SCIENTIFIC JOURNAL

eISSN 2560-4295
UDK 664.9

meat technology

65 Vol. 01 No. 2024 Belgrade

Founder and Publisher
Institute of Meat Hygiene
and Technology

"Meat Technology" is the scientific journal, founded in year 1960, 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 SCOPUS, CABI Database, EBSCO publishing, DOAJ, AGRIS, www.ifocus.my 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).

Founded in 1960.

FOUNDER AND PUBLISHER

Institute of Meat Hygiene and Technology

11000 Belgrade, Kaćanskog 13

P.O. Box 33-49

Phone 381 11 2650-655

e-mail: institut@inmes.rs

www.inmes.rs

DIRECTOR

Vesna Z. Đorđević, PhD

EDITOR IN CHIEF

Vesna Z. Đorđević, PhD

PROOFREADER FOR ENGLISH LANGUAGE

Sheryl Avery, PhD

TECHNICAL EDITION

Danijela Šarčević, PhD

Slaviša Šobot

COMPUTER PROCESSING AND PRINTING

Naučna KMD – Beograd

CIRCULATION

100 copies

PUBLICATION OF THIS JOURNAL IS FINNANCIALLY SUPPORTED BY:

Ministry of Science, Technological Development and Innovations of the Republic of Serbia

www.journalmeattechnology.com

Honorable Editors

Editorial Board Members

Advisory Board Members

Subeditors from Institute of Meat Hygiene and Technology

eISSN 2560-4295
UDK 664.9

INMES
www.inmes.rs

meat technology
scientific journal

Meat Technology Vol. 65 No. 1 P. 1–80 Belgrade 2024

CONTENTS

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

UDK: 637.053
ID: 149439497
https://doi.org/10.18485/meattech.2024.65.1.1

Original scientific paper

Determination of macro- and microelements in mechanically separated meats from different countries of origin and used in the Serbian meat industry

Jasna Đinović-Stojanović[1], Ivana Brankovic Lazić[1], Zoran Petrović[1], Srđan Stefanović[1], Nikola Borjan[1], Munevera Begić[2] and Saša Janković[1]*

[1] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[2] *Faculty of Agriculture and Food Science, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina*

ARTICLE INFO

Keywords:
Mechanically separated meat
Deboned poultry meat
Elements
EFSA opinion
Serbian meat industry

ABSTRACT

According to the European Food Safety Authority (EFSA), calcium (Ca) content is one of the major control parameters for mechanically separated meat (MSM), as this element is an indicator of residual bone in the product. In the current study, the levels of Ca, magnesium (Mg), potassium (K), iron (Fe), copper (Cu) and zinc (Zn) in MSM from different countries (Serbia, Croatia, Bosnia and Herzegovina, France, North Macedonia, Sweden, Denmark and Germany) were determined. Samples were gathered from different meat processing facilities in Serbia. The levels of the six elements were determined by inductively coupled plasma mass spectrometry (ICP-MS). The distribution of the elements in MSMs was examined by applying principal component analysis (PCA). The quality of the MSMs in relation to the Ca content was compared in line both with the Serbian and EU legislation. Furthermore, control of Ca in MSM as well as control of conditions during the process of machine separation meat from bones tissues or from poultry carcasses is necessary to avoid the intake of bones particles in MSM and consequently in meat products.

1. Introduction

Although the production of meat is increasing all over the world, especially in developing countries, the International Agency for Research on Cancer discouraged large consumption of meat and meat products (*IARC*, 2015). From the scientific point of view, special attention should be focused on a special type of meat product, mechanically separated meat (MSM), which is widely used in the meat industry. According to *Regulation (EC) No 853/2004* (2004), MSM is obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means *(Regulation EC,* 2004). Due to its high nutritional value and low cost, MSM enables the production of multi-component products from raw material consisting of protein-rich meat mince from animal carcasses (*Field,* 1981). These meat products have good commercial properties, long shelf life and acceptable price. In order to exclude potential food fraud in the meat industry (*Spink et al.,* 2019), the use of poultry MSM in products should be subject to declaration.

With respect to the increasing use of MSM in the meat industry and the high meat consumption pattern in Serbia, modern consumers in this country have increasing interest in meat quality and safety, especially in relation to their health. According to the European Food Safety Authority (*EFSA, 2013*), calcium (Ca) and total ash content are control parameters for MSM, being indicators of residual bone. The Ca content of MSM is a common indicator of elevated bone content due to the separation

***Corresponding author:** Jasna Djinović-Stojanović, jasna.djinovic@inmes.rs

Paper received: July 2nd 2024. Paper accepted: July 8th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

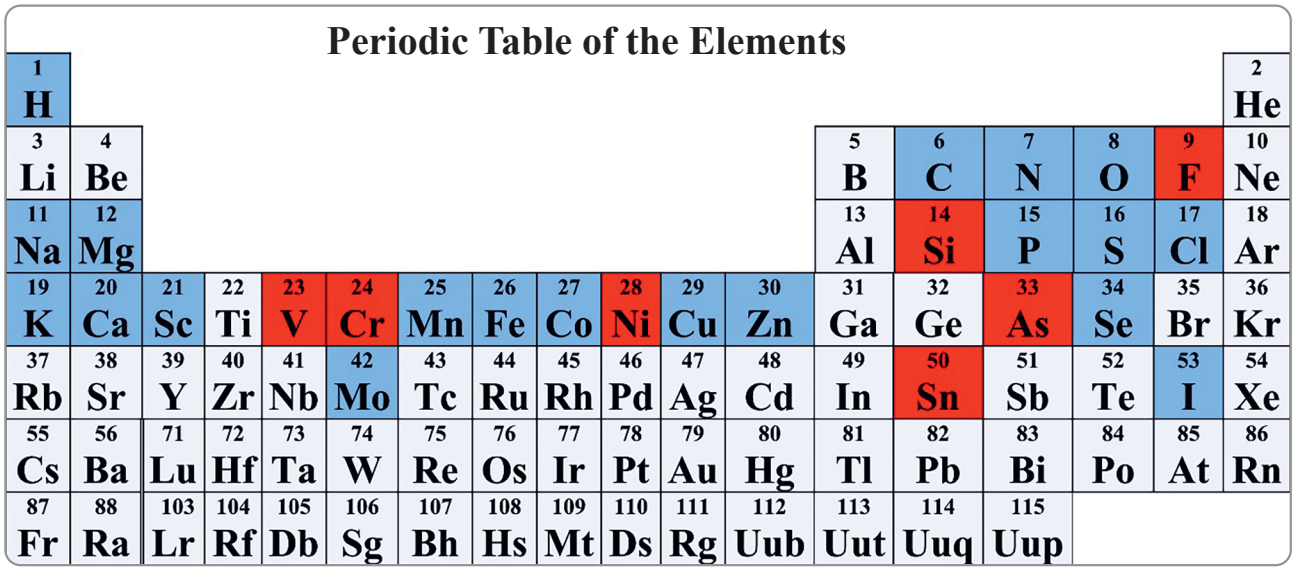

Figure 1. Periodic table highlighting the elements that are essential for life. Essential elements for humans are shown on a blue background, suggested essential elements for humans are on a red background, and nonessential elements for humans are on a grey background (Ali, 2023).

process, and determination of Ca is recommended as the only appropriate chemical parameter which can be used to distinguish MSM from non-MSM products (*EFSA,* 2013). However, according to literature data, Ca content alone does not reliably distinguish low-pressure MSM from minced meat products (*Wubshet et al*., 2019). Detection reliability can be improved by measuring some other elements, like magnesium (Mg), potassium (K) and iron (Fe), which can be significant markers in distinguishing

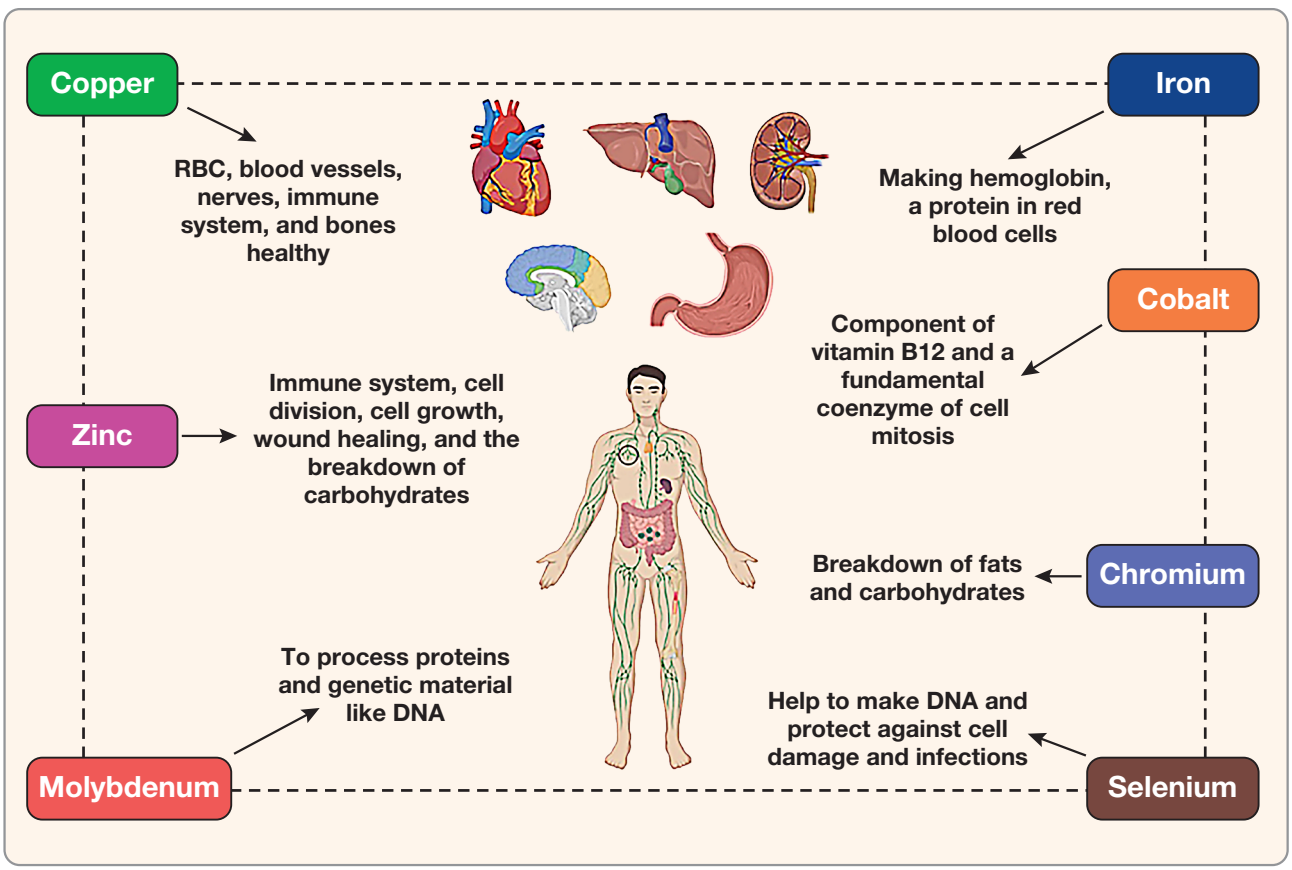

Figure 2. Role of microelements (trace elements) in human health (*Islam et al., 2023*).

MSM from fresh meat (*Dalipi et al*., 2018; *Iammarino et al.*, 2021). Hence, besides the Ca content, from the consumers' point of view, it is useful to determine the contents of some other macro and microelements in MSM. It is well known that some elements are essential for life (Figure 1). Nevertheless *Shyichuk et al.* (2023) indicate Ca content as one of the best indicators of the quality of meat products. This is because meat products often contain other Ca-rich additives, such as chicken fat (Ca content of 150–400 ppm), whey protein (~470 ppm), soy protein (~1780 ppm), articular cartilage (~3800 ppm), milk powder (~9100 ppm). Thus, a high Ca content in a meat product indicates a high amount of non-meat additives (*Shyichuk et al.* 2023).

Numerous trace elements are necessary for the body to continue functioning properly (*Islam et al.,* 2023; *Ali,* 2023*)*. These microelements are essential for many physiological functions, including hormone formation, heartbeat regulation and the formation of blood and bone (Figure 2).

However, the literature data (*Wada,* 2004; *Ali*, 2023) indicate there are some medical conditions and chronic and hereditary diseases caused by deficiency or excess of some macro- and microelements (Tables 1 and 2).

Table 1. Deficiency and excess of macroelements in some medical conditions (Ali, 2023).

Macroelement	Deficiency	Excess
Calcium (Ca^{2+})	• Osteoporosis • Kidney failure • Osteopaenia • Renal disease • Hypoparathyroidism • Fanconi syndrome	• Tuberculosis • Sarcoidosis • Thyroid disease • Chronic kidney disease • Adrenal gland disease
Potassium (K^{+})	• Adrenal gland disorders • Chronic kidney disease • Blood pressure • Liddle syndrome	• Kidney failure • Diabetes • Addison's disease
Magnesium (Mg^{2+})	• Diabetes • Coeliac disease	• Kidney failure • Thyroid problems

Table 2. Deficiency and excess of microelements in some medical conditions (Wada, 2004).

Microelement	Deficiency	Excess
Zinc (Zn^{2+})	*Congenital:* • Acrodermatitis enteropathica *Acquired:* • High-calorie parenteral therapy, enteral nutrition, drugs (chelating agents), inadequate intake	*Acquired:* • Zn fume fever • Zn poisoning
Iron ($Fe^{2+/3+}$)	*Congenital:* • Atransferrinemia *Acquired:* • Iron-deficiency anaemia	*Congenital:* • Haemochromatosis *Acquired:* • Iron poisoning
Copper ($Cu^{1+/2+}$)	*Congenital:* • Menkes disease • Aceruloplasminemia *Acquired:* • High-calorie parenteral therapy • Enteral nutrition	*Congenital:* • Wilson's disease *Acquired:* • Copper fume fever • Copper poisoning • Parkinson's disease

Presently, to the best of our knowledge, there is very little authentic and scientific information available on the content of Ca and other macro- and microelements in MSM used by the meat industry in Serbia (*Tasić et al.,* 2017). Monitoring of MSM is important because it is used in the composition of some meat products that must fulfil the Serbian regulation on minced meat, semi-produced meat and meat products (*Official Gazette RS, 50/*2019 and *34/*2023). Moreover, such data is necessary for future studies on the total dietary intake of specific elements by the Serbian population, and considering the fact that MSM is used in the production of boiled meat products, commonly consumed in Serbia.

Looking at this context, the aim of this paper was to determine the contents of six element (Ca, Mg, K, Fe, copper (Cu) and zinc (Zn)) in MSM originating from different countries, gathered from different meat processing facilities in Serbia. The distribution of the elements in the analysed MSMs was determined by applying principal component analysis (PCA). In addition, the quality of the MSMs in relation to their Ca content was assessed in line with both the Serbian and EU legislation.

2. Materials and Methods

Sample collection

A total of 88 poultry MSM samples from different countries of origin (Serbia, Croatia, Bosnia and Herzegovina, France, North Macedonia, Sweden, Denmark and Germany), were collected in different meat processing facilities in Serbia during 2022. After collection, the MSMs were labelled and stored in polyethylene bags and frozen at −18 °C. Frozen MSMs were thawed at 4 °C and homogenized, then approximately 0.5 g (wet weight) of sample was mineralized by adding 5 mL of nitric acid (67–70%, TraceMetal grade, Fisher Chemical, Belgium) and 5 mL deionized water, purity of 0.067 µS/cm, produced by a Purelab DV35 water purification system (ELGA, Buckinghamshire, UK). Microwave assisted digestion was performed in a MARS 6 iWawe Microwave Digestion System (CEM Technology, USA). After cooling at room temperature, the digests were quantitatively transferred into polypropylene volumetric flasks and diluted to 100 mL with deionized water.

Sample preparation and reagents

Analysis of the following six elements, Ca, K, Mg, Fe, Zn and Cu, was performed by inductively coupled plasma mass spectrometry (ICP-MS) (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). The most abundant isotopes were used for quantification. Operating conditions of the ICP-MS system were: RF power (1550 W); cooling gas flow (14 L min^{-1}); nebulizer flow (1 L min^{-1}); collision gas flow (1 mL min^{-1}); operating mode (Kinetic Energy Discrimination); dwell time (10 ms).

Standards

Standard stock solutions of each element (Ca, K, Mg, Fe, Zn and Cu) were obtained from CPA Chem (Stara Zagora, Bulgaria). The purity of the starting material in standards was 99.999% for each element. For qualitative analysis of the samples, a five-point calibration curve (including zero) was constructed for the ^{44}Ca, ^{39}K, ^{24}Mg, ^{57}Fe, ^{66}Zn and ^{63}Cu isotopes.

Statistical analysis

Statistical analysis of experimental data was performed using software Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). One-way analysis of variance (ANOVA) and Tukey's HSD test for comparison of means were used to analyse differences in the elements' levels in MSMs from the different countries. PCA was used to group the observed samples and to discover any possible correlations among the element levels.

3. Results and Discussion

The contents in the MSMs of the six elements (Ca, Mg, K, Fe, Cu and Zn), expressed in terms of mean, standard deviation (SD), median and range, are presented in Table 3.

According to both Serbian regulation (*Official Gazette RS. 50/2019 and 34/2023*) and the European Food Safety Authority (*EFSA,* 2013), the maximum permitted Ca level in MSM is 1000 mg/kg (100 mg/100 g). In this study, the highest mean Ca level was established in MSMs from Sweden (1115.65 mg/kg), but this was not significantly higher than the mean Ca levels in MSMs from other countries ($p > 0.05$). The mean Ca levels from

Table 3. Levels (mg/kg) of six selected elements (Ca, Mg, K, Fe, Cu and Zn) in mechanically separated meats (MSMs) from different countries

	Element levels (mg/kg)					
Country of MSM origin	**Ca**	**Mg**	**K**	**Fe**	**Cu**	**Zn**
Serbia, n=27						
Mean	888.00 [a]	198.66 [a,b]	2594.76 [a,b]	17.02 [a,b]	0.48 [a]	13.31 [a,b]
SD	620.77	29.64	348.91	3.60	0.14	2.81
Median	684.77	194.88	2487.03	17.01	0.46	12.69
Min	304.88	127.35	1868.04	9.67	0.28	8.36
Max	2852.21	273.75	3450.80	28.69	0.92	19.59
Croatia, n=9						
Mean	532.50 [a]	228.56 [b]	2928.76 [b]	13.88 [a]	0.50 [a]	12.52 [a]
SD	278.14	43.09	465.17	4.10	0.14	2.60
Median	716.36	198.62	2482.41	16.40	0.46	11.97
min	175.69	155.45	2238.04	8.32	0.35	8.57
max	1034.28	306.28	3600.19	19.78	0.82	16.54
Bosnia and Herzegovina, n=17						
Mean	814.32 [a]	214.64 [b]	2886.06 [b]	15.64 [a,b]	0.51 [a]	12.13 [a]
SD	440.65	48.55	538.29	2.48	0.38	3.41
Median	691.76	214.53	2885.10	15.35	0.43	12.13
min	229.63	119.24	1530.35	10.37	0.27	4.08
max	2548.56	319.79	3874.90	20.83	2.55	21.63
France, n=6						
Mean	703.37 [a]	152.88 [a]	2157.74 [a]	15.97 [a,b]	0.48 [a]	17.56 [b]
SD	57.06	12.93	84.25	2.01	0.02	4.57
Median	697.20	156.52	2161.95	16.16	0.48	17.47
min	644.88	134.27	2054.68	13.76	0.47	13.59
max	774.21	164.22	2252.36	17.83	0.50	21.69
North Macedonia, n=7						
Mean	778.26 [a]	210.49 [a,b]	2983.74 [b]	35.99 [d]	0.47 [a]	14.24 [a,b]
SD	90.79	12.07	214.23	3.64	0.06	3.34
Median	800.05	209.17	2988.76	35.35	0.46	13.25
min	658.70	198.60	2716.45	32.41	0.42	11.41
max	854.23	225.00	3241.00	40.84	0.55	19.06

	Element levels (mg/kg)					
Country of MSM origin	**Ca**	**Mg**	**K**	**Fe**	**Cu**	**Zn**
Sweden, n=6						
Mean	1115.65 [a]	193.58 [a,b]	2407.91 [a,b]	22.59 [c]	0.50 [a]	12.77 [a,b]
SD	119.42	20.10	201.59	0.96	0.05	0.92
Median	1119.91	196.96	2437.13	22.50	0.50	12.85
min	977.57	168.90	2145.00	21.50	0.44	11.63
max	1245.23	211.50	2612.38	23.84	0.55	13.77
Denmark, n=6						
Mean	1061.18 [a]	173.18 [a,b]	2091.63 [a]	20.37 [b,c]	0.38 [a]	11.51 [a]
SD	110.66	26.18	146.84	1.29	0.02	1.02
Median	1081.89	170.49	2063.99	20.49	0.38	11.20
min	908.20	149.97	1961.28	18.69	0.35	10.71
max	1172.75	201.76	2277.24	21.81	0.41	12.94
Germany, n=10						
Mean	998.08 [a]	195.37 [a,b]	2363.31 [a,b]	18.16 [a,b,c]	0.35 [a]	11.48 [a]
SD	302.94	18.16	129.13	1.04	0.04	1.32
Median	1034.53	200.60	2352.77	18.29	0.34	11.20
min	507.12	163.51	2141.15	15.72	0.31	9.70
max	1373.97	226.33	2581.47	20.37	0.42	13.72

[a–d] Different superscripts within the same column indicate significantly different means according to Tukey's HSD test; ($p < 0.05$)

this study (from 532.50 to 1115.65 mg/kg) were similar to levels reported by *Miedico et al.* (2022) for high pressure MSM (1019 mg/kg) and low pressure MSM (511 mg/kg). The mean Ca level from Serbian MSMs (888 mg/kg) was similar to that reported by *Tasić at al.* (2017) (721 mg/kg). The mean levels of Mg in MSMs from Croatia and Bosnia and Herzegovina in the current study were statistically higher than the mean level measured in MSMs from France ($p < 0.05$). The use of Ca and Mg derived parameters, such as (Ca^{2+} - Mg^{2+}) and the ratio, Ca^{2+}/Mg^{2+}, could be useful parameters to discriminate between MSM and non-MSM (*Iammarino et al., 2021*). The lowest mean contents of K was established in MSMs from Denmark (2091.63 mg/kg) and France (2157.71 mg/kg), and they were statistically lower than the mean K levels measured in MSMs from Croatia, Bosnia and Herzegovina and North Macedonia. The mean Fe levels in MSMs from Serbia, Bosnia and Herzegovina, France and Germany were not statistically different (range from 15.64 to 18.16 mg/kg) and were close to data obtained by *Miedico et al.* (2022) (from 14.3 to 17.9 mg/kg). The mean Fe level determined in MSMs from North Macedonia (35.99 mg/kg) was statistically higher than mean Fe levels measured in all other MSMs ($p < 0.05$). The mean Cu levels in MSMs from the different countries were not significantly different and were in line with data for high pressure MSMs reported by *Miedico et al.* (2022) (0.415 mg/kg). In French MSMs, the mean Zn level (17.56 mg/kg) was similar to that reported by *Miedico et al.* (2022) (16.3 mg/kg). Zn in MSMs from France was significantly higher than Zn in MSMs from Croatia, Bosnia and Herzegovina, Denmark and Germany ($p < 0.05$).

PCA was applied to the correlation matrix, which included the six parameters for the MSMs from eight different countries (*Hammer et al., 2001*). PCA was applied to group the observed the possible correlations

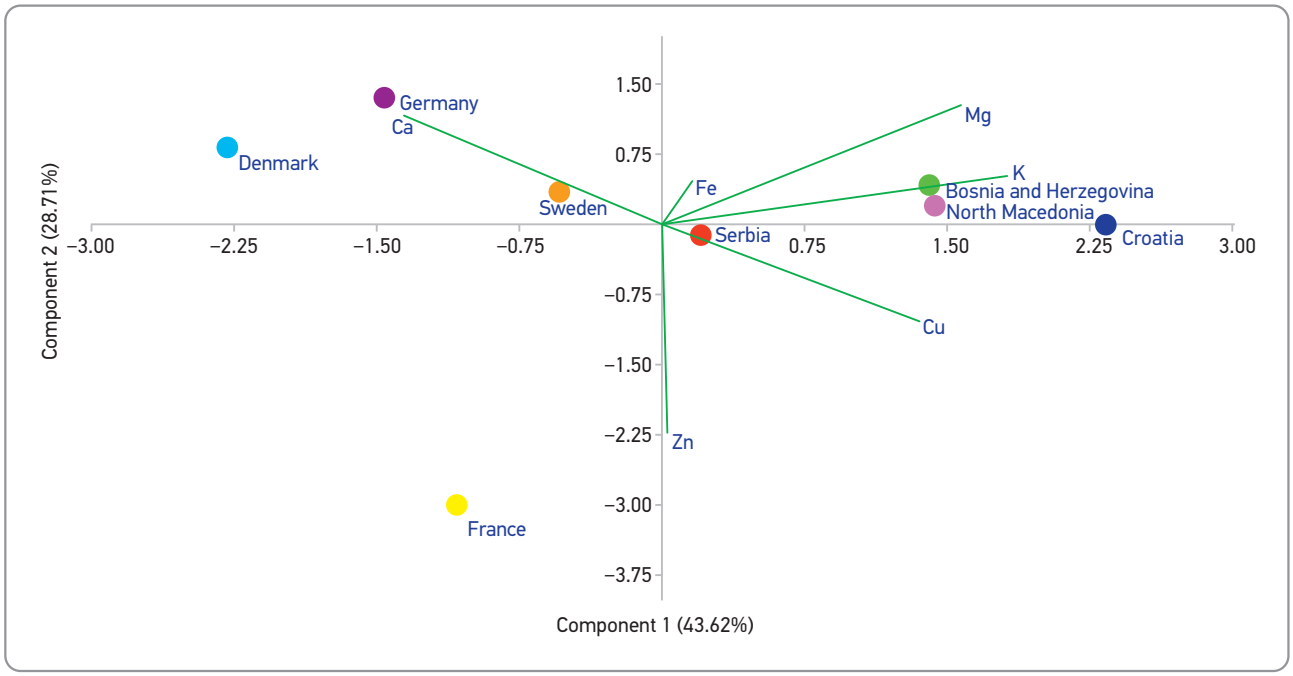

Figure 3. Bi-plot graphic of PCA of Ca, Mg, K, Fe, Cu and Zn levels in mechanically separated meats from different countries.

among the measured Ca, Mg, K, Fe, Cu and Zn levels and the country of MSM origin (Serbia, Croatia, Bosnia and Herzegovina, France, North Macedonia, Sweden, Denmark and Germany) (Figure 3).

The first two components (PC1 and PC2) resulting from the examination of the levels of micro- and macroelements in the MSMs from different countries accounted for 72.33% of the total variance (PC1 43.62%, PC2 28.71%). In the case of PC1, the levels of K and Mg (significant positive correlations) as well as the Ca level (significant negative correlation) contributed the most to the variability of the MSMs. In the case of PC2, significant positive correlations were established for Mg and Ca levels, while a significant negative correlation was established for the Zn level. For the third principal component (PC3), the level of Fe achieved a strong positive correlation, while the Mg level produced a strong negative correlation. For the fourth principal component (PC4), the levels of Ca and Cu achieved strong positive correlations, while the Fe level produced a strong negative correlation. Figure 3 shows the Mg levels were highly positively correlated with K levels, while the Ca and Cu levels were highly negatively correlated.

The influence of different parameters that described the MSMs studied can be evaluated from Figure 3, in which the MSMs from different countries are located on different sides of the graphic. MSMs from Sweden, Denmark and Germany, in which the highest Ca levels were observed, were located on the left upper side of the graphic. The MSMs from Bosnia and Herzegovina, North Macedonia and Croatia were on the opposite side of the graphic (right upper). MSMs from Croatia were located the furthest on that side, since these products contained the highest Mg and K levels. MSMs from Serbia and France were located on the lower side of the graphic. MSMs from France were separated with regard to their high Zn levels.

4. Conclusion

This study aimed to provide information on levels of Ca, Mg, K, Fe, Cu and Zn in MSMs used by the meat industry in Serbia. Data obtained from ANOVA show the country of origin significantly influences the Mg, K, Fe and Zn contents ($p < 0.05$) in MSMs, but there is no statistically significant influence of country of origin on the Ca or Cu content ($p > 0.05$). Ca levels in MSMs from Sweden and Denmark were slightly higher than the EFSA maximum permitted level for Ca in MSM. Furthermore, control of Ca in MSM samples, as well as control of conditions during the process of machine separation of meat from bones and tissues or from poultry carcasses, are both necessary to avoid the occurrence of bone particles in MSM and, consequently, in meat products that contain MSM.

Određivanje makro- i mikroelemenata u mehanički separisanom mesu poreklom iz različitih zemalja koje se koriste u industriji mesa u Srbiji

Jasna Đinović-Stojanović, Ivana Brankovic Lazić, Zoran Petrović, Srđan Stefanović, Nikola Borjan, Munevera Begić i Saša Janković

INFORMACIJE O RADU

Ključne reči:
Mehanički separisanio meso
Otkošteno meso živine
Elementi
EFSA mišljenje
Industrija mesa u Srbiji

APSTRAKT

Prema preporuci evropske agencije za bezbednost hrane (European Food Safety Authority (EFSA), sadržaj kalcijuma (Ca) u mehanički separisanom mesu (mechanically separated meat, MSM) je glavni indicator ostatka kostiju u uzorcima MSM. U ovom radu određen je sadržaj Ca, magnezijuma (Mg), kalijuma (K), gvožđa (Fe), bakra (Cu) i cinka (Zn) u MSM uzorcima iz različitih zemalja (Srbija, Hvatska, Bosna i Hercegovina, Francuska, Severna Makedonija, Švedska, Danska i Nemačka). Uzorci su uzeti iz različitih postrojenja za preradu mesa u Srbiji. Sadržaji šest elemenata određeni su primenom induktivno kuplovane plazme sa masenom spektrometrijom (inductively coupled plasma mass spectrometry, ICP-MS). Distribucija elemenata u MSM uzorcima analizirana je primenom PCA analize (principal component analysis, PCA). Sadržaj kalcijuma koji se odnosi na kvalitet mehanički separisanog mesa, proveren je u skladu sa pravilnikom Republike Srbije i EU propisima. I ubuduće, neophodna je redovna kontrola sadržaja Ca u MSM, kao i kontrola uslova u procesu odvajanja mesa sa kostiju na kojima je to meso ostalo posle otkoštavanja trupa ili sa trupa živine, kako bi se smanjilo unošenje čestica kostiju u MSM, a samim tim i u proizvode od mesa.

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

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-66/2024-03/200050 from 05.02.2024.

References

Ali, A. A. H. (2023). Overview of the vital roles of macro minerals in the human body. *Journal of Trace Elements and Minerals*, 4, 100076.

Dalipi, R., Berneri, R., Curatolo, M., Borgese, L., Depero, L. E. & Sangiorgi, E. (2018). Total reflection X-ray fluorescence used to distinguish mechanically separated from non-mechanically separated meat. *Spectrochimica Acta Part B: Atomic Spectroscopy,* 148, 16–22.

EFSA (European Food Safety Authority), (2013). Scientific opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine. *EFSA Journal*, 11(3), 1–78 3137.

Field, R. A. (1981). Mechanically deboned red meat. In C. O. Chichester, E. M. Mrak, & G. F. Stewart (Vol. Eds.), Advances in Food Research. Vol. 27 (pp. 23–107). Academic Press.

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4 (1), 1–9.

Iammarino, M., Miedico, O., Sangiorgi, E., D'Amore, T., Berardi, G., Accettulli, R., Dalipi, R., Marchesani, G. & Chiaravall, A. E. (2021). Identification of mechanically separated meat in meat products: a simplified analytical approach by ion chromatography with conductivity detection. *International Journal of Food Science and Technology*, 56, 5305–5314.

IARC, International Agency for Research on Cancer. (2015). IARC Monographs evaluate consumption of red meat and processed meat. https://www.iarc.who.int/wp-content/uploads/2018/07/pr240_E.pdf.

Islam, M. R., Akash, S., Jony M. H., Alam, N., Nowrin, F. T., Rahman, M, Rauf, A. & Thiruvengadam, M. (2023). Exploring the potential function of trace elements in human health: A therapeutic perspective. *Molecular and Cellular Biochemistry*, 478 (10). DOI:10.1007/s11010-022-04638-3.

Miedico, O., Nardelli, V., D'Amore, T., Casale, M., Oliveri, P., Malegori, C., Paglia, G. & Iammarino, M. (2022). Identification of mechanically separated meat using multivariate analysis of 43 trace elements detected by inductively coupled mass spectrometry: A validated approach. *Food Chemistry*, 397, 133842.

Regulation (EC) No 853/2004, (2004). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin.

Shyichuk, A., Kowalska, M., Shyychuk, I., Lamkiewicz, J. & Ziółkowska, D. (2023). Determination of calcium in meat products by automatic titration with 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid. *Molecules*, 28, 6592.

Official Gazette RS, 50/2019 and 34/2023. Rulebook on the quality of the minced meat, semi-finished meat and meat products.

Spink, J., Zhang, G., Chen, W. & Speier-Pero, C. (2019). Introducing the food fraud prevention cycle (FFPC): a dynamic information management and strategic roadmap. *Food Control*, 105, 233–241.

Tasić, A., Kureljušić, J., Nešić, K., Roković, N., Vićentijević, M., Radović, M. & Pisinov, B. (2017). Determination of calcium content in mechanically separated meat. *IOP Conference Series: Earth and Environmental Science,* 85(1) 012056 (1–5).

Wada, O. (2004). What are trace elements? - Their deficiency and excess states. *Medicine, Chemistry*. http://www.med.or.jp/english/pdf/2004_08/351_358.pdf

Wubshet, S. G., Wold, J. P., Böcker, U., Sanden, K. W. & Afseth, N. K. (2019). Raman spectroscopy for quantification of residual calcium and total ash in mechanically deboned chicken meat. *Food Control*, 95, 267–273.

Authors ORCID info

Jasna Djinović-Stojanović https://orcid.org/0000-0003-4602-0835
Ivana Branković-Lazić https://orcid.org/0009-0005-5844-9278
Zoran Petrović https://orcid.org/0000-0003-2016-5681
Srđan Stefanović https://orcid.org/0000-0002-8011-5654
Nikola Borjan https://orcid.org/0000-0003-4067-3755
Munevera Begić https://orcid.org/0000-0002-9425-8568
Saša Janković https://orcid.org/0000-0002-5223-6993

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

UDK: 599.325.11(497.113)"2010/2011"
ID: 149433097
https://doi.org/10.18485/meattech.2024.65.1.2

Original scientific paper

Levels and interactions of selected elements (Fe, Mn and Cu) in European hare tissue within different age classes from Serbian agricultural regions

Zoran Petrović[1], Jelena Ćirić, Saša Janković[1], Nikola Borjan[1], Dejana Trbović[1] and Srđan Stefanović[1]*

[1] *Institute of Meat Hygiene and Technology, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Brown hare
Elements
Kidney
Liver
Interactions

ABSTRACT

The contents of the essential elements, iron, manganese and copper, were determined in the kidney and liver of the European brown hare (*Lepus europaeus).* The wild hares assayed were divided into five age classes ranging from 3 months to more than 36 months. The animals were collected during 2010/2011 from 21 different hunting terrains and originated mainly from arable and agricultural biotopes in Serbia. The mutual interactions of the metals obtained from kidneys and livers of 157 individual hares in age groups were calculated. The mean levels of Fe, Mn and Cu (mg/kg, wet weight) registered in kidney (K) and liver (L) were: Fe (K) 103.3±42.1; Fe (L) 138.5±52.7; Mn (K) 1.75±0.66; Mn (L) 2.36±0.85; Cu (K) 3.32±0.62; Cu (L) 4.16±1.40. No statistically significant differences ($p>0.05$) were found between the age groups with regard to the Fe, Mn and Cu contents in the kidneys and liver of brown hares (within the same organs). Statistically significant differences between levels in liver and kidney (between different organs) were registered in all age groups (in favour of higher levels in the liver over the kidney) of hares, except for Fe contents in both organs in the age groups of 3–6 and 12 months. Correlations between the content of elements within the age groups were determined using the Pearson test for normal distributions. The correlation patterns between the essential elements in the hare liver and kidney showed both positive and negative significant correlations among some single or different elements within the same organ and among the elements between the two organs. Within age groups, we registered seven different statistically significant mixed associations (FeK-FeL, CuL-MnL, MnL-FeL, CuK-MnK, MnK-FeL, CuK-FeL, and CuL-FeL).

1. Introduction

In much of the literature, the term "essential metals" has been used to signify those metals required by living organisms, and the absence of which produces specific deficiency symptoms (*Duffus,* 2002). Furthermore, "trace elements" are defined as essential, i.e., Mn, Fe, Cu, Zn, Se, Co, Mo and I, for plants or animals (*Zoroddu et al.,* 2019). The essential elements in herbivore tissues have attracted a great deal of attention in basic and applied biology. These elements are known to play important functions in the body, such as storage, regulation and supply of energy (*Rai et al.,*2021*; Tibbett et al.,*2021*; Nunes et al.,*2022).

Fe, Cu and Mn play important roles in most biological process, be it structural or enzymatic. These elements also play a role in oxygen transport and reduction, as they each take part in the composition or functioning of respiratory pigments. As essential metals in animals, however, they can also create a level of toxicity (*Pajarillo et al.,* 2021*; Jomova et al.,* 2022). The liver and kidney are the sites of metal metabolism in the body. The proteins that are involved in

***Corresponding author:** zoran.petrovic@inmes.rs

Paper received: April 4th 2024. Paper accepted: May 7th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

the fluxes of Fe, Mn and Cu in mammals have been observed mostly in these two organs in both the absorption and the excretion processes. Therefore, it is important to understand the role of these elements in these organs to comprehend the overall status of essential metals in the body (*Fu and Xi,* 2020*; Liu et al.,* 2020*; Cygan and Szczegielniak,* 2021).

Many previous field studies established that different heavy metals (considered as toxic or essential) have to be present in the body and different organs of the European hare **(***Venäläinen et al.,* 1996*; Massanyi et al.,* 2003*; Pedersen et al.,* 2006*; Kolesarova et al.,* 2008*; Dlugaszek et al.,* 2013*; Le Fidalgo et al.,* 2015*; Demirbaş and Erduran,* 2017*; Wajdzik et al.* 2017*; Długaszek,* 2019). Some studies also investigated the distribution and levels of Fe, Mn and Cu in the organs of various animal species, including hares (*Lopez Alonso et al.,* 2004*; Kompiš and Ballova* 2021*; Squadrone et al.* 2022). However, from the literature reviewed for this study, there is limited information on the specific interactions between these elements within or between the target organs in the European hare, including narrower differentiation of age groups. Understanding these interactions is crucial, as Fe, Mn and Cu play essential roles in numerous biochemical and physiological processes. Furthermore, information on trace element levels in animal tissues is a valuable resource in animal toxicology. Fe is vital for oxygen transport and energy production, Mn participates in antioxidant defence and enzyme activation, while Cu is involved in enzyme function and cellular signalling (*Pilarczyk et al.,* 2020*; Kalisińska et al.,* 2023*; Stepanova et al.,* 2023).

By studying the background of Fe, Mn and Cu in animal physiology, we can try to establish a foundation for exploring their interactions within the hare's organs and optimal levels to ensure the normal growth and development of hares. The primary goals of this research were to examine: (1) the levels of Fe, Mn and Cu in kidney and liver of a hare population divided into five age groups that represent the natural life span of this wildlife species; (2) how Fe, Mn and Cu interact in the liver and kidney of European hares within the same and different organs and try to uncover the factors that affect these interactions derived from statistical data. Our focus was on analysing the amounts and patterns of Fe, Mn and Cu in these organs and determining whether there are any connections or interdependencies among the three metals. Furthermore, we aimed to comment on how factors such as diet composition, environmental conditions and especially age factors might impact these interactions.

2. Materials and methods

2.1 Studied wild hares and the collection locations

A total of 157 European brown hares (*Lepus europaeus*) were collected during the regular hunting season in the fall and winter of 2010/2011. The hares were collected mainly from habitats characteristic of the European brown hare, namely near agricultural land and other open areas such as meadows, clearings, plains, bushes and shrubs. The hares were from 21 Serbian regions at the locations shown on the map (Fig. 1). Of the study animals, 84 hares were collected from central Serbia, from the territories of 11 hunting associations (1–11: Užice, Bajina Bašta, Ub, Obrenovac, Mladenovac, Belgrade, Šabac, Ćićevac, Kuršumlija, Vranje and Prokuplje), while 73 hares were from the northern province of Vojvodina, from the territories of 10 hunting associations (12–21: Sonta, Aleksa Šantić, Sombor, Novi Sad, Pančevo, Putinci, Nikinci, Buđanovci, Mali Radinci, Voganj).

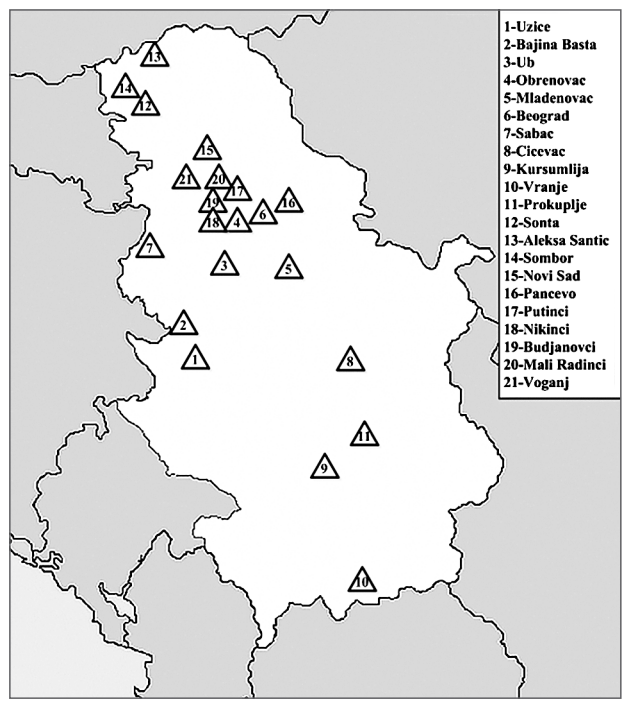

Figure 1. Locations in Serbia where hares were collected

2.2 Age determination of the studied hares

The detailed methodology for determining the age of the studied brown hare population was described in previous studies (*Suchentrunk and Hartl, 1991; Petrović et al., 2013, 2014*). The examined hares were divided into five age groups: 3–6 months, 12 months, 12–24 months, 24–36 months, older than 36 months.

