Clinical Nutrition*, *89* (5), 1699S-1703S.

McEvoy, C. T., Temple, N. & Woodside, J. V. (2012). Vegetarian diets, low-meat diets and health: a review. *Public Health Nutrition*, *15* (12), 2287–2294.

Menzies, K. & Sheeshka, J. (2012). The process of exiting vegetarianism: An exploratory study. *Canadian Journal of Dietetic Practice and Research*, *73* (4), 163–168.

Michelle, S. T., Fishbeck P. S. & Hendrickson C. T. (2015). Energy use, blue water footprint, and greenhouse gas emissions for current food consumption patterns and dietary recommendations in the US. *Environmental Systems & Decisions, 35,* (4) DOI.10.1007/10669–015–9577-y.

Mills, M. R. (1996). The comparative anatomy of eating. (http://www.adaptt.org/documents/Mills%20The%20Comparative%20Anatomy%20of%20Eating1.pdf)

Odusanya, I. A. & Atanda, A. A. (2018). Income-health nexus in sub-Saharan Africa: Evidence from heterogeneous panel models. *The European Journal of Applied Economics*, *15* (1), 94–109.

Pavlicevic, N., Baltic., Z. M., Dimitrijevic M., Karabasil, N., Djordjevic, V., Markovic, R. & Grbic, S. (2014). Polyunsaturated fatty acids in the fish meat and their significance for human health. *Meat Technology, 55* (1), 1–7.

Petti, A., Palmieri, B., Vadalà, M. & Laurino, C. (2017). Vegetarianism and veganism: not only benefits but also gaps. A review. *Progress in Nutrition*, *19* (3), 229–242.

Ponzio, E., Mazzarini, G., Gasperi, G., Bottoni, M.C. & Vallorani, S. (2015). The vegetarian habit in Italy: Prevalence and characteristics of consumers. *Ecology of Food and Nutrition*, *54* (4), 370–379.

Provenza, F.D., Meuret, M. & Gregorini, P. (2015). Our landscapes, our livestock, ourselves: Restoring broken linkages among plants, herbivores, and humans with diets that nourish and satiate. *Appetite*, *95*, 500–519.

Ramalanjaona, B. J., Sorrento, C., Pagano, A. S. & Marquez, S. (2016). "You are what you eat" OR "You eat what you are?" Comparative Mammalian Gastrointestinal Anatomy. *The FASEB Journal*, *30 (1 Supplement)*, 1044–1046.

Rogne, T., Tielemans, M. J., Chong, M. F., Yajnik, C. S., Krishnaveni, G. V., Poston, L., Jaddoe, V. W., Steegers, E. A., Joshi, S., Chong, Y. S., Godfrey K. M., Yap, F., Yahyaoui, R., Thomas, T., Hay, G., Hogeveen, M., Demir, A., Saravanan, P., Skovlund, E., Martinussen, M. P., Jacobsen, G. W., Franco O. H., Bracken, M. B. & Risnes K. R. (2017). Associations of maternal vitamin B12 concentration in pregnancy with the risks of preterm birth and low birth weight: A systematic review and meta-analysis of individual participant data. *American Journal of Epidemiology*, *185* (3), 212–223.

Rohrmann, S., Overvad, K., Bueno-de-Mesquita, H. B., Jakobsen, M. U., Egeberg, R., Tjonneland, A., Nailler, L., Boutron-Ruault, M., Clavel-Chapelon, F., Krogh, V., Palli, D., Panico, S., Tumino, R., Ricceri, F., Bergmann, M. M., Boeing, H., Li, K., Kaaks, R., Khaw, K., Wareham, N. J., Crowe, F. L., Key, T. J., Naska, A., Trichopoulou, A., Trichopoulos, D., Leenders, M., Peeters, P. H. M., Engeset, D., Parr, C. L., Skeie, G., Jakszyn, P., Sanchez, M., Huerta, J. M., Redondo, M. L., Barricarte, A., Amiano, P., Drake, I., Sonestedt, E., Hallmans, G., Johansson, I., Fedirko, V., Romieux, I., Ferrari, P., Norat, T., Vergnau, A. C., Riboli, E. & Linseisen, J. (2013). Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. *BMC Medicine*, *11* (1)*,* 63.