2.3 Sample preparation

From the 157 hares, a total of 157 kidneys and 157 livers were taken, i.e., a total of 314 organs. The entire liver and both kidneys were removed from each animal. The livers and kidneys were stored at −20 °C until analysis. After each entire organ was subjected to a standard homogenization process (the two kidneys from each animal were processed together), 1 g of homogenate was digested with 8 ml HNO_3 (65 %v/v, analytical grade, JT Baker, Center Valley, USA) and 2 ml H_2O_2 (30 %, analytical grade, Kemika, Zagreb, Croatia) using the acidic microwave digestion method. Homogenates were digested in a microwave digestion device (Milestone TC, EVISA, EU) with temperature control. The digestion program started at a power of 1,000 W and was then ramped up to 200 °C for 10 minutes. The digests were then held at 1,000 W and a temperature of 180 °C for 20 minutes. Digests were then stored frozen at −18 °C and thawed in a refrigerator for 2 h before elemental analysis.

2.4 Elemental analysis (iron, manganese and copper)

The elements were measured with an atomic absorption spectrophotometer (VARIAN SpectrAA 220): Fe at 248.3 nm, LOD (1.0 mg/kg), Mn at 279.5 nm, LOD (0.5 mg/kg) and Cu at 324.8 nm, LOD (0.1 mg/kg). The accuracy of the method was tested with the standard reference material (BCR No. 186—Community Bureau of Reference) and by a recovery test with spiked samples. The recoveries of the reference material were ±10 % of the certified mean values. The recoveries in the different sample materials were 96 %–101 % in kidney and 98 %–102 % in liver for Fe; 88–96 % in kidney and 81 %–94 % in liver for Mn; 95 %–98 % in kidney and 92 %–95 % in liver for Cu; the coefficient of variation was between 4 % and 9 %. Calibrations were prepared from commercial solutions in HNO_3 (0.2 %) containing 1,000 mg/l of each element (JT Baker). All results are reported on a wet weight (w.w.) basis.

2.5 Statistical analysis

The analysis was performed using the MINITAB software package (MINITAB INC, USA), version 17.0. Levels in organs were expressed as means, standard deviations, minimum and maximum. Before selecting the appropriate statistical test, the best individual distribution of the data series was determined (16 different distributions were analysed) based on the lowest value of the Anderson-Darling coefficient and the highest p-value (above 0.05) for the final selection of the distribution that best fit the normal distribution. One-way ANOVA and post hoc HSD Tukey test were used to examine statistical differences of element contents in organs between age groups. The significance of the correlations of Fe, Mn and Cu levels between organs was calculated using Pearson's correlation (Ps). Differences were considered statistically significant if the p-value was ≤ 0.05. Boxplots were used to represent both measures of central tendency and the variability of the data.

3. Results

The measured contents of the three elements (mg/kg weight) in the kidney and liver of the hares examined are shown in Table 1.

3.1. Iron (Fe) levels in kidney and liver

Statistically significant differences (Table 1) between the Fe content in the kidneys and liver were not registered within the age groups of hares of 3–6 months and 12 months ($p > 0.05$). Later in the hare's life, looking at the mean values for the kidneys from this study, the Fe decreased slightly and then remained relatively constant. The mean Fe contents in the liver of the different age groups showed an increasing trend throughout the hares' life (Table 1). The Fe contents in the liver of hares of the other age groups (12–24 months, 24–36 months, and over 36 months.) were statistically significantly higher than in the kidneys ($p \leq 0.05$). No statistically significant differences were found between the Fe contents in the kidneys and liver between the different age groups ($p>0.05$). The mean Fe levels (Table 2) including the whole population studied for kidney (K) and liver (L) were 103.3 mg/kg and 138.5 mg/kg, respectively.

3.2 Manganese (Mn) levels in kidney and liver

The mean Mn levels in the examined kidneys (1.75 mg/kg) were lower than those in the liver (2.36 mg/kg), and the difference in the determined Mn levels between the two organs was statistically significant ($p=0.001$). Statistically significant differences between the Mn content in the kidneys compared to the liver were also found in all age groups

(p=0.001–0.022). No statistically significant differences were found between the age groups with regard to the Mn content in the kidneys and livers of hares (Table 1). The mean Mn levels in the kidneys and liver (Table 1) of the studied individuals by age groups (3–6 months,12 months, 12–24 months, 24–36 months, older than 36 months) were, respectively: for kidney: 1.96 mg/kg, 1.88 mg/kg, 1.59 mg/kg, 1.65 mg/kg and 1.55 mg/kg; for the liver: 2.47 mg/kg, 2.58 mg/kg, 2.39 mg/kg, 2.15 mg/kg and 2.30 mg/kg. The lowest Mn level in the kidneys was 0.54 mg/kg (Table 3, sampling site 7) and was recorded in a hare aged 24–36 months from the area of Šabac (Table 1; MnK 24–36 m). The highest measured Mn level in the kidneys was 2.70 mg/kg in a hare collected from Buđanovci (Table 3, sampling

Table 1. Overview of the content (mg/kg) of the analysed elements (Fe, Mn and Cu) in the kidneys (K) and liver (L) of brown hares according to age groups

Age	Statistical measure	Fe/K	Fe/L	Mn/K	Mn/L	Cu/K	Cu/L
3–6 months (n=28)	mean	110.2	128.7	1.96	2.47	3.33	4.26
	SD	39.4	50.5	0.80	0.78	0.66	1.48
	min	29.5	44.8	1.0	0.93	2.45	0.73
	max	203.8	269.2	2.70	3.79	5.20	8.06
12 months (n=41)	mean	109.2	132.0	1.88	2.58	3.32	4.27
	SD	51.3	62.5	0.79	0.99	0.79	1.60
	min	37.6	43.01	0.72	0.42	1.34	0.92
	max	324.1	313.1	2.28		5.34	9.34
12–24 months (n=22)	mean	107.9	145.6	1.59	2.39	3.41	3.93
	SD	36.2	41.8	0.42	0.73	0.48	0.99
	min	62.4	81.5	0.79	0.55	2.28	1.97
	max	186.4	237.3	2.23	4.13	4.33	6.32
24–36 months (n=51)	mean	94.5	140.7	1.65	2.15	3.26	4.16
	SD	34.6	46.2	0.55	0.73	0.55	1.47
	min	42.5	65.2	0.54	0.90	1.76	2.07
	max	163.9	298.7	2.64	3.97	4.43	8.89
36+ months (n=15)	mean.	92.5	155.0	1.55	2.30	3.34	3.94
	SD	47.1	64.2	0.47	1.04	0.45	0.93
	min	39.6	75.9	0.62	0.50	2.73	2.20
	max	176.8	283.9	2.31	4.11	4.24	6.19

Legend: K – kidney, L – liver, SD – standard deviation, n – number of hares included. The results are expressed on the basis of wet weight (w.w).

Table 2. Statistically significant differences between the element contents in kidney (K) and liver (L) according to hare age groups

Age (months)	FeK/FeL	MnK/MnL	CuK/CuL
3–6 months	p=0.134**	p=0.001*	p=0.003*
12 months	p=0.054**	p=0.003*	p=0.001*
12–24 months	p=0.002*	p=0.001*	p=0.029*
24–36 months	p=0.001*	p=0.001*	p=0.001*
36+ months	p=0.004*	p=0.022*	p=0.038*

Legend: * statistically significant differences between the metal contents in kidney (K) and liver (L) according to age groups ($p \leq 0.05$); ** Differences were not registered.

Table 3. Overview of the content (mg/kg) of the analysed elements (Fe, Mn and Cu) in the kidneys (K) and liver (L) of brown hares according to sampling sites

Locality	Statistical measure	Fe/K	Fe/L	Mn/K	Mn/L	Cu/K	Cu/L
1. Užice (n=10)	mean ± SD	100.1±48.3	144.7±51.7	1.95±0.82	2.45±0.57	3.32±0.47	3.72±0.60
	min – max	29.5–186.4	89.9–267.7	0.99–2.12	1.83–3.24	2.75–4.40	2.74–4.86
2. Bajina Bašta (n=6)	mean ± SD	103.1±45.6	171.8±61.9	1.62±0.53	1.64±1.47	3.00±0.49	2.84±2.67
	min – max	39.6–155.1	89.5–273.8	0.92–2.10	0.42–3.46	2.35–3.71	0.91–7.54
3. Ub (n=10)	mean ± SD	104.2±45.8	153.9±50.6	1.24±0.63	3.26±0.79	3.90±0.40	3.93±0.49
	min – max	58.3–185.3	81.5–258.4	0.62–2.62	1.82–4.13	3.29–4.59	3.24–4.68
4. Obrenovac (n=6)	mean ± SD	95.5±44.1	146.8±67.5	1.61±0.30	2.07±0.55	2.82±0.37	3.87±0.45
	min – max	46.3–176.8	85.7–232.9	1.14–20.01	1.38–3.00	2.14–3.21	3.44–4.60
5. Mladenovac (n=10)	mean ± SD	120.1±36.9	169.3±58.4	1.46±0.31	2.39±0.88	3.14±0.41	4.10±0.76
	min – max	54.4–181.8	89.0–269.2	1.00–2.05	1.36–3.67	2.45–3.97	2.66–5.17
6. Belgrade (n=7)	mean ± SD	144.2±88.2	216.6±106.2	1.60±0.38	1.98±0.76	3.42±0.28	3.43±1.73
	min – max	54.5–324.1	62.1–313.1	0.95–1.97	0.98–3.30	2.96–3.86	0.92–6.13
7. Šabac (n=9)	mean ± SD	92.0±23.2	121.6±26.5	1.48±0.68	2.28±0.20	2.47±0.44	3.22±0.98
	min – max	63.5–125.4	81.6–161.4	0.54–2.49	1.94–2.50	1.76–3.09	2.07–4.76
8. Ćićevac (n=7)	mean ± SD	64.8±16.5	153.6±8.15	1.83±0.30	1.77±0.20	3.36±0.36	3.36±0.36
	min – max	53.3–91.5	153.1–164.7	1.40–2.12	1.50–2.06	3.16–4.23	2.90–4.02
9. Kuršumlija (n=6)	mean ± SD	93.3±23.6	122.2±57.7	1.61±0.5	2.63±0.36	3.08±0.59	3.82±0.52
	min – max	61.8–131.7	48.3–213.5	1.0–2.20	2.22–3.14	2.54–4.05	3.42–4.80
10. Vranje (n=6)	mean ± SD	88.4±38.5	118.4±40.9	1.85±0.66	2.89±1.0	2.98±0.37	4.15±0.85
	min – max	42.0–155.1	74.6–173.8	0.66–2.42	1.38–3.97	2.52–3.64	2.53–4.76
11. Prokuplje (n=7)	mean ± SD	74.9±24.9	95.0±45.3	2.16±0.95	2.81±1.3	2.81±1.10	4.42±1.67
	min – max	37.6–101.2	43.0–163.3	1.45–2.28	1.27–5.08	1.34–4.36	2.03–6.64
12. Sonta (n=7)	mean ± SD	88.2±14.9	138.8±18.5	1.46±0.16	1.73±0.85	3.27±0.36	4.02±1.37
	min – max	68.8–113.4	118.5–173.3	1.16–1.62	0.96–3.08	2.65–3.58	2.85–6.19
13. Aleksa Šantić (n=9)	mean ± SD	126.3±42.11	149.3±68.1	1.63±0.24	1.98±0.78	3.74±0.46	3.90±1.39
	min – max	57.7–203.4	44.8–253.3	1.39–2.06	0.90–3.26	3.20–4.82	1.36–5.81
14. Sombor (n=9)	mean ± SD	85.5±23.9	110.4±40.2	2.18±0.35	2.65±0.90	3.07±0.45	3.74±1.39
	min – max	50.6–123.3	64.4–186.3	1.55–2.54	0.93–4.11	2.60–3.74	0.73–5.28
15. Novi Sad (n=6)	mean ± SD	69.3±25.7	99.9±21.6	2.03±0.33	2.77±0.74	3.70±0.23	7.49±1.38
	min – max	42.5–106.2	65.2–122.7	1.72–2.65	1.70–3.77	3.31–3.93	5.98–9.34
16. Pančevo (n=9)	mean ± SD	94.3±22.3	126.7±29.2	1.43±0.17	2.26±0.69	3.06±0.50	4.43±1.28
	min – max	69.0–141.5	78.0–160.6	1.14–1.60	1.35–3.65	2.25–3.60	2.75–6.94
17. Putinci (n=6)	mean ± SD	119.3±32.0	158.3±28.5	1.87±0.32	2.34±0.65	3.84±0.36	5.95±1.80
	min – max	74.2–163.6	115.5–196.4	1.21–2.04	1.54–3.06	3.46–4.43	3.71–8.84
18. Nikinci (n=9)	mean ± SD	138.9±29.4	116.1±34.9	1.62±1.15	1.67±0.41	3.41±0.32	3.64±0.34
	min – max	100.1–178.4	76.0–177.9	0.65–2.50	1.06–2.43	2.99–4.07	3.18–4.07
19. Buđanovci (n=6)	mean ± SD	80.0±22.1	122.1±32.0	1.73±0.70	2.42±0.51	3.94±1.13	4.89±0.98
	min – max	54.9–116.9	87.5–166.9	1.01–2.70	1.69–2.95	2.51–5.34	3.87–6.74
20. Mali Radinci (n=6)	mean ± SD	140.9±33.8	143.1±37.5	2.04±0.81	3.08±0.72	3.73±0.18	5.25±.1.50
	min – max	91.1–196.2	105.7–165.0	1.94–2.38	2.51–4.38	3.45–3.90	4.01–8.06
21. Voganj (n=6)	mean ± SD	134.9±35.2	124.2±42.6	1.69±0.38	2.77±0.47	3.33±0.22	4.73±1.21
	min – max	80.9–176.9	86.4–183.9	1.16–2.12	3.00–5.12	3.11–3.72	3.00–6.32
N= 157	(mean ± SD)	103.3±42.1	138.5±52.7	1.75±0.66	2.36±0.85	3.32±0.62	4.16±1.40
	min – max	29.5–324.1	43.0–313.1	0.54–2.70	0.42–5.12	1.34–5.34	0.73–9.34

Legend: K – kidney, L – liver, SD – standard deviation, n – number of samples, N – samples analysed in total; the results are expressed on the basis of wet weight (w.w)

site 19), an individual aged 3–6 months (Figure 3, MnK 3–6m). The lowest Mn level in the liver was 0.42 mg/kg and was recorded in a hare from Bajina Bašta (Table 3, sampling site 2), an individual 12 months old (Figure 3., MnL 12m). The highest recorded Mn level in the liver was 5.08 mg/kg in a one-12 months -old individual from Prokuplje (Table 1, sampling site 11) (Figure 3, MnL 12m).

3.3 Copper (Cu) levels in kidney and liver

The mean Cu levels (3.32 mg/kg) in the examined kidneys of the brown hare were lower than those in the liver (4.16 mg/kg), and the difference in the determined Cu levels between the two organs was statistically significant (p=0.001). Statistically significant differences between the Cu content in the kidneys compared to the liver were also found in all age groups (p=0.001−0.038). No statistically significant differences were found between the age groups with regard to the Cu content in the kidneys and liver of hares (Table 1). The mean Cu levels in the kidneys and liver (Table 1) of the studied individuals by age groups (3–6 months, 12 months, 12–24 months, 24–36 months and older than 36 months) were: for kidney: 3.31 mg/kg, 3.32 mg/kg, 3.41 mg/kg, 3.26 mg/kg and 3.34 mg/kg; for the liver: 4.26 mg/kg, 4.27 mg/kg, 3.93 mg/kg, 4.16 mg/kg and 3.94 mg/kg. The lowest measured Cu level in the kidneys was 1.34 mg/kg (Table 3, sampling site 11) and was recorded in a hare aged 12 months from the Prokuplje area (Table 1; CuK 12 m). The highest measured Cu level in the kidneys was 5.34 mg/kg in a hare collected from the Buđanovci area (Table 3, sampling site 19), an individual aged 12 months (Fig 4, Cu K12 m). The lowest Cu level in the liver

Table 4. Mean element levels (mg/kg) measured in hare organs by other authors

Element (mg/kg)	Kidney	Liver	Country	Authors
Fe	119[a] (total) 130[a] immature 108[a] adults	198[a](total) 236[a] immature 179[a] adults	Poland	Myslek and Kalisinska, 2006
	480 [f]	600 [f]	Poland	*Wajdzik et al., 2017**
	242,6 [e] /345,9 [b] (215.35–257.79)[e] (223.56–668.43)[b]	207,1 [e] /307,9 [b] (172.02–233.61)[e] (172.02–233.61)[b]	Croatia	Linšak et al., 2022
Mn	2.00 [a](total) 2.19[a] immature 1.98[a] adults	2.51[a](total) 2.62[a] immature 2.50[a] adults	Poland	Myslek and Kalisinska, 2006
	6.00[a]	4.80[a]	Turkey	Demirbaş and Erduran, 2017
Cu	2.6 [a] 6.6 [b]	3.1 [a] 5.8 [b]	Poland	Krelowska-Kulas et al., 1994
	(3.76 – 4.64) [a] (4.64 – 5.32) [b]	(4.64 – 5.32) [a] (4.61 – 5.15) [b]	Finland	Venäläinen et al.,1996
	3.85(total) 3.90[a] immature 3.78[a] adults	3.97(total) 3.95[a] immature 3.99[a] adults	Poland	Myslek and Kalisinska, 2006
	2.60 (juvenile)[c] 2.89 (adults)[c]	2.96 (juvenile)[c] 3.04 (adults)[c]	Spain	Le Fidalgo et al., 2015
	1.91 [a]	2.34 [a]	Turkey	Demirbaş and Erduran, 2017
	15[f]	14.7[f]	Poland	*Wajdzik et al., 2017**
	14[d]	13.8[d]	Croatia	*Lazarus et al., 2022**

Legend: The letters a-f indicate the environmental conditions of the sampling area. a –unpolluted areas, b – industrial areas; c – cultivated lands, d – natural gas treatment plant, e – coastal unpolluted area, f – mixed area (agricultural and industrial); *- results expressed as dry matter content, the data given in parentheses indicate the interval of findings.

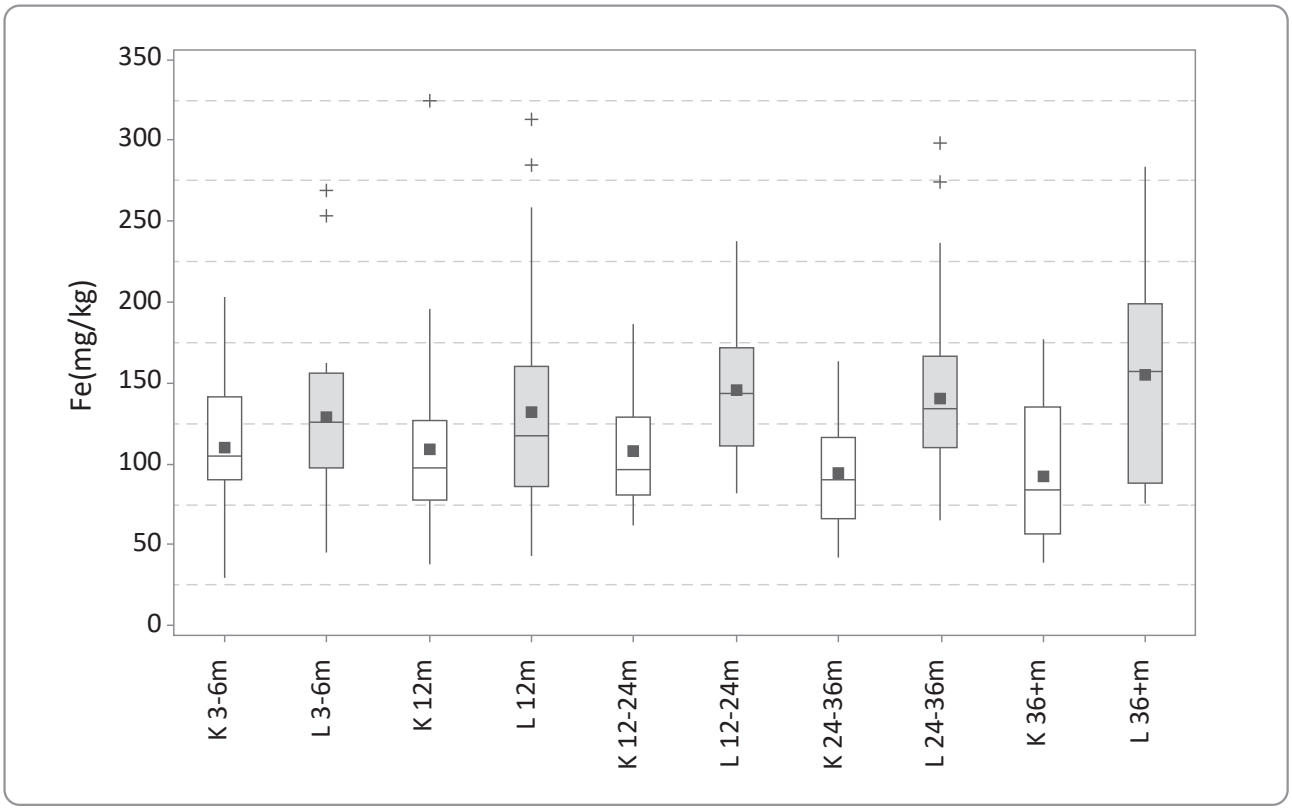

Figure 2. Iron (Fe) content (mg/kg, w.w.) in kidney (K) and liver (L) of hares grouped by age.

Legend: × indicates above-average values, ■ indicates mean values; a horizontal line within the box indicates median values

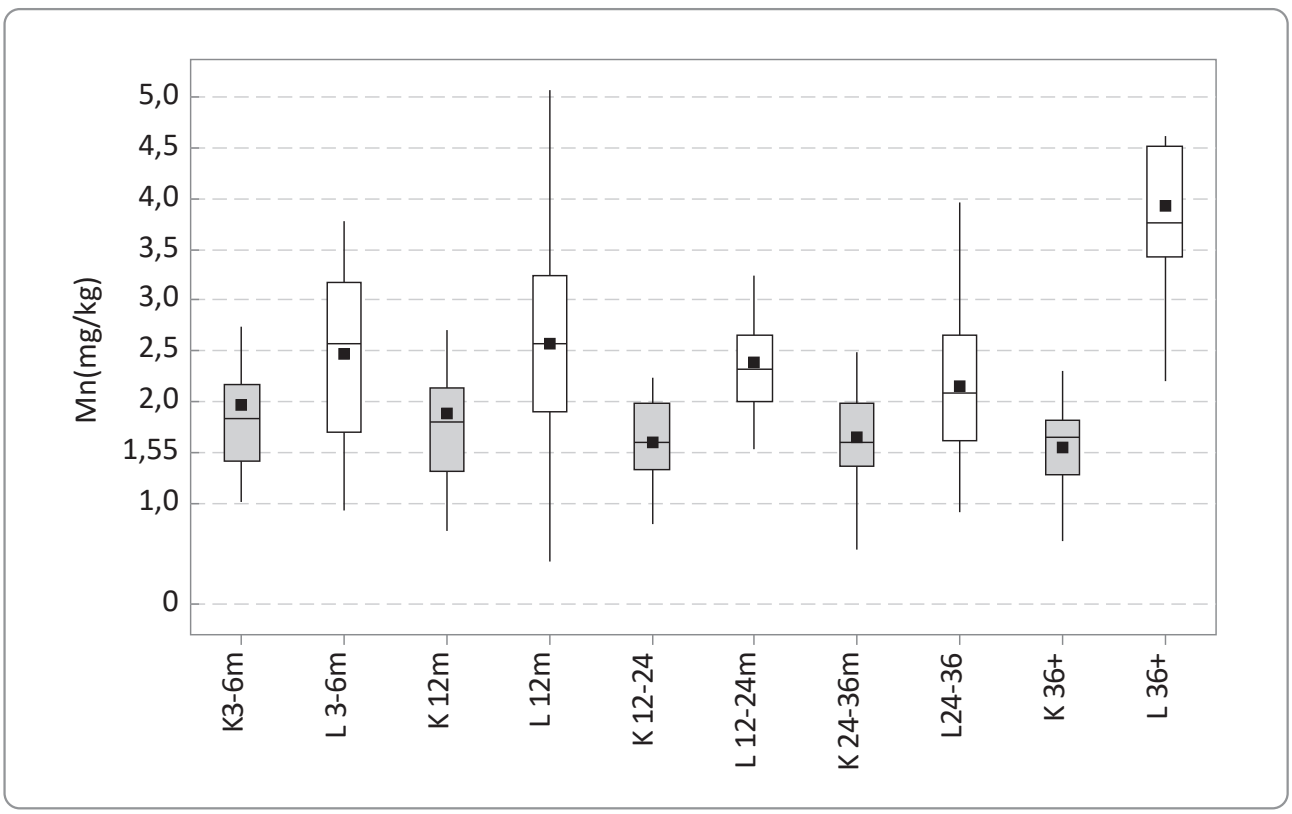

Figure 3. Manganese (Mn) content (mg/kg, w.w.) in kidney (K) and liver (L) of hares grouped by age.

Legend: × indicates above-average values, ■ indicates mean values; a horizontal line within a box indicates median values

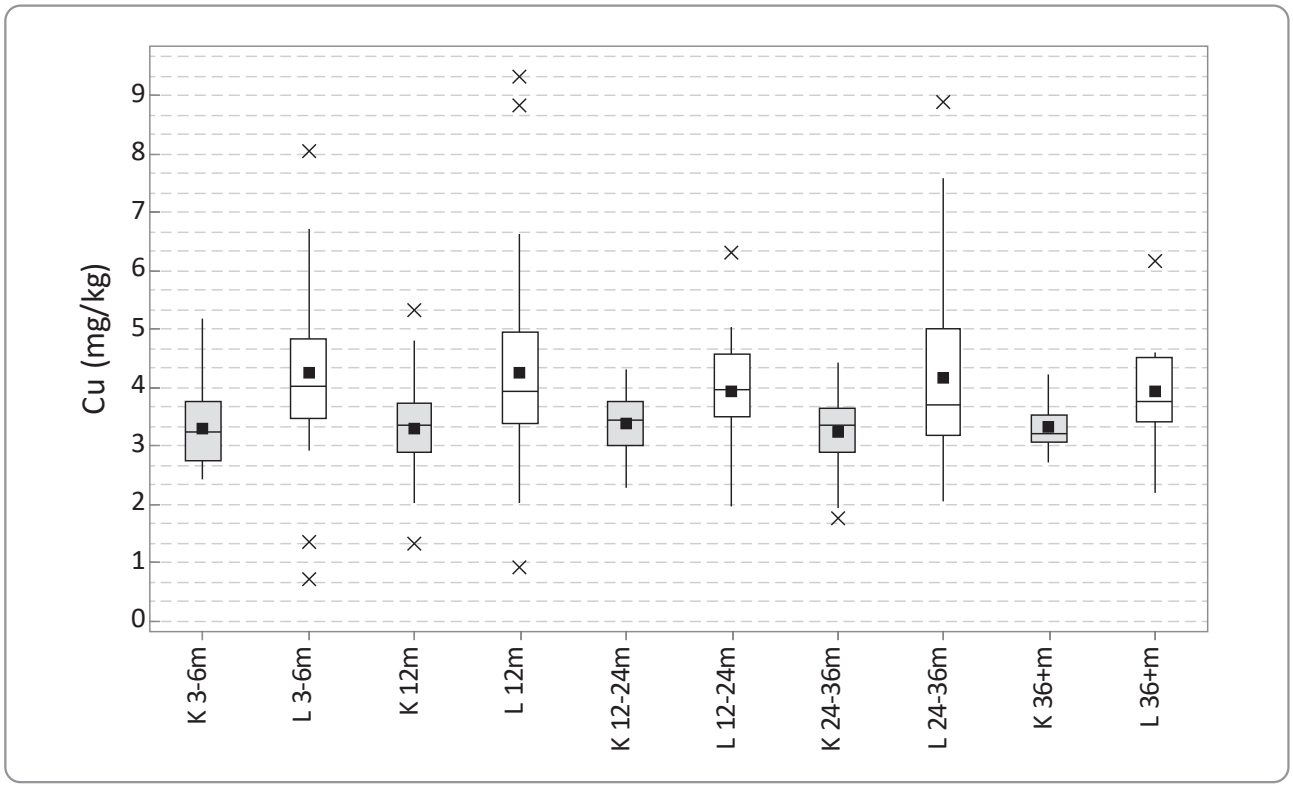

Figure 4. Copper (Cu) content (mg/kg, w.w.) in kidney (K) and liver (L) in hares grouped by age

Legend: × indicates above-average values, ■ indicates mean values; a horizontal line within a box indicates median values

was 0.73 mg/kg was recorded in a hare from Sombor (Table 3, sampling site 14), an individual 3–6 months old (Figure 4., CuL 3–6m). The highest recorded Cu level in the liver was 9.34 mg/kg in a one-year-old individual from the Novi Sad area (Table 1, sampling site 15) (Figure 4., CuL 12m).

The graphical representations of the Fe, Mn and Cu levels (mg/kg, w.w.) in the kidneys and liver of the examined hares according to age groups (3–6 months, 12 months, 12–24 months, 36 months and older than 36 months) can be found in Figs. 2–4.

3.4. Interactions between Fe, Mn and Cu in the kidney and liver of European hares

The correlation patterns between the essential elements in the liver and kidney of our hares were both positive and negative significant correlations among the single or different elements within the same organ and among the elements between the two organs (Table 5 and Table 6).

All registered correlations between liver and kidney were found when the Fe, Mn and Cu levels were correlated considering the whole population of hares studied (Table 5). All the mixed correlations between the three essential metals were found when the correlation matrix was formed by age groups (Table 6). The data analysis revealed a total of 12 different statistically significant associations (Table 5 and Table 6). Of these, seven different statistically significant correlations were observed for all samples analysed (Table 5) and all related to the association of different elements between kidney and liver.

Of these, only two common ones (FeK-FeL and MnK-FeL) occurred in correlations by age group (Table 6). The first common association, FeK-FeL, occurred in most age groups (3–6 m, Ps=0.56; 12 m, Ps=0.70; 24–36 m Ps=0.46), but was not found in groups of 12–24 m and over 36 m. Another common correlation, MnK-FeL, appeared once in the 12–24 m animals (Table 6). Within the age groups, the CuL-MnL correlation should be emphasised, which occurred four times (in the age groups: 3–6 m, Ps = 0.49; 12 m, Ps = 0.52; 24–36 m, Ps = 0.45 and 36 m+ , Ps = 0.68), but was not detected in the 12–24 m age group. The MnL-FeL correlation occurred twice (in the age groups 12 m, Ps= −0.31 and 12–24 m, Ps = −0.52). Other registered connections by age groups appeared once: CuK-MnK in 3–6 m, MnK-FeL in 12 m, CuK-FeL in 12–24 m and CuL-FeL in 24–36 m.

Of the seven correlation associations registered in the entire brown hare population studied, five out of a total of 12 were registered (Table 5), namely FeK-CuL, MnK-MnL, MnK-CuL, CuK-MnL and CuK-CuL, but did not appear in any correlation matrix formed by age groups. Most of these correlations were weak in relation to the whole population (Table 5), although they were weak to moderately strong within some age groups (± Ps correlation coefficient of −0.31 to 0.7). Weak to moderate negative correlations were also recorded in some age groups (Ps from −0.31 to −0.52). Note that all statistically significant correlations in

Table 5. Pearson (Ps) correlations between levels of different elements in kidney (K) and liver (L) of European hare within the whole examined population (n = 157)

Kidney – Liver		
Element/organ correlation	**Correlation coefficient**	**p value**
FeK-FeL	0.49	0.001[d]
FeK-CuL	−0.25	0.002[c]
MnK-FeL	−0.16	0.043[a]
MnK-MnL	0.21	0.010[b]
MnK-CuL	0.20	0.013[a]
CuK-MnL	0.19	0.018[a]
CuK-CuL	0.32	0.001[d]

Legend: [a]p ≤ 0.05, [b]p ≤ 0.01, [c]p ≤ 0.005, [d]p ≤ 0.001. Unless otherwise stated, the significance level is $p \leq 0.05$.

Table 6. Pearson (Ps) correlations between levels of elements in kidney (K) and liver (L) of European hare within particular age classes

Kidney-Liver, Kidney-Kidney, Liver-Liver (3–6 months. n=28)		
Element/organ correlation	**Correlation coefficient**	**p value**
FeK-FeL	0.56	0.002[d]
CuK-MnK	0.68	0.001[a]
CuL-MnL	0.49	0.009[b]
Kidney-Liver, Liver-Liver (12–24 months, n=22)		
Element/organ correlation	**Correlation coefficient**	**p value**
FeK-FeL	0.70	0.001[d]
CuL-MnL	0.52	0.001[a]
MnL-FeL	−0.31	0.049[a]
Kidney-Liver, Liver-Liver (12–24 months, n=22)		
Element/organ correlation	**Correlation coefficient**	**p value**
MnK-FeL	−0.47	0.026[a]
CuK-FeL	−0.47	0.029[a]
MnL-FeL	−0.52	0.014[a]
Kidney-Liver, Liver-Liver (24–36 months, n=51)		
Element/organ correlation	**Correlation coefficient**	**p value**
FeK-FeL	0.46	0.001[d]
CuL-FeL	−0.39	0.005[c]
CuL-MnL	0.45	0.001[d]
Liver-Liver (36+ months, n=15)		
Element/organ correlation	**Correlation coefficient**	**p value**
CuL-MnL	0.68	0.007[b]

Legend: [a]p ≤ 0.05, [b]p ≤ 0.01, [c]p ≤ 0.005, [d]p ≤ 0.001

the 12–24 month age group (MnK-FeL, Ps =−0.47; CuK-FeL, Ps= −0.46 and MnL-FeL, Ps= −0.52) were of medium strength and negatively correlated. The two recorded MnL-FeL correlations in the 12 m and 12–24 month age groups were negative. The largest number of mixed correlations between elements and between age groups appeared within the liver data (CuL-MnL, MnL-FeL and CuL-FeL) seven times, then between the liver and kidney data (CuK-MnL, MnK-FeL and CuK-FeL) five times, but only once within the kidney data (CuK-MnK).

4. Discussion

Our study documents Fe, Mn and Cu levels in kidney and liver within hare age groups and sampling sites as well as interactions within age and correlations between organs.

4.1. Iron (Fe) levels in kidney and liver

The content of Fe in the kidneys and liver did not differ in hares of 3–6 months and 12 months old. These results could be interpreted to mean that the content in these two organs is evenly built up until the animals are about 12 months old. Comparing the results of the present study with those of other studies (Table 4), it can be seen that the mean metal levels in our hares were lower than in those from Poland (*Myslek and Kalisinska,* 2006*; Wajdzik et al.,* 2017) and Croatia (*Linsak et al.,* 2017). In our study, the age difference between the studied groups was sharper and the mean Fe levels in the kidneys and liver (Table 1) of the studied individuals by age groups (3–6 months, 12 months, 12–24 months, 36 months and older than 36 months) were: for kidney (110.2 mg/kg; 109.2 mg/kg; 107.9 mg/kg; 94.5 mg/kg and 92.5 mg/kg) and for the liver (128.7 mg/kg; 132.0 mg/kg; 145.6 mg/kg; 140.7 mg/kg and 155.0 mg/kg), respectivly. The results for the Fe content in the kidneys of hares from the present study in relation to the age of 6 months to 24 months were similar to those from Poland (*Myslek and Kalisinska,* 2006). Other measured mean Fe levels in both organs were lower with respect to the studies selected for comparison (Table 4). It should be noted that comparisons of results should only be made between values expressed in the same way (i.e., wet weight or dry weight). In the study by *Falandysz et al.,* 1994), the Fe levels in the kidneys and liver of rabbits from farms in northern Poland were 27–83 mg/kg in kidney and 50–180 mg/kg in liver (similar to the present study, taking into account the interval results from different hunting areas).

In an older study (*Ferguson et al.,* 1962), the Fe levels in various organs (lung, kidney, liver, spleen) of laboratory-bred rabbits were investigated. The results for the kidney were 36–300 mg/kg and for the liver were 43–540 mg/kg. If we look at the Fe values determined in the kidneys and liver of the brown hares from the sampling sites in Serbia (Figure 2) and compare them with the study by *Ferguson* (1962), there are some similarities in the range. This comparison could lead us to the conclusion that the data from our study are close to the physiological Fe values in the examined organs of hares of different age groups from Serbia. The higher Fe levels (outliers) found in some hares (Figure 2) could be attributed to the state of the sample before homogenization, i.e., the high Fe could be due to excess blood on the surface of the organs.

4.2. Manganese (Mn) levels in kidney and liver

If we compare the Mn levels in the organs of brown hares from our study with the results of *Mislek and Kalisinska* (2006) from Poland, there was a very high degree of agreement, both in terms of the mean value (our study shows 1.75 mg/kg in the kidneys and 2.36 mg/kg in the liver, the Polish study 2.00 mg/kg and 2.51 respectively) and in terms of the levels in younger and older individuals. The Mn content in the kidneys and liver of young brown hares aged 3–6 months (Table 1) show, for example, that the levels (w.w.) in the kidney (1.96 mg/kg) and the liver (2.47 mg/kg) corresponded very well with those of younger individuals from Poland (kidney 2.19 mg/kg and liver 2.62 mg/kg). This similarity was also seen in older individuals. Thus, Mn levels of our study in hares aged 24–36 months (kidney 1.65 mg/kg, liver 2.15 mg/kg) were close to the Mn levels in older individuals from the Polish study of 2006 (Table 4), wherein the Mn levels in kidney and liver were 1.98 mg/kg and 2.50 mg/kg, respectively. Compared to the Turkish study (*Demirbaş and Erduran*, 2017), their registered mean Mn levels (kidney 6.00 mg/kg and liver 4.80 mg/kg) were somewhat lower in our study and also in the Polish study (*Mislek and Kalisinska,* 2006). In cattle and cervids, both ruminants, normal liver Mn levels were within the following ranges: 2.5–6.0 and 2.5–8.0 mg/kg (*Puls,* 1994).

Mn levels in the organs of brown hares can be influenced by the environment, as Europe consists of many land masses with different geomorphology and climate (*Vidus-Rosin et al.,* 2011*; Canova et al.,* 2020*; Kitowski et al.,* 2017*; Fattorini et al.,* 2021*; Buglione et al.,* 2022). Normal Mn levels in the kidneys of wild animals are lower than in the liver, but in some cases, the opposite is true (Table

4; *Demirbaş and Erduran,* 2017). The comparison of the results obtained in this study with regard to the Mn content in the kidney and liver of the European brown hare is consistent with other studies that have looked at the content of this element in various organs and animal species in the past. In the digestive system, the highest Mn levels were found in the stomach, followed by the liver and the kidneys, and the lowest levels were encountered in the intestines (*Ertl et al.,* 2016*; Kalisinska and Budis,* 2019). The Mn levels found in the organs of brown hares in this study were not so high as to have harmful effects (*Hackländer,* 2022*; Kompiš and Ballová,* 2021*; Selimovic and Arnold,* 2022).

Sporadically detected low Mn levels (0.42 mg/kg), e.g. in the liver of a 12-month-old hare (Figure 3) from the Bajina Basta region (Table 3, sampling point 2), could indicate a deficiency of this element in the diet or could be related to the health status of the individual. In fact, it is likely that brown hares suffer from malnutrition when they live in areas with monocultures that are poor in plant biodiversity (*Schai-Braun et al.,* 2015). It is well known that malnutrition has a negative impact on the survival rate of brown hares (*Edvards et al.,* 2000).

It appears from this study that the mean Mn levels in the organs of the brown hare by age classes are close to the physiological level and are not significant when the reported levels are considered in the context of the effect of this metal from the environment on the hare individuals studied, i.e. the toxicity of the registered amounts of Mn per individual. These results are in line with previous studies, where it was stated that the gastrointestinal and hepatobiliary systems play crucial roles in regulating and maintaining Mn organ levels within a relatively narrow physiologic range *(Aschner and Aschner* 2005*; Foster et al.,* 2015*; Zeman et al.* 2015).

From the literature reviewed, it appears there are not many field studies that have investigated Mn levels in different tissues and organs of the same animals and in different age groups of brown hares. Therefore, the data from our study are valuable data to show reference ranges for Mn levels in kidney and liver of European hares as they age.

4.3. Copper (Cu) levels in kidney and liver

A comparison of the mean Cu contents in the kidneys and liver of brown hares from our study with other reference studies (Table 4) shows a great similarity with the data from the Polish studies on hares from unpolluted areas (*Krelowska-Kulas et al.,* 1994*; Myslek and Kalisinska,* 2006) and slightly lower Cu levels in the kidneys and liver compared to in the organs of hares from unpolluted areas in Finland (*Venaelaeinen et al.,* 1996). In our study, Cu levels in kidney and liver were slightly higher on average, but still similar to levels in the Spanish study from hares collected from cultivated areas (*Le Fidalgo et al.,* 2015). Compared to the Turkish study that examined hares from unpolluted areas (*Demirbaş and Erduran*, 2017), the mean Cu levels in both examined organs in brown hares from Serbia were almost double.

In all hare age groups, some liver Cu levels (Fig. 4) were were outliers from the majority of the levels measured in the given population, i.e., 75% of the data from the third quartile and above the maximum levels (marked with the symbol x as outliers). This can be interpreted to mean that the excess Cu accumulates in the liver of these herbivores and in cases where Cu intake exceeds physiological limits (*Woolliams et al.,* 1983*; Grace et al.,* 1998). When comparing two groups of reared rabbits, one fed a basic diet (10 mg Cu/kg) and the other fed a pelleted diet with the addition of $CuSO_4 \times 5H_2O$ (140 mg/kg Cu — four times more than the maximum 35 mg/kg permitted by the EU regulation), *Skoivanova et al.* (2002) found 4.62 mg/kg in the liver of the control group, while the liver of rabbits fed a high dose of Cu contained 118.5±31.8 mg/kg of Cu. Moreover, the nutrient requirement for a rabbit in terms of Cu intake is 10 mg/kg per day (*Mateos and De Blas,* 1998). According to some studies, the Cu content in the diet had no significant effect on the Cu content in the liver (*Korish and Attia,* 2020*; López-Alonso and Miranda,* 2020*; Taylor et al.,* 2020). In conclusion, Cu supplementation increases liver Cu levels, while a non-supplemented diet prevents Cu accumulation in liver.

4.4. Interactions between Fe, Mn and Cu in the kidney and liver of European hares

There have been a number of previous studies on herbivorous species that revealed a large number of different correlations and interactive effects of essential element content in different tissues (*Goyer,* 1997*; Medvedev,* 1999*; Lopez et al.,* 2002a*,* 2004b*; Myslek and Kalisińska,* 2006). The explanation for the correlations found in this study seems to be related to a similar homeostatic mechanism of the so-called "cationic metals", a group of elements that are essential for body function (*Fairbrother et al.,* 2007). From these data alone, it is not possible to draw fundamental conclusions about the mutual kinetics of these elements (*Rahil-Khazen et al.,* 2002). A number of the elements studied produced interactive effects, evident in the brown hares from this study as well as

in the herbivores from the earlier studies mentioned, with one element being able to influence the levels of another element in a predictable way.

5. Conclusions

The levels of Fe, Mn and Cu in kidneys and livers of brown hares collected in Serbian agricultural regions are within physiological limits and are comparable to other studies from other countries with similar biotopes and environmental conditions. The mean levels of all three investigated elements (Fe, Mn, Cu) between age groups within the same organ do not change significantly during the life span of the brown hare.

The content of Mn and Cu in hare liver is higher than in hare kidney in all age groups examined. For Fe, there are no statistically significant differences in the contents in these two organs in the first year of life, while after the first year of life, Fe contents in the liver and kidney are statistically significantly different, as is also the case for Mn and Cu.

The results of this study show that the correlations of the levels of the tested elements between different organs observed in the whole population of brown hares studied have a quantitatively lower strength than the strength of the relationship (Ps correlation coefficient) between different or within the same organs when the correlation matrix is formed separately for each age group. This finding suggests that a more precise age categorisation of brown hares gives a better picture of the registered associations and interactive effects. In this study, it was also observed that some correlations seen for the whole population did not occur between individual age groups in brown hares.

Određivanje nivoa i interakcije elemenata (Fe, Mn i Cu) u tkivu divljeg zeca u različitim starosnim grupama iz poljoprvrednih regiona Srbije

Zoran Petrović, Jelena Ćirić, Saša Janković, Nikola Borjan, Dejana Trbović i Srđan Stefanović

INFORMACIJE O RADU

Ključne reči:
Mrki zec
Elementi
Bubreg
Jetra
Interakcije.

APSTRAKT

Određivan je sadržaj gvožđa, bakra i mangana u bubrezima i jetri evropskog divljeg zeca (Lepus europaeus). Ispitani zečevi su bili podeljeni u 5 starosnih grupa u rasponu od 3 meseca do starijih od 36 meseci. Prikupljeni su sa 21 različitog lovnog terena Vojvodine i Centralne Srbije, u blizini uglavnom obradivih i poljoprivrednih područja u Srbiji tokom 2010/2011. Studija je obuhvatila rezultate ispitanih koncentracija navedenih elemenata i međusobne interakcije u populaciji od ukupno 157 jedinki. Srednje vrednosti koncentracija Fe, Mn i Cu (mg/kg, vlažna masa) registrovane u bubrezima i jetri iznosile su: Fe (bubreg) 103,3±42,1, Fe (jetra) 138,5 ±52,7; Mn (bubreg) 1,75±0,66 Mn (jetra) 2,36±0,85; Cu (bubreg) 3,32±0,62, Cu (jetra) 4,16±1,40. Nisu bile registrovane statistički značajne razlike ($p>0,05$) između starosnih grupa u pogledu sadržaja Fe, Mn i Cu u bubrezima i jetri zečeva (unutar istog organa). Statistički značajne razlike između koncentracija elemenata u jetri i bubrezima (između različitih organa) registrovane u svim starosnim grupama u korist jetre u odnosu na bubreg zečeva osim koncentracije Fe u oba organa u starosnim grupama od 3–6 i 12 meseci. Obrasci korelacije između esencijalnih elemenata u jetri i bubrezima divljeg zeca u ovoj studiji pokazali su postojanje pozitivnih i negativnih statistički značajnih korelacionih povezanosti između pojedinačnih ili različitih elemenata unutar istog tkiva i pojedinih elemenata između različitih tkiva. Unutar starosnih grupa registrovano je 7 različitih statistički značajnih asocijacija (FeB-FeJ, CuJ-MnJ, MnJ-FeJ, CuB-MnB, MnB-FeJ, CuB-FeJ, CuJ-FeJ). Korelacione povezanosti između sadržaja elemenata u okviru organa unutar i između starosnih grupa određivane su primenom Pirsonovog testa za normalnu distribuciju.

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

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-66/2024-03/200050 from 05.02.2024.

References

Aschner, J. L., & Aschner, M. (2005). Nutritional aspects of manganese homeostasis. *Molecular Aspects of Medicine*, 26, 353–362.

Buglione, M., Filippo, G., Conti, P., & Fulgione, D. (2022). Eating in an extreme environment: diet of the European hare (*Lepus europaeus*) on Vesuvius. *The European Zoological Journal*, 89, 1201–1214.

Canova, L., Gazzola, A., Pollini, L., & Belsterei, A. (2020). Surveillance and habitat diversity affect European brown hare (*Lepus europaeus*) density in protected breeding areas. *European Journal of Wildlife Research*, 66, 66.

Cygan-Szczegielniak, D. (2021). The levels of mineral elements and toxic metals in the *Longissimus lumborum* muscle, hair and selected organs of red deer (*Cervus elaphus* L.) in Poland. *Animals*, 11 (5), 1231.

Demirbaş, Y., & Erduran, N. (2017). Concentration of selected heavy metals in brown hare (*Lepus europaeus*) and wild boar (*Sus scrofa*) from central Turkey. *Balkan Journal of Wildlife Research*, 30, 26–33.

Długaszek, M. (2019). Studies on relationships between essential and toxic elements in selected body fluids, cells and tissues. *Chemico-Biological Interactions*, 297, 57–66.

Dlugaszek, M., & Kopczynski, K. (2013). Elemental composition of muscle tissue of wild animals from central region of Poland. *International Journal of Environmental Research*, 7, 973–978.

Duffus, J. H. (2002). Heavy Metals—A Meaningless Term? IUPAC Technical Report. 74, 793–807.

Edwards, P. J., Fletcher, M. R., & Berny, P. (2000). Review of the factors affecting the decline of the European brown hare, *Lepus europaeus* (Pallas, 1778) and the use of wildlife incident data to evaluate the significance of paraquat. *Agriculture, Ecosystems & Environment*, 79, 95–103.

Ertl, K., Kitzer, R., & Goessler, W. (2016). Elemental composition of game meat from Austria. *Food Additives & Contaminants: Part B*, 9, 120–126.

Fairbrother, A., Wenstel, R., Sappington, K., & Wood, W. (2007). Framework for metals risk assessment. *Ecotoxicology and Environmental Safety*, 68, 145–227.

Falandysz, J. (1991). Manganese, copper, zinc, iron, cadmium, mercury and lead in muscle meat, liver and kidneys of poultry, rabbit and sheep slaughtered in the northern part of Poland. *Food Additives & Contaminants*, 8, 71–83.

Falandysz, J., Kotecka W., & Kannan K. (1994). Mercury, lead, cadmium, manganese, copper, iron and zinc concentrations in poultry, rabbit and sheep from the northern part of Poland. *Science of the Total Environment*, 141, 51–57.

Fattorini, S., Mantoni, C., Di Biase, L., & Pace, L. (2021). Mountain biodiversity and sustainable development. In: Leal Filho, W., Azul, A. M., Brandli, L., Lange Salvia, A., Wall, T. (eds) Life on Land. Encyclopedia of the UN Sustainable Development Goals. Springer, Cham.

Ferguson B. A., Akahoshi Y., Laing P. G., & Hodge E. S. (1962). Trace metal ion concentration in the liver, kidney, spleen, and lung of normal rabbits. *The Journal of Bone and Joint Surgery*, 44–A.

Foster, M. L., Bartnikas, T. B., Johnson, L. C, Herrera, C., Pettiglio, M. A., Keene, A. M., Taylor, M. D., & Dorman, D. C. (2015). Pharmacokinetic evaluation of the equivalency of gavage, dietary, and drinking water exposure to manganese in F344 rats. *Toxicological Sciences*, 145, 244–251.

Fu, Z., & Xi, S. (2020). The effects of heavy metals on human metabolism. Toxicology Mechanism and Methods, 30, 167–176.

Goyer, R. A. (1997). Toxic and essential metal interactions. *Annual Review of Nutrition*, 17, 37–50.

Grace, N. D., Knowles, S. O., Rounce, J. R., West, D., & Lee, J., 1998. Effect of increasing pasture copper concentrations on the copper status of grazing Romney sheep. *New Zealand Journal of Agricultural Research*, 41, 377–386.

Hackländer, K. (2022). European hare *Lepus europaeus* Pallas, 1778. In: Hackländer, K., Zachos, F. E. (eds) Handbook of the Mammals of Europe. Handbook of the Mammals of Europe. Springer, Cham.

Jomova, K., Makova, M., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., Rhodes C. J., & Valko, M. (2022). Essential metals in health and disease. *Chemico-Biological Interactions*, 367, 110173.

Kalisinska E., Budis H. (2019). Manganese, Mn. In: Kalisińska E., editor. Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: An Ecotoxicological Assessment of the Northern Hemisphere. Springer; Cham, Switzerland: 213–246.

Kalisinska, E., Lanocha-Arendarczyk, N., °C Podlasinska, J. (2021). Current and historical nephric and hepatic mercury concentrations in terrestrial mammals in Poland and other European countries. *Science of the Total Environment*, 775, 145808.

Kitowski, I., Jakubas, D., Wiącek,D., & Sujak, A. (2017). Inter-colony differences in hepatic element concentrations of European flagship farmland bird, the rook *Corvus frugilegus*, breeding in rural habitats in East Poland. *Agriculture, Ecosystems & Environment*, 250, 123–132.

Kolesarova, A., Slamecka, J., Jurcik, R., Tataruch, F., Lukac, N., Kovacik, J., Capcarova, M., Valent, M., & Massanyi, P. (2008). Environmental levels of cadmium, lead and mercury in brown hares and their relation to blood metabolic parameters. *Journal of Environmental Science and Health, Part A*, 43, 646–650.

Kompiš, M., & Ballová, Z. K. (2021). The influence of preferred habitat and daily range of the European hare on its contamination by heavy metals, 2021: A case study from the West Carpathians. *Environmental Science and Pollution Research*, 28, 52093–52105.

Korish, M. A., & Attia Y. A. (2020). Evaluation of heavy metal content in feed, litter, meat, meat products, liver, and table eggs of chickens. *Animals*, 22 (10), 727.

Krelowska-Kulas M., Kudelka W., Stalinski Z., & Bieniek J. (1994). Content of metals in rabbit tissues. *Nahrung*, 38, 393–396.

Lazarus, M., Orct, T., Sekovanić, A., Skoko, B., Petrinec, B., Zgorelec, Ž., Kisić, I., Prevendar-Crnić, A., Jurasović, J., & Srebočan, E. (2022). Spatio-temporal monitoring

of mercury and other stable metal(loid)s and radionuclides in a Croatian terrestrial ecosystem around a natural gas treatment plant. *Environmental Monitoring and Assessment*, 194, 481.

Le Fidalgo, B., L., C., Goicoa A., & Espino, L. (2015). Accumulation of zinc, copper, cadmium and lead in liver and kidney of the Iberian hare (*Lepus granatensis*) from Spain. *Journal of Veterinary Sciences*, 2, 15–20.

Linšak, Ž., Gobin, I., Linšak, D. T., & Broznić, D. (2022). Effects of long-term lead exposure on antioxidant enzyme defense system in organs of brown hare (*Lepus europaeus* Pallas) as a bioindicator of environmental pollution in Croatia. *Biological Trace Element Research*, 200, 5091–5103.

Liu, T., Liang, X., Lei, C., Huang, Q., Song, W., Fang, R., Li, C., Li, X., Mo, H., Sun, N., L, H., & Liu, Z. (2020). High-fat diet affects heavy metal accumulation and toxicity to mice liver and kidney probably via gut microbiota. *Frontiers in Microbiology*, 11, 1604.

Lopez Alonso, M., Prieto Montaña, F., Miranda, M., Castillo, C., Hernandez, J., & Benedito, J. S. (2004). Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *BioMetals*, 17, 389–397.

López-Alonso M., & Miranda M. (2020). Copper supplementation, A challenge in cattle. *Animals*, 10, 1890.

López-Alonso, M. & Benedito, J. L., Miranda, M., Castillo, C., Hernández, J., & Shore, R. F. (2002). Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution. *Archives of Environmental Contamination and Toxicology*, 42, 165–172.

Lopez-Alonso, M., Montaña, F. P., Miranda, M., Castillo, C., Hernandez J., & Benedito, J. L. (2004). Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *BioMetals*, 17, 389–397.

Mason, C. F., & Stephenson, A. (2001). Metals in tissues of European otters (*Lutra lutra*) from Denmark, Great Britain and Ireland. *Chemosphere*, 44, 351–353.

Massanyi, P., Tataruch, F., Slamecka, J., Toman, R., & Jurcik, R. (2003). Accumulation of lead, cadmium, and mercury in liver and kidney of the brown hare *Lepus europaeus* in relation to the season, age, and sex in the west Slovakian lowland. *Journal of Environmental Science and Health*, 38, 1299–309.

Massányi, P., Toman, R., Uhrín, V., & Renon, P. (1995). Distribution, of cadmium in selected organs of rabbits after an acute and chronic administration. *Italian Journal of Food Science*, 7, 311–316.

Mateos, G. G., & De Blas, C. (1998). Minerals, vitamins and additives. In: De Blas C., Wiseman J. (ed). The Nutrition of the Rabbit. CABI Publishing, Wallingford, 145–174.

Medvedev, N., 1999. Level of heavy metals in Karelian wildlife, 1989–91. *Environmental Monitoring and Assessment*, 56, 177–93.

Myslek P., & Kalisinska E. (2006). Contents of selected heavy metals in the liver, kidneys and abdominal muscle of the brown hare (*Lepus europaeus* Pallas) in Central Pomerania, Poland. *Polish Journal of Veterinary Sciences*, 9, 31–41.

Nunes da Silva, M., Machado, J., Osorio, J., Duarte, R., & Santos, C. S. (2022). Non-essential elements and their role in sustainable agriculture. *Agronomy*, 12, 888.

Pajarillo, E. A. B., Lee, E., & Kang, D. K. (2021). Trace metals and animal health: Interplay of the gut microbiota with iron, manganese, zinc, and copper. *Animal Nutrition*, 7, 750–761.

Pedersen, S., & Lierhagen, S. (2006). Heavy metal accumulation in arctic hares (*Lepus arcticus*) in Nunavut, Canada. *Science of the Total Environment*, 368, 951–955.

Petrović, Z., Teodorović, V., Dimitrijević, M., Borozan S., Beuković, M., & Milićević, D. (2013). Environmental Cd and Zn concentrations in liver and kidney of european hare from different Serbian regions: Age and tissue differences. *Bulletin of Environmental Contamination and Toxicology*, 90, 203–207.

Petrović, Z., Teodorović, V., Djurić, S., Milićević, D., Vranić, D., & Lukić, M. (2014). Cadmium and mercury accumulation in European hare (*Lepus europaeus*): age-dependent relationships in renal and hepatic tissue. *Environmental Science and Pollution Research*, 21, 14058–14068.

Pilarczyk, B., Tomza-Marciniak, A., Pilarczyk, R., Udała, J., Kruzhel, B., & Ligocki, M. (2020). Content of essential and non-essential elements in wild animals from western Ukraine and the health risks associated with meat and liver consumption. *Chemosphere*, 244, 125506.

Puls, R. (1994). Mineral Levels in Animal Health: Diagnostic Data, 2nd edn. Sherpa International, Clearbrook, BC.

Rahil-Khazen, R., Bolann, B. J., & Ulvik, R. J. (2002). Correlations of trace element levels within and between different normal autopsy tissues analysed by Inductively coupled plasma atomic emission spectrometry (ICP-AES). *BioMetals*, 15, 87–98.

Rai, G. K., Bhat, B. A., Mushtaq, M., Tariq, L., Rai, P. K., Basu, U., … & Bhat, J. A. (2021). Insights into decontamination of soils by phytoremediation: A detailed account on heavy metal toxicity and mitigation strategies. *Plant Physiology*, 173, 287–304.

Schai-Braun, S. C., Reichlin, T. S., Ruf, T., Klansek, E., Tataruch, F., Arnold, W., et al. (2015). The European hare (*Lepus europaeus*): A picky herbivore searching for plant parts rich in fat. *PLoS One*, 10, e0134278.

Selimovic, A., Tisier M. L., & Arnold, W. (2023). Maize monoculture causes niacin deficiency in free-living European brown hares and impairs local population development. *Frontiers in Ecology and Evolution*, 10 – 2022.

Squadrone, S., Robetto, S., Orusa, R., Griglione, A., Falsetti, S., Paola, B., Abete, M. C. (2022). Wildlife hair as bioindicators of metal exposure. *Biological Trace Element Research*, 200, 5073–5080.

Stepanova, M. V., Sotnikova L. F., & Zaitsev, S. Y. (2023). Relationships between the content of micro- and macroelements in animal samples and diseases of different etiologies. *Animals*, 13, 852.

Suchentrunk, F. R. W., & Hartl, G. B. (1991). On eye lens weights and other age criteria of the Brown hare (*Lepus europaeus* Pallas, 1778). *Zeitschrift für Säugetierkunde*, 56, 365–374.

Taylor, A. A., Tsuji, J. S., Garry, M. R., McArdle, M. E., Goodfellow, W. L., Adams, W. J., & Menzie, C. A. (2020). Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper. *Environmental Management*, 65, 131–159.

Tibbett, M., Green, I., Rate, A., De Oliveira, V. H., & Whitaker, J. (2021). The transfer of trace metals in the soil-plant-arthropod system. *Science of the Total Environment*, 20, 779, 146260.

Venäläinen, E. R., Niemi, A., & Hirvi, T. (1996). Heavy metals of hares in Finland. 1980–82 and 1992–93. *Bulletin of Environmental Contamination and Toxicology*, 56, 251–258.

Vidus-Rosin, A., Meriggi, A., Cardarelli, E., Mariani, M. A., Chiara Corradelli, C., Barba, A. (2011). Habitat overlap between sympatric European hares (*Lepus europaeus*) and Eastern cottontails (*Sylvilagus floridanus*) in northern Italy. *Acta Theriologica*, 56, 53–61.

Wajdzik, M., Halecki, W., Kalarus, K., Gąsiorek, M., & Pająk, M. (2017). Relationship between heavy metal accumulation and morphometric parameters in European hare (*Lepus europaeus*) inhabiting various types of landscapes in southern Poland. *Ecotoxicology and Environmental Safety*, 145, 16–23.

Woolliams, J. A., Suttle, N. F., Wiener, G., Field, A. C., & Woolliams, C. (1983). The long-term accumulation and depletion of copper in the liver of different breeds of sheep fed diets of differing copper content. *Journal of Agricultural Sciences*, 100, 441–449.

Zeman, T., Buchtova, M., Docekal, B., Misek, I., Navratil, J., Mikuska, P., Šerý, O., & Večeřa, Z. (2015). Organ weight changes in mice after long-term inhalation exposure to manganese oxides nanoparticles. *Journal of Physics: Conference Series*, 617, 012018.

Zoroddu, M. A., Aaseth, J., Crisponi, G., Medici, S., Peana, M., & Nurchi, V. M. (2019). The essential metals for humans: a brief overview. *Journal of Inorganic Biochemistry*, 195, 120–129.

Authors ORCID info

Zoran Petrović https://orcid.org/0000-0003-2016-5681
Jelena Ćirić https://orcid.org/0000-0002-8118-7676
Saša Janković https://orcid.org/0000-0002-5223-6993
Nikola Borjan https://orcid.org/0000-0003-4067-3755
Dejana Trbović https://orcid.org/0000-0001-9357-7789
Srđan Stefanović https://orcid.org/0000-0002-8011-5654

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

UDK: 637.514.5.05:637.518
ID: 149457161
https://doi.org/10.18485/meattech.2024.65.1.3

Original Scientific Paper

Effect of different protein sources (plant, cricket powder and microalgae) on the technological and functional properties and sensory characteristics of pork meatballs

Maria Momchilova[1], Dilyana Gradinarska-Ivanova[2], Dinko Yordanov[2], Gabor Zsivanovits[1] and Natalia Pats[3]*

[1] *Institute of Food Preservation and Quality, Food Technology Division, 4000 Plovdiv, 154 Vasil Aprilov Blvd., Agricultural Academy of Bulgaria, Bulgaria*
[2] *University of Food Technologies, Technological Faculty, Department of Meat and Fish Technology, 26 Maritsa Blvd., 4002 Plovdiv, Bulgaria*
[3] *Educational Establishment „Grodno State Medical University", Grodno, Belarus*

ARTICLE INFO

Keywords:
Spirulina powder
Soy flour
Lupin flour
Cricket powder
Colour parameters
Textural traits
Emulsion stability
Sensory evaluation

ABSTRACT

This study demonstrates the potential use of soy flour, spirulina powder, cricket powder, buckwheat flour and lupin flour as alternative protein sources in a minced meat product (meatballs) by comparing the reformulated meatballs with control meat-only samples. We analysed the use of the same amount of each of the selected protein sources on the technological and functional characteristics and the sensory perception of raw and cooked meatballs. Higher pH and better emulsion stability was observed in the soy flour, spirulina and cricket powder samples compared to the meat-only sample. In the texture profile, greater hardness and springiness of the samples made with buckwheat flour, soy flour and spirulina powder was found compared to the meat-only sample, but lesser values for the same parameters when cricket powder or lupin were added. The results obtained indicated that spirulina and cricket powder are promising ingredients for the innovative formulation of meat products and are suitable for application in a mixed design approach.

1. Introduction

Consumer interest in healthy and nutritionally complete foods, both of animal and plant origin, is constantly growing. Simultaneously, in the context of resource scarcity, global climate change, environmental pollution and increasing food demand, the strategies for more efficient and sustainable agri-food systems have prompted researchers and producers to explore different protein sources that could be used for obtaining new, healthy, sustainable and natural foods with a balanced nutritional composition (*Markard et al.,* 2012; *Velasco-Muñoz et al.,* 2021). Meat products, being both sources of a wide variety of important nutrients (proteins, lipids, minerals and vitamins) (*Jiménez-Colmenero and Delgado Pando,* 2013*; Lorenzo and Pateiro* 2013*; Lorenzo et al.,* 2014) and recognisable, widely consumed and valued foods due to their taste qualities, can be seen as a suitable object of composition modification with a view to the manufacture of innovative products with improved nutritional benefits (*dos Santos et al.,* 2016*; Lorenzo et al.,* 2016; *Domínguez et al.,* 2017; *Heck et al.,* 2017). Meat product reformulation through the addition of various plant products and proteins is not a new invention; however, this trend is nowadays oriented to the technical and economic benefits but also to the enrichment of the finished products with various natural sources of biologically active compounds (*Eisinaite et al.,* 2016) that reduce the risk of a number of socially

***Corresponding author:** Maria Momchilova, masha821982@abv.bg

Paper received: January 24th 2024. Paper accepted: March 20th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

significant diseases (*Neuhouser,* 2019). Out of all plant proteins, soy protein products are the most widely used in the food industry, the meat industry in particular (*Asgar et al.,* 2010). Regardless of all proven technological and health benefits of soy protein preparations (isolates, concentrates, texturisers, granules and flours), they are classified as allergenic foods (*Spychaj et al.,* 2018). Furthermore, there have been concerns in recent years that soy production is one of the causes of deforestation in South America's rainforests, and that it is one of the infamous genetically modified foods rejected by many consumers in Europe. These are the reasons for the growing number of studies searching for other, more sustainable meat alternatives (*Altmann et al.,* 2019*; Grahl et al.,* 2018).

Lupin flour (hereafter called lupin) and buckwheat flour are possible sources of plant protein in the technology of various meat products owing to their similarities with soy (*Danowska-Oziewicz and Kurp,* 2017) and their good emulsifying and gelling properties (*Yang et al.,* 2021*; Janssen et al.,* 2007). Buckwheat has been recognised as a promising functional food source and is cultivated in various countries worldwide (*Ohsawa et al.,* 2020*; Pinski et al.,* 2023). Therefore, incorporating buckwheat in product formulations can make them attractive to the food market on account of their health benefits, and these products can become suitable food for people with gluten intolerance (*Sofi et al.,* 2022). The addition of lupin to foods can enhance their nutritional value by improving their protein content and well-established sustainability parameters, which is regarded as a crucial factor in the promotion of healthier food environments (*Abreu et al.,* 2023). Other foods rich in high-quality proteins and referred to as "foods of the future" for their potential to address the challenge of feeding the world's growing population are insects and microalgae (*Koyande et al.,* 2019*; Ruskova et al.,* 2023). Both of them fall within the scope of the so-called "novel foods", thus attracting growing interest not only from a nutritional perspective, but also from the point of view of the European Union's circular economy strategy and the reduction of greenhouse emissions, since they offer a way of securing a sufficient supply of protein in a sustainable manner.

This study demonstrates the potential use of soy flour, buckwheat flour, lupin (lupin flour), cricket powder and spirulina powder as alternative protein sources in a minced meat product (meatballs) by comparing the reformulated samples with control, meat-only samples. We aimed to compare the use of the same amounts of each of the five selected meat protein substitutes on the technological and functional characteristics of raw and cooked meatballs and on their sensory perception.

2. Materials and Methods

Six different meatball types were prepared for the study. The following recipe was used as the basic formulation: lean pork meat (shoulder blade): 50%; semi-fat pork: 50%; potable water: 20%; sodium chloride: 1.8%. The formulation without additives was used as a control. Soy flour, buckwheat flour, lupin flour, cricket powder and dry spirulina powder were added in 1% concentrations to the other five meatball types, respectively. Before the addition, the dry additives had been hydrated in water in a 1:3 w/v ratio. The protein additives were purchased from retail shops, cricket powder was supplied by EntoSynergy Ltd (Bulgarevo, Bulgaria), and the meat raw materials were supplied by the AGO–MES meat manufacturing company (Asenovgrad, Bulgaria).

The samples were prepared in the following production sequence: the meat was ground using a meat grinder with a grid diameter of 6 mm and divided into six equal parts; the necessary salting materials, water, and a protein supplement were added to each part in a mixer as indicated in Figure 1; 0.060 kg meatballs were formed from each obtained meat batter and were then packed on polyvinyl chloride plates and stored at 4±1 °C. At 24 h after the meatballs were prepared, the raw meatballs were analysed according to the following physicochemical parameters: pH, emulsifying capacity and colour characteristics. After roasting the meatballs to a temperature of 72°C in the centre, they were examined to determine their thermal weight loss (cooking yield) and textural parameters and were subjected to sensory evaluation.

pH analysis

The pH determinations were carried out on a prepared aqueous extract of the sample (1:9 w/v), using a pH meter (MS 2004, Microsyst, Bulgaria).

Colour analysis

The colour parameters lightness, (L*), redness, (a*), yellowness, (b*), chroma (C), and hue (h) were determined spectrophotometrically using a Minolta Chroma meter (model CR 410, Osaka, Japan) in the CIELab system.

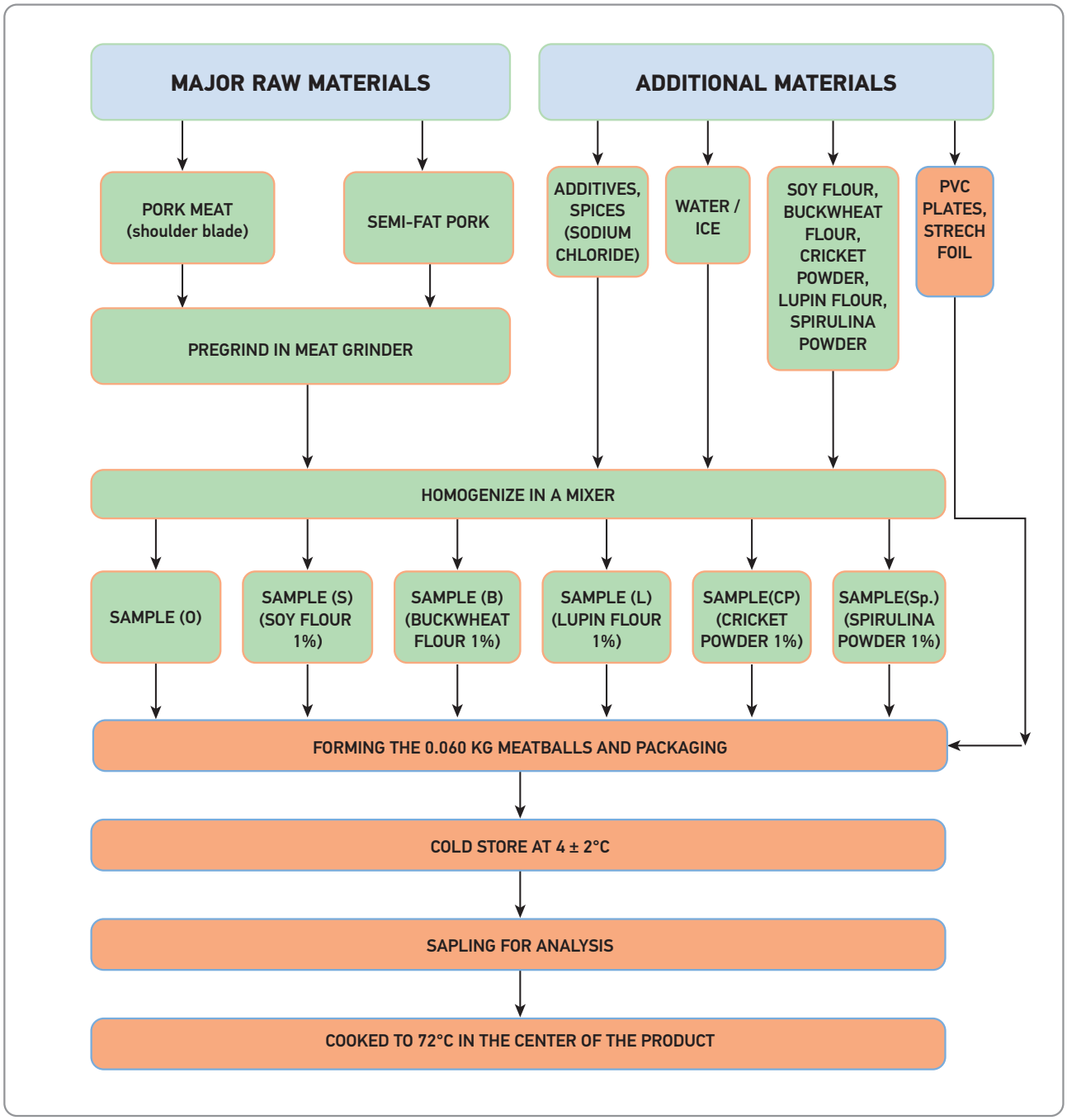

Figure 1. Flow chart of the meatball preparation with the addition of different protein sources to the samples

Emulsion stability

For determination of the emulsion stability, the method described by *Zorba and Kurt* (2006) was used. Thirty grams of each sample before and after heat treatment were weighed into a centrifuge tube and heated in a water bath at 70 °C for 30 minutes. Immediately after heating, the tubes were centrifuged at 2000 rpm min $^{-1}$ for 10 minutes, and the separated water and oil were weighed and used to calculate the emulsion stability (ES).

Cooking yield

The cooking yield was determined as the percentage of weight loss in the samples after cooking according to the method described previously (*Murphy et al.*, 1975).

Texture profile analysis (TPA)

A TA-XT Plus texture analyser (Stable Micro Systems, Surrey, UK) was used to analyse the texture profile of the finished heat-treated meatballs under

the following measurement conditions: sample size: 40±2 mm in diameter and 25±2 mm in height; diameter of the compression cylinder: 50 mm, compression speed: 2 mm s^{-1}; degree of deformation: 8 mm; and relaxation time between two compressions: 5 s. The hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness of the samples were calculated on the basis of the obtained values (*Bourne,* 1978*; Bourne,* 2002; *Kim et al.,* 2009).

Sensory evaluation

The sensory evaluation was performed in a sensory laboratory, with precautions taken to ensure that each panellist would make an independent evaluation. The analysis was performed on six meatball samples, each sample designated by a 3-digit number and randomly assigned to trained panellists. The meatballs were evaluated for appearance, colour, aroma, consistency, taste, aftertaste, saltiness and overall sensory evaluation. Each sensory parameter was rated along a structured 7-point scale with values ranging from *dislike extremely* (1), *dislike very much* (2), *dislike* (3), *acceptable* (4), to *like* (5), *like very much* (6) and *like extremely* (7) (*Kırkın et al.,* 2019).

Statistical analysis

All the data obtained were statistically analysed by one-way analysis of variance (ANOVA) using the Statgraphics 16 software product. Significant ($p \leq 0.05$) differences between the treatments were determined using Duncan's post hoc test. All experiments were performed in triplicate, and the data presented in the tables and figures were expressed as means±standard deviation (SD).

3. Results and Discussion

The addition of the different protein sources, although in small amounts (1%), had a significant effect on the pH values of the meatballs (Table 1). The highest pH values were measured in the soy samples, followed by the spirulina and cricket powder samples ($p<0.05$). In contrast, the addition of the other two plant flours, buckwheat and lupin, led to lower pH values of the samples, even below the measured value for the meat-only control sample. An increase in pH with the addition of spirulina, soy or insect powder was also reported by other authors who studied the effect of such additives following their addition to sausages (*Kim et al.,*2016*; Marti-Quijal et al.,* 2019). The changes in the pH values could have resulted directly from the pH of the individual ingredients, but it is important to point out that the use of additives that can increase the pH of the meat batter is desirable from the point of view of the water holding capacity of the meat product; hence, a higher yield and better consistency during heat treatment are obtained. In contrast, a low pH can cause protein denaturation which affects protein solubility, water holding capacity and colour (*Cornfort,* 1994).

Each one of the additives used, due to its own colour and its hydration before being added to the meat batter, led to changes in the general colour characteristic of the meatballs with the additives compared

Table 1. Effect of the addition of different protein sources on the pH value and the colour characteristics of raw pork meatballs

Sample	Parameter					
	pH	lightness (L*)	redness (a*)	yellowness (b*)	chroma (C)	hue (h)
O	6.16±0.01^{c}	52.89±7.48^{b}	10.72±1.12^{b}	5.73±0.54^{ab}	12.18±0.88^{b}	28.32±4.19^{a}
S	6.36±0.01^{f}	60.82±6.20^{c}	10.91±3.66^{b}	7.23±1.97^{bc}	13.10±4.11^{bc}	33.97±2.23^{ab}
B	6.12±0.01^{a}	55.51±2.61^{bc}	12.11±1.08^{b}	7.93±0.98^{c}	14.37±1.31^{bc}	32.41±2.93^{ab}
L	6.14±0.01^{b}	56.31±6.49^{bc}	11.89±1.30^{b}	8.76±0.97^{c}	14.77±1.39^{bc}	36.41±3.27^{b}
CP	6.23±0.01^{d}	53.68±6.86^{bc}	12.19±1.44^{b}	8.79±1.78^{c}	15.09±1.74^{c}	35.69±5.60^{ab}
Sp	6.30±0.01^{e}	33.55±3.12^{a}	−1.14±1.03^{a}	4.87±0.73^{a}	5.08±0.79^{a}	102.97±10.96^{c}

Note: Results are mean values for the respective samples after triplicate measurements of the individual parameters.

$^{a-e}$**:** Values bearing the same superscripts were not statistically different ($P > 0.05$).

Sample description: sample O: control meatballs without additives; sample S: soy flour sample; sample B: buckwheat flour sample; sample L: lupin flour sample; sample CP: cricket powder sample; sample Sp: spirulina powder sample.

to the meatballs without additives (Table 1). Thus, for instance, the highest L* values were recorded for the soy and lupin samples while the lowest lightness was observed for the spirulina sample, where, despite its good hydration, the dark green-blue colour of the spirulina strongly affected all colour parameters of the end product. The negative a* and b* values measured in these meatballs were attributed to the presence of phycocyanin (blue colour) and chlorophyll pigments (green colour) in the composition of *Spirulina platensis* (*Danesi et al.,*2004*; Marrez et al.,* 2013; *Marti-Quijal et al.,* 2019).

The cricket powder sample also showed higher *L**, *a** and *b** values compared to meatballs without additives (Table 1), and this was consistent with the results obtained by *Smarzyński et al.* (2019), who observed higher *L*, a*, b** when cricket powder was used in pork pâté. Although the values obtained for the red colour component remained statistically indiscernible (p>0.05) except for the spirulina sample, the highest a* values were measured in the cricket powder (12.19±1.44) and buckwheat (12.11±1.08) samples. This was in conformity with the results reported by other researchers who studied the effect of the addition of insect powder (*Kim et al.,* 2016*; Han et al.,* 2023) and buckwheat flour and flakes (*Shin et al.,* 2017*; Salejda et al.,* 2022) in the production of pork or poultry sausages. On the basis of the comparison of the *C* and *h* values of the meatballs, they were arranged in the following order with regard to the degree of colour changes in relation to the control meatballs without additives: buckwheat < soy < cricket powder < lupin < spirulina.

The emulsion stability of the meatballs prior to their heat treatment is presented in Table 2. The lowest emulsion stability values were reported for the lupin flour (78.23±1.14) and buckwheat flour (79.70±3.35) samples, which led to significant water losses during the subsequent heat treatment. The low pH values of these samples were a good indicator of the stability of the meat emulsions obtained (*Ho et al.,* 2022). The best emulsion stability was observed in the meat batter of the soy (85.78±1.27) and spirulina (85.32±1.24) samples, without any statistically significant difference between them (p>0.05). This corresponds to the high gelling and emulsifying capacity reported for proteins in spirulina (*Hamed et al.,* 2015*; Bernaerts et al.,* 2019), which makes the latter a competitive technological and functional ingredient compared to some commercial proteins used as emulsifiers in meat products, such as sodium caseinate, whey proteins and soy protein preparations (*Teuling et al.,* 2019).

Regarding the weight losses after heat treatment, represented via the finished product yield (Table 2), the investigated protein sources led to differences in this parameter as well. The lowest losses were found for the meatballs without a hydrated additive, followed by the samples with spirulina, cricket powder and lupin. The higher protein content in the additives used was probably one of the reasons for the differences in the yields (*Kolb et al.,* 2004*; Christaki et al.,* 2011). According to *Kim et al.* (2016), the higher yield obtained when using insect powder in meat products is due to the lower moisture content and higher protein content in

Table 2. Effect of the addition of different protein sources on the emulsion stability, textural parameters and cooking yield of pork meatballs

Sample	Parameter							
	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness (N)	Adhesiveness (N mm)	Emulsion stability, %	Cooking yield, %
O	52.23±17.22^{ab}	6.52±2.15^{ab}	0.55±0.02^{a}	33.53±7.46^{cd}	29.93±7.21^{cd}	−0.03±0.00^{b}	82.27±3.69^{bc}	60.51±2.56^{d}
S	65.58±18.45^{bc}	7.80±2.31^{bc}	0.54±0.06^{a}	30.43±3.00^{cd}	27.22±2.30^{cd}	−0.08±0.05^{ab}	85.78±1.27^{c}	58.63±3.21^{bc}
B	76.82±20.83^{c}	9.58±2.58^{c}	0.49±0.02^{a}	39.92±7.22^{d}	35.56±7.97^{d}	−0.08±0.10^{ab}	79.7±3.35^{ab}	54.39±3.32^{a}
L	43.21±6.31^{ab}	5.39±0.78^{ab}	0.54±0.03^{a}	23.39±4.45^{bc}	21.22±4.78^{bc}	−0.13±0.08^{ab}	78.23±1.14^{a}	58.63±1.69^{bc}
CP	41.75±14.07^{a}	5.21±1.76^{a}	2.43±2.69^{ab}	18.23±11.35^{ab}	16.69±10.27^{ab}	−0.17±0.20^{a}	82.43±1.18^{bc}	59.01±2.10^{b}
Sp	55.07±19.75^{ab}	6.87±2.47^{ab}	4.35±3.74^{b}	13.81±9.46^{a}	12.94±8.41^{a}	−0.97±0.05a	85.32±1.24^{c}	60.22±1.76^{cd}

Note: Results are mean values for the respective samples after triplicate measurements of the individual parameters.

$^{a-e}$: Values within the same column bearing the same superscripts were not statistically different (p>0.05)

Sample description: sample O: control meatballs without additives; sample S: soy flour sample; sample B: buckwheat flour sample; sample L: lupin flour sample; sample CP: cricket powder sample; sample Sp: spirulina powder sample.

the composition of these products, whereas the lower weight losses in our meatballs containing spirulina could be attributed to this product's high protein and polysaccharide content (*Backers and Noll,* 1998). The use of high protein additives that contain fibre in meat products leads to higher yields due to the improved water immobilisation capacity (*Steenblock et al.,* 2001*; Choe et al.,* 2011). Most probably, the similar technological properties of soy and lupin, related to binding the added water and affecting the texture of meat products (*Asgar et al.,* 2010), made the yields of the samples containing these additives statistically indiscernible. The greatest weight loss, and hence, the lowest yield, was observed in the meatballs made with the addition of buckwheat flour (Table 2). This was consistent with the lowest emulsion stability found for these samples. According to *Pires et al.* (2017), problems in the structure and consistency of finished sausages occurred when the emulsion stability was below 85%, as was the case with our buckwheat flour meatballs.