Rosi, A., Mena, P., Pellegrini, N., Turroni, S., Neviani, E., Ferrocino, I., Di Cagno, R., Ruini, L., Ciati, R., Angelino, D., Maddock, J., Gobbetti, M., Brighenti, F., Del Rio, D. & Scazzina, F. (2017). Environmental impact of omnivorous, ovo-lacto-vegetarian, and vegan diet. *Scientific Reports*, *7* (1), 6105.

Rozin, P., Hormes, J. M., Faith, M. S. & Wansink, B. (2012). Is meat male? A quantitative multi-method framework to establish metaphoric relationships. *Journal of Consumer Research*, *39* (3), 629–643.

Sans, P. & Combris, P. (2015). World meat consumption patterns: An overview of the last fifty years (1961–2011). *Meat Science*, *109*, 106–111.

Satija, A., Bhupathiraju, S.N., Spiegelman, D., Chiuve, S.E., Manson, J. E., Willett, W., Rexrode, K. M., Rimm, E. B.& Hu, F. B. (2017). Healthful and unhealthful plant-based diets and the risk of coronary heart disease in U.S. adults. *Journal of the American College of Cardiology, 70* (4),411–422.

Spencer, C. (1996). The heretic's feast: A history of vegetarianism. UPNE, Lebanon, New Hampshire, USA.

Tom, M. S., Fischbeck, P. S. & Hendrickson, C. T. (2015). Energy use, blue water footprint, and greenhouse gas emissions for current food consumption patterns and dietary recommendations in the US. *Environment Systems and Decisions*, *36* (1), 92–103.

Vranken, L., Avermaete, T., Petalios, D. & Mathijs, E. (2014). Curbing global meat consumption: emerging evidence of a second nutrition transition. *Environmental Science and Policy*, *39*, 95–106.

Woo, K. S., Kwok, T. C. & Celermajer, D. S. (2014). Vegan diet, subnormal vitamin B-12 status and cardiovascular health. *Nutrients*, *6* (8), 3259–3273.

Yip, C. S. C., Lam, W. & Fielding, R. (2018). A summary of meat intakes and health burdens. *European Journal of Clinical Nutrition*, *72* (1), 18–29.

Yokoyama, Y., Nishimura, K., Barnard, N.D., Takegami, M., Watanabe, M., Sekikawa, A., Okamura, T. & Miyamoto, Y. (2014). Vegetarian diets and blood pressure: a meta-analysis. *JAMA Internal Medicine*, *174* (4), 577–587.

Paper received: 3.05.2018.
Paper accepted: 15.05.2018.

Guidelines for Authors

Meat Technology (ISSN 0494-9846) is a scientific journal that publishes:

Original scientific papers (papers which present previously unpublished results of authors' own investigations using scientific methodology);

Review papers (papers which include original, detailed and critical overview of a research problem or an area to which the author has significantly contributed, as evidenced by auto citations);

Brief or preliminary papers (full-format original scientific papers or of preliminary character);

Reviews (of books, scientific conferences etc.)

Eligible for publishing are those papers, which have not been previously published, presented or considered for publication in another journal, except as abstracts presented at scientific conferences. The first author is both responsible for meeting these criteria and for obtaining agreement to publish from all of the co-authors.

Procedure

Papers are subject to anonymous reviews (two at least), while the decision to accept the paper for publishing is reached by the editor-in-chief, together with subeditors and the members of the editorial board.

Papers should be submit on: www.journalmeattechnology.com or via mail: danijela.sarcevic@inmes.rs.

Accepted papers are subject to proofreading. The editorial board reserves the right to minor corrections of the manuscript. Where major corrections are necessary, the first author will be notified, and the paper sent for revision, with a set deadline. After all corrections, authors are requested to submit a *Statement by authors* via email to danijela.sarcevic@inmes.rs.

Language

Papers must be written in English (British English spelling). The text should be clear, concise and grammatically correct.

Preparation and submission of manuscripts

The paper should be edited in Microsoft Word software, using Times New Roman font, size 12 pt, line spacing 1.5 and margins of 2 cm. Papers are submitted in electronic form by email: danijelas@inmes.com or institute@inmes.com.

All papers must contain the following (Title, Author line, Affiliation, Corresponding Author, Abstract):

Title In lowercase, bold, font size 14 pt, the title is descriptive of the study.

Author line Below the title, list the names of the authors (first name, last name, lowercase, italic, font size 12 pt). Numbers following names in superscript refer to the authors' institutions.

Affiliation At the bottom of the first page, include authors' affiliations according to the numbers in superscript. The names and addresses of the institutions authors are affiliated to should be given (italics, font size 10 pt, the main words capitalized).

Corresponding Author In a new line immediately below the Affiliation, the name and e-mail of the corresponding author should be provided (font size 12 pt, not italicised).

Abstract This should contain 150-250 words with a maximum of five key words below, separated by commas (both abstract and key words are in italics, font size 12 pt). The abstract should be typed below the title and author line.

Papers belonging to a category other than original scientific papers can contain sections titled by choice of the author. Original scientific papers should contain the following sections in this order: Introduction, Materials and Methods, Results and Discussion (combined or separate), Conclusion, Acknowledgement (optional) and References. Section titles are typed in lowercase with main words capitalised, font size 12 pt, bold.

Introduction This should contain a clear description of the investigated subject and aim of the research with short citations of the relevant literature (usually not more than 10 years old);

Materials and Methods This section describes materials and methods used and outlines the design of the experiment;

Results and Discussion The results should be processed by statistical methods appropriate to the study; they should be clear and concise using tables, graphs, photographs, illustrations and other. The same result(s) must not be presented in both table and graph. Discussion must be related to the results presented, avoiding repetitions of already stated facts, and using comparison of obtained results and relevant literature data related to similar groups of products, comparable analytical methods etc.

Numbers in-text and in tables/figures. Use decimal points, not decimal commas. Avoid starting and ending sentences with numbers (re-write the sentence).

Figures are numbered consecutively with cardinal Arabic numerals (Figure 1; Figure 2; Figure 3 etc.). Each figure is referred to in the text using consecutive cardinal Arabic numbers. Figures can be graphs, illustrations, flow diagrams, photographs, maps etc. Figure titles are placed below the figures, centre aligned (in sentence case with the figure number in bold, a full-stop after the figure number, the first word of the title capitalised). Figures and tables are submitted separately, in an appendix.

Tables are numbered consecutively with cardinal Arabic numerals (Table 1; Table 2; Table 3 etc.). Each table is referred to in the text using consecutive cardinal Arabic numerals. Table titles are placed above the tables, centre aligned (in sentence case with the table number in bold, a full-stop after the table number, the first word in the title capitalised). Tables have only three full horizontal lines, one at the top, one at the bottom and one under the column headings. Use superscript letters for table footnotes. Tables should be fully understandable without reference to the text. Figures and tables are submitted separately, in an appendix.

If tables or figures originate from other sources, the author is required to state the source of such data (author, year of publication, journal etc.). Notes should be placed at the bottom of the page containing cited material.

The author should apply the International System of Units (SI system) and current regulation on measuring units and measuring instruments. Symbols for units derived by division are given as negative exponents, e.g. 10 g L^{-1}; 250 V cm^{-2}.