The texture analysis demonstrated that the buckwheat samples showed the highest hardness, gumminess, springiness and chewiness, together with the lowest values for the cohesiveness parameter (Table 2). Buckwheat proteins have the ability to increase the hardness of the product, similarly to soy proteins (*Bejosano and Corke,* 1998), and in our study, the significant increase in these textural parameters was also a consequence of the deteriorated emulsion stability and the water loss during the heat treatment of these samples. The use of spirulina in the composition (formulation) of the meatballs resulted in numerically lower but statistically indiscernible values for hardness, springiness and adhesiveness, and higher cohesiveness values compared to the soy sample. However, the chewiness and gumminess of the spirulina samples were significantly lower than the soy and all other samples. A similar trend towards a decrease in hardness was also observed by *Marti-Quijal et al.* (2018), who replaced soy with spirulina in the production of cooked turkey breast, as well as by *Parniakov et al.* (2018), who reported a decrease in the values of the textural parameters, with the exception of adhesiveness, in chicken rotti made with the addition of spirulina. Among our meatball types, the lowest hardness and gumminess were observed in the cricket powder sample, which was in contrast to the increase in the hardness of emulsion sausages found by *Kim et al.* (2016). Other researchers, who established a decrease in the hardness and cohesiveness and an increase in the springiness of meat batter after 10% substitution of lean meat with cricket powder (*Ho et al.,* 2022), suggested that different insect protein sources and different meat product preparation technologies could have an impact on the textural properties of the finished products. As a result of incorporating cricket powder in a hydrated state into the meatball batter (as in our study), the higher water content of the product can induce a decrease in the shear force, hardness, springiness and chewiness compared to the meat-only control (*Grahl et al.,* 2018). The highest cohesiveness was obtained

Table 3. Effect of the addition of different protein sources on the sensory descriptors of roasted pork meatballs

Sample	Parameters						
	Appearance	Colour	Aroma	Consistency	Taste	Aftertaste	Saltiness
O	6.7±0.48^{a}	6.7±0.48^{c}	6.2±0.63^{b}	5.4±1.26^{a}	6.6±0.52^{bc}	5.9±1.10^{b}	6.0±0.82^{b}
S	6.3±0.48^{a}	6.2±0.79^{bc}	6.2±1.03^{b}	5.7±0.82^{a}	6.8±0.42^{c}	6.2±1.03^{b}	5.9±0.57^{b}
B	6.4±0.70^{a}	5.8±0.79^{b}	5.0±0.82^{a}	5.9±0.74^{a}	5.8±1.32^{ab}	6.2±1.15^{b}	5.4±0.97^{b}
L	6.6±0.70^{a}	5.8±0.92^{b}	4.7±1.06^{a}	5.6±0.52^{a}	5.2±1.55^{a}	5.9±0.99^{b}	5.4±0.70^{b}
CP	6.3±0.82^{a}	6.2±0.79^{bc}	6.2±0.92^{b}	5.2±0.79^{a}	6.2±0.79^{bc}	6.1±0.99^{b}	5.5±0.71^{b}
Sp	6.2±0.92^{a}	5.0±1.05^{a}	6.0±0.94^{b}	5.9±0.74^{a}	4.9±0.99^{a}	4.9±0.88^{a}	4.4±0.52^{a}

Note: Results are mean values for the respective sample after five measurements of the individual parameters.

$^{a-e}$**:** Values within the same column bearing the same superscripts are not statistically different ($P > 0.05$)

Sample description: sample O: control meatballs without additives; sample S: soy flour sample; sample B: buckwheat flour sample; sample L: lupin flour sample; sample CP: cricket powder sample; sample Sp: spirulina powder sample.

in our samples with cricket powder and spirulina, which was in conformity with the results reported by *Kim et al.* (2016), who investigated the addition of new protein sources to emulsion-type meat sausages and also recorded an increase in cohesiveness compared to the control. Gumminess and chewiness parameters give an idea of the structural and mechanical properties that affect performance of the products during consumption. In the soy sample, these parameters were closest to the meat-only sample, whereas the spirulina and cricket powder samples showed the lowest gumminess and chewiness. In view of the fact that the low hardness and springiness of meat products can result in a lower quality product from the consumers' point of view, the cricket powder meatball was the least desirable of our formulations with regard to this parameter. This is consistent with the data of *Han et al.* (2023), who investigated the effect of cricket powder addition to meat sausages on their texture and emulsifying capacity.

Each one of the protein sources added affected the colour, taste and texture of the resultant reformulated meatballs. However, any difference in colour and taste of reformulated products is usually perceived as undesirable by consumers (*Jeon,* 2006; *Prakash and Kumari,* 2011; *Beheshtipour et al.,* 2013). Therefore, the soy and cricket powder samples were evaluated as being the most acceptable in terms of colour and taste (Table 3), due to their score proximity to the meatballs without additives. As had been expected, the spirulina sample received the lowest scores for these parameters because the green colour of microalgae affects consumer perception adversely (*Becker,* 2007; *Fradique et al.,* 2013). Furthermore, heat treatment of spirulina meatballs even increased the darkened colour. In addition to the dark, almost black colour of these meatballs, an earthy aftertaste and musty algae odour were also detected, similarly to the sensory results obtained by *Grahl et al.* (2018). Interestingly, the spirulina sample was rated as the saltiest among our products, probably due to the sodium and potassium ions contained in spirulina (*Janda et al., 2023*), and which are detected by the ion channels on the tongue and amplify the saltiness sensation. Lower aroma and taste grades were also given to the lupin and buckwheat samples, although both were rated positively, as liked and liked very much, respectively.

In terms of the degree of overall liking and acceptance by sensory panellists, the meatballs were ranked in the following ascending order: lupin < buckwheat < spirulina < cricket powder < soy < control. (Figure 2).

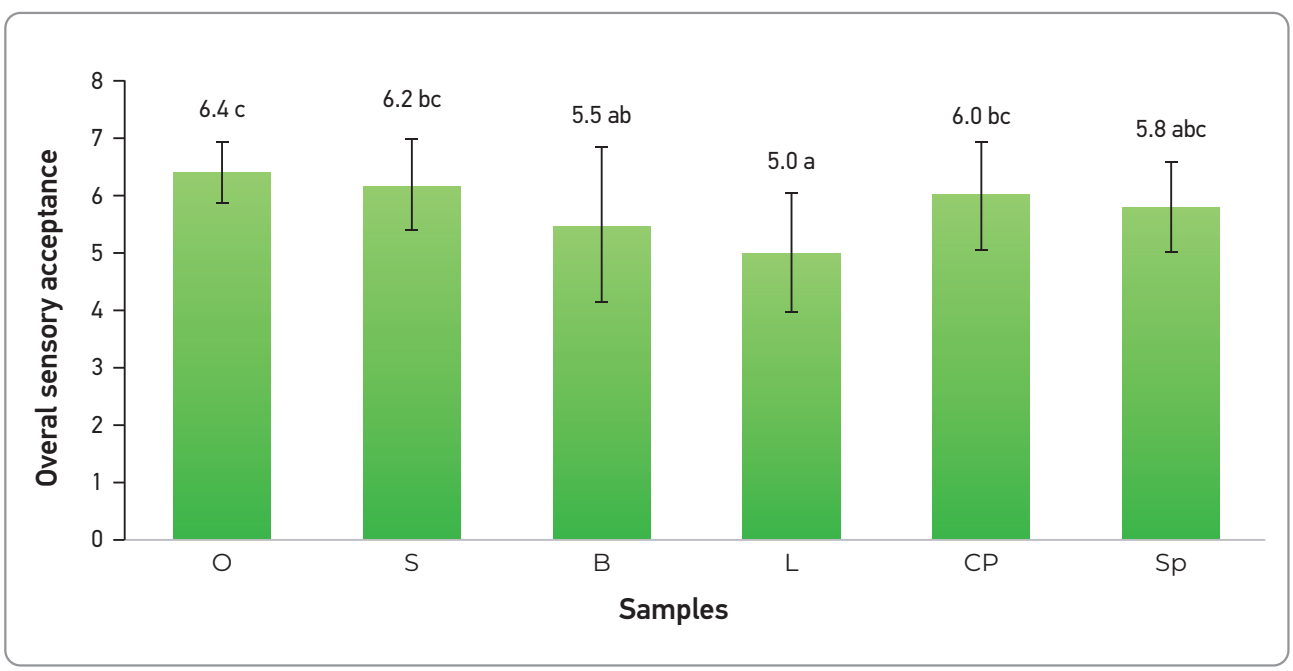

Figure 2. Overall sensory acceptance of the control and reformulated meatballs with different added protein sources

Sample description: sample O: control meatballs without additives; sample S: soy flour sample; sample B: buckwheat flour sample; sample L: lupin flour sample; sample CP: cricket powder sample; sample Sp: spirulina powder sample.

4. Conclusion

The experimental data provides objective evidence that the different protein sources, added in 1% amounts to the meat batter of reformulated pork meatballs, led to different emulsion stability and water holding capacity in the meat batter, as well as to modifications in the textural characteristics of the finished products. The inclusion of soy, spirulina or cricket powder as protein sources contributed to better emulsion stability and lower losses compared to the lupin and buckwheat samples. In the texture profiling, greater hardness and springiness of the buckwheat flour, soy flour and spirulina samples were observed compared to the control meatballs without additives; however, values of the same parameters, compared with the control, were lower with the addition of cricket powder or lupin. Significant differences were recorded regarding the colour parameters (L*, a*, b*, C and h), these colour differences were directly dependent on the protein source used, and they had impacts on the sensory evaluation. The results obtained indicate that spirulina and cricket powder are promising ingredients for the innovative formulation of minced meat products and are suitable for application in a mixed design approach.

Uticaj različitih izvora proteina (povrće, brašna od cvrčka i mikroalge) na tehno-funkcionalna svojstva i senzorne karakteristike svinjskih ćufti

Marija Momčilova, Diljana Gradinarska-Ivanova, Dinko Jordanov, Gabor Živanovič i Natalija Pats

INFORMACIJE O RADU

Ključne reči:
Spirulina u prahu
Sojino brašno
Brašno lupina
Prah od cvrčka
Parametri boje
Teksturne karakteristike
Stabilnost emulzije
Senzorna procena

APSTRAKT

Ova studija je pokazala potencijalnu upotrebu spiruline u prahu, praha od cvrčka, brašna od heljde i lupine kao alternativnog proteina u proizvodu od mlevenog mesa (mesne ćufti) upoređujući preformulisane uzorke sa kontrolnim uzorcima napravljenim od soje i sa uzorcima samo od mesa. Analizirali smo upotrebu jednake količine svakog od odabranih izvora proteina na tehnološke i funkcionalne karakteristike sirovih i kuvanih ćufti i njihovu senzornu percepciju.Uočeno je povećanje pH vrednosti i stabilnosti emulzije u uzorcima sojinogbrašna, spiruline i praha od cvrčkau poređenju sa uzorkom samo sa mesom. U profilu teksture utvrđeno je povećanje čvrstoće i elastičnosti uzoraka napravljenih od heljdinog brašna, sojinog brašna i spiruline u prahu u poređenju sa uzorkom samo od mesa i smanjenje istih parametara kada su dodani praha od cvrčka i lupina.Dobijeni rezultati ukazuju da su spirulina u prahu i praha od cvrčka obećavajući sastojci za inovativnu formulaciju proizvoda od mesa i pogodni za primenu u mešovitom dizajnerskom pristupu.

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

Acknowledgment: The research was carried out under project TN 15 of the Agricultural Academy, Task 1, entitled “Investigation of the possibilities of using different protein sources in semi-finished meat products. Effect on the quality characteristics and technological properties”. The researchers also thank the AGO–MES company in Asenovgrad, Bulgaria, for the meat raw materials supplied and EntoSynergy Ltd in Bulgarevo, Bulgaria for the cricket powder supplied.

References

Abreu, B., Lima, J., & Rocha, A. (2023). Consumer Perception and Acceptability of Lupin-Derived Products: A Systematic Review. *Foods*, 12 (6),1241.

Altmann, B. A., Neumann, C., Rothstein, S., Liebert, F., & Mörlein, D. (2019). Do dietary soy alternatives lead to pork quality improvements or drawbacks? A look into micro-alga and insect protein in swine diets. *Meat Science*, 153, 26–34.

Asgar, M. A., Fazilah, A., Huda, N., Bhat, R., & Karim, A. A. (2010). Nonmeat protein alternatives as meat extenders and meat analogs. Comprehensive Reviews in *Comprehensive Reviews in Food Science and Food Safety*, 9 (5), 513–529.

Backers, T., & Noll, B. (1998). Dietary fibres move into meat processing. *Fleischwirtschaft,* 78 (4), 319–320.

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25 (2), 207–210.

Beheshtipour, H., Mortazavian, A. M., Mohammadi, R., Sohrabvandi, S., & Khosravi-Darani, K. (2013). Supplementation of *Spirulina platensis* and *Chlorella Vulgaris* algae into probiotic fermented milk. *Comprehensive Reviews in Food Science and Food Safety*, 12 (2), 144–154.

Bejosano, F. P., & Corke, H. (1998). Amaranthus and buckwheat protein concentrate effects on an emulsion-type meat product. *Meat Science*, 50 (3), 343–353.

Bernaerts, T. M., Gheysen, L., Foubert, I., Hendrickx, M. E., & Van Loey, A. M. (2019). The potential of microalgae and their biopolymers as structuring ingredients in food: A review. *Biotechnology Advances,* 37 (8), 107419. A

Bourne, M. (2002). Food Texture and Viscosity: Concept and Measurement. 2nd ed. Academic Press, Geneva, New York. US.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology*, 32, 62–66.

Choe, J. H., Kim, H. Y., Han, D. J., Kim, Y. J., Park, J. H., Ham, Y. K., & Kim, C. J. (2011). Effect of goldenrod (*Solidago virgaurea*) leaf and stem powder on physical and sensory characteristics of emulsion-type sausages. *Food Science of Animal Resources*, 31 (5), 668–675.

Christaki, E., Florou-Paneri, P., & Bonos, E. (2011). Microalgae: a novel ingredient in nutrition. *International Journal of Food Sciences and Nutrition*, 62 (8), 794–799.

Cornforth, D. (1994). Color—its basis and importance. In Quality Attributes and Their Measurement In Meat, Poultry and Fish Products. Boston, MA: Springer US. pp. 34–78.

Danesi, E. D. G., Rangel-Yagui, C. D. O., Carvalho, J. C. M. D., & Sato, S. (2004). Effect of reducing the light intensity on the growth and production of chlorophyll by *Spirulina platensis*. *Biomass and Bioenergy*, 26 (4), 329–335.

Danowska-Oziewicz, M., & Kurp, L. (2017). Physicochemical properties, lipid oxidation and sensory attributes of pork patties with lupin protein concentrate stored in vacuum, modified atmosphere and frozen state. *Meat Science*, 131, 158–165.

Domínguez, R., Pateiro, M., Agregán, R., & Lorenzo, J. M. (2017). Effect of the partial replacement of pork backfat by microencapsulated fish oil or mixed fish and olive oil on the quality of frankfurter type sausage. *Journal of Food Science and Technology,* 54 (1), 26–37.

dos Santos Alves, L. A. A., Lorenzo, J. M., Gonçalves, C. A. A., Dos Santos, B. A., Heck, R. T., Cichoski, A. J., & Campagnol, P. C. B. (2016). Production of healthier bologna type sausages using pork skin and green banana flour as a fat replacers. *Meat Science*, 121, 73–78.

Dubey, R. P., & Pooja, K. (2011). Preparation of low-fat and high-protein frozen yoghurt enriched with papaya pulp and Spirulina. *Trends in Biosciences,* 4 (2), 182–184.

Eisinaite, V., Vinauskiene, R., Viskelis, P., & Leskauskaite, D. (2016). Effects of freeze-dried vegetable products on the technological process and the quality of dry fermented sausages. *Journal of Food Science,* 81 (9), 2175–2182.

Fradique, M., Batista, A. P., Nunes, M. C., Gouveia, L., Bandarra, N. M., & Raymundo, A. (2013). *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source. *LWT-Food Science and Technology*, 50 (1), 312–319.

Grahl, S., Palanisamy, M., Strack, M., Meier-Dinkel, L., Toepfl, S., & Mörlein, D. (2018). Towards more sustainable meat alternatives: How technical parameters affect the sensory properties of extrusion products derived from soy and algae. *Journal of Cleaner Production*, 198, 962–971.

Grahl, S., Strack, M., Weinrich, R., & Mörlein, D. (2018). Consumer-oriented product development: the conceptualization of novel food products based on spirulina (*Arthrospira platensis*) and resulting consumer expectations. *Journal of Food Quality*, 2018.

Hamed, I., Özogul, F., Özogul, Y., & Regenstein, J. M. (2015). Marine bioactive compounds and their health benefits: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 14, 446–465.

Han, X., Li, B., Puolanne, E., & Heinonen, M. (2023). Hybrid Sausages using pork and cricket flour: Texture and oxidative storage stability. *Foods,* 12 (6), 1262.

Heck, R.T., Guidetti, R., Etchepare, M. A., dos Santos, L. A. A., Cichoski, A. J., Ragagnin, C., Smanioto, J., Lorenzo, J. M., Wagner, R., & Campagnol, P. C. B. (2017). Is it possible to produce a low-fat burger with a healthy n - 6/n - 3 PUFA ratio without affecting the technological and sensory properties? *Meat Science,* 130, 16–25.

Ho, I., Peterson, A., Madden, J., Huang, E., Amin, S., & Lammert, A. (2022). Will it cricket? Product development and evaluation of cricket (*Acheta domesticus*) powder replacement in sausage, pasta, and brownies. *Foods,* 11 (19), 3128.

Janda-Milczarek, K., Szymczykowska, K., Jakubczyk, K., Kupnicka, P., Skonieczna-Żydecka, K., Pilarczyk, B., Tomza-Marciniak, A., Ligenza. A., Stachowska, E., & Dalewski, B. (2023). Spirulina supplements as a source of mineral nutrients in the daily diet. *Applied Sciences*, 13 (2), 1011.

Janssen, F., Pauly, A., Rombouts, I., Jansens, K. J., Deleu, L. J., & Delcour, J. A. (2017). Proteins of amaranth (*Amaranthus* spp.), buckwheat (*Fagopyrum* spp.), and quinoa (*Chenopodium* spp.): A food science and technology perspective. *Comprehensive Reviews in Food Science and Food Safety,* 16 (1) 39–58.

Jeon, J. K. (2006). Effect of Chlorella addition on the quality of processed cheese. *Journal of the Korean Society of Food Science and Nutrition,* 35 (3), 373–377.

Jiménez Colmenero, F., Delgado Pando, G. (2013). Fibre-enriched meat products. In: Fibre-Rich and Wholegrain Foods: Improving Quality. Eds. J. A. Delcour & K. Poutanen, Woodhead Publishing Limited. UK, pp. 329–347.

Kim, E. J., Corrigan, V. K., Hedderley, D. I., Motoi, L., Wilson, A. J. & Morgenstern, M. P. (2009). Predicting the sensory texture of cereal snack bars using instrumental measurements. *Journal of Texture Studies*, 40 (4), 457–481.

Kim, H.W., Setyabrata, D., Lee, Y. J., Jones, O. G., & Kim, Y. H. B. (2016). Pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient in emulsion sausages. *Innovative Food Science & Emerging Technologies*, 38, 116–123.

Kırkın, C., Inbat, S. M., Nikolov, D., & Yildirim, S. (2019). Effects of tarragon essential oil on some characteristics of frankfurter type sausages. *AIMS Agriculture and Food*, 4 (2), 244–250.

Kolb, N., Vallorani, L., Milanovic, N., & Stocchi, V. (2004). Evaluation of marine algae wakame (*Undaria pinnatifida*) and kombu (*Laminaria digitata* japonica) as food supplements. *Food Technology and Biotechnology*, 42 (1), 57–61.

Koyande, A. K., Chew, K. W., Rambabu, K., Tao, Y., Chu, D. T., & Show, P. L. (2019). Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness,* 8 (1), 16–24.

Lorenzo, J. M., & Pateiro, M. (2013). Influence of type of muscles on nutritional value of foal meat. *Meat Science*, 93, 630–638.

Lorenzo, J. M., Munekata, P. E. S., Pateiro, M., Campagnol, P. C. B., & Domínguez, R. (2016). Healthy Spanish salchicho´n enriched with encapsulated n-3 long chain fatty acids in konjac glucomannan matrix. *Food Research International,* 86, 289–295.

Lorenzo, J. M., Sarriés, M. V., Tateo, A., Polidori, P., Franco, D., & Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: a review. *Meat Science*, 96 (4), 1478–1488.

Markard, J., Raven, R., & Truffer, B. (2012). Sustainability transitions: An emerging field of research and its prospects. *Research Policy*, 41 (6), 955–967.

Marrez, D. A., Naguib, M. M., Sultan, Y. Y., Daw, Z. Y., & Higazy, A. M. (2013). Impact of culturing media on biomass production and pigments content of *Spirulina platensis*. *International Journal of Advanced Research*, 1 (10), 951–961.

Marti-Quijal, F.J., Zamuz, S., Galvez, F., Roohinejad, S., Tiwari, B.K., Gómez, B.,Barba, F.J., & Lorenzo, J. M. (2018). Replacement of soy protein with other legumes or algae in turkey breast formulation: Changes in physicochemical and technological properties. *Journal of Food Processing and Preservation*, 42 (12), e13845.

Marti-Quijal, F. J., Zamuz, S., Tomašević, I., Gómez, B., Rocchetti, G., Lucini, L., Remize, F., Barba. F. J., & Lorenzo, J. M. (2019). Influence of different sources of vegetable, whey and microalgae proteins on the physicochemical properties and amino acid profile of fresh pork sausages. *LWT,* 110, 316–323.

Murphy, E. W., Criner, P. E., & Gray, B. C. (1975). Comparison of methods for calculating retentions of nutrients in cooked foods. *Journal of Agricultural and Food Chemistry*, 23 (6), 1153–1157.

Neuhouser, M. L. (2019). The importance of healthy dietary patterns in chronic disease prevention. *Nutrition Research,* 10, 5317.

Ohsawa, R., Matsui, K., & Yasui, Y. (2020). Current research and future prospects in common buckwheat (*Fagopyrum esculentum*). *Breeding Science*, 70 (1), 1.

Parniakov, O., Toepfl, S., Barba, F. J., Granato, D., Zamuz, S., Galvez, F., & Lorenzo, J. M. (2018). Impact of the soy protein replacement by legumes and algae based proteins on the quality of chicken rotti. *Journal of Food Science and Technology,* 55, 2552e59.

Pinski, A., Zhou, M., & Betekhtin, A. (2023). Editorial: Advances in buckwheat research. *Frontiers in Plant Science*, 14, 1190090.

Pires, M. A., Munekata, P. E. S., Baldin, J.XC., Rocha, Y. J. P., Carvalho, L. T., dos Santos, I. R., Barros, J. C., & Trindade, M. A. (2017). The effect of sodium reduction on the microstructure, texture and sensory acceptance of Bologna sausage. *Food Structure,* 14, 1–7.

Ruskova, M., Petrova, T., & Goranova, Z. (2023). Edible insects-new meat alternative: a review. *Journal of Central European Agriculture,* 24 (1), 260–267.

Salejda, A. M., Olender, K., Zielińska-Dawidziak, M., Mazur, M., Szperlik, J., Miedzianka, J., Zawiślak, I., Kolniak-Ostek, J., & Szmaja, A. (2022). Frankfurter-type sausage enriched with buckwheat by-product as a source of bioactive compounds. *Foods*, 11 (5), 674.

Shin, H. B., Kim, H. Y., & Chun, J. Y. (2017). Quality characteristics of emulsion-type chicken sausages added different level of buckwheat powder. *Korean Journal of Poultry Science,* 44 (2), 135–141.

Smarzyński, K., Sarbak, P., Musiał, S., Jeżowski, P., Piątek, M., & Kowalczewski, P.Ł. (2019). Nutritional analysis and evaluation of the consumer acceptance of pork pâté enriched with cricket powder-preliminary study. *Open Agriculture,* 4 (1), 159–163.

Sofi, S. A., Ahmed, N., Farooq, A., Rafiq, S., Zargar, S. M., Kamran, F., Tanveer, D. A., Shabir, M. A., Dar, B. N., & Mousavi, K. A. (2023). Nutritional and bioactive characteristics of buckwheat, and its potential for developing gluten-free products: An updated overview. *Food Science & Nutrition,* 11 (5), 2256–2276.

Spychaj, A., Pospiech, E., Iwańska, E., & Montowska, M. (2018). Detection of allergenic additives in processed meat products. *Journal of the Science of Food and Agriculture,* 98 (13), 4807–4815.

Steenblock, R. L., Sebranek, J. G., Olson, D. G., & Love, J. A. (2001). The effects of oat fiber on the properties of light bologna and fat-free frankfurters. *Journal of Food Science,* 66 (9), 1409–1415.

Teuling, E., Schrama, J. W., Gruppen, H., & Wierenga, P. A. (2019). Characterizing emulsion properties of microalgal and cyanobacterial protein isolates. *Algal Research,* 39, 101471.

Velasco-Muñoz, J. F., Mendoza, J. M. F., Aznar-Sánchez, J. A., & Gallego-Schmid, A. (2021). Circular economy implementation in the agricultural sector: Definition, strategies and indicators. *Resources, Conservation and Recycling*, 170, 105618.

Yang, J., Lee, J., & Sung, J. (2021). Influence of acid treatment on flavonoid content and biological activity in tartary buckwheat grains and its application for noodles. *LWT*, 145, 111488.

Zorba, Ö., & Kurt, Ş. (2006). Optimization of emulsion characteristics of beef, chicken and turkey meat mixtures in model system using mixture design. *Meat Science*, 73 (4), 611–618.

Authors ORCID info

Maria Momchilova https://orcid.org/0000-0003-0328-6844
Dilyana Gradinarska-Ivanova https://orcid.org/0000-0003-2373-4333
Dinko Yordanov https://orcid.org/0000-0002-9300-6588
Gabor Zsivanovits https://orcid.org/0000-0003-3278-6119
Natalia Pats https://orcid.org/0000-0001-8726-6845

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

UDK: 637.55.04:639.111.1
ID: 149530121
https://doi.org/10.18485/meattech.2024.65.1.4

Original scientific paper

The influence of hunting region and deer species on the content of volatile compounds in deer meat

Marija Starčević[1], Branislav Baltić[2], Aleksandra Tasić[3], Milica Laudanović[1], Srđan Stefanović[2], Jelena Janjić[1] and Snežana Ivanović[3]*

[1] *Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18*
[2] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11 000 Belgrade, Serbia*
[3] *Scientific Institute of Veterinary Medicine of Serba, Janisa Janulisa 14, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Red deer
Fallow deer
Roe deer
Meat quality
Volatile compound

ABSTRACT

The aim of this study was to assess the effect of region (lowland vs. mountain region) on the content of volatile compounds of red deer (Cervus elaphus), fallow deer (Dama dama) and roe deer (Capreolus capreolus). A total of forty eight female carcasses of three species (16 red deer, 16 fallow deer, and 16 roe deer) were collected from lowland and a mountain region, so from each region, 8 red deer, 8 fallow deer, and 8 roe deer were collected. In our study, higher contents of the aldehydes, ketones, and alcohols responsible for off-flavours of meat were found in our fallow deer meat than in red deer and roe deer meat. Moreover, in our study, region affected most of the content of aldehydes, heterocyclic and phenolic compounds, aromatic hydrocarbons, and some ketones, alcohols, and esters. Higher content of volatile compounds responsible for off-flavours was detected in mountain deer meat than in deer meat from the lowland region.

1. Introduction

During the last few decades, the meat of deer has been regularly consumed in European countries with predominant species being red deer, roe deer, and fallow deer (*Sorriano et al*., 2020). According to the Food and Agriculture Organization of the United Nations, annual game meat production has increased worldwide from approximately 1.89 million tons in 2010 to more than 2.03 million tons in 2022, and the increase is related to enhanced consumer interest in game meat (*FAOSTAT*, 2022). In Serbia, the number of deer was 6.127 in 2013 and 8.928 in 2021, while the number of roe deer in the same period ranged from 120.000 to 145.000. The number of hunting deer was 1.052 in 2013 and 1.172 in 2021, while the hunting of roe deer in the same period ranged from 9.000 to 11.000 animals (*www.stat.gov.rs*). One of the reasons for higher interest of consumers for game meat lies in the fact that game meat is generally considered as „healthy“ due to its high content of proteins (more than 22%), minerals, vitamins, and lower lipid content than 3% (*Costa et al.*, 2016). Moreover, game meat is a good source of unsaturated fatty acids, long chain n-3 polyunsaturated fatty acids, and conjugated linoleic acid (CLA) and it is regarded as „natural meat“, since animals mainly feed on pasture free of hormones, antibiotics, and other substances (*Soriano et al*., 2020). Game meat consumption differs among European countries and it is the highest in France (5.7 kg of game/person/year) and the lowest in Slovenia (0.56 kg of game/person/year) (*Mesinger et al*., 2023). Moreover, it is assessed that only 2–4% of the population consumes game meat and the reason for lower representation in the diet of Europeans could be ascribed to its high price, low availability, eating habits that not include game meat, and lack

***Corresponding author:** Marija Starčević, *marijadok@gmail.com*

Paper received April 4th 2024. Paper accepted April 8th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

of recepies using game meat (*Soriano et al*., 2020). Game meat is generally regarded as expensive and exotic, and it is not often available on the market (*Hoffman and Wiklund*, 2006).

Consumer's attitude toward the use of game meat in diet depends on sensory characteristics of meat such as colour, tenderness, and specific flavour (*Soriano et al*., 2020). The sensory characteristics of meat could be affected by many factors such as diet, gender, age, body condition, season, and climate (*Dannenberger et al*., 2013; *Kudrnáčová et al*., 2018; *Soriano et al*., 2020). Aroma is one of the most important attribute when consumer is making a decision to purchase meat (*Bosse et al*., 2017). During processing of meat different volatile organic compounds are formed that give meat characteristic flavour and their precursors significantly affect the final aroma of cooked meat (*Wojtasik-Kalinowska et al.,* 2023).

To the best of our knowledge, there are insufficient data in the published literature that evaluate the effect of region, with respect to altitude/terrain, on the content of volatile compounds in deer meat. Therefore, the aim of this study was to assess the effect of region (lowland vs. mountain region) on the content of volatile compounds of red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*).

2. Materials and Methods

A total of forty eight female carcasses of three species (16 red deer (*Cervus elaphus*), 16 fallow deer (*Dama dama*), and 16 roe deer (*Capreolus capreolus*)) were collected during the hunting season in October of 2019. Animals were approximately two years old as estimated by tooth eruption (*England & Wales Best Practise Guide*, 2019). Free-roaming deer of the three species were shot in two hunting districts, one a lowland and one a mountain region, so from each region, 8 red deer, 8 fallow deer, and 8 roe deer were collected. The lowland region is Karadjordjevo in Vojvodina (112 m above sea level, with average daily temperature of 14.6°C) and the mountain region is Deli Jovan in Eastern Serbia (from 700 to 1150 m above sea level, with average daily temperature of 13.0°C). The animals in the lowland region had access to 4120 hectares of free roaming area, consisting of oak (*Quercus robur*), ash (*Fraxinus excelsior*), elm (*Ulmus campestris*), poplar (*Populus alba*), and willow (*Salix babylonica*) forest and pastures. The mountain region hunting area comprises approximately 13000 ha, with predominantly beech (*Fagus silvatica*), oak (*Quercus robur*), acacia (*Robinia pseudoacacia*), and hornbeam (*Carpinus betulus*) forest. From December to March, deer were provided with the same additional feed that consisted of roughage feed (65% fodder beet and 35% alfalfa hay) and concentrate feed (whole kernel corn) placed in separated troughs. From March to December, deer were provided with additional concentrate feed (whole kernel corn) in order to provide all the necessary macro- and micronutrients that enable animals to maintain satisfactory health and achieve good performance results. The free-roaming animals had access to salt blocks during the whole year. Considering the differences in deer species (red deer, fallow deer, and roe deer) and hunting region (lowland and mountain region), six experimental groups were formed, each containing 8 carcasses.

Animals were shot from hunting stands and approximately in the head and neck region, immediately exsanguinated on the ground, hung onto the side of the truck, transported to the facility and then eviscerated and skinned within 1 h. Carcasses were held in chilled storage at 4°C for 24 h prior to sampling. At 24 h post mortem, meat samples (M. *longissimus lumborum*) were taken from the right side of each carcass behind the last rib, packed in polyethylene bags, and kept at –18°C in a thin layer for no longer than 10 days until analyses of the content of volatile organic compounds.

The day before analysis of the content of volatile organic compounds, meat samples were defrosted overnight at 4°C. Volatile compounds were analysed according to the procedure described by *Ivanović et al*. (2020).

Statistical analysis of the results was conducted with GraphPad Prism software version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Two-way analysis of variance (ANOVA) with Tukey's multiple comparison test was performed to test the effect of region (mountain region vs. lowland) and game species (fallow deer, red deer, and roe deer) as the main effects, and their interactions on the volatile content of deer meat. All parameters were described by means and standard error of means (SEM). Values of $p<0.05$ were considered significant.

3. Results

The effects of region (lowland vs. mountain region) and game species (fallow deer, red deer, and roe deer) on specific volatile substances in M. *longissimus lumborum* of our fallow deer, red deer, and roe deer are shown in Table 1.

Table 1. The effect of region (lowland vs. mountain region) and game species (fallow deer, red deer, and roe deer) on the content of volatile organic compounds in deer meat (n=8).

	Fallow deer		Red deer		Roe deer			p value (ANOVA)		
VOC (µg/kg)	**Mountain**	**Lowland**	**Mountain**	**Lowland**	**Mountain**	**Lowland**	**SEM**	**R**	**S**	**R×S**
Aldehydes										
Hexanal	2.9^{6a}	3.0^{4a}	Nd	Nd	1.6^{9b}	1.7^{0b}	0.375	ns	***	ns
Furfural	nd	Nd	0.02	0.016	nd	nd	0.004	ns	***	ns
Heptanal	0.3^{2a}	0.0^{7b}	0.0^{8b}	0.0^{8b}	0.5^{9c}	0.5^{3c}	0.091	**	***	**
Octanal	0.5^{8a}	0.0^{4b}	0.5^{6a}	0.6^{0}a	0.6^{9}a	0.6^{9}a	0.081	***	***	***
Phenylacetaldehyde	0.1^{2a}	0.0^{7ab}	0.1^{4a}	0.1^{0a}	0.0^{1b}	0.0^{9ab}	0.050	**	ns	**
Benzaldehyde	nd	Nd	Nd	Nd	0.02	0.02	0.006	ns	***	ns
Ketones										
2-butanone	12.0^{5a}	$21.^{7c}$	6.6^{3ab}	4.9^{1b}	1.9^{1b}	4.0^{3b}	4.111	*	***	**
2,3-butanedione	0.6^{4a}	1.2^{2a}	0.5^{6a}	0.7^{8a}	2.2^{4b}	1.6^{2b}	0.447	***	ns	**
2-heptanone	0.1^{7a}	0.2^{4a}	1.3^{1b}	1.1^{8b}	0.0^{5a}	0.0^{3a}	0.280	ns	***	ns
3-methyl-2(5H)-furanone	0.7^{2a}	0.9^{2a}	0.2^{9b}	0.3^{5b}	0.2^{4b}	0.1^{8b}	0.148	ns	***	ns
Heterocyclic compounds										
Furan	0.0^{8a}	2.1^{0b}	1.1^{2c}	1.3^{0c}	3.0^{8d}	3.3^{2d}	0.232	***	***	***
β-butyrolactone	3.1^{9a}	0.5^{3b}	4.1^{5c}	1.2^{3d}	0.0^{9b}	0.0^{9b}	0.257	***	***	***
2-pentylfuran	nd	0.30^{0a}	Nd	Nd	0.02^{1b}	0.02^{3b}	0.021	***	***	***
2-methyl pyrazine	3.2^{4a}	3.2^{4a}	Nd	Nd	0.6^{0b}	1.1^{9c}	0.198	**	***	***
2,5-dimethyl pyrazine	2.2^{5a}	0.9^{0b}	1.1^{9c}	1.3^{2c}	0.0^{8d}	0.0^{9d}	0.115	***	***	***
2,6-dimethyl pyrazine	1.4^{3a}	1.4^{8a}	1.0^{4b}	1.0^{4b}	0.0^{8c}	0.0^{8c}	0.163	ns	***	ns
Thiophene	nd	Nd	Nd	Nd	nd	nd	0.000	ns	ns	ns
Phenolic compounds										
Guaiacol	0.2^{7a}	0.3^{0a}	Nd	Nd	0.7^{4b}	0.5^{9c}	0.057	*	***	**
Aromatic hydrocarbons										
1,2-dimethoxybenzene	0.3^{4a}	Nd	0.9^{8b}	1.0^{4b}	0.2^{0ac}	0.0^{5c}	0.147	**	***	**
Sulphuric compounds										
2,5-dimethyl thiophene	0.11	0.02	0.07	0.07	0.03	0.03	0.050	ns	ns	ns
2-methyl thiophene	1.3^{2a}	1.3^{9a}	3.5^{1b}	3.8^{3b}	0.0^{8c}	0.0^{8c}	0.309	ns	**	ns
2-buthanethiol	0.5^{0a}	0.4^{7a}	1.0^{5b}	0.9^{9b}	0.3^{4a}	0.3^{5a}	0.127	ns	***	ns
2-methyl-3-furanthiol	nd	Nd	0.4^{2a}	0.4^{3a}	nd	nd	0.072	ns	***	ns
Alcohols										
2-butanol	10.4^{4a}	13.2^{0b}	3.2^{1c}	3.3^{5c}	5.1^{8d}	5.4^{1d}	0.523	***	***	***
2-pentanol	0.0^{5a}	0.05	Nd	Nd	0.0^{6a}	0.0^{6a}	0.010	ns	***	ns
3-methyl-1-butanol	38.5^{7a}	37.8^{3a}	28.5^{9b}	26.5^{1c}	30.2^{6d}	31.3^{6d}	0.582	**	***	***
2,3-butanediol	1.4^{7a}	1.7^{0a}	3.3^{7b}	3.3^{5b}	7.5^{5c}	6.6^{4c}	0.559	ns	***	*
1-octen-3-ol	0.8^{5a}	1.0^{2b}	0.6^{8c}	0.5^{9c}	0.3^{5d}	0.4^{0d}	0.087	ns	***	**

VOC (µg/kg)	Fallow deer		Red deer		Roe deer		SEM	p value (ANOVA)		
	Mountain	Lowland	Mountain	Lowland	Mountain	Lowland		R	S	R×S
Organic acids										
Propionic acid	2.6^{6a}	0.6^{3b}	1.3^{2c}	1.2^{2c}	0.6^{3b}	0.6^{0b}	0.401	***	***	***
3-methylbutanoic acid	1.2^{5a}	1.5^{3a}	2.5^{2b}	2.5^{5b}	0.2^{8c}	0.7^{2ac}	0.390	ns	***	ns
Hexanoic acid	nd	nd	0.0^{7a}	0.0^{7a}	nd	nd	0.012	ns	***	ns
Nonanoic acid	0.1^{3ab}	0.1^{5a}	0.1^{1ab}	0.1^{2ab}	0.0^{8b}	0.0^{8b}	0.027	ns	***	ns
Esters										
Isopropenyl acetate	nd	nd	0.01	0.02	nd	nd	0.005	ns	***	ns
Ethyl acetate	nd	0.04	Nd	Nd	nd	nd	0.014	**	**	**
Isobutyl acetate	0.2^{0a}	1.5^{0b}	1.1^{4c}	1.2^{0c}	0.8^{3d}	0.9^{2d}	0.072	***	***	***
Butyl acetate	5.4^{4a}	5.6^{3a}	Nd	Nd	0.9^{4b}	0.9^{0b}	0.091	ns	***	**
2-methylbutyl acetate	9.1^{3a}	8.7^{9a}	6.5^{2b}	6.4^{1b}	4.2^{7c}	4.5^{1c}	0.242	ns	***	*
3-methylbutyl acetate	0.0^{8a}	0.1^{1a}	0.0^{2b}	0.0^{2b}	0.0^{2b}	0.0^{1b}	0.019	ns	***	ns
Hexyl acetate	0.6^{4a}	0.5^{9a}	0.2^{3b}	0.3^{3c}	0.4^{2d}	0.3^{7dc}	0.049	ns	***	***
Ethyl butanoate	12.9^{6a}	13.7^{6a}	10.3^{0b}	11.2^{9c}	9.2^{1d}	7.8^{5e}	0.474	ns	***	ns
Ethyl isovalerate	nd	nd	Nd	Nd	nd	nd	0.000	ns	ns	ns
Ethyl 2-methylbutanoate	1.1^{5a}	1.2^{6a}	0.3^{3b}	0.2^{0bc}	0.0^{9cd}	0.0^{6d}	0.076	ns	***	**
Ethyl octanoate	1.34	1.42	0.09	0.11	1.07	1.19	0.067	**	***	ns
Alkanes										
Heptane	0.1^{1a}	0.0^{8a}	2.2^{6b}	3.2^{7c}	3.7^{0d}	3.9^{0d}	0.17	***	***	***

Data are means and standard error of means (SEM); R – Region; S – species; R×S – interaction between region and species; VOC – volatile organic compounds; nd – not detected; Within a row, means with a different superscript letter differ (a, b, c, d, e – $p < 0.05$); ns – not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

In our deer meat, aldehydes and ketones were found only in small quantities (aldehydes ranged from 0 to 3.04 µg/kg, and ketones ranged from 0 to 21.70 µg/kg). On the other hand, the most abundant compounds determined in our study were alcohols (ranged from 0 to 38.65 µg/kg). In our study, species affected all examined compounds ($p<0.01$), except for phenylacetaldehyde, 2,3-butanedione, thiophene, 2,5-dimethyl thio-phene, and ethyl isovalerate. Higher contents of the most abundant compounds among the aldehydes (hexanal), ketones (2-butanone), and alcohols (2-butanol and 3-methyl-1-butanol) were found in our fallow deer meat than in red deer and roe deer meat ($p<0.001$). Moreover, in our study, region affected most of the content of aldehydes, heterocyclic and phenolic compounds, aromatic hydrocarbons, and some ketones, alcohols, and esters ($p<0.05$).

4. Discussion

Some volatile compounds from the aldehydes and heterocyclic compound groups were not detected in the deer meat. The main precursors of these particular volatile compounds are unsaturated fatty acids, as the main constituent of phospholipids (*Martin et al*., 2002). Products of lipid oxidation and degradation are aldehydes, ketones, alcohols, hydrocarbons, and furans, while during the Maillard reaction, sulphuric and heterocyclic compounds are formed (*Neethling et al.,* 2016). *Bhadury et al.* (2021) has shown that many volatile compounds that were assumed to be created during thermal processes are also detected in raw meat. Packaging systems for meat and storage time may also affect lipid oxidation processes and, thus, formation of volatile compounds (*Wojtasik-Kalinowska*,2023).