Common abbreviations:

- CFU colony forming units, capitalised, common and so is never spelled out
- kg kilogram, common and so is never spelled out
- L litre, common and so is never spelled out
- Longissimus dorsi (LD) is redundant and so is not used. For the whole muscle, use Longissimus thoracis et lumborum (LTL). The correct terms for the two parts of this muscle are Longissimus thoracis (LT) or Longissimus lumborum (LL).
- mL millilitre, common and so is never spelled out
- µm micrometre, common and so is never spelled out
- mol mole, common and so is never spelled out
- M molar, common and so is never spelled out
- PCR polymerase chain reaction, common and so is never spelled out
- SD standard deviation, capitalised, common and so is never spelled out
- SE standard error, capitalised, common and so is never spelled out
- sp. species (singular), common and so is never spelled out (not capitalised, full-stop)
- spp. species (plural), common and so is never spelled out (not capitalised, full-stop)
- UV ultraviolet, capitalised, common and so is never spelled out
- aw water activity
- h hour(s)
- min minute(s)
- 25°C (no gap after the numeral)
- 20±1°C (no gaps between numbers, sign and unit in-text and in tables/figures)
- p<0.05, p<0.01 (not italicised, not capitalised, no gaps)
- n=120 (no gaps between the letter, sign and numerals in-text and in tables/figures)
- found in 20.05% of cats…(no gap in-text)

Conclusion This section provides a review of the most important facts obtained during the research.

Acknowledgement This should contain the number of the project i.e. title of the program under which the research was conducted, as well as the name of the institution that funded the project or program. The acknowledgement is written after the conclusion, before the references.

In-text references

Each publication cited in the text must be listed in References. The citations in the text need to be arranged in the following way:

If there is only one author of the cited paper, the author's surname and the year of publication is stated in the brackets (*Thomas and Brooks*, 2008). In case the same author has more publications in the same year, additional letters are added next to the year (*Thomas and Brooks*, 2008a; *Thomas and Brooks*, 2008b).

If there are two authors of the publication, surnames of authors and year of publication is written in the brackets (*Thomas and Fenwick*, 2008).

If there are three or more authors, the surname of the first author is stated in the brackets, followed by abbreviation "et al." and year of publication (*Thomas et al*., 2008).

If multiple references are cited within the same brackets, citations should be in chronological order, and then in alphabetical order if necessary, e.g. (*ISO*, 1995; *Devine et al*., 2001; *Abayas and Zhao*, 2012). These citations are separated with semi-colons.

Citations which are in the text but are not within brackets are not in italics.

References

The reference list (Times New Roman font size 12 pt) should include recent international publications. If the original literature cited is not available, the authors should quote the source used. The references should be sorted in alphabetical order and should be cited exactly the way they appear in the original publication. Sources and volume numbers are italicised, but issue numbers are not in italics.

Examples:

Journals:

Givens, D. I., Kliem, K. E. & Gibbs, R. A. (2006). The role of meat as a source of n3 polyunsaturated fatty acids in the human diet. *Meat Science*, *74* (1), 209218.

Books:

Bao, Y. & Fenwick, R. (2004). Phytochemicals in health and disease, CRC Press, Los Angeles, USA.

Books with authored chapters:

Marasas, W. F. O. (1996). Fumonisins: History, worldwide occurrence and impact. In: Fumonisins in food, advances in experimental medicine and biology. Eds. L. S. Jackson, J. W. DeVries & L. B. Bullerman, Plenum Press, New York, pp. 118.

PhD and MSc theses:

Radeka, S. (2005). Grape mash maceration and varietal aroma of Malvazija istarska wine, PhD Thesis, Faculty of Agriculture, University of Zagreb, Croatia.

Laws, regulations, decrees:

Serbia. (2010). Regulation on general and special conditions of hygiene of food at any stage of production, processing and transport. *Official Gazette of the Republic of Serbia*, 72.

European Union. (2013). **Commission regulation (EU) No 1019/2013 of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 as regards histamine in fishery products. *L 282*, 46–47.**

Symposiums, Congresses:

Harvey, J. (1992). Changing waste protein from a waste disposal problem to a valuable feed protein source: a role for enzymes in processing offal, feathers and dead birds. Alltech's 8th Annual Symposium, Nichdasville, Kentucky, Proceedings, 109−119.

Citations with organisations as authors:

Food and Drug Administration. (1995). Decomposition and histamine-raw frozen tuna and mahi-mahi; canned tuna; and related species; availability of revised compliance policy guide, Federal Registration, *60*, (1), 39754–39756.

European Food Safety Authority. (2011). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal 9* (10), 2393.