During the storage of meat and meat products, lipid oxidation of mainly phospholipids may occur, and this phenomenon, known as warmed-over flavour (WOF), negatively affects meat quality. Meat with those changes has "rancid" and "metallic" tastes that are a consequence of many synthesized volatile compounds, such as hexanal, 2,3-octanedione, and trans-4,5-epoxy-(E)-2-decenal (*Kosowska et al.,* 2017). Although aldehydes and ketones are the predominant compounds in meat of domestic ruminants (*Villa Lobos- Del Gado et al*., 2014, *Moran et al*., 2022), in our deer meat those groups of volatiles substances were found only in small quantities likely due to the short period of storage before the analysis. The most abundant volatile compounds in our deer meat were alcohols and esters. Many factors affect volatile compounds in meat, such as breed, age, sex, rearing conditions, diet, and supplementation (*Wojtasik-Kalinowska*, 2023). Diet affects the volatile compounds in ruminant meat, and thus, a grain-based diet leads to a higher content of aldehydes and lactones, while the meat of grass-fed animals has higher contents of various phenols, terpenes, indoles, and sulphur compounds (*Bleicher et al*., 2022). The total fatty acid composition and fatty acid ratios in meat are characteristic of the animal species (*Neethling et al*., 2016). Thus, the content of PUFAs in deer meat, as the main precursors for volatile compound formation, depends on species. In our study, species affected all examined compounds, except for phenylacetaldehyde, 2,3-butanedione, thiophene, 2,5-dimethyl thiophene, and ethyl isovalerate. Higher contents of the most abundant compounds among the aldehydes (hexanal), ketones (2-butanone), and alcohols (2-butanol and 3-methyl-1-butanol) were found in our fallow deer meat than in red deer and roe deer meat.

Moreover, in our study, region affected most of the content of aldehydes, heterocyclic and phenolic compounds, aromatic hydrocarbons, and some ketones, alcohols, and esters. Higher contents of aldehydes, phenolic compounds, aromatic hydrocarbons, or-ganic acids, and 3-methyl-1-butanol were found in mountain deer than in deer from the lowland region. Discrepancies were observed for ketones (2-butanone, 2,3-butanedione), furan, 2-pentylfuran, 2-methyl pyrazine, 2-butanol, esters, and heptane, where higher levels were found in lowland deer than in deer from the mountain region. Higher content of volatile compounds found in deer from mountain region than in lowland region could be due to the fact that our lowland deer primarily grazed. Deer from lowland region had access to pastures, while mountain deer grazed to a lesser extent and primarily consumed concentrate feed. Pasture diets are richer in n-3 PUFA, as protective compounds for lipid oxidation and fat-soluble antioxidants like carotene and tocopherol that prevent lipid oxidation and formation of volatile compounds (*Neethling et al.,* 2016).

Unsaturated aldehydes and ketones are most responsible for off-flavours because of their low threshold (*Neethling et al.,* 2016). Since low levels of aldehydes and ketones were detected, the studied deer meat likely would have a low level of off-flavours. The most abundant aldehyde determined in our study was hexanal, originating from linoleic and arachidonic acid and having a rancid fragrance and grassy or green aroma (*Martin et al.,* 2002). In other studies, hexanal was the most abundant aldehyde (*Moran et al*., 2022, *Wei et al*., 2014), while *Ivanović et al*., 2020. did not detect hexanal in fallow and roe deer meat. It is important to note that during meat storage, the abundance of aldehydes in raw meat varies due to increased lipid oxidation, leading to rancid odour notes (*Dominguez et al.,* 2019). Although our meat samples were not stored for a long period, during long storage durations, long-chain aldehydes could degrade to short-chain aldehydes, such as hexanal (*Moran et al.,* 2022). In our study the most abundant ketone compound was 2-butanone, characterized by acetone-like odour. Ketones in meat are usually derived from the oxidation of free fatty acids (*Moran et al.,* 2022). In the group of alcohols, 3-methyl-1-butanol, with its pungent fragrance and associated with off-flavour, was detected as being the most abundant. Alcohols found in deer meat are mainly secondary products from aldehydes and likely generated from lipid oxidation (*Bueno et al.,* 2019).

Fallow deer had higher content of hexanal, 2-butanone, and 3-methyl-1-butanol than red deer and roe deer, indicating the potential lower sensory acceptability of fallow deer meat. Furthermore, the content of hexanal was similar in deer meat from the two regions. Moreover, 2-butanone was more abundant in lowland deer than in deer from the mountain region, while a higher level of 3-methyl-1-butanol was found in mountain deer than in lowland deer. These discrepancies in the amounts of the above-mentioned compounds that contribute to off-flavours made it difficult to conclude how region could affect the acceptability of deer meat.

5. Conclusions

With regard to volatile compounds responsible for off-flavours of meat, we detetcted higher volatile levels in fallow deer meat than in red deer and roe deer meat, as well as in mountain deer meat than in deer meat from the lowland region. Overall, considering the results of our study, we found that hunting region and deer species affects the volatile compounds responsible for off-flavours of meat.

Uticaj regiona lovišta i vrste jelenske divljači na sadržaj isparljivih jedinjenja u mesu jelena

Marija Starčević, Branislav Baltić, Aleksandra Tasić, Milica Laudanović, Srđan Stefanović, Jelena Janjić i Snežana Ivanović

INFORMACIJE O RADU

Ključne reči:
Evropski jelen
Jelen lopatar
Srndać
Kvalitet mesa
Isparljiva jedinjenja

APSTRAKT

Cilj ovog istraživanja bio je da se proceni uticaj regiona lovišta (ravničarski, odnosno planinski region) na sadržaj isparljivih jedinjenja u mesu evropskog jelena (Cervus elaphus), jelena lopatara (Dama dama) i srndaća (Capreolus capreolus). Sakupljeno je ukupno četrdeset osam trupova ženki tri vrste (po 16 trupova evropskog jelena, jelena lopatara i srndaća), tako da je iz ravničarskog i planinskog kraja prikupljeno po 8 trupova evropskog jelena, jelena lopatara i srnadaća. U našem istraživanju u mesu jelena lopatara utvrđen je veći sadržaj aldehida, ketona i alkohola odgovornih za neprijatan ukus mesa u poređenju sa mesom evropskog jelena i srndaća. Pored toga, region lovišta uticao je na sadržaj većine aldehida, heterocikličnih i fenolnih jedinjenja i estara. Veći sadržaj isparljivih jedinjenja odgovornih za neprijatan ukus utvrđen je u mesu jelenske divljači iz planinskog kraja nego iz ravničarskog kraja.

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

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-66/2024-03/200143).

References

ANON, (2008). *England & Wales Best Practise Guide*. Records & Surveys: Aging by teeth., Accessed May 2019 https://www.thedeerinitiative.co.uk/uploads/guides/110.pdf.

Bhadury, D., Nolvachi, Y., Marriott, P. J., Tanner, J. & Tuck, K. L. (2021). Detection of Volatiles from Raw Beef Meat from Dif-ferent Packaging Systems Using Solid-Phase Microextraction GC–Accurate Mass Spectrometry. *Foods*, 10, 2018.

Bleicher, J., Ebner, E. E. & Bak, K. H. (2022). Formation and Analysis of Volatile and Odor Compounds in Meat—A Review. *Molecules*, 27 (19), 6703.

Bosse, R., Wirth, M., Becker, T., Weiss, J. & Gibis, M. (2017). Determination of volatile marker compounds in raw ham using headspace-trap gas chromatography. *Food Chemistry*, 219, 249–259.

Bueno, M., Resconi, V. C., Campo, M. M., Ferreira, V. & Escudero, A. (2019). Development of a robust HS-SPME-GC-MS method for the analysis of solid food samples. Analysis of volatile compounds in fresh raw beef of differing lipid oxidation degrees. *Food Chemistry*, 281, 49–56.

Costa, H., Mafra, I., Oliveira, M. B. P. P. & Amaral, J. S. (2016). Game: Types and Composition. In: Caballero B, Finglas P, Toldrá F, editors. *The Encyclopedia of Food and Health.* Oxford: Academic Press, 177–183.

Dannenberger, D., Nuernberg, G., Nuernber, K., & Hagemann, E. (2013). The effects of gender, age and region on macro and micronutrient contents and fatty acid profiles inthe muscles of roe deer and wild boar in Mecklenburg-Western Pomerania (Germany). *Meat Science*, 94, 39–46.

Daszkiewicz, T. & Mesinger, D. (2018). Fatty acid profile of meat (*Longissimus lumborum*) from female roe deer (*Capreolus capreolus L.*) and red deer (*Cervus elaphus L.*). *International Journal of Food Properties*, 21 (1), 2276–2282.

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W. & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, 8, 429.

FAOSTAT, (2019). Livestock primary, production quantity, game meat. Accessed August 2021 http://www.fao.org/faostat/en/#data/QCL.

Hoffman, L. C. M & Wiklund, E. (2006). Game and venison-meat for the modern consumer. *Meat Science*, 74 (1), 197–208.

Ivanović S., Pavlović M., Pavlović I., Tasić A., Janjić J. & Baltić Ž. M. (2020). Influence of breed on selected quality parameters of fresh goat meat. *Archives Animal Breeding,* 14, 63 (2), 2019–2029.

Kim, K.W., Kim, H. J., Kim, H. J., Lee, S. S., Lee, E. D., Kim, D. K., Lee, S. H., Jang, A., Lee, J. (2020). Effect of Feeding Regime on Meat Quality of Elk Deer Loin during Aging. *Journal of Food and Nutrition Research,* 8 (7), 355–361.

Kosowska, M., Majcher, M. & Fortuna, T. (2017). Volatile compounds in meat and meat products. *Food Science and Technology,* 37 (1), 1–7.

Kudrnáčová, E., Bartoň, L., Bureš, D., & Hoffman, L. C. (2018). Carcass and meat characteristics from farm-raised and wild fallow deer (Dama dama) and red deer (Cervus elaphus): A review. *Meat Science*, 141, 9–27.

Martin, L., Timon, L. M., Petron, J. M., Ventanas, J. & Antequera, T. (2002). Evolution of volatile aldehydes in Iberian ham matured under different processing condition. *Meat Science,* 54, 323–327.

Mesinger, D., Ocieczek, A., Kozirok, W. & Owczarek, T. (2023). Attitudes of Young Tri-City Residents toward Game Meat in the Context of Food Neophobia and a Tendency to Look for Diversity in Food. *International Journal of Environmental Research and Public Healthy* 21(20), 3815.

Moran, L., Vivanco, C., Lorenzo, J. M., Barron L. J. R. & Aldai, N. (2022). Characterization of volatile compounds of cooked wild Iberian red deer meat extracted with solid phase microextraction and analysed by capillary gas chromatography — mass spectrometry. *LWT*, 163, 113472.

Nagy, J., Szabó, A., Donkó, T., Bokor, J., Romvári, R., Repa, I. & Fébel, H. (2019). Body composition and venison quality of farmed red deer (Cervus elaphus) hinds reared on grass, papilionaceous or mixed pasture paddocks. *Archives Animal Breeding,* 62, 227–239.

Serrano, M. P., Maggiolino, A., Landete-Castillejos, T., Pateiro, M., Barbería, J. P., Fierro, Y., Domínguez, R., Gallego, L., Garcia, A., De Palo, P. & Lorenzo, J. M. (2020). Quality of main types of hunted red deer meat obtained in Spain compared to farmed venison from New Zealand. *Scientific Reports — Nature,* 10, 12157.

Neethling, J., Hoffman, L.C. & Muller, M. (2016). Factors influencing the flavour of game meat: A review. *Meat Science*, 113, 139–153.

Razmaite, V., Siukscius, A., Sveistiene, R., Bliznikas, S. & Svirmickas, G. J. (2017). Comparative evaluation of longissimus and semimembranosus muscle characteristics from free-living and farmed red deer (Cervus elaphus) in Lithuania. *Zoology and Ecology.* 27 (2), 176–183.

Soriano, A., Murillo, P., Perales, M., Sánchez-García, C., Murillo, J. A. & García Ruiz, A. (2020). Nutritional quality of wild Iberian red deer (Cervus elaphus hispanicus) meat: Effects of sex and hunting period. *Meat Science,* 168, 108189.

Švrčula, V., Košinová, K., Okrouhlá, M., Chodová, D. & Hart, V. (2019). The effect of sex on meat quality of fallow deer (Dama dama) from the farm located in the Middle Bohemia. *Italian Journal of Animal Science,* 18 (1), 498–504.

Vigano, R., Demartini, E., Riccardi, F., Corradini, A., Besozzi, M., Lanfranchi, P., Chiappini, P. L., Cottini, A. & Gaviglio, A. (2018). Quality parameters of hunted game meat: Sensory analysis and pH monitoring. *Italian Journal Food Safety,* 8 (1), 7724.

Wojtasik-Kalinowska, I., Szpicer, A., Binkowska, W., Hanula, M., Marcinkowska-Lesiak, M. & Poltorak, A. (2023). Effect of Processing on Volatile Organic Compounds Formation of Meat—Review. *Applied Sciences*, 13 (2), 705.

Authors ORCID info

Marija Starčević https://orcid.org/0000-0002-0857-5218
Branislav Baltić https://orcid.org/0000-0002-3455-2909
Aleksandra Tasić https://orcid.org/0000-0002-8361-5697
Milica Laudanović https://orcid.org/0009-0008-6381-2803
Srđan Stefanović https://orcid.org/0000-0002-8011-5654
Jelena Janjić https://orcid.org/0000-0003-3351-7199
Snežana Ivanović https://orcid.org/0009-0000-9556-6936

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

UDK: 639.3(497.11)"2012/2021"
ID: 149401353
https://doi.org/10.18485/meattech.2024.65.1.5

Original scientific paper

Examination of the volume and value of fish and fish products imports into Serbia from 2012 to 2021

Jelena Janjić[1], Milorad Mirilović[1], Branislav Vejnović[1], Spomenka Đurić[1], Tihana Vujanić[1], Milica Laudanović[1] and Branislav Baltić[2]

[1] Faculty of Veterinary Medicine University of Belgrade, Bulevar oslobođenja 18, Belgrade, Serbia
[2] Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia

ARTICLE INFO

Keywords:
Catch
Aquaculture
Serbian market
Consumption

ABSTRACT

Serbia is partially supplied with fish from its own aquaculture and fishing, both commercial and recreational, which averaged 6.70 tons for the period from 2012 to 2021. The majority of fish in the market during the same period came from imports (an average of 34,090 tons). Out of the total catch of fish and fish products on the market in Serbia, 92.80% consisted of marine fish and seafood, while only 7.14% consisted of freshwater fish. The volume of imported sea fish and fish products followed this descending order: hake > tuna > seafood > canned fish > fish fillets > herring > sardines > mackerel > other seafood. Among freshwater fish, trout was the most commonly imported, followed by other species of fish and carp. The average total value of fish imports from 2012 to 2021 was €86.030 million, and the average import price of fish was €2.50/kg. With the import of fish and fish products, and fish from domestic production and catches, the fish market in Serbia was supplied with 41,270 tons of fish during the period studied, which means that the annual per capita fish consumption in Serbia was about 7 kilograms.

1. Introduction

The importance of fish in human nutrition is well known (proteins, fats, minerals, vitamins) and that is why this type of food is appreciated by the largest number of consumers (*Phogat et al.,* 2022). Despite the exceptional nutritional value, the consumption of fish is very variable in different parts and countries of the world, which depends on numerous factors (supply, demand, habits, price). According to data from 2023, the catch of fish from natural resources (oceans, seas, rivers, lakes) and fish from aquaculture (freshwater, seas, brackish waters) amounted to 186 million tons (MT), of which 90.6 MT came from catches and 96 MT from aquaculture (*FAO,* 2022). The volume of fish on the world market can be increased by the production of fish from aquaculture, while the catch of fish from natural resources will stagnate due to the protection of the fish stock, especially the catch of the most commonly caught fish species (hake, small blue fish) and the prevention of changes in the water ecosystem. Overfishing of natural resources is protected by limiting the catch of fish in each fishing area. Fishing areas in oceans and seas (with the exception of coastal territorial waters of states) are divided by international agreements to countries that have fishing fleets (*Ivanović et al.*, 2015). The world's largest producer of fish (catch and aquaculture) is China with 67.8 MT (38% of world production), and among the five largest producers are Indonesia (16.7 MT), India (10.9 MT), Vietnam (6.4 MT), and Bangladesh (6.3 MT). 58.8 million people are employed in the primary fishing sector (catch and production in aquaculture), and a total of about 600 million people work in the secondary sector (processing, transport, traffic).

***Corresponding author:** Jelena Janjić, *jelena.janjic@vet.bg.ac.rs*

Paper received: April 4th 2024. Paper accepted: April 12th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

China, Chile and Norway had the biggest increase in fish production, and Egypt and Nigeria had the biggest decrease in production. From 1961 to 2022, the increase in fish production in aquaculture grew at an average annual rate of 3%. In the same period, the population of people in the world grew at an average annual rate of 1.6%. It is believed that by 2030, fish production in aquaculture will increase by 14% and will amount to 106 MT (*Anon.,* 2022).

In contrast to the world trends of fish production in aquaculture in Serbia, fish production is declining. In 2023, 149 carp ponds, 77 trout ponds, and three for the cultivation of catfish were registered in Serbia. In 2023, around 2,000 workers were employed in primary production. The area under carp ponds was 13,750 hectares in 1997, 8,411 hectares in 2010, and 5,527 hectares in 2022. In 1997, there were 146,933 square meters under trout ponds, in 2018 it was 81,411 square meters, and in 2022 it was 60,135 square meters. In addition to the reduction of the area under ponds, there was also a drop in fish production per unit area, which is particularly pronounced in carp ponds, where fish production per hectare fell below one ton (*Anon.,* 1997; 2010; 2018; 2022). Serbian fisheries share the fate of livestock production in Serbia (reduction in the number of cattle, pigs, poultry, import of frozen meat, import of meat products). In order to meet the needs of fish for the market, Serbia imports fish and fishery products from as many as 65 countries in the world, mostly from Spain, Norway, Thailand, Vietnam and Croatia. The volume of fish exports from Serbia is negligible and refers to the re-export of processed fish (smoked, packaged). With fish from its own production, fish from commercial and recreational fishing, Serbia cannot meet the needs of the market, so fish and fish products are imported. From these two sources (fish from Serbia and fish from imports), the quantity of fish is ensured so that the consumption of fish in Serbia is equal to a third of the average consumption of fish in the world (*Anon.,* 2019*; Baltić et al.,* 2023).

One of the most significant and largest fish farms in Europe, Ečka (covering 1,700 ha), was in operation for over 120 years and closed in 2023. Today, Serbia is supplied with fish from aquaculture (carp and trout farms), commercial and recreational fishing, and primarily, from the import of marine fish.

The aim of this paper is to examine the volume, import, and value of fish and fish products (marine and freshwater) in Serbia from 2012 to 2021 (ten years).

2. Materials and Methods

Data on the volume and value of fish and fish products in Serbia were obtained from the Statistical Office of the Republic of Serbia, Department for Dissemination and Public Relations (*www.stat.gov.rs*). The volume and value of imports were prepared based on tariff numbers assigned to each type of fish or fish product. Based on these data, the volume of imports and the value of fish were classified into two main groups: marine and freshwater fish. Marine fish were further categorized according to the volume and value of fish and fish products into eight groups (hake, tuna, canned fish, sardines, herring, mackerel, fish fillets, and seafood). Seafood (mollusks, crustaceans, and shellfish) was also included in the marine fish group. Freshwater fish were classified into three groups (carp, trout, and other freshwater fish species).

The average total value (€) of fish imports, classified into three groups (marine fish, seafood, and freshwater fish) is presented. The average values of imports per kilogram (€/kg) for total fish and by groups are also shown.

The results obtained were compared by statistical analysis using GraphPad Prism software, version 9.00 for Windows (GraphPad Software, San Diego, California USA, *www.graphpad.com*). The mean values, and measures of volume and value of fish and fish products imports for the ten-year period were calculated. Trends were computed, and all results are presented tabularly and graphically using Microsoft Excel 2010.

3. Results and Discusion

Globally, the fish market is supplied by catches from open waters (oceans, seas, lakes, rivers) and fish farmed in aquaculture. In 2023, the volume of fish caught in open waters was 90.6 million tons. This level has been maintained for over 30 years because a larger catch would threaten the survival of the most commonly caught species of fish (small pelagic fish, hakes) and would lead to disturbances in the ecosystems of open waters (seas, oceans). Today, 96 million tons of fish are harvested from aquaculture (freshwater, saltwater, and brackish water). Although aquaculture was known more than 2000 years ago, it was not given significant attention until the 1960s. This is the period when there was a rapid increase in the world human population (the population boom), with the number

of inhabitants increasing by one billion every 12 to 14 years, reaching 8 billion in 2023 (*Baltić & Marković*, 2017).

A particularly rapid increase in fish production in aquaculture was recorded from the 1990s. By that time, the nutritional value of fish as food with a well-balanced content of macronutrients such as proteins (high content, good digestibility, essential amino acids) and fats (low content, favorable ratio of n-3/n-6 fatty acids) was already known, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Micronutrients and vitamins also significantly contribute to the nutritional value of fish (A, D, B_{12}, folic acid, and choline), as do important minerals (Ca, Zn, Se, I, Cu, F, Mg, Mn, and Cr) (*Ghaly et al*., 2013; *Khalili & Sampels*, 2018; *Innes & Calder*, 2020; *Boyd et al.*, 2022; *Phogat et al.*, 2022; *Tacon*, 2023). Fish is recommended in human diets because it protects against various non-communicable diseases, especially cardiovascular diseases, preterm born and mentally ill children (*Luo et al*., 2022). The global fish catch in 2023 was 90.6 million tons, and aquaculture production was 96 million tons, meaning that the total fish supplied to the market in 2023 was 186.6 million tons (*Ali et al.*, 2022).

The largest catch and production of fish in 2023 were in China, followed by Indonesia, Vietnam, and the USA. Out of the total catch and production of fish, 166 million tons were intended for human consumption, 15 million tons for animal feed, and 4 million tons were used for other purposes. Out of the total catch and production of fish, 65 million tons were subject to trade (export/import) (*Baltić et al*., 2023).

The average total import (Table 1) of fish and fish products into Serbia from 2012 to 2021 was 34,090±3,421 tons with a coefficient of variation of 10.04%. Among marine fish, the most commonly imported types were various types of hake, tuna, then canned fish (sardines, tuna), small pelagic fish (sardines, herring, mackerel), fillets, and other types of marine fish (scorpionfish, sea bream, sea bass, salmon). The import of seafood such as mollusks, crustaceans, and shellfish was also significant (4,104±1,045 tons). Among freshwater fish, trout was most commonly imported (1,519±351.3 tons), while the import of carp and other types of freshwater fish (bighead carp, grass carp, catfish, pike) was much lower. The import of marine fish (Figure 1) was significantly higher (92.86%) than the import of freshwater fish (7.14%).

Table 1. Average fish imports (tons), total and by species, for the ten-year period from 2012 to 2021.

Origin	Total import	$\overline{X}$	Measures of variation				
			Sd	Se	X_{min}	X_{max}	C_v (%)
		34090	**3421**	**1082**	**29490**	**41071**	**10.04**
Marine	Hake	7755	1752	554	5065	11238	22.59
	Tuna	4858	601,1	190,1	4219	5934	12.37
	Canned fish	3485	1320	417,4	1993	5701	37.88
	Sardines	2369	354	111,9	1682	2915	14.95
	Herring	2604	503,6	159,2	1773	3382	19.34
	Mackerel	2367	431,5	136,5	1788	2896	18.23
	Fillets	2755	1044	330,3	1316	4836	37.90
	Other types of marine fish	1361	574,4	181,7	458	2053	43.09
	Seafood	4104	1045	330,4	2908	6545	25.46
Freshwater	Carp	450	348,7	110,3	83	1143	77.50
	Trout	1519	351,3	111,1	940	1830	23.13
	Other types of freshwater fish	463	214,6	67,87	209	912	46.35

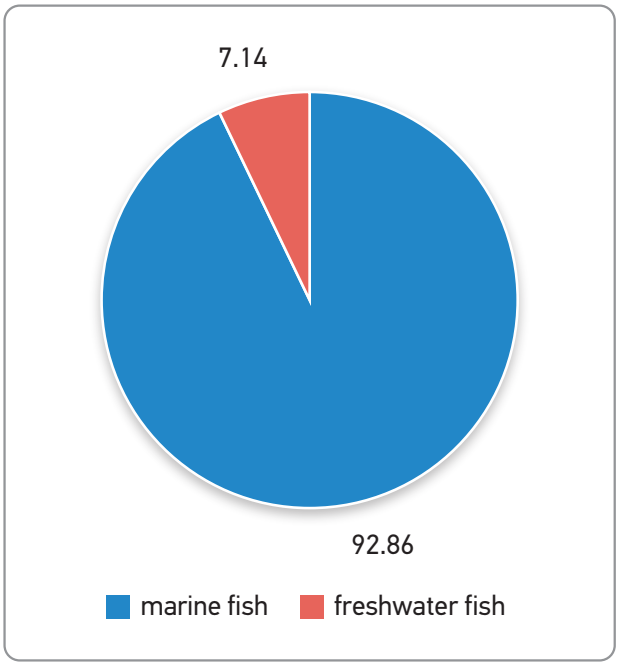

Figure 1. Average share (%) of imports of marine and freshwater fish species for the ten-year period from 2012 to 2021.

Data on the average share of individual marine fish species and fish products, as well as seafood, for the ten-year period from 2012 to 2021 are shown in Figure 2. Approximately a quarter of the import of marine fish and fish products consisted of hake (24.52%), while the least common imports were other types of marine fish (4.21%).

In Serbia, fish is most commonly imported frozen. Freezing (−18 °C to −30 °C) is, in fact, the most common method of preserving fish and making it available for sale. Only small quantities of the highest quality fish species (salmon, sea bass, sea bream, Peter's fish, tuna fillets) are imported chilled on ice (−1 °C) and are intended for specialized restaurants and the most modernly equipped supermarkets (*Zhu et al.*, 2021). These fish also command the highest prices, as do various types of seafood (crabs, shellfish, some types of mollusks), which are imported frozen or on ice. Among fish products, canned small pelagic fish (sardines) and canned large pelagic fish (tuna) are the most commonly imported. Canned fish accounted for 11.02%, or 3,485 tons, of the total fish imports (Figure 2). Cans are thermally processed products (113 °C to 160 °C) in hermetically sealed containers (most often cans) that do not require special storage conditions during distribution and sale, have a long shelf life (over a year), and are well accepted by consumers (*Tsironi et al.*, 2019).

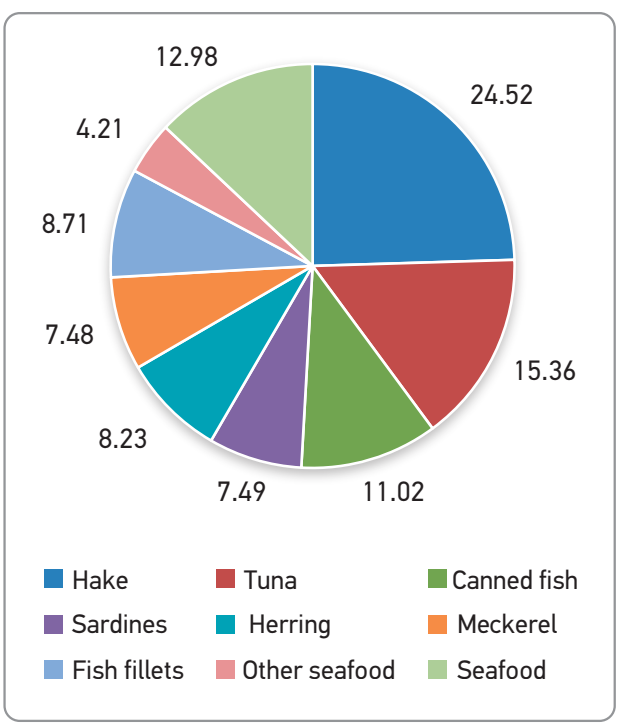

Figure 2. Average share (%) of imports of individual marine fish species and fish products, as well as seafood, for the ten-year period from 2012 to 2021.

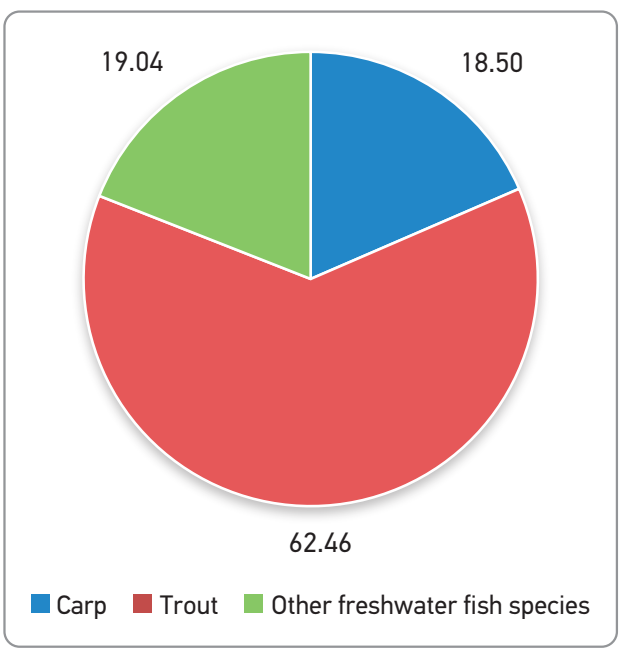

Figure 3. Average share (%) of imports of individual freshwater fish species for the ten-year period from 2012 to 2021.

Freshwater fish are most commonly imported live from neighboring countries (Bosnia and Herzegovina, Croatia, and North Macedonia). Less than a quarter of freshwater fish imports consisted

of carp (18.50%) and other freshwater fish species (19.04%), with a significantly larger import of trout (62.46%) (Figure 3).

Figure 4 shows the trend of the average total fish imports, and total imports of marine fish, seafood, and freshwater fish (000 tons) for the ten-year period from 2012 to 2021. The trends of total imports (y = 0.9296x + 28.978), and imports of marine fish (y = 0.6302x + 24.06) and seafood (y = 0.2624x + 2.661) were increasing, which was particularly pronounced in 2020 and 2021, while the trend of freshwater fish imports was stagnant (y = 0.0289x + 2.2731).

The value of fish and fish product imports is shown in Table 2. The average import value in the observed period was €86.030±22.100 million, with a coefficient of variation of 25.69%. The average import value of marine fish was €74.883±18.884 million, seafood €4.957±1.545 million, and freshwater fish €6.189±2.024 million.

The trend of the value of fish and fish product imports (million €) for the ten-year period from 2012 to 2021 is shown in Figure 5. The total value of fish and fish product imports increased from 2012 to 2021, as demonstrated by the trend equation y = 6.4523x + 50.542. A similar trend was observed for the import of marine fish and fish products, where the trend equation was y = 5.4658x + 44.822. The trends of seafood imports (y = 0.4322x + 2.5793) and freshwater fish imports (y = 0.5549x + 3.1369) were aligned, as shown by the trend equations.

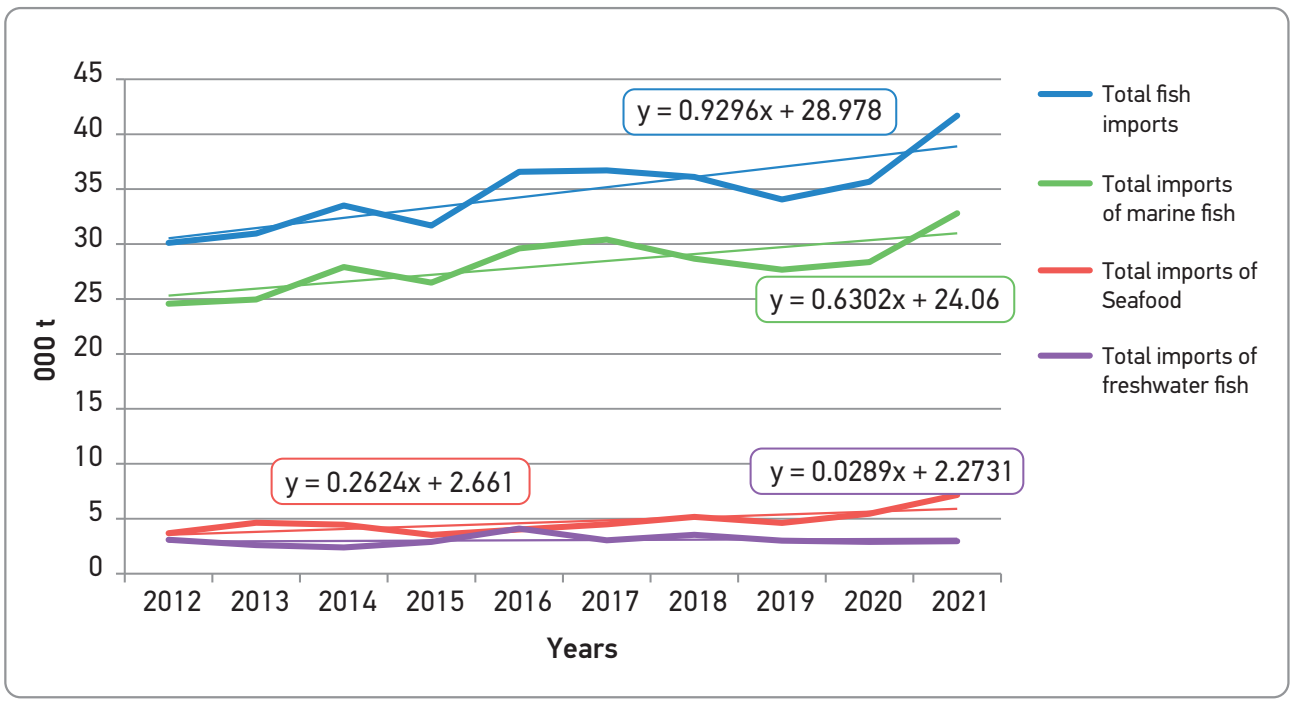

Figure 4. The trend of fish imports (000 tons) for the ten-year period from 2012 to 2021.

Table 2. Average values (000 €) of fish imports, total and by species, for the ten-year period from 2012 to 2021.

Import value (€)	$\overline{X}$	Measures of variation				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Total	86030	22100	6989	63665	138515	25,69
Marine	74883	18884	5972	55916	120401	25,22
Seafood	4957	1545	488,6	3345	8463	31,17
Freshwater	6189	2024	640,1	3655	9651	32,71

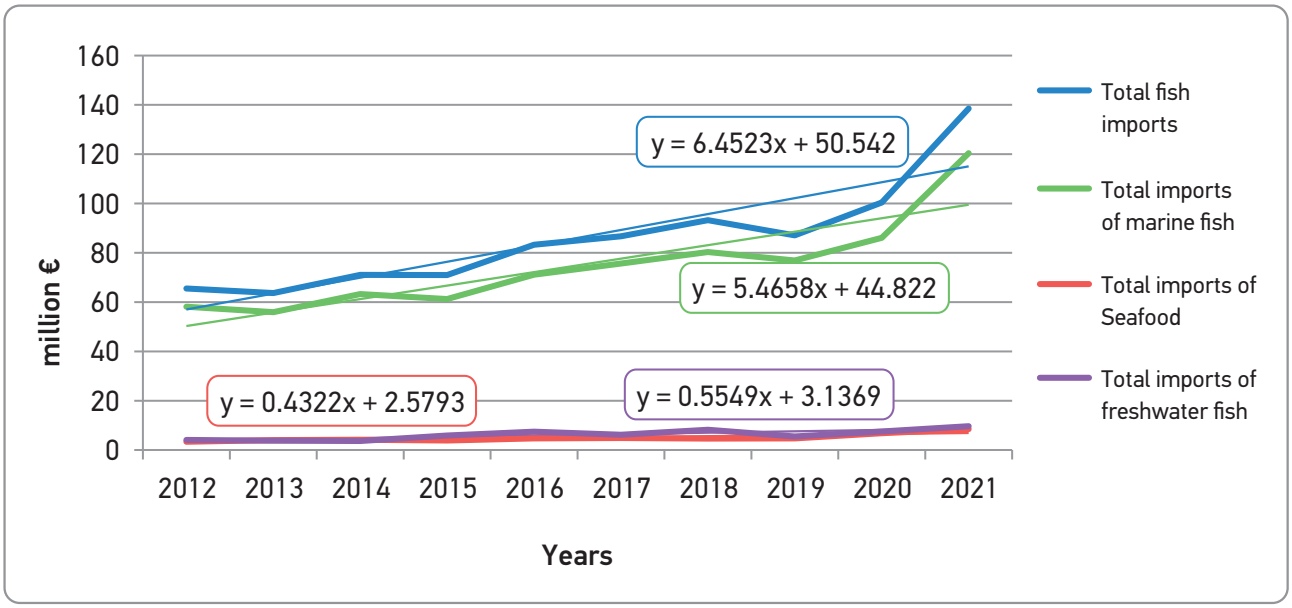

Figure 5. The trend in the value of fish and fish product imports (million €) for the ten-year period from 2012 to 2021.

The average import value of fish and fish product imports, expressed in €/kg for the examined period, was €2.49±0.39/kg, for marine fish €2.44±0.39/kg, for seafood €3.55±0.66/kg, and for freshwater fish €2.56±0.36/kg (Figure 6).

The trend in the value of fish and fish product imports (€/kg) for the examined period is shown in Figure 7. The largest increase in import value per kg was recorded for seafood imports (y =X0.2047x + 2.4293).

The total value of fish and fish product imports, as well as the value of marine fish, seafood, and freshwater fish imports, increased during the examined period, as defined by the equations (y = 0.1152x + 1.8593, y = 0.1144x + 1.8107, y = 0,2047x + 2,4293, and y = 0.079x + 2.1293, respectively).

Serbia is a fish-importing country, especially of marine fish. As evident from the data collected since 2012, the import of fish to Serbia has been constantly

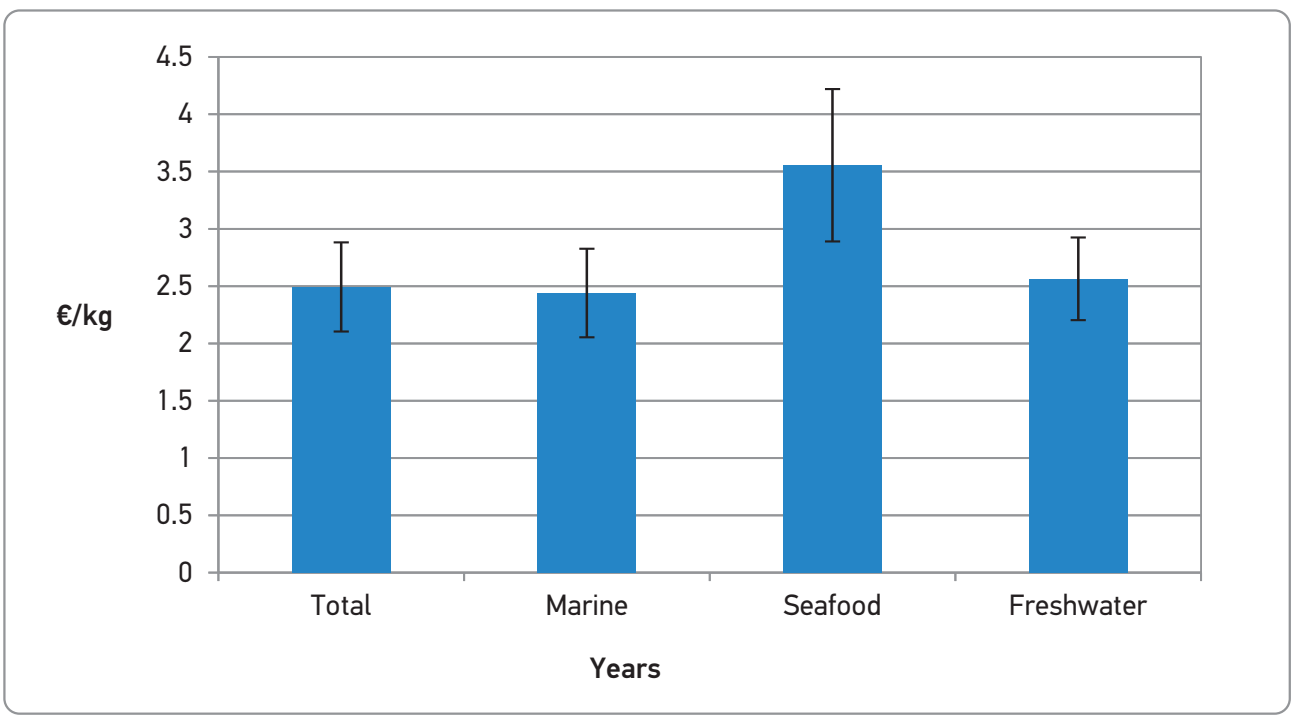

Figure 6. Average import values (€/kg) of fish and fish products for the ten-year period from 2012 to 2021.

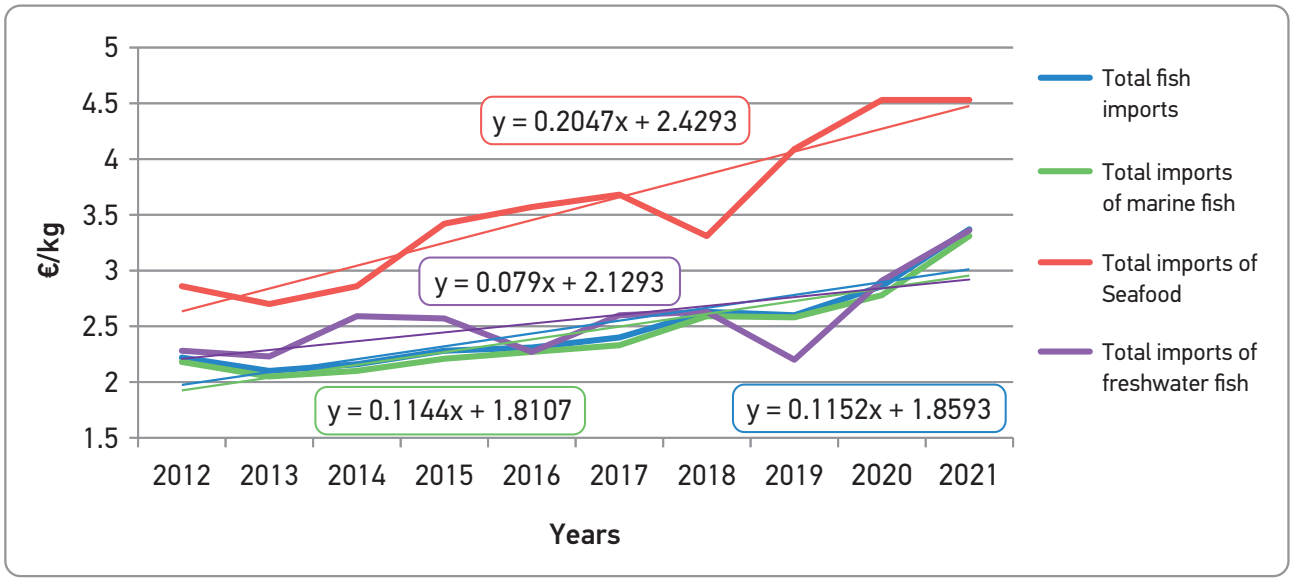

Figure 7. The trend in the value of fish and fish product imports (€/kg) for the ten-year period from 2012 to 2021.

increasing. One of the reasons is the decreased fish production in Serbia, particularly lower carp fish production. The causes of the decline in carp production are primarily related to the drastic reduction in the area of carp ponds. From 2011–2013, there were over 8,500 ha of carp ponds, which decreased to less than 6,500 ha by 2019–2021. Additionally, the low yield of carp per ha, which was less than one ton per ha from 2012–2021, has significantly contributed to the reduction in carp production. This is far less than the potential, which is three to five tons in semi-intensive farming and up to 10 tons in intensive farming conditions (*Marković*, 2010; *Ivanović et al.*, 2015; *Baltić et al.*, 2023). The average catch of carp fish in Serbia from 2012 to 2021 was 5,491 tons, and of trout, 1,206 tons, totaling 6,697 tons. With commercial and recreational fishing (carp, bighead carp, bream, goldfish), the local fish supply on the market was increased by 1,183 tons. In the statistical yearbooks of Serbia, commercial and recreational fishing covers only the four most commonly caught species. With fish imports (an average of 34,090 tons), the fish market in Serbia was supplied with 41,270 tons of fish and fish products, meaning that fish consumption in Serbia (six million inhabitants) was around 7 kg per inhabitant annually (*Baltić et al.*, 2023; *www.stat.gov.rs*).

If the average price of imported freshwater fish is €2.65/kg, then the value of fish production in aquaculture in Serbia and from catches would be €18.687 million, which would increase the value of fish on our market to €104.666 million. Among the total value of fish on the market, fish from aquaculture and catches in Serbia accounted for only 18.85% in the study period. This is a consequence of the low value of fish production in Serbia and its small share in the total volume and value of fish on the Serbian market. The situation of fisheries in Serbia is related to the unfavorable state of agricultural production, especially in the primary animal production sector. Agricultural production, including fisheries in Serbia, does not have sufficient state support (subsidies, taxes relief), and without it, regardless of land (water) resources (14,000 ha under carp ponds and 100,000 ha of infertile land that could be used for fish production in Vojvodina), animal feed production, and the possibility of improving fish farming technology, an increase in fish production in aquaculture in Serbia cannot be expected (*Baltić et al.*, 2023; *Baltić et al.*, 2023).

Fish consumption worldwide has been growing annually by 3% since 1961, while the population growth rate was 1.6%, which has led to an increase in fish consumption. The average annual fish consumption worldwide was 9.9 kg in 1961, reaching 20.5 kg in 2019. It is believed that annual fish consumption worldwide will be 21.4 kg per inhabitant by 2030, and aquaculture fish production will increase to 106 million tons. One of the reasons for greater fish consumption is to meet the nutritional needs of the growing number of people worldwide, but also is due to the increasing significance and awareness of the nutritional value of fish in human diets. Iceland has the highest fish consumption in the world (91 kg per inhabitant annually), while Afghanistan has the lowest (0.24 kg per inhabitant annually) (*Baltić et al.*, 2023).

Due to the decreasing volume of fish production in aquaculture and fish catches in open waters in Serbia since 2012, the volume of fish imports has increased. The largest volume of fish imports pertains to the import of marine fish, seafood, and fish products (canned fish). With the increase in the volume of imports and the rising price of fish on the global market, the value of fish imports has been continuously increasing. Thanks primarily to fish imports, the consumption of fish in Serbia per inhabitant per year over the last ten years has been about seven kilograms, which is a third of the average fish consumption per inhabitant worldwide. Serbia has the potential to significantly increase fish production in aquaculture, especially carp fish in the region of Vojvodina.

Ispitivanje obima i vrednosti ribe i proizvoda od ribe u Srbiji od 2012. do 2021. godine

Jelena Janjić, Milorad Mirilović, Branislav Vejnović, Spomenka Đurić, Tihana Vujanić, Milica Laudanović i Branislav Baltić

INFORMACIJE O RADU

Ključne reči:
Ulov
Akvakultura
Tržište Srbije
Potrošnja

APSTRAKT

Srbija se delimično snabdeva ribom iz sopstvene akvakulture i ribolova, komercijalnog i rekreativnog, što u proseku iznosi 6,70 tona za period od 2012. do 2021. godine. Najveći deo ribe na tržištu u istom periodu je iz uvoza (u proseku 34.090 tona). Od ukupnog ulova ribe i ribljih proizvoda, 92,80% čine morska riba i morski plodovi, dok svega 7,14% čini slatkovodna riba. Obim uvezene morske ribe i ribljih proizvoda ima opadajući redosled: oslić > tunjevina > morski plodovi > konzerve od ribe > riblji fileti > haringa > sardine > skuša > ostali morski plodovi. Od slatkovodne ribe najviše se uvozi pastrmka, a zatim ostale vrste riba i šaran. Prosečna ukupna vrednost uvoza ribe od 2012. do 2021. godine iznosila je 86.030 miliona evra, a prosečna uvozna cena ribe 2,5 evra/kg. Pored uvoza ribe i ribljih proizvoda, kao i ribe iz domaće proizvodnje i ulova, tržište ribe u Srbiji snabdeveno je sa 41.270 tona ribe, što znači da je godišnja potrošnja ribe po stanovniku u Srbiji oko 7 kilograma.

Disclosure statement: No potential conflict of interests was reported by authors,

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-66/2024-03/200143).

References

Ali, A., Wei, S., Ali, A., Khan, I., Sun, Q., Xia, Q., Wang, Z., Han, Z., Liu, Y., & Liu, S., (2022). Research progress on nutritional value, preservation and processing of fish—A review. *Foods*, 11 (22), 3669.

Anon, 2019. https://agrosmart.net/2019/10/08/srbija-ima-15-000--hektara-ribnjaka-a-mogla-bi-100-000/

Anon, (1997, 2010, 2018, 2022). Statistical Yearbook.

Anon, (2022). https://www.geeksforgeeks.org/top-10-fish-producing-countries-in-the-world/.

Baltić, B., Aksentijević, K., Bogunović, D., Starčević, M., Mitrović, R., Mrdović, B., & Janjić, J. (2023). Investigation of the volume of fish production and catch in Serbia from 2012 to 2021. *Meat Technology,* 64 (2), 329–333.

Baltić, M. Ž., Janjić, J., Glišić, M., Bošković, M., Baltić, B., Tasić, A., & Nedić, D. (2023). Ulov i proizvodnja ribe u svetu i Srbiji. *28. Godišnje savjetovanje doktora veterinarske medicine Republike Srpske (Bosna i Hercegovina)*, Trebinje, 15–17. jun 2023, pp.112–113.

Boyd, C. E., McNevin, A. A., & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food security,* 14 (3), 805–827.

FAO (2022). https://www.aquafeed.com/newsroom/reports/new-record-for-global-aquaculture-production-faos-2022-sofia-reports/#:~:text=Reports-,New%20record%20for%20global%20aquaculture%20production%2C%20FAO's%202022%20SOFIA%20reports,214%20million%20tonnes%20in%202020.

Ghaly, A. E., Ramakrishnan, V. V., Brooks, M. S., Budge, S. M. & Dave, D. (2013). Fish processing wastes as a potential source of proteins. Amino acids and oils: A critical review. *Journal of Microbiology Biochemistry and Technology,* 5 (4), 107–129.

Innes, J. K. & Calder, P. C. (2020). Marine omega-3 (N-3) fatty acids for cardiovascular health: an update for 2020. *International Journal of Molecular Sciences,* 21 (4), 1362.

Ivanović, J., Baltić, Ž. M., Janjić, J., Marković, R., Bošković, M., Đorđević, V., & Dokmanović, M. (2015). Obim i struktura ulova i proizvodnje ribe u Srbiji od 2006. do 2012. godine. *Veterinarski Glasnik,* 69 (5–6), 453–465.

Khalili Tilami, S. & Sampels, S. (2018). Nutritional value of fish: lipids, proteins, vitamins, and minerals. *Reviews in Fisheries Science & Aquaculture,* 26 (2), 243–253.

Luo, J., Amenyogbe, E., Fu, W. J., Huang, J. S. & Chen, G. (2022). Hepatic transcriptome profiles reveal the hepatoprotective effects of dietary quercetin and sodium quercetin-5′-sulfonates supplementation in hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus polyphekadion*♂). *Aquaculture*, 560, 738483.

Marković, Z. (2010). Šaran, Gajenje u ribnjacima i kaveznim sistemima. Prof. dr Zoran Marković, 152, Beograd.

Phogat, S., Dahiya, T., Jangra, M., Kumari, A. & Kumar, A. (2022). Nutritional benefits of fish consumption for humans: A review. *International Journal of Environment and Climate Change*, 12 (12), 1443–1457.

Tacon, A. G. (2023). Contribution of fish and seafood to global food and feed supply: An analysis of the FAO food balance sheet for 2019. *Reviews in Fisheries Science & Aquaculture,* 31 (2), 274–283.

Tsironi, T., Anjos, L., Pinto, P.I., Dimopoulos, G., Santos, S., Santa, C., Manadas, B., Canario, A., Taoukis, P., & Power, D. (2019). High pressure processing of European sea bass (*Dicentrarchus labrax*) fillets and tools for flesh quality and shelf life monitoring. *Journal of Food Engineering,* 262, 83–91.

Zhu, Z., Li, T. & Sun, D.W. (2021). Pressure-related cooling and freezing techniques for the food industry: Fundamentals and applications. *Critical Reviews in Food Science and Nutrition,* 61 (17), 2793–2808.

Authors ORCID info

Jelena Janjić https://orcid.org/0000-0003-3351-7199
Milorad Mirilović https://orcid.org/0000-0003-2771-7514
Branislav Vejnović https://orcid.org/0000-0002-6328-7446
Spomenka Đurić https://orcid.org/0000-0002-2992-7534
Tihana Vujanić
Milica Laudanović https://orcid.org/0009-0008-6381-2803
Branislav Baltić https://orcid.org/0000-0002-3455-2909

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

UDK: 637.56'81: 597.551.214
621.798-982
ID: 149517321
https://doi.org/10.18485/meattech.2024.65.1.6

Original scientific paper

Influence of modified atmosphere packaging on the shelf life and quality of chilled common carp (*Cyprinus carpio*) steaks

Jelena Babić Milijašević[1], Vesna Đorđević[1], Jasna Đinović-Stojanović[1], Srđan Stefanović[1], Zoran Petrović[1] and Milan Milijašević[1]*

[1] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11 000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Common carp
Cyprinus carpio
FFA
TVB-N
Sensory assessment
Shelf life

ABSTRACT

The objective of this study was to investigate the impact of modified atmosphere packaging (MAP1: 80% O_2 + 20% CO_2 and MAP2: 90% CO_2 + 10% N_2) on selected chemical and sensory attributes of common carp (*Cyprinus carpio*) steaks stored at 3 ± 0.5 °C, and to establish the shelf life of the products. Samples were assessed on days 1, 3, 5, 7, 9, 11, 13, 15 and 17. Carp steaks stored in a CO_2-enriched atmosphere exhibited lower pH values throughout the entire storage period than steaks in the other atmospheres. The increase in TVB-N values followed this order: MAP2 < control < MAP1. From day 9 of storage, FFA contents were significantly higher ($p < 0.01$) in MAP2 fish compared to control and MAP1 fish. The presence of oxygen (in MAP1 and control fish) led to an elevation in total volatile basic nitrogen (TVB-N) compared to fish packaged in the absence of oxygen. Based primarily on sensory, but also chemical parameters, it was determined that carp steaks packaged in modified atmosphere with 80% O_2 + 20% CO_2 remained acceptable for up to 15 days of storage, whereas carp steaks packaged under 90% CO_2 + 10% N_2, as well as carp steaks stored on flaked ice in air, remained unchanged until the end of the study (17 days) .

1. Introduction

Fish, owing to its nutritional richness, plays a pivotal role in human diets. What makes fish especially appealing to consumers is its abundance of proteins, minerals and vitamins, alongside it being a notable source of essential fatty acids crucial for averting various human ailments. With such attributes, fish stands out as one of the most nutritionally significant food sources. In recent times, there has been a global surge in consumer preference for fresh fish over frozen or processed fish. This shift has induced the advancement of modified atmosphere packaging (MAP) for fish and fish products, ensuring prolonged shelf life and the preservation of key freshness indicators (*Gimenéz et al.*, 2002).

The shelf life of any food product, including fresh fish, is characterized by the post-packaging duration within which the product remains safe for consumption. During this period, the sensory characteristics (colour, odour, flavour and texture) and nutritional quality of the product must remain consistent and acceptable to consumers (*Huss*, 1995).

The assessment of fish quality can be conducted through sensory evaluations, microbial analyses, or chemical techniques, such as the measurement

***Corresponding author:** Jelena Babić Milijašević, *jelena.babic@inmes.rs*

Paper received: April 15th 2024. Paper accepted: Jun 14th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

of volatile compounds, lipid oxidation, determination of ATP breakdown products and the presence of biogenic amines (*Gulsun et al*., 2009). The collective quantity of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is termed total volatile base nitrogen (TVB-N). Its level in fish flesh is commonly used as a parameter for estimating spoilage and as an indicator of fish freshness. These compounds are generated during the degradation of proteins and non-protein nitrogen components, primarily due to the metabolic activity of spoilage bacteria in fish and the action of endogenous enzymes (*Connell*, 1990). These processes contribute to alterations in the textural and sensory properties of fish muscle.

Hydrolytic changes in lipids result in the release of free fatty acids (FFA), which are highly susceptible to oxidative processes. Fish oil contains significant quantities of polyunsaturated fatty acids, leading to the initiation of oxidation reactions and the formation of hydroperoxides and other potentially detrimental secondary oxidation by-products. The peroxide value (PV) is considered an indicator of the primary oxidation rate, while the thiobarbituric acid (TBA) value serves as an indicator of secondary oxidation (*Ježek and Buchtová*, 2012). The alterations in lipid composition in fish and shellfish contribute to the deterioration of quality during prolonged storage, especially under unfavourable conditions.

The shelf life of fresh chilled fish is relatively short, typically lasting about 2 to 3 days at ambient temperatures of 2 ± 2 °C. It has been demonstrated that packaging fish in a modified atmosphere significantly prolongs the product's shelf life.

In Serbia, carp is the most commonly retailed freshwater fish. Fish are typically sold live, fresh chilled and unpacked (with a shelf life of 2 to 3 days), vacuum-packed (with a shelf life of 5 to 7 days), or frozen. Vacuum packaging is the preferred method of fish packaging. Modified atmosphere packaging with various gas mixtures is rarely used for fish in Serbia. Experimental monitoring data on freshwater fish packaged under modified atmosphere are generally limited.

The objective of this research was to observe changes in selected chemical and sensory parameters of common carp (*Cyprinus carpio*) steaks packaged in a modified atmosphere during storage at 3 ± 0.5 °C and to determine the shelf life of the products.

2. Materials and Methods

2.1. Sampling

Fourteen common carp (*Cyprinus carpio*) of average body weight of 2.50 ± 0.30 kg were obtained from a fishpond where a semi-intensive rearing system was used. Fish were transported live to the fish slaughtering and processing facility, where they were stunned, slaughtered, scaled, and the carcasses were cut into steaks 2 cm thick and of 220 g average weight. The 81 carp steaks were divided into three groups.

One group of fish was placed on top of flaked ice placed in polystyrene boxes with outlets for water drainage. The ice:fish ratio was 3:1 and was maintained constantly throughout the experiment. The flaked ice was changed daily. This experimental group, fish on ice, was used as control. The other two groups of carp steaks were both packaged in modified atmospheres, but with different gas ratios: MAP1: 80% O_2 + 20% CO_2 and MAP2: 90% CO_2 + 10% N_2. The packaging machine used was a Variovac (Variovac Primus, Zarrentin, Germany), and the packaging material was foil OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene, Dynopack, Polimoon, Kristiansand, Norway) with low gas permeability (degree of permeability for O_2 — 3.2 cm^3/m^2/day at 23 °C, for N_2 — 1 cm^3/m^2/day at 23 °C, for CO_2 — 14 cm^3/m^2/day at 23 °C and for steam 15 g/m^2/day at 38 °C). The ratio of gas:fish in the package was 2:1. All carp steaks were stored in the same conditions at 3 ± 0.5 °C and on 1, 3, 5, 7, 9, 11, 13, 15 and 17 days of storage, chemical and sensory testing was performed.

2.2. Chemical analyses

Muscle pH was measured by Cyber Scan pH-510 digital pH-meter (EUTECH Instruments, Netherland).

The TVB-N was determined in triplicate by using the official steam distillation method according to Commission Regulation (EC) 2074/2005 and was expressed as mg TVB-N/100 g.

The FFA content, expressed as % of oleic acid, was determined in accordance with EN ISO 660:2020.

The PV, expressed in milliequivalents of peroxide oxygen per kg of fat, was determined by the EN ISO 3960:2017 method.

2.3. Sensory evaluation

Sensory analysis was conducted by six experienced panellists from our laboratory staff, in the sensory evaluation laboratory, at room temperature (20 °C) and with adequate lighting. Each piece of fish was removed from the packaging 10 min before the evaluation and presented on a tray. These trays were coded by randomly chosen 3-digit numbers. Each panellist analysed the fish steaks individually for overall acceptability, with regard to odour, flesh colour and texture, using a 1–5 intensity scale, with 5 corresponding to the most liked sample and 1 corresponding to least liked sample. Fish was defined as unacceptable when a score of <2 points was recorded by at least of 50% of the panellists. Fish steaks from all three fish groups were evaluated throughout the 17-day storage period on each sampling day.

2.4. Statistics

The mean values and standard deviations for chemical and sensory data were calculated by using column statistics with the processing of six values for each analysed group. Significant differences between groups were calculated by using one-way ANOVA. When a significant F was found, additional post-hoc tests with Tukey's adjustment were performed. Differences were considered as significant when the p-value was ≤ 0.05. All analyses were performed using the program Microsoft Office Excel (2016).

3. Results and Discussion

Figure 1 shows the pH of common carp steaks packaged in different atmospheres. In MAP1 fish, a significant ($p < 0.01$) increase in pH was noted between day 5 (pH: 6.49 ± 0.03) and day 9 (pH: 6.63 ± 0.06) of the study. Afterwards, the pH began to decrease, reaching 6.30 ± 0.04 by day 17. Conversely, a decrease in pH was observed in MAP2 fish throughout the entire storage period, with the lowest pH of 6.19 ± 0.02 recorded on day 15. During the storage period, the pH of control (iced and in air) fish fluctuated and ranged from 6.51 ± 0.09 to 6.63 ± 0.03. Compared to control fish, those packaged in MAP containing 90% CO_2 + 10% N_2 exhibited lower pH throughout the storage period, while the pH in MAP1 fish was significantly lower ($p < 0.01$) after 9 days of storage. The mean pH values for

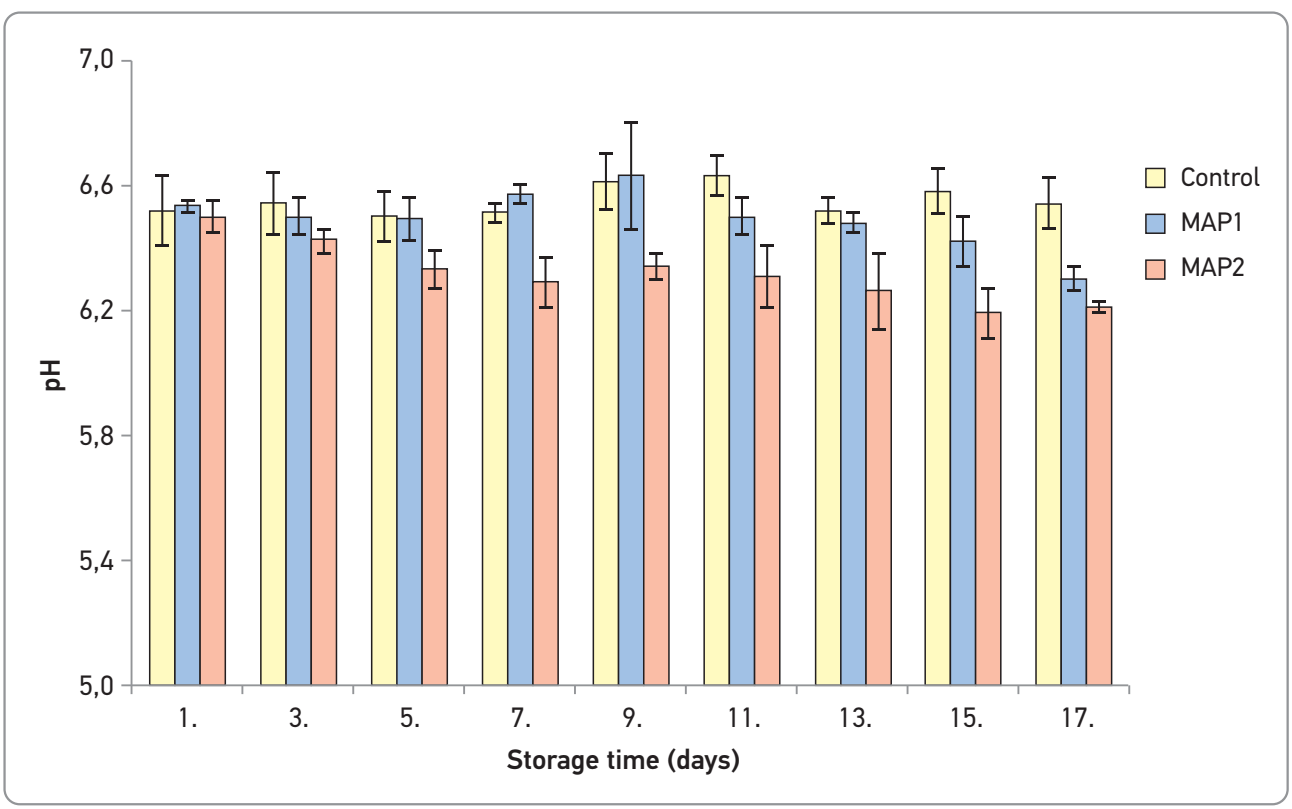

Figure 1. pH of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

control carp steaks and carp steaks packaged in MAP1 and MAP2 during storage were 6.55 ± 0.08, 6.49 ± 0.06, and 6.32 ± 0.07, respectively.

The pH of live fish muscle tissue typically hovers around 7.0, but *post-mortem* pH generally ranges from 6.0 to 7.1, depending on factors such as the season, fish species and other variables. The increase in lactic acid production during glycolysis under anaerobic conditions causes a decrease in the *post-mortem* pH of fish muscle, influencing the quality of fish meat (*Ashie et al*., 1996).

As shown in Figure 1, the lowest pH was recorded in carp steaks packaged in the atmosphere with 90% CO_2. Some other studies (*Milijašević et al*., 2010; *Provincial et al*., 2010, *Babić Milijašević et al*., 2023) have also reported significantly lower pH in fish packaged in modified atmospheres with a higher percentage of CO_2, attributed to the dissolution of CO_2 in fish muscle and leading to an increase in carbonic acid production. However, *Stenstrom* (1995) concluded that decrease of pH can be caused by acidic metabolic products produced by various bacteria, particularly lactobacilli. The moderate increase in pH of MAP1 fish after five days of storage may be caused by the higher quantity of basic compounds produced by the activity of fish spoilage bacteria (*Ruiz-Capillas* and *Moral*, 2001), which had favourable growth conditions provided by the high concentration of O_2 in this gas mixture (80% O_2).

The pH of common carp muscle and its variations under different experimental conditions in our study are in accordance with the findings of other studies (*Masniyom et al*., 2002; *Goulas and Kontominas*, 2007; *Babić et al*., 2014). In contrast, *Arashisar et al*. (2004) did not find significant differences among pH of rainbow trout fillets packaged in different atmospheres.

Figure 2 gives TVB-N values (mg/100 g) for the common carp steaks packaged in different atmospheres. The levels of TVB-N in carp steaks were practically indistinguishable ($P > 0.05$) at the beginning of the study. However, as the storage period progressed, there was an observable increase in TVB-N values in all experimental groups. Figure 2 illustrates how TVB-N values in carp steaks were significantly influenced by the atmospheric conditions used. The increase in TVB-N values followed this order: MAP2 < control < MAP1, with levels ranging from 12.35 ± 0.46 to 18.31 ± 0.48 mg N/100 g in MAP2 fish, from 12.38 ± 0.25 to 20.82 ± 1.45 mg N/100 g

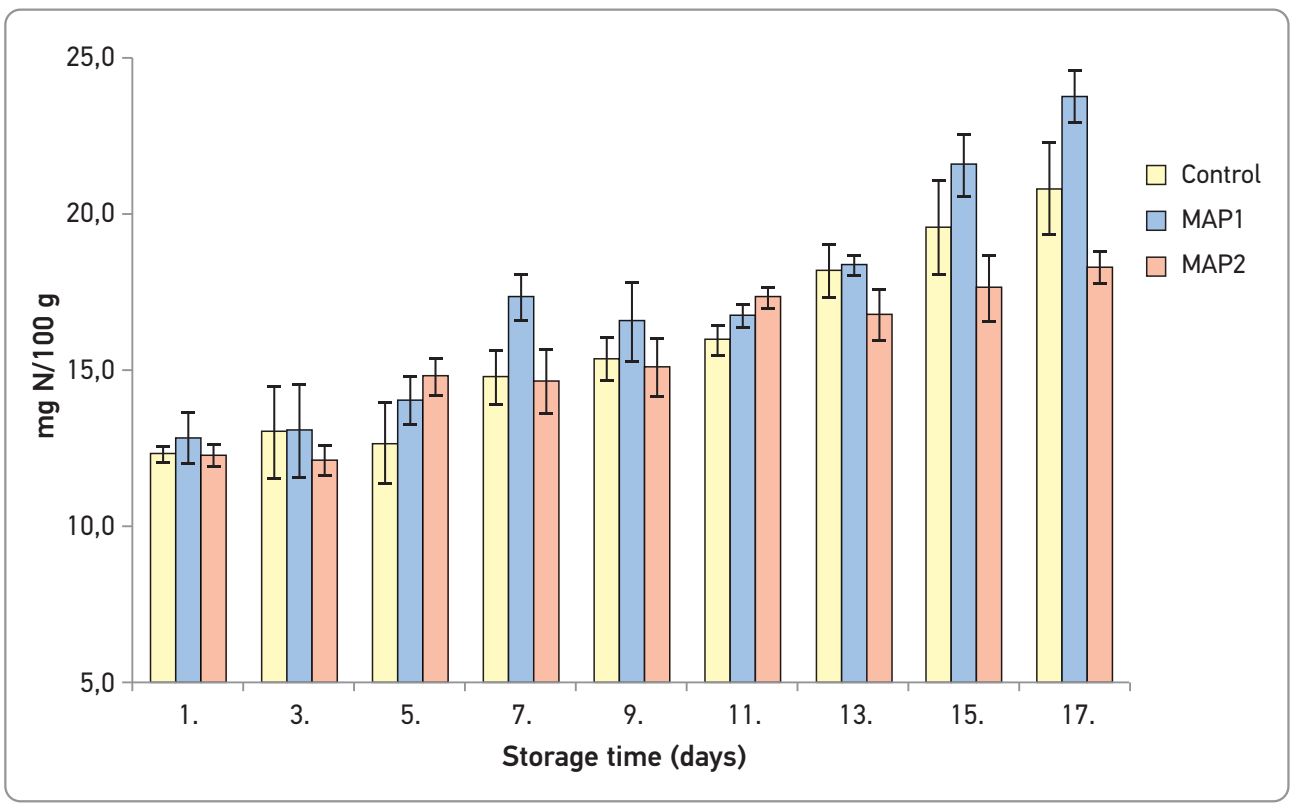

Figure 2. Total volatile base nitrogen content (mg/100 g) of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

in control fish, and from 12.40 ± 1.48 mg N/100 g to 23.77 ± 0.84 mg N/100 g in MAP1 fish during the 17-day storage period. Notably, TVB-N levels in MAP2 fish changed to lesser extent than did those in MAP1 and control fish. From day 11 onward, TVB-N values in MAP2 fish were significantly lower ($p < 0.01$) than in control fish, and compared to MAP1 fish, values were lower ($p< 0.01$) starting from day 7 of storage until the end of the study.

From day 7 onwards, the gas composition of MAP2 significantly ($p < 0.01$) delayed the formation of TVB-N compared to MAP1 fish. These discrepancies in TVB-N values may be attributed to the higher CO_2 content in MAP2 compared to MAP1 (90% versus 20%). Previous studies by *Masniyom et al*. (2013) suggested that higher CO_2 concentrations potentially inhibit the growth of predominantly Gram-negative microorganisms and reduce bacterial deamination capacity, thereby leading to a decrease in volatile compound production. Similar findings were reported by *Milijašević et al*. (2010) and *Babić et al*. (2014) for carp steaks stored under MAP, corroborating the results of the present study. Sea bass samples kept under higher CO_2 concentrations also exhibited lower TVB-N values (*Masniyom et al*., 2002). Generally, control sea bass showed higher TVB-N values compared to samples stored in CO_2-enriched atmospheres throughout the storage period (*Masniyom et al*., 2002). In our research, control fish (iced and in air) exhibited lower TVB-N values than fish packaged in MAP1. This could be explained by the presence of a high concentration of oxygen (80%) in our MAP1 packaging, which could have facilitated aerobic bacterial growth and subsequent increases in TVB-N due to bacterial decomposition of fish flesh.

While some researchers have recommended the TVB-N limit of 25 to 35 mg N/100g as an indicator for rejecting commercial fresh whole fish and processed fish products (*Connell*, 1990), no specified limit for acceptability of common carp has been established by Commission Regulation (EC) 2074/2005. In their study, *Ježek and Buhtova* (2010) proposed 20 mg N/100g in carp meat as the highest acceptable limit for TVB-N. In comparison with our study, TVB-N levels in MAP2 fish consistently remained below this limit specified by *Ježek and Buhtova* (2010) throughout the entire storage period. However, this limit was exceeded in our study by control fish (day 17) and MAP1 fish (day 15).

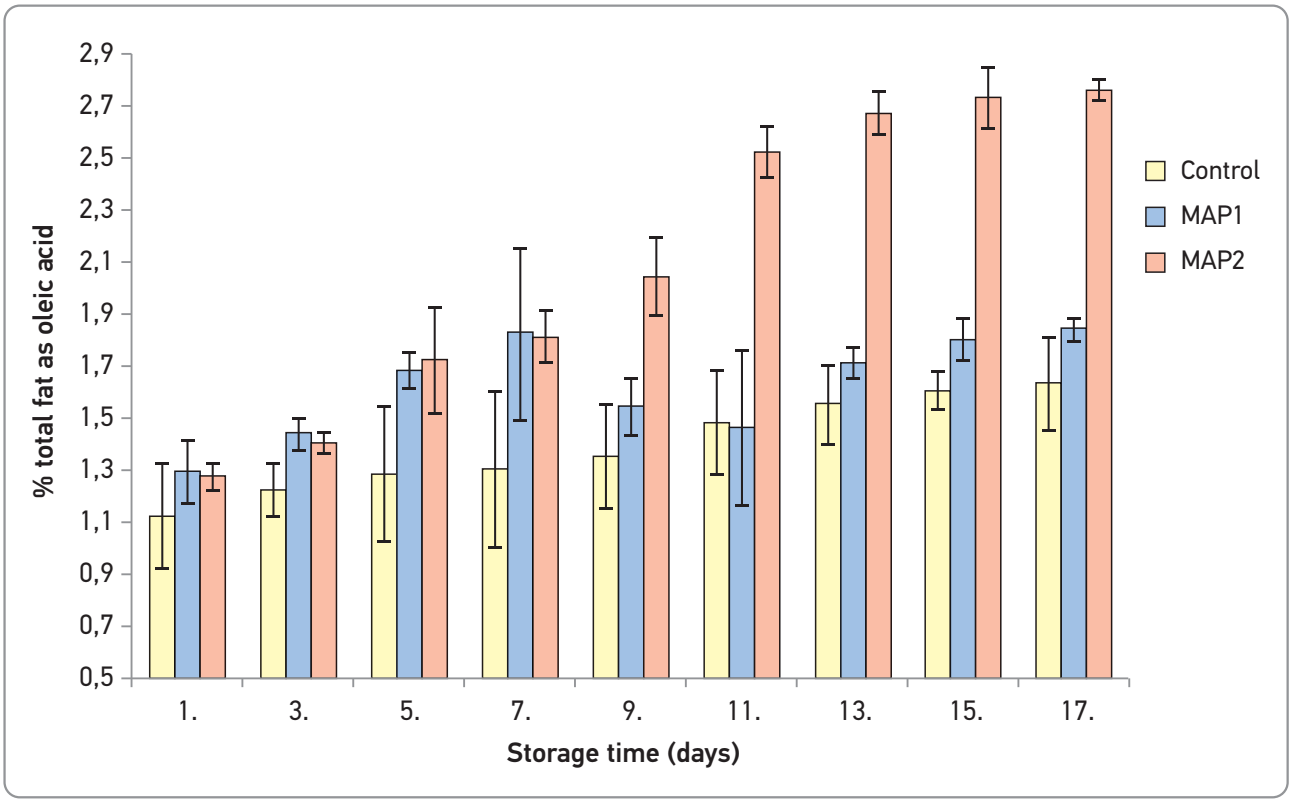

Figure 3. Free fatty acid content of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

Figure 3 shows the FFA contents (in % total fat as oleic acid) in common carp steaks packaged in the different atmospheres. Throughout the entire storage period, control fish had lower FFA contents compared to fish packaged in MAP. Simultaneously, the growth of FFA values in control fish was less pronounced (1.63 ± 0.18 on day 17) than in the MAP fish. From day 1 to day 7, there were no significant ($p > 0.05$) differences between the FFA contents of fish packaged in MAP1 and MAP2. However, the production of these degradation products followed a different line in the two types of packaging. In MAP2 fish, a highly significant ($p \leq 0.01$) increase in FFA was observed from day 9 (2.04 ± 0.1) until the end of the study (2.76 ± 0.04). On the other hand, in MAP1 fish, a significant decrease in FFA was determined between storage day 7 (1.82 ± 0.11) and day 11 (1.46 ± 0.06). From that day onward, the FFA content increased in the MAP1 fish until the end of the study (1.84 ± 0.12).

The process of lipid hydrolysis is followed by the release of FFAs. In our study, storage of carp steaks on flaked ice and in air had the smallest impact on FFA production. The significantly lower FFA level in MAP1 fish ($p \leq 0.01$) compared to MAP2 fish starting from day 9 can be attributed to the rapid conversion of FFA to oxidation products, due to the presence of O_2 in MAP1. Similar results were reported by *Ježek and Buhtova* (2012) for silver carp stored under MAP, supporting the results of our study. According to *Ozyurt et al.*, (2009) FFAs interact with myofibrillar proteins and negatively affect muscle texture. This author found a good correlation between FFA formation and the loss of freshness in fish. Otherwise, *Fagan et al*. (2004) pointed out that FFA levels have no effect on the sensory quality of fish.

Figure 4 shows the PV of common carp steaks packaged in the different atmospheres. Lipid oxidation in fish depends on several factors, such as fish species, storage temperature and lipid composition. This oxidation is often main reason for the short shelf life of fish and fish products. The PV was used to determine primary products of lipid oxidation, mainly hydroperoxides.

During the first five days of storage, PV was not detected in both, unpackaged and packaged fish. Later on, PV was lower in the control fish than in fish packaged in MAP. PV in fish packaged in the oxygen-rich atmosphere (80%) produced the highest PV from day 7 to day 13. At the end of the study (days 15 and 17), PVs were higher in fish packaged

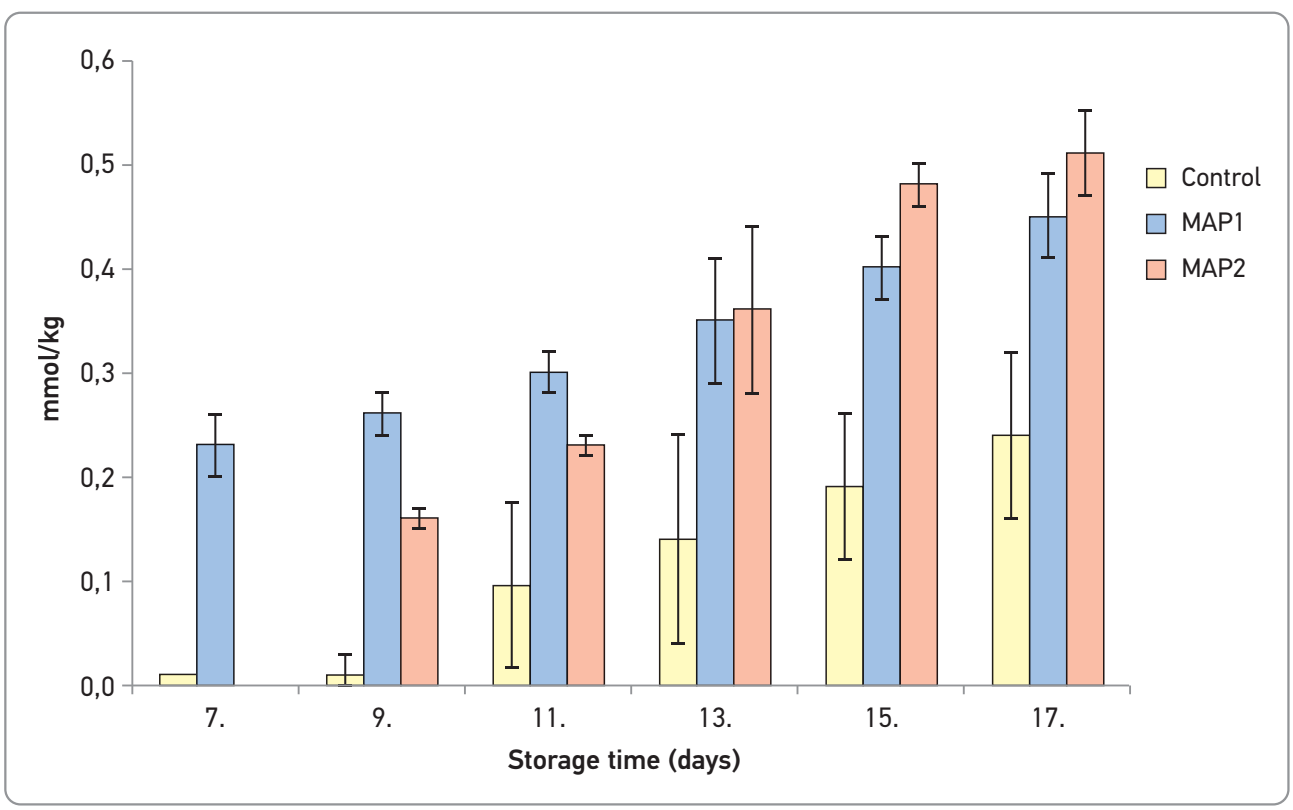

Figure 4. Peroxide value of common carp steaks packaged under different conditions during storage at 3 °C.

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2.

Table 1. Sensory evaluation of carp steaks packaged under different conditions during storage at 3˚C

Sensory parameter	Packaging conditions	Storage time (days)								
		1	3	5	7	9	11	13	15	17
Odour	Control	5.0±0.0^{a}	5.0±0.0^{a}	4.8±0.3^{a}	4.7±0.2^{a}	4.5±0.1^{a}	4.2±0.9^{a}	3.6±0.5^{b}	3.4±0.8^{b}	3.2±0.6^{b}
	MAP 1	5.0±0.0^{a}	5.0±0.0^{a}	4.8±0.3^{a}	4.1±0.4^{b}	3.6±0.3^{b}	3.5±0.0^{b}	3.3±0.2^{b}	2.6±0.4^{c}	1.2±0.2^{d}
	MAP2	5.0±0.0^{a}	5.0±0.0^{a}	4.9±0.2^{a}	4.8±0.2^{a}	4.6±0.3^{a}	4.0±0.4^{b}	3.8±0.5^{b}	3.5±0.4^{b}	2.8±0.5^{c}
Flesh texture	Control	5.0±0.0^{a}	5.0±0.0^{a}	4.8±0.2^{a}	4.6±0.5^{a}	4.4±0.4^{a}	3.7±0.5^{b}	3.6±0.8^{b}	3.2±0.5^{b}	3.1±0.2^{b}
	MAP1	4.9±0.5^{a}	4.9±0.5^{a}	4.8±0.7^{a}	4.5±0.4^{a}	4.0±0.0^{a}	3.8±0.8^{a}	3.5±0.2^{a}	2.7±0.4^{b}	2.5±0.3^{b}
	MAP2	4.8±0.2^{a}	4.1±0.0^{b}	4.0±0.0^{b}	3.7±0.2^{b}	3.6±0.7^{b}	3.5±0.9^{b}	3.2±0.6^{b}	2.9±0.6^{b}	2.7±0.4^{b}
Flesh colour	Control	5.0±0.0^{a}	5.0±0.0^{a}	4.9±0.1^{a}	4.8±0.2^{a}	4.7±0.3^{a}	4,6±0.3^{a}	4,3±0.3^{a}	3.7±0.2^{b}	3.5±0.7^{b}
	MAP 1	5.0±0.0^{a}	4.2±0.3^{b}	3.7±0.4^{b}	3.7±0.4^{b}	3.6±0.4^{b}	3.3±0.4^{b}	3.4±0.3^{b}	2.5±0.1^{c}	1.8±0.6^{d}
	MAP 2	5.0±0.0^{a}	5.0±0.0^{a}	4.8±0.2^{a}	4.6±0.6^{a}	4.1±0.6^{a}	3.6±0.7^{b}	3.6±0.5^{b}	3.4±0.6^{b}	2.6±0.4^{c}
Overall acceptability	Control	5.0±0.0^{a}	4.9±0.7^{a}	4.8±0.7^{a}	4.8±0.4^{a}	4.5±0.6^{a}	4.4±0.6^{a}	4.2±0.8^{a}	3.8±0.7^{a}	3.3±0.5^{a}
	MAP 1	4.9±0.3^{a}	4.6±0.7^{a}	4.2±0.3^{a}	3.7±0.4^{a}	3.6±0.4^{a}	3.5±0.2^{a}	3.5±0.4^{a}	2.6±0.4^{b}	1.2±0.2^{c}
	MAP 2	5.0±0.0^{a}	5.0±0.0^{a}	4.8±0.8^{a}	4.5±0.3^{a}	4.7±0.5^{a}	4.2±0.6^{b}	4.1±0.6^{b}	3.6±0.4^{b}	2.7±0.6^{c}

Legend: Control: kept on ice and in air. MAP1: 80% O_2 + 20% CO_2. MAP2: 90% CO_2 + 10% N_2. Same lowercase letters in a row indicate no significant differences ($p > 0.05$).

in the MAP2 atmosphere without oxygen. In their research, *Ruiz-Capillas and Moral* (2001) suggested that lipid oxidation depends on the synergy effect between CO_2 and O_2. For that reason, lipid oxidation in the atmosphere with 40% O_2 could be more intensive than in the atmosphere with 60% O_2. The fluctuations in PV that were recorded in our research are in line with the results of *Ježek and Buhtova* (2007), and they indicate the fact that PV cannot be considered as a suitable indicator of fish freshness.