Software:

STATISTICA (Data Analysis Software System) (2006). v.7.1., StatSoft, Inc., USA (www.statsoft.com).

Websites:

Technical report on the Food Standards Agency project G010008 (2002). Evaluating the risks associated with using GMOs in human foods, University of Newcastle, UK (http://www.foodsafetynetwork.ca/gmo/gmnewcastlereport.pdf).

LIST OF REVIEWERS

As an Editor in chief of scientific journal “Meat Technology”, I would like to express my gratitude to professors, scientists and researchers for their contribution of reviewing in our journal. In this volume we present the list of reviewers.

Milan Z. Baltic, PhD
University in Belgrade, Faculty of Veterinary Medicine, Departman of Hygiene and Technology of Animal Origin

Iva Steinhauserova, PhD
Faculty of Veterinary Hygiene and Ecology, Departman of Meat Hygiene and Technology, Brno, Czech Republic

Andrej Kirbis, PhD
Univeristy in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Republic of Slovenia

Igor Tomasevic, PhD
University in Belgrade, Faculty of Agriculture, Department for Technology of Animal Products, Belgrade, Republic of Serbia

Vesna Z. Djordjevic, PhD
Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

Tomaz Polak, PhD
University in Ljubljana, Faculty of Biotechnology, Department of Meat Technology and Food Risk, Ljubljana, Republic of Slovenia

Sava Buncic, PhD
University of Novi Sad, Faculty of Agriculture, Department of Veterinary medicine, Novi Sad, Republic of Serbia

Olgica Ceric, PhD
Food and Drug Administartion – FDA, Veterinary Laboratory Investigation and Response Network, New Hampshire, USA

Luca Cocolin, PhD
Università degli Studi di Torino, Faculty of Agriculture, Department of Agricultural, Forest and Food Sciences, Torin, Italy

Galia Zamaratskia, PhD
Swedish University of Agricultural Science, Department of Food Sceinece, Uppsala, Sweden

Lea Demsar, PhD
University in Ljubljana, Faculty of Biotechnology, Department of Meat Technology and Food Risk, Ljubljana, Republic of Slovenia

Aurelija Spiric, PhD
Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

Antonia Ricci, PhD
National Laboratory for *Salmonella,* Departman for Food Safety, Risk Analysis/OIE Referential Laboratory for *Salmonella*, Padua, Italy

Irina Tchernukha, PhD
The Gorbatov’s All Russian Meat Research Institute, Moscow, Russia

Vlado Teodorovic, PhD
University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

Olivera Djuragic, PhD
Institute for Food Technology, Novi Sad, Republic of Serbia

Dragan Momcilovic, PhD
Food and Drug Administartion – FDA, Centrs for Veterinary Medicine, Rockville, USA

Tomas Alter, PhD
Faculty of Veterinary Medicine, Institute of Meat Hygiene and Technology, Institute of Food and Milk Hygiene, Berlin, Germany

Lidija Peric, PhD
University of Novi Sad, Faculty of Agriculture, Novi Sad, Republic of Serbia

Marija Jokanovic, PhD
University in Novi Sad, Faculty of Technological Science, Novi Sad, Republic of Serbia

Petrovic Jelena, PhD
Scientific Institute for Veterinary medicine, Novi Sad, Republic of Serbia

Zorica Jugovic-Knezevic, PhD
University in Belgrade, Faculty of Technological Science, Belgrade, Republic of Serbia

Breda Jakovec-Strajn, PhD
University in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Slovenia

Rebeka Garsija, PhD
Animal Plant Health Agency, Dartford, United Kingdom

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

664.9

MEAT technology : scientific journal / editor in chief Vesna Z. Djordjevic. - Vol. 57, No. 1 (2016) - . - Belgrade : Institute of Meat Hygiene and Technology, 2016- (Beograd : Naučna KMD). - 30 cm

Dva puta godišnje. - Je nastavak:
Tehnologija mesa = ISSN 0494-9846
ISSN 2466-4812 = Meat technology (Belgrade)
COBISS.SR-ID 225196812