The sensory evaluation results for carp steaks are outlined in Table 1. It is evident that carp steaks packaged in MAP1 received significantly lower scores ($P < 0.05$) for all sensory attributes by day 15 than the fish stored under other conditions. On day 17, the rancid odour detected in MAP1 fish caused the odour score to fall below the acceptability threshold of 2. On the last day of the study, a diminished intensity of the pink cream colour of carp muscle was observed, alongside surface slime.

Throughout the storage period, both control and MAP2 fish showed a decrease in sensory attribute scores, yet they remained within acceptable levels. Notably, despite being deemed acceptable, the texture of MAP2 fish consistently received lower ratings. This was attributed to a softened texture observed from day 3 onwards. This soft texture could be ascribed to the CO_2 percentage in the MAP2 fish. Dissolution of CO_2 in the fish muscle's aqueous phase led to a decrease in pH and subsequent loss of meat juice, adversely affecting product consistency. Furthermore, the absence of O_2 and the higher CO_2 percentage in the MAP2 gas mixture resulted in a greyish hue, which received relatively low scores from our panellists. According to the odour scores, common carp steaks packaged under 80% O_2 + 20% CO_2 would likely have a shelf life of up to 15 days at 3 °C. Common carp steaks packaged in 90% CO_2 + 10% N_2 and those kept on flaked ice in air would likely be acceptable (from an odour perspective) for 17 days.

4. Conclusion

In conclusion, packaging common carp steaks in a 90% CO_2 + 10% N_2 atmosphere slowed down both proteolytic reactions and secondary lipid oxidation compared to packaging in an 80% O_2 + 20% CO_2 gas mixture. Based primarily on odour scores, it was concluded that common carp steaks packaged in a modified atmosphere with 80% O_2 + 20% CO_2 remained acceptable for up to 15 days of storage at 3 °C. In contrast, common carp steaks packaged in 90% CO_2 + 10% N_2 and those kept on flaked ice in air remained unchanged until the end of the study (17 days).

Uticaj pakovanja u modifikovanu atmosferu na održivost i odabrane parametre kvaliteta ohlađenih odrezaka šarana (*Cyprinus carpio*)

Jelena Babić Milijašević, Vesna Đorđević, Jasna Đinović-Stojanović, Srđan Stefanović, Zoran Petrović i Milan Milijašević

INFORMACIJE O RADU

Ključne reči:
Šaran
Cyprinus carpio
FFA
TVB-N
Senzorna svojstva
Održivost

APSTRAKT

Cilj ovog rada bio je da se ispita uticaj pakovanja u modifikovanu atmosferu (MAP1: 80%O2 + 20%CO2 i MAP2: 90%CO2 + 10%N2) na odabrane hemijske i senzorske parametre kvaliteta odrezaka šarana (Cyprinus carpio) i da se odredi njihova održivost. Uzorci su ispitivani 1, 3, 5, 7, 9, 11, 13, 15 i 17 dana eksperimenta. Odresci šarana upakovani u modifikovanu atmosferu sa većim procentom ugljen-dioksida imali su nižu pH vrednost tokom eksperimenta. TVB-N vrednosti su se povećavale sledećim redosledom: MAP2 < kontrola < MAP1. Od devetog dana eksperimenta vrednosti FFA bile su značajno veće ($p < 0,01$) u MAP 2 uzorcima u poređenju sa kontrolnim i uzorcima upakovanim u MAP1. Prisustvo kiseonika u MAP1 uzorcima i kod uzoraka čuvanih na ledu dovelo je do povećanja TBA vrednosti.

Rezultati senzorskih i hemijskih ispitivanja su pokazali da odresci šarana pakovani u atmosferu sa 80 posto kiseonika i 20 posto ugljen-dioksida ostaju nepromenjeni do petnaestog dana, a uzorci pakovani u atmosferu sa 90 posto ugljen-dioksida, kao i uzorci čuvani na ledu do sedamnaestog dana skladištenja.

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

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, (Grant No. 451-03-66/2024-03/200050 dated 05.02.2024).

References

Arashisar, Ş., Hisar, O., Kaya, M. & Yanik, T. (2004). Effect of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. *International Journal of Food Microbiology,* 97, 209–214.

Ashie, I. N. A., Smith, J. P. & Simpson, B. K. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition*, 36 (1–2), 87–121.

Babic Milijasevic, J., Milijasevic, M., Lilic, S., Djinovic-Stojanovic, J., Nastasijevic, I., Geric, T. (2023). Effect of vacuum and modified atmosphere packaging on the shelf life and quality of gutted rainbow trout (*Oncorhynchus mykiss*) during refrigerated storage. *Foods,*12, 3015.

Babić, J., Dimitrijević, M., Milijašević, M., Đorđević, V., Petronijević, R., Grbić, S., Spirić, A. (2014). Effect of modified atmospheric conditions and vacuum packaging on selected chemical parameters that define freshness of rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*). *Hemijska Industrija,* 68, 69–76.

Connell, J. J. (1990). Methods of assessing and selecting for quality. In: Connell JJ (ed) Control of fish quality. Fishing News Books, Oxford, pp 122–150.

European Union, (2005). Regarding to Total Volatile Basic Nitrogen (TVB-N) limit values for certain categories of fishery products and analysis methods to be used. Commission Regulation (EC) 2074/2005. *Official Journal of the European Union*, L 338, 36.

Fagan, J. D., Gormley, T. R. & Ut Mhuircheartaigh, M. M. (2004). Effect of modified atmosphere packaging with freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. *Innovative Food Science and Emerging Technologies,* 5, 205–214.

Gimenéz, B., Roncalés, P., & Beltrán, J. A. (2002). Modified atmosphere packaging of filleted rainbow trout. *Journal of the Science of Food and Agriculture,* 82, 1154–1159.

Goulas, A. E., & Kontominas, M. G. (2007). Effect of modified atmosphere packaging and vacuum packaging on the shelf-life of refrigerated chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *European Food Research and Technology,* 224, 545–553.

Gülsün, Ö., Esmeray, K., Serhat, Ö., & Fatih, Ö. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry,* 114 (2), 505–510.

Huss, H. H. (1995). FAO Fisheries technical paper 348. Food and Agriculture Organization of the United Nations, Rome.

Jayasingh, P., Cornforth, D., P., Brennand, C. P., Carpenter, C. E., & Whittier, D., R. (2002). Sensory evaluation of ground beef stored high-oxygen modified atmosphere packaging. *Journal of Food Science, 9,* 3493–3496.

Ježek, F., & Buchtová, H. (2012). Effect of modified atmosphere packaging on the course of physical and chemical changes in chilled muscle tissue of silver carp (*Hypophthalmichthys molitrix* V.). *Polish Journal of Veterinary Sciences* 15 (3), 439–445.

Ježek, F., & Buchtová, H. (2007). Physical and chemical changes in fresh chilled muscle tissue of common carp (*Cyprinus carpio* L.) packed in a modified atmosphere. *Acta Veterinaria Brno,* 76, 83–92.

Ježek, F., & Buchtová, H. (2010). Shelf-life of chilled muscle of common carp (*Cyprinus carpio* L.) packaged in carbon monoxide enriched modified atmosphere. *Acta Veterinaria Brno,* 79, 117–125.

Masniyom, P., Benjakul, S., & Visessanguan, W. (2002). Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging, *Journal of the Science of Food and Agriculture*, 82, 873–880.

Masniyom, P., Benjama, O. & Maneesri, J. (2013). Effect of modified atmosphere and vacuum packaging on quality changes of refrigerated tilapia (*Oreochromis niloticus*) fillets. *International Food Research Journal,* 20 (3), 1401–1408.

Milijašević, M., Babić, J., Baltić, M. Ž., Spirić, A., Velebit, B., Borović, B., & Spirić, D. (2010). Uticaj različitih smeša gasova na promene nekih mikrobioloških i hemijskih parametara u odrescima šarana (*Cyprinus carpio*) upakovanih u modifikovanu atmosferu. *Meat Technology,* 51 (1), 66–70.

Ozyurt, G., Kuley, E., Ozkutuk, S. & Ozogul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and golband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry,* 114, 505–510.

Provincial, L., Gil, M., Guillen, E., Alonso, V., Roncales, P., & Beltran, J. A. (2010). Effect of modified atmosphere packaging using different CO_2 and N_2 combinations on physical, chemical, microbiological and sensory changes of fresh sea bass (*Dicentrarchus labrax*) fillets. *International Journal of Food Science & Technology*, 45, 1828–1836.

Ruiz-Capillas, C. & Moral, A. (2001). Residual effect of CO_2 on hake (*Merluccius merluccius* L.) stored in modified and controlled atmospheres. *European Food Research and Technology*, 212, 413–420.

Stenstrom, I. J. (1985). Microbial flora of cod fillets (*Gadus morhua*) stored at 2°C in different mixtures of carbon dioxide and nitrogen/oxygen. *Journal of Food Protection,* 48, 585–589.

Authors ORCID info

Jelena Babić Milijašević https://orcid.org/0009-0003-8923-7046
Vesna Đorđević https://orcid.org/0009-0008-5187-4089
Jasna Đinović-Stojanović https://orcid.org/0000-0003-4602-0835
Srđan Stefanović https://orcid.org/0000-0002-8011-5654
Zoran Petrović https://orcid.org/0000-0003-2016-5681
Milan Milijašević https://orcid.org/0000-0003-4269-236X

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

UDK: 637.56.055:576.89
ID: 149494025
https://doi.org/10.18485/meattech.2024.65.1.7

Original scientific paper

Use of attribute agreement analysis (AAA) in the validation of sensory evaluation methods: Case study for the visual determination of parasites in fish

Zoran Petrović[1], Jelena Ćirić[1], Jelena Babić Milijašević[1], Milan Milijašević[1], Mirjana Lukić[1], Jelena Jovanović[1] and Aleksandra Nikolić[1]*

[1] *Institute of Meat Hygiene and Technology, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Attribute agreement analysis (AAA)
Sensory methods
Validation
Fish
Parasites

ABSTRACT

Validation of sensory evaluation employs a process similar to any other method validation procedure in analytical chemistry. However, the parameters often measured in sensory testing are different, because the human sensory apparatus and brain are the instruments being calibrated. A total of 10 fish samples were examined by an expert 5-member evaluation panel for the visual presence of parasites in frozen fish. From each frozen hake sample, a group of six slices of fish muscle was formed by longitudinal sectioning. The total of 60 sliced samples were divided into 10 plates with six samples each. The first plate contained six slices, each of which contained parasites. The attribute agreement analysis showed strong agreement in the overall ratings. It was found that at least 75% of all tests achieved the highest level of agreement. The results of the tests were presented in the form of tables and graphs summarizing the subjective test results using Fleiss' Kappa and Cohen's Kappa statistics.

1. Introduction

Attribute agreement analysis is a statistical method used to determine if trained expert sensory panels are using a particular scale consistently and in the same way (*MoreSteam,* 2024). This method is used in the validation of sensory tests and is widely adopted in the food industry. In order to use attribute agreement analysis, it is essential that the objects, reference standards, and the rating scales themselves are precisely defined. The use of such sensory expert panels is highly regulated, and their fitness for purpose and the methods they use must be scientifically validated (*Djekic et al.,* 2021*; Sipos et al.,* 2021).

Sensory evaluation methods, such as the one validated in this case study, usually employ the use of trained expert panels who are experienced in the subject area. Such panels are often used in the food and drink industries to assess product attributes, and in new product development and quality control, as well as being required for the labeling and marketing of products. Use of such expert sensory panels is highly regulated, and their fitness for purpose and the methods they employ have to be scientifically validated (*Barbieri et al.,* 2020*; Da Costa et al.,*2020*; Mihafu et al.,* 2020*; Mohammadi-Moghaddam and Firoozzare,* 2021*; Gupta et al.,* 2022).

When a consistent and valid method is developed and used, not only is the capability of the product to meet consumer expectations and preferences increased, but the improvements and changes made to the product can be scientifically shown to result in an improved sensory output (*Quintão et al.,*2020*; Stone et al.,* 2020*; Vivek et al.,* 2020). This is important to both the producer and the consumer of the product, as it demonstrates the validity and trustworthiness of the sensory results. Furthermore, the use of validated methods will

***Corresponding author:** Zoran Petrović, zoran.petrovic@inmes.rs*

Paper received: April 4th 2024. Paper accepted: May 7th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

strengthen the possibility of making and defending scientific claims about a product (*Pavli et al.,*2020*; Rose and Johnson,* 2020*; Sürücü and Maslakci,* 2020). The use of qualitative methods of sensory evaluation, such as for parasites in fish, requires certainty that the method is reproducible and repeatable and, more importantly, that the assessors have a high agreement rate (*Freitas et al.,* 2020*; Jurica et al.,* 2021).

Attribute agreement analysis has a lot of benefits, key among which is its ability to remove subjectivity in the validation process. First of all, validation using this method is not dependent on the knowledge of a particular expert in a certain field. Also, attribute agreement analysis removes individualism. This is because what may be of high severity to one assessor may not necessarily be so to another. By matching the severity ratings and looking at the percentage agreement, it is possible to tell if the assessors are in agreement in as far as qualitative grading is concerned (*Xiong et al.,* 2020). This method is used in the validation of sensory tests and is widely adopted in the food industry (*Carpenter et al.,* 2000). In order to use attribute agreement analysis, it is essential that the objects, reference standards, and the rating scales themselves are precisely defined (*Hubbard,* 2012).

The focus in this paper was on how attribute agreement analysis can be used to validate a sensory examination method. The goal of validating the sensory method was to demonstrate the appropriateness of adopting such a method in order to fulfil accreditation requirements (*ISO 17025,* 2017). For the parasites in fish, we wanted to show that the method is consistent, precise, and less subjective or more sensitive (or specific) than alternative methods (*Zhang et al.,* 2022).

In addition, the validation should show that the detection method actually leads to the correct conclusion regarding the presence or absence of the parasite in the fish. For example, the method should be able to show that the tested fish sample, which was declared parasite-free, actually does not contain any parasites. If, on the other hand, the sample is classified as parasite-positive, the validation should show that the method provides a correct result.

2. Materials and Methods

Prior to validating a method using attribute agreement analysis, the first step of training the assessors using an appropriate presentation, known as a consensus building session, was conducted. This session aimed to set the standards of the method and reduce the variability of the data.

2.1. Sample preparation

The validation sample consisted of 60 slices made from two groups of frozen hake one of which tested positive and the other negative for parasites.

The frozen fish were thawed at room temperature for 6 h until they became a suitable texture for cutting fillets in the semifrozen state (−3 °C). From each fish sample, a group of six slices of fish muscle was formed by longitudinal sectioning. The total of 60 sliced samples were divided onto 10 plates with six samples each. The first plate contained six slices, each of which contained visible parasites (Figure 1).

The other plates with serial numbers 2–10 each contained six slices without parasites (Figure 2).

The visual inspection was carried out by a sensory panel consisting of five trained appraisers, doctors of veterinary medicine. The visual inspection results were recorded by each appraiser individually

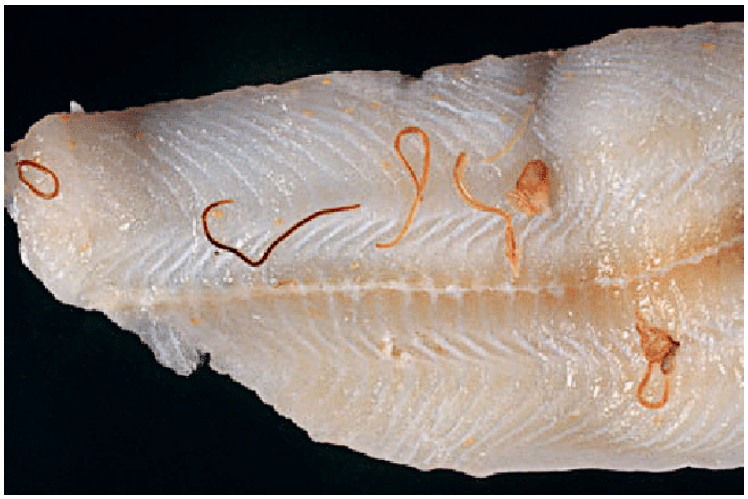

Figure 1. Fish samples with visible parasites situated on plate.

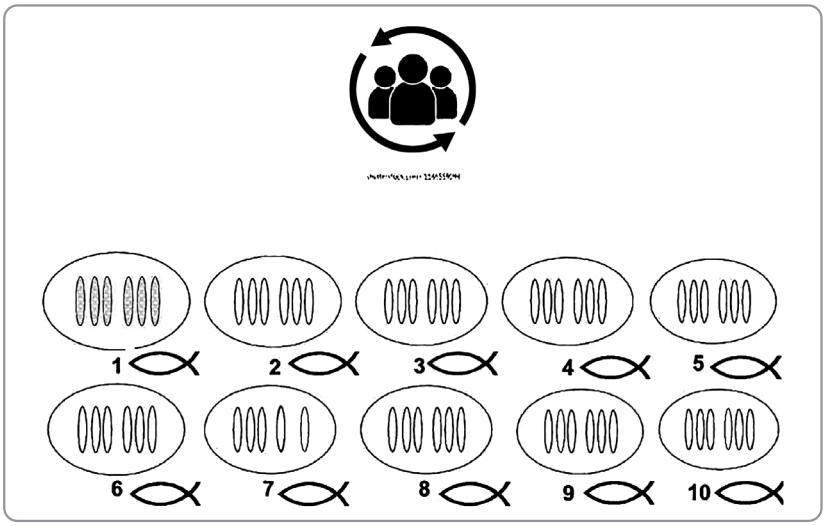

Figure 2. Scheme of validation experiment

and after the experiment were processed using appropriate statistical tools.

2.2. Temperature control

Temperature of filets was measured using digital thermometer, model, TESTO 926-1 (Germany), equipped with a wi-fi puncture probe.

2.3. Environmental and room conditions

When performing the method validation, the recommendations of the ISO 17025:2017 standard, point 6.3, were observed, and the ambient conditions were monitored and recorded. The room temperature was 20±1 °C and the relative humidity of the room (rH) was 64%. The room was illuminated with the prescribed ambient lighting of 220 lux. The light intensity in the room was measured with a lux light meter (MMS Med Lab, UK).

2.4. Statistical analysis

The obtained data were statistically processed in the statistical package MINITAB INC. VER. 17, USA (*Minitab,* 2024), using the tool within the option: STAT/QUALITY TOOLS/ATTRIBUTE AGREEMENT ANALYSIS.

In our study, this MINITAB tool was used to assess the agreement of subjective ratings or classifications given by the five appraisers; the tool can be used for nominal and ordinal data. The attribute agreement analysis worksheet was used to create the worksheet for this study (Figure 3).

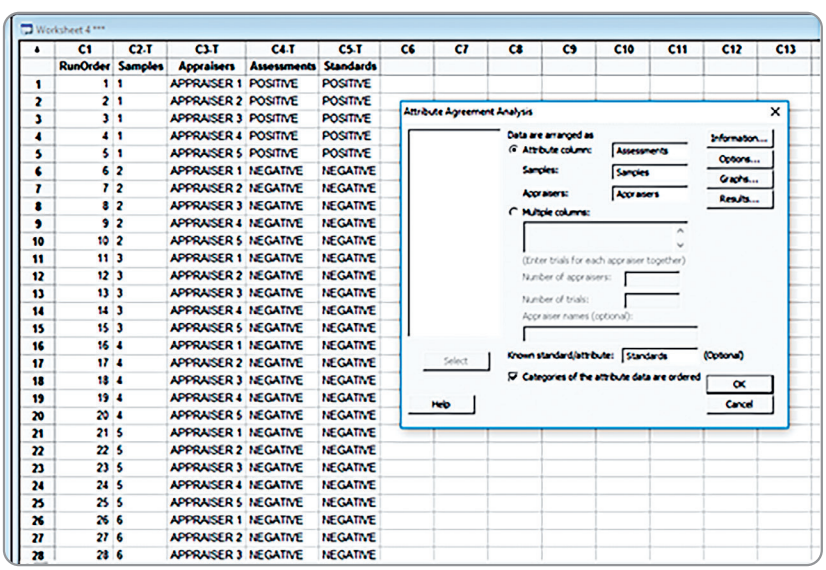

Figure 3. Layout of the statistical tool used

The data set was structured so that it was stacked in an attribute column. The results obtained from each appraiser were entered as text (positive/negative) data. For the data in the attribute column, all responses were grouped into one column and columns were set up with grouping indicators for the appraiser and the sample number. The grouping indicators were used to define each sample. The confidence level for the interval estimate of the percentage agreement between appraisers and between each appraiser and the standard was set at 95%. We specified a column for known standards/attributes (expected outcome) in the main dialog to estimate how often each appraiser's judgments deviated from the known standard or attribute values.

3. Results and Discussion

The attribute agreement analysis output included graphical and numeric output in the forms shown in the text below. The statistical programme displayed three assessment agreement tables: Each appraiser vs standard; between appraisers, and; all appraisers vs standard.

3.1 Attribute agreement analysis for assessment reports

Samples: 10	Appraisers: 5
Replicates: 1	Total runs: 50
Date of study:	10 2023
Reported by:	Head of sensory panel
Name of product:	Fish
Misc:	Method validation — Visual determination of parasites in fish

3.2 Each appraiser vs standard

Tables 1 to 4 show output tables from the MINITAB statistical programme, using operation 3.2. The parameter analyzed in a statistical test lies between the endpoints of the confidence limit interval. In this case, as shown in Table 1, 74.11% to 100% of the appraisers correctly identified the positive samples. In Table 2, and in accordance with the findings in Table 1, there were no deviations in the overall evaluation of the fish samples or of those fish samples with parasites that were previously declared as standard.

Table 1. Assessment agreement

Appraiser	# Inspected	# Matched	Percent	95% CI
APPRAISER 1	10	10	100.00	(74.11, 100.00)
APPRAISER 2	10	10	100.00	(74.11, 100.00)
APPRAISER 3	10	10	100.00	(74.11, 100.00)
APPRAISER 4	10	10	100.00	(74.11, 100.00)
APPRAISER 5	10	10	100.00	(74.11, 100.00)

Matched: Appraiser's assessment across trials agrees with the known standard.

Table 2. Assessment disagreement

Appraiser	# NEGATIVE/POSITIVE	Percent	# POSITIVE/NEGATIVE	Percent	# Mixed Percent
APPRAISER 1	0 0.00	0	0.00	0	0.00
APPRAISER 2	0 0.00	0	0.00	0	0.00
APPRAISER 3	0 0.00	0	0.00	0	0.00
APPRAISER 4	0 0.00	0	0.00	0	0.00
APPRAISER 5	0 0.00	0	0.00	0	0.00

NEGATIVE/POSITIVE: Assessments across trials = NEGATIVE / standard = POSITIVE.
NEGATIVE/POSITIVE: Assessments across trials = POSITIVE / standard = NEGATIVE.
Mixed: Assessments across trials are not identical.

Fleiss' kappa and Cohen's kappa scores are included in the statistic programme's output tables (Table 3 and Table 4, respectively). The higher the kappa score, the greater the agreement between appraisers, and the better the validation of the test (MINITAB, 2024). The statistical software calculates Cohen's kappa when two appraisers rate a single trial, or when one appraiser rates two trials. The Fleiss kappa coefficient theoretically ranges from −1 to +1. Values close to 1 indicate a strong agreement between the overall rating and the individual appraisers. Tables 3 and 4 show kappa coefficient values of 1 for each appraiser, indicating their full agreement with the overall rating. In our study, the results of Cohen's kappa test (Table 4) were identical to the Fleiss Kappa indices. A test of significance and its p-value are displayed to indicate the significance of each result (P=0.00008).

3.3 Between appraisers

Figures 4 to 6 show the statistical programme's output data, using the appropriate statistical operation (Figure 3). In Figure 4, we plotted the results between the appraisers, who in this case each gave an identical answer by looking at all groups of fish slices distributed on 10 plates. The first plate contained six positive samples from the same sample population (in this study, this plate was also declared to be the standard), which gave us confidence intervals with the population parameter (positive parasite findings).

Table 3. Fleiss' kappa statistics

Appraiser	Response	Kappa SE Kappa	Z	P(vs > 0)
APPRAISER 1	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 2	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 3	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 4	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 5	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008

Table 4. Cohen's kappa statistics

Appraiser	Response	Kappa SE Kappa	Z	P(vs > 0)
APPRAISER 1	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 2	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 3	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 4	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008
APPRAISER 5	POSITIVE	1 0.316228	3.16228	0.0008
	NEGATIVE	1 0.316228	3.16228	0.0008

Between Appraisers

Assessment Agreement

# Inspected	# Matched	Percent	95% CI
10	10	100.00	(74.11, 100.00)

Matched: All appraisers' assessments agree with each other.

Fleiss' Kappa Statistics

Response	Kappa	SE Kappa	Z	P(vs > 0)
POSITIVE	1	0.1	10	0.0000
NEGATIVE	1	0.1	10	0.0000

Figure 4. Results of comparison between appraisers
Matched: All appraisers' assessments agree with each other.

3.3.1 Assessment Agreement

The software is able to determine any deviation of a particular appraiser from the standard. However, this was not calculated in our study, as the current data are from only a single trial for each appraiser.

3.4 All appraisers vs standard

3.4.1 Assessment Agreement

The software output also displays a graph of the confidence intervals (CI), comparing each appraiser against the standard, as shown in Figure 6. This figure shows the CIs, comparing each appraiser (1–5) against the standard (fish samples on plate No. 1 (Figure 1) and that were declared to be positive (so did contain parasites).

It should also be noted that each matching percentage is associated with a confidence interval (Figure 6). The results of the statistical operation, presented in Table 1, show complete agreement of the results of the evaluation of all samples by the appraisers, including the samples declared as the standard (the first plate with all six positive samples) at the confidence level of 95% within the corresponding confidence interval (CI).

All Appraisers vs Standard

Assessment Agreement

# Inspected	# Matched	Percent	95% CI
10	10	100.00	(74.11, 100.00)

Matched: All appraisers' assessments agree with the known standard.

Fleiss' Kappa Statistics

Response	Kappa	SE Kappa	Z	P(vs > 0)
POSITIVE	1	0.141421	7.07107	0.0000
NEGATIVE	1	0.141421	7.07107	0.0000

Cohen's Kappa Statistics

Response	Kappa	SE Kappa	Z	P(vs > 0)
POSITIVE	1	0.141421	7.07107	0.0000
NEGATIVE	1	0.141421	7.07107	0.0000

Figure 5. Results of comparison between all appraisers vs standard
Matched: Appraiser's assessment across one trial agrees with the known standard

Measurements in sensory analysis (by humans) are subjective assessments by people rather than direct physical measurements. In these situations, the quality characteristics are difficult to define and evaluate (*Nute,* 2010). To obtain meaningful classifications, more than one appraiser should classify the response measure. If the appraisers agree, there is a possibility that the ratings are correct. If the appraisers disagree, the usefulness of the rating is limited. In this method, each of the appraisers rates or grades a series of samples. The sensory measurement depends on not only the human factors of the sensory appraisers, such as their experience and acuteness of sense, but also on the laboratory environment, the experimental condition, and the presentation of the test samples. In practice, the traditional validation methods are usually time-consuming and costly, and validation of sensory methods often becomes just an administrative burden to the quality assurance personnel.

4. Conclusions

It is clear that the validation of sensory evaluation methods is important, as it separates the field of sensory science from an industry that relies heavily on guesswork and subjectivity.

A statistical kappa analysis was performed to determine the degree of agreement between each subjective assessment given by the appraisers and the actual parasitic status (positive or negative) of the fish samples. At the end of the analysis, the subjective assessment that had the highest agreement with the objective measurement was determined. It was found that, statistically, at least 75% of all appraisals achieved the highest level of agreement with the objective status of the fish samples, which allowed the attribute's discriminatory ability in describing sensory differences to be verified. Therefore, this case study validated the sensory evaluation method, based on attribute agreement.

One of the main advantages of attribute agreement analysis is that it provides a quick and accurate method for evaluating both the sensory method and the attributes. In addition, the graphical representation provided by the MINITAB statistical programme provides information about the quality of the appraisers and whether the scale is correctly calibrated or not.

By assessing the strength of agreement among appraisers using well-categorized and standardized measurement criteria, attribute agreement analysis helps us to establish the degree of the quality of the sensory evaluation. This makes the evaluation

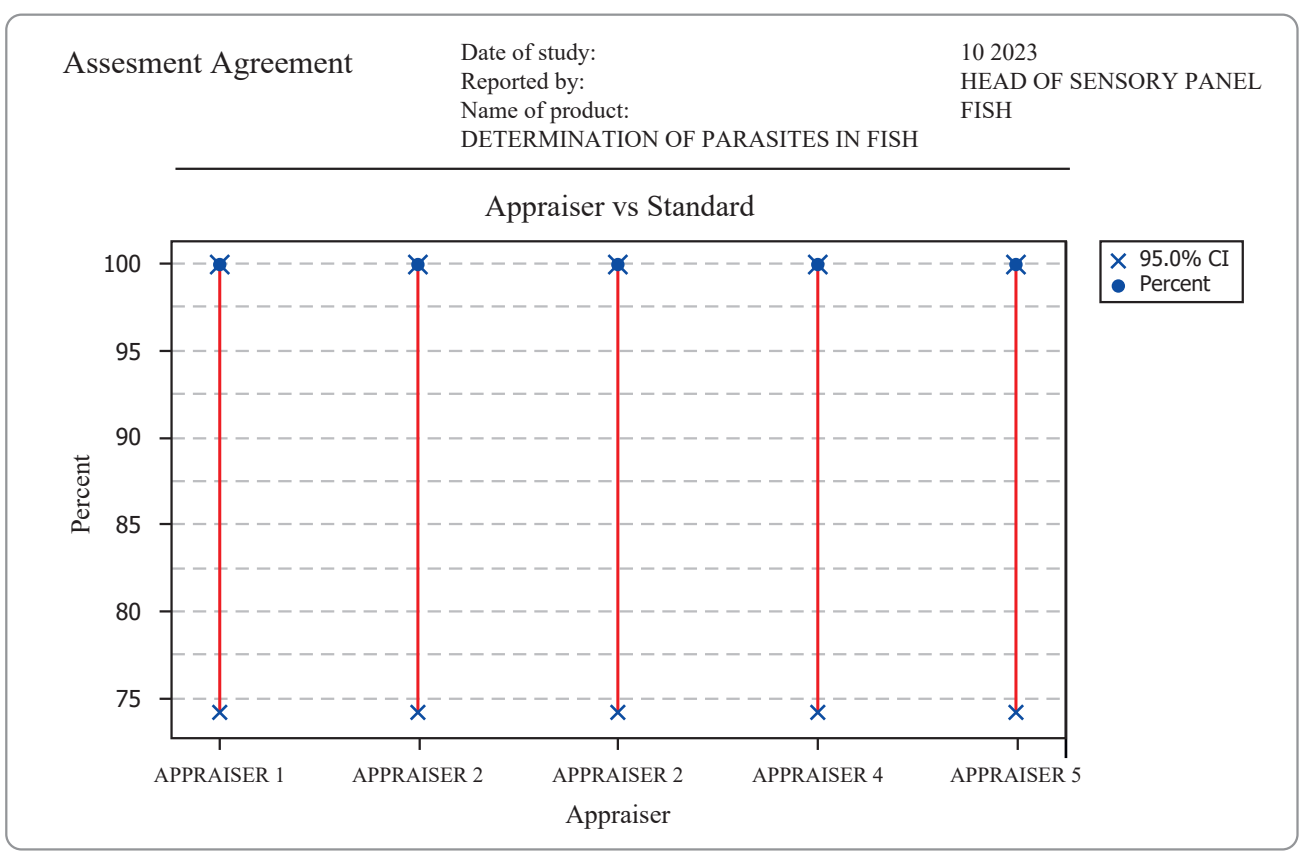

Figure 6. Graphical output for the confidence intervals comparing each appraiser against the standard

results more reliable and acceptable. In our case, the method should be able to show that no visible parasites are actually present in cases where the fish sample tested is declared parasite-free. If, on the other hand, a fish sample is classified as parasite-positive, the validated method should also produce a correct result stating that.

Finally, this tool can be used to check whether the measurement error is at an acceptable level before performing a data analysis. The attribute agreement analysis quantifies three types of variations: variations within the repeated measurements of a single appraiser (repeatability); variations between the measurements of different appraisers, (reproducibility) including corresponding confidence intervals for both of the measuring characteristics, and; variations between an appraiser's measurements and a reference or standard.

Upotreba Attribute Agreement Analysis – AAA u validaciji senzornih metoda ispitivanja: Studija slučaja za vizuelno određivanje parazita u ribama

Zoran Petrović, Jelena Ćirić, Jelena Babić Milijašević, Milan Milijašević, Mirjana Lukić, Jelena Jovanović i Aleksandra Nikolić

INFORMACIJE O RADU

Ključne reči:
Analiza saglasnosti atributa (AAA)
Senzorne metode
Validacija
Ribe
Paraziti

APSTRAKT

Validacija senzornih metoda ispitivanja prema zahtevima treba da koristi proces sličan svakom drugom postupku validacije metoda u npr. hemijskim ispitivanjima. Međutim, parametri koji se često mere u senzornom testiranju su različiti, jer su ljudski senzorni aparat i um instrumenti koji se "kalibrišu". Ukupno 10 uzoraka ribe je ispitano od strane petočlanog panela za procenu vizuelnog prisustva parazita u smrznutom osliću. 1 uzorak ribe je izabran iz prve grupe koja je imala parazite, dok je 9 uzoraka riba izabrano iz druge grupe bez parazita. Od svakog smrznutog uzorka oslića, uzdužnim sečenjem formirana je grupa od 6 tankih odsečaka mišića ribe. Test uzorci za ispitivanje su obuhvatili 60 isečenih uzoraka koji su raspoređeni u 10 tanjira sa po 6 narezanih uzoraka. Prvi tanjir je sadržao 6 tankih fileta, od kojih je svaki sadržao parazite. Rezultati primenjenog statističkog pristupa pokazali su jako slaganje u ukupnim ocenama. Statističkom obradom u ovoj studiji utvrđeno je da je najmanje od 75% do 100 % (interval poverenja) svih pojedinačnih testova članova panela postiglo najviši nivo saglasnosti. Rezultati statističkih testova su predstavljeni u obliku tabela i grafikona koji sumiraju subjektivne rezultate testa korišćenjem Fleiss Kappa i Cohen Kappa statistike.

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

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, (Grant No. 451-03-66/2024-03/200050 dated 05.02.2024).

References

Barbieri, S., Brkić Bubola, K., Bendini, A., Bučar-Miklavčič, M., Lacoste, F., Tibet, U., … & Gallina Toschi, T. (2020). Alignment and proficiency of virgin olive oil sensory panels: The OLEUM approach. *Foods*, 9 (3), 355.

Carpenter, R. P. Lyon, D. H., & Hasdell, T. A. (2000). Guidelines for Sensory Analysis in Food Product Development and Quality Control. In: Putting sensory analysis into practice. Eds. Carpenter, R. P. Lyon,D. H., Hasdel, T. A., 129–141, *Springer Science & Business Media*, US.

Da Costa, G. M., De Paula, M. M., Costa, G. N., Esmerino, E. A., Silva, R., de Freitas, M. Q., … & Pimentel, T. C. (2020). Preferred attribute elicitation methodology compared to conventional descriptive analysis: A study using probiotic yogurt sweetened with xylitol and added with prebiotic components. *Journal of Sensory Studies*, 35 (6), e12602.

Djekic, I., Lorenzo, J. M., Munekata, P. E., Gagaoua, M., & Tomasevic, I. (2021). Review on characteristics of trained sensory panels in food science. *Journal of Texture Studies*, 52 (4), 501–509.

Freitas, J., Vaz-Pires, P., & Câmara, J. S. (2020). From aquaculture production to consumption: Freshness, safety, traceability and authentication, the four pillars of quality. *Aquaculture*, 518,734857.

Gupta, M. K., Torrico, D. D., Ong, L., Gras, S. L., Dunshea, F. R., & Cottrell, J. J. (2022). Plant and dairy-based yogurts: a comparison of consumer sensory acceptability linked to textural analysis. *Foods*, 11 (3), 463.

Hubbard, M. R. (2012). Statistical Quality Control for the Food Industry, Springer Science & Business Media, US.

Jurica, K., Brčić Karačonji, I., Lasić, D., Bursać Kovačević, D., & Putnik, P. (2021). Unauthorized food manipulation as a criminal offense: Food authenticity, legal frameworks, analytical tools and cases. *Foods,* 10 (11), 2570.

Mihafu, F. D., Issa, J. Y., & Kamiyango, M. W. (2020). Implication of sensory evaluation and quality assessment in food product development: A review. *Current Research in Nutrition and Food Science Journal*, 8 (3), 690–702.

MINITAB, (2024). https://support.minitab.com/en-us/minitab/21/help-and-how-to/quality-and-process-improvement/measurement-system-analysis/supporting-topics/attribute-agreement-analysis/what-is-an-attribute-agreement-analysis-also-called-attribute-gage-r-r-study/ assessed March of 16th.

Mohammadi-Moghaddam, T. & Firoozzare, A. (2021). Investigating the effect of sensory properties of black plum peel marmalade on consumers acceptance by Discriminant Analysis, *Food Chemistry: X*, 11, 100126.

MoreSteam, Attribute Agreement Analyss, https://www.moresteam.com/help/engineroom/attribute-agreement-analysis, assessed March of 7th, 2024.

Nute, G. R. (2010). Sensory Evaluation of Meat Products. In Handbook of Meat Processing, Eds. F. Toldrá. 457–468, Blackwell Publishing.

International Organization for Standardization. (2017). General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017). https://www.iso.org/standard/.

Pavli, F. G., Argyri, A. A., Chorianopoulos, N. G., Nychas, G. J. E., & Tassou, C. C. (2020). Effect of Lactobacillus plantarum L125 strain with probiotic potential on physicochemical, microbiological and sensorial characteristics of dry-fermented sausages. *LWT*, 118, 108810.

Quintão, C., Andrade, P., & Almeida, F. (2020). How to Improve the Validity and Reliability of a Case Study Approach? *Journal of Interdisciplinary Studies in Education*, 9 (2), 264–275.

Rose, J., & Johnson, C. W. (2020). Contextualizing reliability and validity in qualitative research: toward more rigorous and trustworthy qualitative social science in leisure research. *Journal of Leisure Research*, 51 (4) 432–451.

Sipos, L., Nyitrai, Á., Hitka, G., Friedrich, L. F., & Kókai, Z. (2021). Sensory Panel Performance Evaluation—Comprehensive Review of Practical Approaches. *Applied Sciences*, 11 (24), 11977.

Stone, H., Bleibaum, R. N., & Thomas, H. A. (2020). Sensory evaluation practices. In organizing and operating sensory sciece capabilites, 23, Publisher Elsevier Science,

Sürücü, L., & Maslakci, A. (2020). Validity and reliability in quantitative research. Business & Management Studies: *An International Journal*, 8 (3), 2694–2726.

Vivek, K., Subbarao, K. V., Routray, W., Kamini, N. R., & Dash, K. K. (2020). Application of fuzzy logic in sensory evaluation of food products: A comprehensive study. *Food and Bioprocess Technology*, 13, 1–29.

Xiong, Y., Li, W., & Liu, T. (2020). Risk early warning of food quality safety in meat processing industry. *International Journal of Environmental Research and Public Health*, 17 (18), 6579.

Zhang, Z., Sun, Y., Sang, S., Jia, L., & Ou, C. (2022). Emerging approach for fish freshness evaluation: principle, application and challenges. *Foods,* 11 (13),1897.

Authors ORCID info

Zoran Petrović https://orcid.org/0000-0003-2016-5681
Jelena Ćirić https://orcid.org/0000-0002-8118-7676
Jelena Babić Milijašević https://orcid.org/0009-0003-8923-7046
Milan Milijašević https://orcid.org/0000-0003-4269-236X
Mirjana Lukić https://orcid.org/0009-0001-7749-864X
Jelena Jovanović https://orcid.org/0000-0003-0301-729X
Aleksandra Nikolić https://orcid.org/0009-0009-0012-3418

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

UDK: 639.3.043:669.018.674
ID: 149393673
https://doi.org/10.18485/meattech.2024.65.1.8

Original Scientific Paper

Trend analysis of heavy metal contamination and arsenic levels in complete feed for fish and other complete animal feeds

Biljana Pećanac[1], Jelena Janjić[2], Vesna Đorđević[3], Tatjana Baltić[3], Srđan Stefanović[3], Milica Laudanović[2] and Jelena Ćirić[3]*

[1] Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan", Branka Radicevica 18, 78000 Banja Luka, Bosna and Herzegovina
[2] University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobodjenje 18, 11000, Serbia
[3] Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia

ARTICLE INFO

Keywords:
Fish feed
Animal feed
Heavy metal
Level
Toxic

ABSTRACT

The aim of this research was to analyse the levels of toxic heavy metals (Mercury, Lead and Cadmium) and a toxic metalloid (Arsenic) in fish feed and animal feed from Bosnia and Herzegovina for the period from 2013 to 2018. Data from the National Veterinary Inspection Sector were provided for this study (n=438). The mean levels of As, Hg, Pb and Cd in all fish feed samples were 0.90±0.50 mg/kg, 0.02±0.05 mg/kg, 0.42±0.70 mg/kg and 0.16±0.11 mg/kg, respectively. During 2013–2018, the mean levels of As, Hg, Pb and Cd in animal feed (other complete feed) were 0.42±1.22 mg/kg, 0.01±0.02 mg/kg, 0.75±2.18 mg/kg and 0.10±0.12 mg/kg, respectively. In animal feeds sampled between 2013–2018, mean annual Pb levels increased the most among the four elements studied. In contrast, mean annual As, Hg and Cd levels in animal feeds continuously decreased during the study period. The mean annual Pb level in fish feed decreased, but continuous increases were observed in mean annual Hg and Cd levels during the study. The results show that the levels of toxic elements in fish feed and animal feed require attention and deserve a high priority monitoring program, as most feeds complied with the regulated maximum allowed concentrations of As, Hg, Pb and Cd in Bosnia and Herzegovina and in the European Union, but some did not.

1. Introduction

Agricultural and industrial development has been responsible for much heavy metal contamination of soils and waters. The major routes of heavy metal input to agricultural soils include animal manures, fertilizer and livestock products (*Nicholson et al.,* 1999). Hazards from the group of industrial-chemical pollutants include heavy metals and organic chemical contaminants. Due to the protection of consumer health, a number of heavy metals in foods is limited by the regulations in most European countries. This primarily refers to mercury, lead, cadmium, arsenic, and in some cases to other heavy metals such as zinc, tin, copper and iron. Animal feed and feed materials can be contaminated with heavy metals. This is very important, because these elements, after ingestion by animals, can then transfer along the food chain to be present in foods of animal origin, causing them to be a risk for human health (*Adamse et. al*., 2017).

Pb, Hg, Cd and As are the most toxic elements for animal health (*Bampidis et al.,* 2013; *Suttle*, 2010). Heavy metal toxicity can occur in animals from high ambient air concentrations near emission sources, or by eating contaminated feed (*Pandey and Madhuri*, 2014; *Castro-Gonzalez et al*., 2008). These

***Corresponding author:** Biljana Pećanac, *biljana.pecanac@virs-vb.com*

Paper received: March 15th 2024. Paper accepted: April 1st 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

metals are toxic since they are bioaccumulated in animal liver and kidney with potent effects of mutagenicity, carcinogenicity, teratogenicity and immunosuppression. Pb is a toxic, bioaccumulative heavy metal that has no known biological function. It accumulates in high concentrations in bones, teeth, liver, lungs, kidneys, brain and spleen, and it poses a serious risk to the health of the human population (*Castro-Gonzalez et al*., 2008). The most pronounced toxic effects of Pb are expressed on the nerve, haematological, cardiovascular and kidney systems (*Agency for Toxic Substance and Disease Registry*, 2005). Cd is not essential to any organism, and it is also not known to have any biological function in mammals. Hg is a cumulative poison, so symptoms of poisoning depend on the frequency or quantity of the input (*National Research Council (NRC)*, 2005; *Lopez-Alonso*, 2012; *Mandal and Suzuki*, 2002). As accumulates in the liver, from which it slowly releases and distributes into the stomach, intestines, the nervous system, and skin, and it tends to be deposited in bones and skin and permanently in the teeth (*Gwalteney-Brant et al.,* 2002).

Within the European Union (EU), the maximum allowable concentrations (MAC) of As, Pb, Hg and Cd in animal feed are regulated in Directive 2002/32/EC, last amended by Regulation 2015/186 (*Commission Regulation (EU) 2015*). Along with the EU regulation, national monitoring of toxic element concentrations in animal feed and fish feed is very important. It is necessary to have insight into the contamination and levels of toxic elements in the feed for different animal species. However, there are no data about heavy metal levels in animal feed or fish feed in Bosnia and Herzegovina. Accordingly, the aim of this study was to analyse the trends of heavy metal contamination and As levels in fish feed and animal feed between 2013 and 2018 and compare the levels found with literature data.

2. Materials and Methods

2.1 Sampling

Data from the National Veterinary Inspection Sector were provided for this study. Data covered the monitoring results of four toxic elements (As, Pb, Hg and Cd) in fish feed and animal feed in Bosnia and Herzegovina between 2013 and 2018. Animal feed that is submitted for heavy metal testing is partly sampled by the border veterinary inspection, and partly originates from internal traffic, in accordance with the prescribed competences for the purpose of controlling the production and circulation of animal feed in the country and ensuring the appropriate control and health correctness of animal feed. The number of samples (n=438) of fish feed and complete animal feed examined annually between 2013 and 2018 is presented in Table 1.

2.2 Toxic element analysis

All chemicals used were analytical grade purity. Digestion of feed was performed using a microwave closed system, MW 3000 (Anton Paar GmbH, Graz, Austria). Digestion was carried out with programs suitable for preparing samples of feed. After digestion, the content of toxic elements (As, Pb, Cd) in the feeds was determined by atomic absorption spectrometry using a Perkin Elmer Analyst 700 with the MHS system (Shelton, USA). Quality of analyses was controlled using certified reference material. The concentrations determined in the reference material were within the tolerances specified in the delivered certificate.

The amount of Hg was determined by direct burning on the Hg analyser, AMA-254. The principle of Hg determination is based on the quantification of Hg from homogenized samples that are weighed in a

Table 1. Number of fish and complete animal feeds examined in the study

Year	Fish feed	Animal feed	Total
2013	3	–	3
2014	58	25	83
2015	73	3	76
2016	68	16	84
2017	34	42	76
2018	32	84	116
Total			**438**

container. The sample thus prepared is transferred to the incineration furnace where it is dried and decomposed in a stream of oxygen at 850 °C. The decomposed products pass through a catalytic furnace at 700 °C where nitrogen oxides and sulphur are retained. Hg is captured on the amalgamator. The amalgamator is heated for a short period, and the Hg vapour released is transported to the measuring cells. Hg atoms absorb the radiation emitted by the Hg lamp, and based on the absorption of light at the appropriate wavelength, the concentration of Hg in the sample is determined. In proportion to the increase in the number of atoms, the amount of light absorbed by the atoms also increases, and by measuring the amount of absorbed light, it is possible to determine the amount of Hg in the sample.

The levels of heavy metals and As were compared with the MAC in animal feed established by the EU (*Commission Regulation (EU)*, 2015) and by Bosnia and Herzegovina (*Official Gazette of Bosnia and Herzegovina*, no. 72/11, 70/16).

2.3 Statistical analysis

All samples were collected and analysed in triplicate, and the results were expressed as mean±standard deviation. Analysis was elaborated using GraphPad Prism version 7.00 software. The coefficient of determination (R^2) was used to evaluate the significance of potential trends of the elements' levels in the feeds between 2013 and 2018 (Microsoft Office, Excel, 2010). The trends with R^2 values exceeding 0.30 were considered significant (*Adamse et al.,* 2017).

3. Results and discussion

The mean heavy metal and As levels in the fish feed samples analysed between 2013 and 2018 are presented in Table 2. In general, the heavy metals detected in fish feed samples did not exceed the MAC regulated in Directive 2002/32/EC, last amended by Regulation 2015/186 (*Commission Regulation (EU)*, 2015) and the Official Gazette of Bosnia and Herzegovina (no. 72/11, 70/16). However, mean Hg levels in some fish feeds were above the MAC of 0.1 mg/kg (Table 2). The mean annual levels of As, Hg, Pb and Cd in all fish feed samples were 0.90±0.50 mg/kg, 0.02±0.05 mg/kg, 0.42±0.70 mg/kg and 0.16±0.11mg/kg, respectively. The elements detected at the highest levels in the fish feeds from Bosnia and Herzegovina were As and Pb, which ranged from 0.18–2.98 mg/kg and 0.03–4.30 mg/kg, respectively.

The As, Hg, Pb and Cd concentration in complete animal feed samples were in the range of 0.10–6.39 mg/kg, 0.01–0.09 mg/kg, 0.10–13.69 mg/kg and 0.02–0.54 mg/kg, respectively. According to the EU (*Commission Regulation (EU)*, 2015) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16), the MAC of Pb in complete animal feed is 5 mg/kg, so some animal feed samples in the present

Table 2. The heavy metal and arsenic levels in fish feeds (mg/kg) (2013–2018)

Parameter	Elements			
	As	Hg	Pb	Cd
Mean±SD	0.90±0.50	0.02±0.05	0.42±0.70	0.16±0.11
Median	0.71	0.01	0.20	0.12
Range	0.18–2.98	0.01–0.52	0.10–4.30	0.04–0.65

Legend: According to the European Union (Commission Regulation (EU), 2015) and the Official Gazette of Bosnia and Herzegovina (no. 72/11, 70/16), the MAC of As, Hg, Pb and Cd in fish feed is 10 mg/kg, 0.2 mg/kg, 5 mg/kg and 1 mg/kg, respectively.

Table 3. The heavy metal and arsenic levels in complete animal feeds (mg/kg) (2013–2018)

Parameter	Elements			
	As	Hg	Pb	Cd
Mean±SD	0.42±1.22	0.01±0.02	0.75±2.18	0.10±0.12
Median	0.06	0.002	0.25	0.06
Range	0.01–6.39	0.01–0.09	0.10–13.69	0.02–0.54

Legend: According to the European Union (Commission Regulation (EU), 2015/186) and the Official Gazette of Bosnia and Herzegovina (no. 72/11, 70/16), the MAC of As, Hg, Pb and Cd in complete animal feed is 2 mg/kg, 0.1 mg/kg, 5 mg/kg and 1 mg/kg, respectively.

study did not conform with the prescribed MAC (Table 3). As in fish feeds, Pb was the toxic element detected at the highest levels in complete animal feeds (Table 3).

According to *EFSA* (2015), As in animal feed originates from geological sources, industrial activities and specific feed additives. Also, drinking water can be one of the inorganics As sources (*Mandal*, 2002; *Lopez- Alonso*, 2012; *Bampidis at al*., 2013). As contents in the fish feed varied greatly in the examined years (Figure 1). As was not detected in fish feed samples in 2013 or 2014. The mean As level in fish feed was significantly (R^2=0.885) higher in 2015, 2016 and 2017 than in 2018. The mean annual As level in fish feed followed the order 2013<2014<2018<2017<2016<2015. According to the *Commission Regulation (EU)* (2015) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16), the MAC of As in complete animal feed is 2 mg/kg, so some complete animal feed samples in the present study did not conform with the prescribed MAC. *Adamse et al*. (2017) showed that seaweed accumulates As. Similar results were presented in a study by *Makkar et al*. (2016). *Wang* (2013) showed that feed additives for animal production can have a high risk of unacceptable As levels. According to a study by EFSA (*European Food Safety Authority*, 2015), high levels of As were found in fishmeal. Total As concentrations in complete feeds were shown by the EFSA contaminants panel (*European Food Safety Authority*, 2015), when the mean As level in complete feeds for beef cattle, broilers and pigs (grower/finishers) was 0.36 mg/kg, 0.34 mg/kg and 0.31 mg/kg, respectively. In the current study, As levels between 2013 and 2018 were higher in fish feed than in complete animal feed. Mean annual As levels did not vary significantly in complete animal feeds from 2013 to 2018 (Figure 1). As was once used for disease control in farm animals, but now, in Europe, its use is forbidden in animal production (*Li et al.*, 2005).

Trend analysis of Pb levels in complete animal and fish feeds between 2013 and 2018 is shown in Figure 2. The highest mean annual Pb level was found in complete animal feed from 2017. Each year of the study, the mean Pb level was below 2 mg/kg. In the fish feed samples, mean Pb levels decreased during 2013–2018. However, Pb levels in complete animal feed increased in that period. The reason for this upward trend of Pb levels is unexplained. Pb is a very toxic element and is an indicator of environmental pollution caused by anthropogenic factors (*Sager*, 2007). As a toxic element, Pb levels in mineral supplements and premix for animals can be higher than in other livestock feedstuffs. The EFSA contaminants panel (2004a) stated Pb levels in complete feeds for beef, poultry, broilers and pigs were 1.14 mg/kg, 1.16 mg/kg, 0.52 mg/kg and 1.03 mg/kg, respectively. In the study by *Wang*

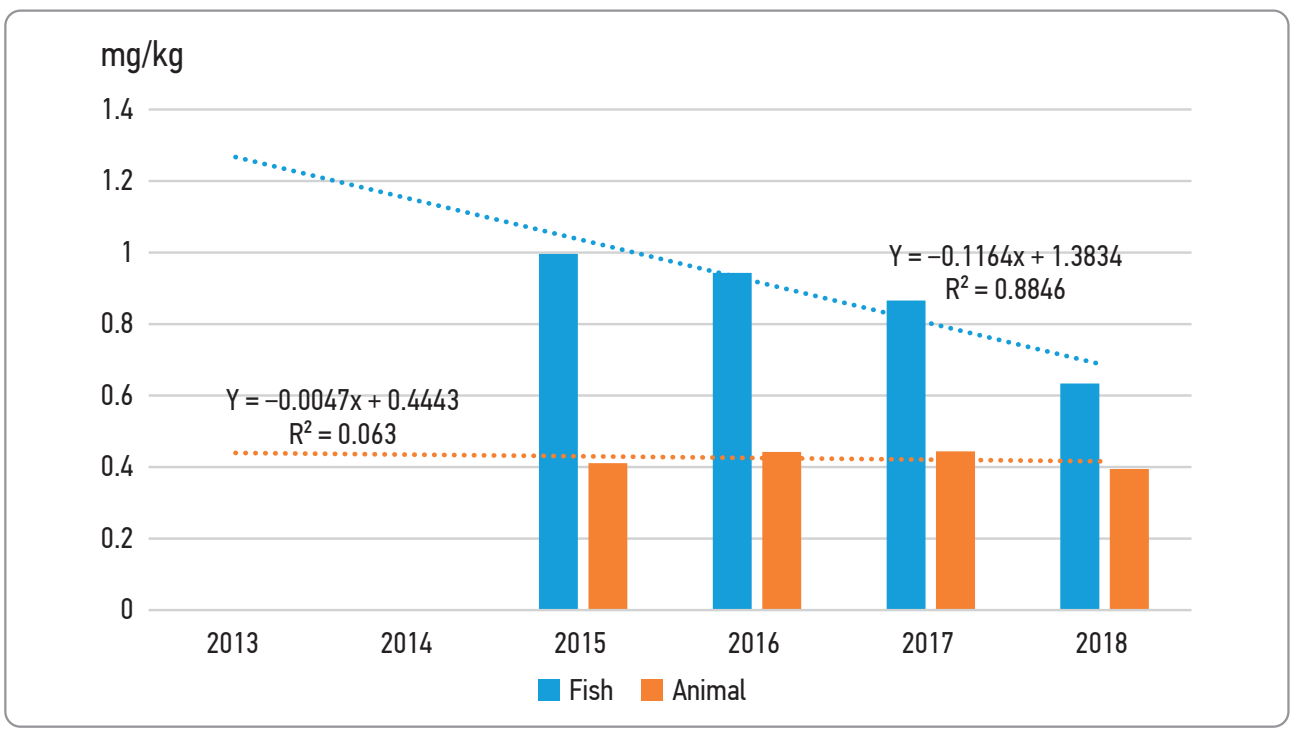

Figure 1. The mean levels of Arsenic in complete animal and fish feeds, between 2013 and 2018.

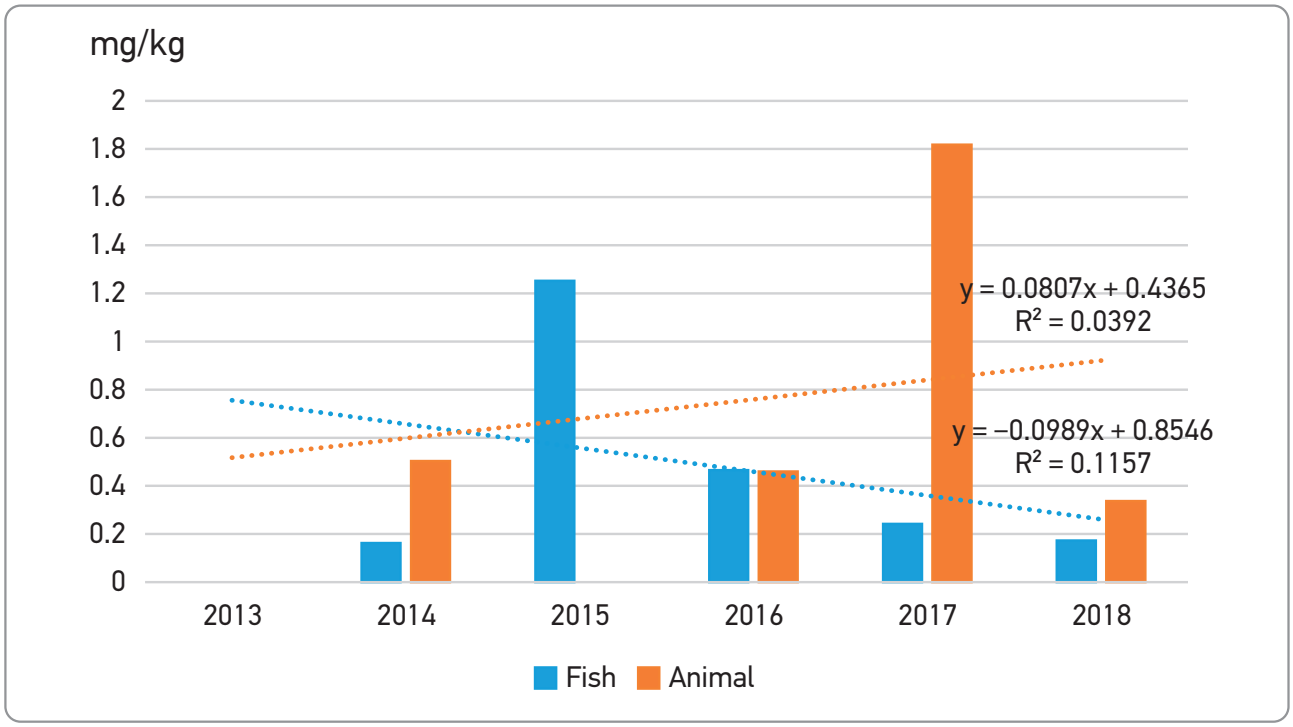

Figure 2. The mean levels of Lead in complete animal and fish feeds, between 2013 and 2018.

et al. (2013), a high correlation between Pb levels in the environment and in animal manure was found. During 2013–2018, Pb levels increased in the complete animal feed in our study, which could be due to the increased use of mineral supplements in animal nutrition. The monitoring program for Pb contamination in the environment showed that Pb levels decreased in all European countries (*European Food Safety Authority*, 2015).

Significantly decreasing mean annual levels of Hg were found in complete animal feed samples (R^2=0.60) for all the tested period, 2013–2017

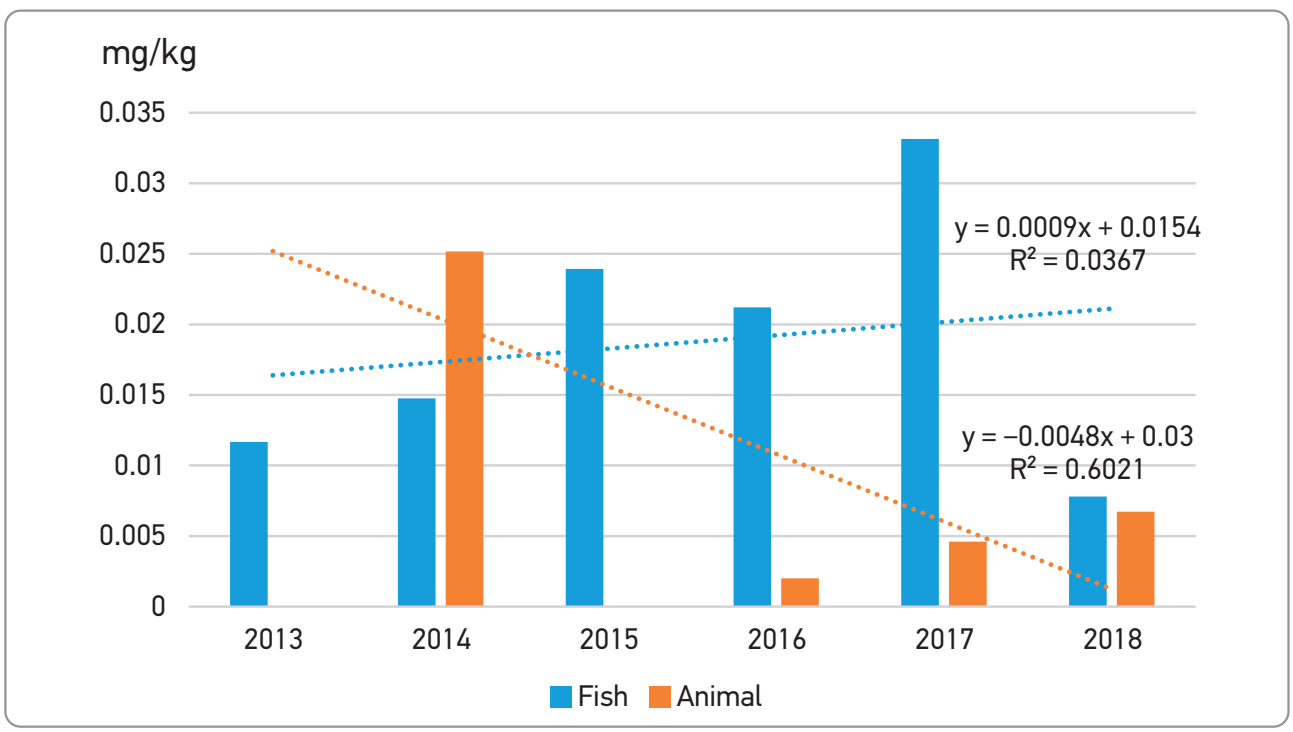

Figure 3. The mean levels of Mercury in complete animal and fish feeds, between 2013 and 2018.

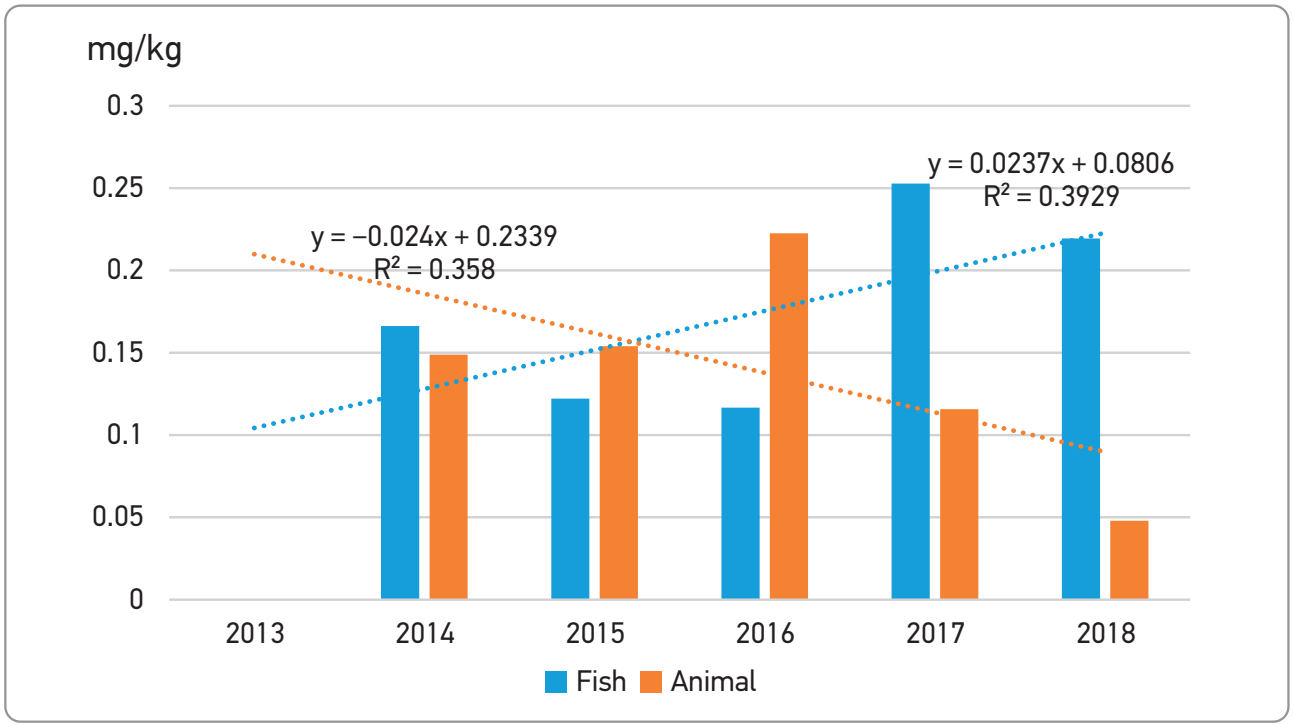

Figure 4. The mean levels of Cadmium in complete animal and fish feeds, between 2013 and 2018.

(Figure 3). However, mean annual levels of Hg in fish feed increased during this period. Compared with the other examined years, in complete animal feed samples during 2017, a higher mean Hg level was found. Similarly, to Pb, Hg is a toxic element and indicator of environment pollution (*Nicholson et al*., 2017; *Li et al.*, 2005). Fish feed and other fish meals are the major sources of Hg in animal nutritional products utilized in livestock production (*Lopez- Alonso*, 2012). The Hg concentrations in feed supplements and additives are generally low. According to EFSA (*European Food Safety Authority*, 2008), higher Hg concentrations were found in rodent complete feeds (0.050 mg/kg), poultry complete feeds (0.039 mg/kg) and pig complete feeds (0.032 mg/kg). In the present study, mean annual Hg levels in both fish and complete animal feeds were below the MAC (0.2 mg/kg and 0.1 mg/kg, respectively) established by the EU (*Commission Regulation (EU)*, 2015) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16). In a previous study (*Adamse et al*., 2017), Hg levels in animal feed decreased from 2007–2013.

The mean annual Cd levels in fish feed and animal feed between 2013 and 2018 are presented in Figure 4. Significantly decreasing mean Cd levels were found in complete animal feed samples (R^2=0.35) during these years. However, mean Cd levels significantly increased in fish feed during 2013–2018 (R^2=0.39). Mean annual Cd levels in the fish and complete animal feeds were below the MAC (1.0 mg/kg) established by the EU (*Commission Regulation (EU) 2015/186*) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16). High Cd levels in fish feed could be due to agricultural activities (use of fertilizers) (*Lopez-Alonso*, 2012, *Rajaganapathy et al.*, 2011, *Amlund et al*., 2012). Also, according to *McBride* (1998) and *Dai et al*. (2016), mineral supplements can contain high Cd levels. The ESFA contaminants panel (2004b) stated Cd levels in complete feeds for poultry, broilers, ruminants and pigs were 0.16 mg/kg, 0.19 mg/kg, 0.11 mg/kg and 0.09 mg/kg, respectively. *Adamse et al*. (2017) showed that Cd levels significantly increased between 2007 and 2013 in feed material of marine origin. One of the most sensitive animal species for Cd toxicity is the pig (*King et al*., 1992).

4. Conclusion

It is necessary to have insight into the contamination and levels of toxic elements in the feed for different animal species. However, there are no data about heavy metal levels in animal feed or fish feed in Bosnia and Herzegovina. Accordingly, the aim of this study was to analyse the trends of heavy metal contamination and As levels in fish feed and animal

feed between 2013 and 2018 and compare the levels found with literature data. The results show that the levels of toxic elements in fish feed and animal feed require attention and deserve a high priority monitoring program, as most feeds complied with the regulated maximum allowed concentrations of As, Hg, Pb and Cd in Bosnia and Herzegovina and in the European Union, but some did not.

Analiza trenda kontaminacije teškim metalima i nivoa arsena u kompletnoj hrani za ribe i drugih potpunih smeša za ishranu životinja

Biljana Pećanac, Jelena Janjić, Vesna Đorđević, Tatjana Baltić, Srđan Stefanović, Milica Laudanović i Jelena Ćirić

INFORMACIJE O RADU

Ključne reči:
Hrana za ribe
Hrana za životinje
Teški metali
Nivo
Toksičnost.

APSTRAKT

Cilj ovog istraživanja bio je utvrditi nivo toksičnih metala (olovo, kadmijum, živa i arsen) u hrani za ribe i stočnoj hrani iz Bosne i Hercegovine za period od 2013. do 2018. godine. Podaci Sektora nacionalne veterinarske inspekcije su korišćeni za ovu studiju (n=438). Srednji nivoi As, Hg, Pb i Cd u svim uzorcima hrane za ribe bili su 0,90±0,50 mg/kg, 0,02±0,05 mg/kg, 0,42±0,70 mg/kg i 0,16±0,11 mg/kg, pojedinačno. Tokom 2013–2018, srednji nivoi As, Hg, Pb i Cd u stočnoj hrani (ostala potpuna hrana za životinje) bili su 0,42±1,22 mg/kg, 0,01±0,02 mg/kg, 0,75±2,18 mg/kg i 0,10±0,12 mg. /kg, pojedinačno.. U hrani za životinje uzorkovanoj između 2013–2018, srednji nivoi Pb su se najviše povećali. Nasuprot tome, srednji nivoi As, Hg i Cd u stočnoj hrani kontinuirano su opadali tokom perioda istraživanja. Srednji nivo Pb u hrani za ribe se smanjio, ali su uočeni kontinuirani porasti srednjih nivoa Hg i Cd tokom studije. Rezultati pokazuju da nivoi toksičnih elemenata u hrani za ribe i stočnu hranu zahtevaju pažnju i zaslužuju program praćenja visokog prioriteta, iako As, Pb i Hg nisu prekoračili maksimalno dozvoljene koncentracije regulisane Direktivom 2002/32/EC, poslednjom izmenjenom Uredbom. 2015/186 i Službeni glasnik Bosne i Hercegovine (72/11, 70/16).

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

References

Adamse, P., Van der Fels-Klerx, H. J. & de Jong, J. (2017). Cadmium, lead, mercury and arsenic in animal feed and feed materials–trend analysis of monitoring results. *Food Additives & Contaminants: Part A*, 34 (8), 1298–1311.

Agency for Toxic Substance and Disease Registry, (2005). Toxicological Profile for Lead, U.S. Department of Health and Humans Services, Public Health Service, Centres for Diseases Control, Atlanta, GA.

Amlund, H., Berntssen, M. H. G., Lunestad, B. T. & Lundebye, A. K. (2012). Aquaculture feed contamination by persistent organic pollutants, heavy metals, additives and drug residues. In: Fink-Gremmels J., editor. Animal feed Contamination, Effects on Livestock and Food Safety. Cambridge: *Woodhead Publishing Series in Food Science, Technology and Nutrition*, 215, 205–229.

Bampidis, V. A., Nistor, E. & Nitas, D. (2013). Arsenic, cadmium, lead and mercury as undesirable substances in animal feeds. *Scientific Papers Animal Science and Biotechnologies*, 46 (1), 17–22.

Castro-Gonzalez, M. I. & Mendez-Armenta M. (2008). Heavy metals: Implications associated to fish consumption. *Environmental Toxicology and Pharmacology*, 26, 263–271.

Commission Regulation (EU) 2015/186 of 6 February 2015 amending Annex I to Directive 2002/32/ EC of the European Parliament and of the Council as regards maximum levels for arsenic, fluorine, lead, mercury, endosulfan and Ambrosia seeds. *Official Journal of the European Union*, 31/11.

Dai, S. Y., Jones, B., Lee, K. M, Li, W., Post, L. & Herrman, T. J. (2016). Heavy metal contamination of animal feed in Texas. *Journal of Regulatory Science*, 1, 21–32.

EFSA (European Food Safety Authority, (2004b). Opinion on the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to cadmium as undesirable substance in animal feed. Adopted on 2 June 2004. *EFSA Journal*, 72, 1–24.

EFSA (European Food Safety Authority), (2008). Mercury as undesirable substance in animal feed. Scientific opinion of the Panel on Contaminants in the Food Chain. Adopted on 20 February 2008. *EFSA Journal*, 654, 1–74.

EFSA (European Food Safety Authority), (2015). Opinion of the scientific panel on contaminants in the food chain on a request from the Commission related to arsenic as undesirable substance in animal feed. *EFSA Journal*, 180, 1–35.

EFSA (European Food Safety Authority) (2004a). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to lead as undesirable substance in animal feed. Adopted on 2 June 2004. *EFSA Journal,* 71, 1–20.

Gwalteney-Brant, S. M. (2002). Heavy metals. Handbook of Toxicologic Pathology. *Academic Press*, New York, 701–732.

King, R. H., Brown, W. G., Amenta, V. C. M., Shelley, B. C., Handson, P. D., Greenhill, N. B. & Willcock, G. P. (1992). The effect of dietary cadmium intake on the growth performance and retention of cadmium in growing pigs. *Animal Feed Science and Technology*, 1992, 37 (1–2), 1–7.

Li, Y., McCrory, D. F., Powell, J. M., Saam, H. & Jackson-Smith, D. (2005). A survey of selected heavy metal concentrations in Wisconsin dairy feeds. *Journal of Dairy Science*, 88, 2911–2922.

Lopez-Alonso, M. (2012). Animal feed contamination by toxic metals. In: Fink-Gremmels J, editor. Animal feed contamination, effects on livestock and food safety. Cambridge: Woodhead Publishing Series in Food Science, Technology and Nutrition. 215, 183–204.

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F. & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, 212, 1–17.

Mandal, B. K. & Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta*, 58 (1), 201–235.

McBride, M. B. (1998). Growing food crops on sludge-amended soils: Problems with the US Environmental Protection Agency method of estimating toxic metal transfer. *Environmental Toxicology and Chemistry*, 17 (11), 2274–2281.

National Research Council (NRC), (2005). Mineral tolerance of animals. 2nd revised ed. Washington (DC): National Research Council of the National Academies, *The National Academies Press*.

Nicholson, F. A., Chambers, B. J., Williams, J. R. & Unwin, R. J. (1999). Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresource Technology*, 70 (1), 23–31.

Official Gazette of Bosnia and Herzegovina, (2016). Regulation of unwanted supplements in feed for animals. *Official Gazette of BIH*, no. 72/11, 70/16. http://www.fsa.gov.ba/fsa/images/pravnipropisi/hrPravilnik_o_ne%C5%BEeljenim_tvarima_u_hrani_za_%C5%BEivotinje_R01_70-16.pdf 2016

Pandey, G. & Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, 2 (2), 17–23.

Rajaganapathy, V., Xavier, F., Sreekumar, D. & Mandal, P. K. (2011). Heavy metal contamination in soil water and fodder and their presence in livestock and products: a review. *Environmental Science & Technology*, 4, 234–249.

Sager, M. (2007). Trace and nutrient elements in manure, dung and compost samples in Austria. *Soil Biology and Biochemistry*, 39 (6), 1383–1390.

Suttle, N. F. (2010). Mineral nutrition of livestock. Cabi.

Wang, H., Dong, Y., Yang, Y., Toor, G. S. & Zhang, X. (2013). Changes in heavy metal contents in animal feeds and manures in an intensive animal production region of China. *Journal of Environmental Sciences*, 25 (12), 2435–2442.

Authors ORCID info

Biljana Pećanac
Jelena Janjić https://orcid.org/0000-0003-3351-7199
Vesna Đorđević https://orcid.org/0009-0008-5187-4089
Tatjana Baltić https://orcid.org/0000-0002-0496-3133
Srđan Stefanović https://orcid.org/0000-0002-8011-5654
Milica Laudanović https://orcid.org/0009-0008-6381-2803
Jelena Ćirić https://orcid.org/0000-0002-8118-7676

Guidelines for the Authors

"Meat Technology" (ISSN 0494-9846) is a scientific journal 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.

Authors of papers are obliged to respect scientific and ethical principles and rules when preparing and publishing articles in accordance with international standards.

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.

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 authors" on mail danijelas@inmesbgd.com.

Language

Papers must be written on English (British English spelling).

Editing of the manuscripts

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

- danijela.sarcevic@inmes.rs or
- meat.technology@inmes.rs, or via
- www.journalmeattechnology.com.

The text should be clear, concise, grammatically correct and should contain the following sections:

The title (lowercase, bold, font size 14 pt). Below the title, names of the authors (first name, last name, lowercase, italic, font size 12 pt). Numbers following names in superscript refer to the authors' institution.

At the bottom of the first page, put affiliation according to the number in superscript, name and address of the institutions authors are employed in should be given (italic, font size 10 pt, the main words capitalized). In the new line, the name and e-mail of the corresponding author should be provided (font 12).

Abstract on English and Serbian should contain 150–250 words with key words (maximum 5, italic, font 12). The English abstract should be typed below the title and names of the authors, and om Serbian below the conclusion.

The original scientific paper should contain the following chapters: introduction, material and methods, results and discussion (combined or separate), conclusion, notes (optional) and references. Chapter names are typed in lowercase, font size 12, bold.

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

MATERIAL AND METHODS: this chapter describes material and methods used and outlines the design of the experiment;

RESULTS AND DISCUSSION: The results should be processed by statistical methods appropriate to the experiment; they should be clear and concise using tables, graphs, photographs, illustrations and other. The same result should not be presented through both table and graph. Discussion should be related to presented results avoiding repetitions of already stated facts, using comparison of obtained results and relevant literature data related to similar group of products, comparable analytical method et sim.

When in the text, literature is cited by giving author's last name, last name with "and", if the cited literature is published by two authors, or, in the case of

more than two authors by "et al." abbreviation after the surname of the first author (italic). Cited literature with the year of publishing should be in brackets.

Figures and illustrations are numerated with the same number as given in the text of the paper. Titles of the tables are written above the tables; titles of the graphs and illustrations are printed below (in lowercase). Tables, graphs and figures are submitted separately, in the appendix.

If tables, graphs or figures originate from other sources, the author is required to state the source of such data (author, year of publishing, 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.

CONCLUSION: provides the review of the most important facts obtained during the research.

It is important for authors to send **Disclosure statement:** No potential conflict of interest was reported by authors.

Acknowledgement: should contain title and number of the project i.e. title of the program from which is the research carried out and described in the paper, as well as the name of the institution that funded the project or program and should be written after conclusion, before references.

REFERENCES: (Times New Roman 12 pts) should include recent international publications. If the original literature cited has not been available, the authors should quote the source used. The references should be numerated in alphabetical order and should be cited exactly the way they appear in the original publication. Sources, volume and issue numbers should be written in italic.

Example:

- 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), 209–218.

- Books:

Bao, Y., Fenwick, R. (2004). Phytochemicals in Health and Disease, CRC Press, Los Angeles.

- Books with more 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 thesis:

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

- 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.

- 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).

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,* 2008). In case the same author has more publications in the same year, additional letters are added next to the year (*Thomas*, 2008a; *Thomas*, 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 more references are cited within the same brackets, citations should be done in chronological order.

Papers belonging to the category other than original scientific papers can contain chapters titled by choice of the author.

Papers are submitted by e-mail:

- danijela.sarcevic@inmes.rs,
- meat.technology@inmes.rs

or on

- www.journalmeattechnology.com

EDITORIAL BOARD

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.

Antonella Dalle Zotte, University of Padova, Department of Animal Medicine, Production and Health, Padova, Italy

Mohammed Gagaoua, PEGASE, INRAE, Institut Agro, Saint-Gilles, France

Rubén Domínguez Valencia, Meat Technology Center, San Cibrao das Viñas — Ourense, Spain

Oxana Kuznecova, V. M. Gorbatov Federal Research Center of Food System Moscow, Russia

Irina Tchernukha, V. M. Gorbatov Federal Research Center of Food System Moscow, Russia

Iva Steinhauserova, University of Veterinary and Pharmaceutical Sciences, Brno, Faculty of Veterinary Hygiene and Ecology, Brno, Czech

Tomas Alter, Free University Berlin, Faculty of Veterinary Medicine, Berlin, Germany

Sabine Leroy, Nacional Institute for Agricultural Research, Research Center Klermon-Feran, France

Meltem Serdaroğlu, Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey

Lazo Pendovski, Ss Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine, Skopje, Republic of North Macedonia Republic

Muhamed Smajlović, University in Sarajevo, Faculty of Veterinary Medicine, Bosnia and Herzegovina

Tomaž Polak, University in Ljubljana, Faculty of Biotechnology, Ljubljana, Republic of Slovenia

Željko Sladojević, Veterinary Institute „Dr Vaso Butozan", Banja Luka, Bosnia and Herzegovina

Snježana Mandić, University in Banja Luka, Technological Faculty, Banja Luka, Republic of Srpska

Milenko Šarić, University in Banja Luka, Faculty of Agriculture, Banja Luka, Republic of Srpska

Meho Bašić, University in Tuzla, Faculty of Technological Sciences, Bosnia nad Herzegovina

Goce Cilev, University St. Kliment Ohridski, Veterinary Faculty, Bitola, Republic of North Macedonia

Urška Jamnikar Ciglenečki, University in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Republic of Slovenia

Lea Demšar, University in Ljubljana, Faculty of Biotechnology, Ljubljana, Republic of Slovenia

Urška Henigman, University in Ljubljana, Faculty of Biotechnology, Ljubljana, Republic of Slovenia

Sheryl Avery, Scientific & Editorial English Services, Hamliton, New Zeland

Olgica Cerić, Food and Drug Administration – FDA, Veterinary Laboratory Investigation and Response Network, New Hampshire, USA

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

Galia Zamaratskia, Swedish University of Agricultural Sciences, Uppsala, Sweden

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

Ilija Đekić, University in Belgrade, Faculty of Agriculture, Belgrade, Republic of Serbia

Breda Jakovac-Strajn, University in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Republic of Slovenia

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

Vladimira Pistenkova, Faculty of Veterinary Hygiene and Ecology, Brno Czech Republic

Andrej Kirbiš, University in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Republic of Slovenia

Zlatko Jusufhodžić, Veterinary Institute Bihać, Bihać, Bosnia and Herzegovina

Marija Bošković Cabrol, University of Padua, Marie Skłodowska-Curie Postdoctoral Researcher, Padua, Italy

Biljana Pećanac, Public Institution Veterinary Institute of the Republic od Srpska „Dr Vaso Butozan", Banja Luka, Republic of Srpska

Anne Leskoviz, Ecole Nationale Superieure Agronomique de Tolouse, Toulouse, France

Martin Bouwknegt, Nacional Institute for Public Health and Environment, Eindhoven, Netherlands

Jacques-Antonie Hennekinne, Laboratory for Food Safety, ANSES – Agency National Securite Sanitarie de l' Alimentation, de l'Environnment et du Travial, Maisons-Alfort, France

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