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

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A R T I C L E I N F O

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A B S T R A C T

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

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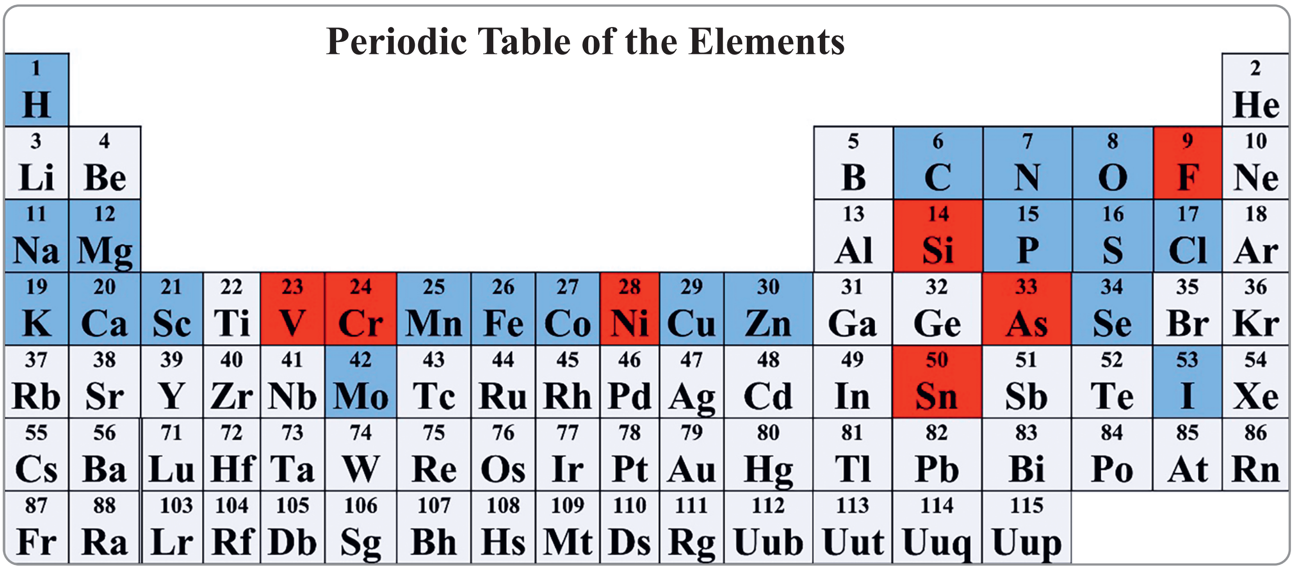

Figure 1. Periodic table highlighting the elements that are essential for life. Essential elements for humans are shown on a blue background, suggested essential elements for humans are on a red background, and nonessential elements for humans are on a grey background (Ali, 2023).

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

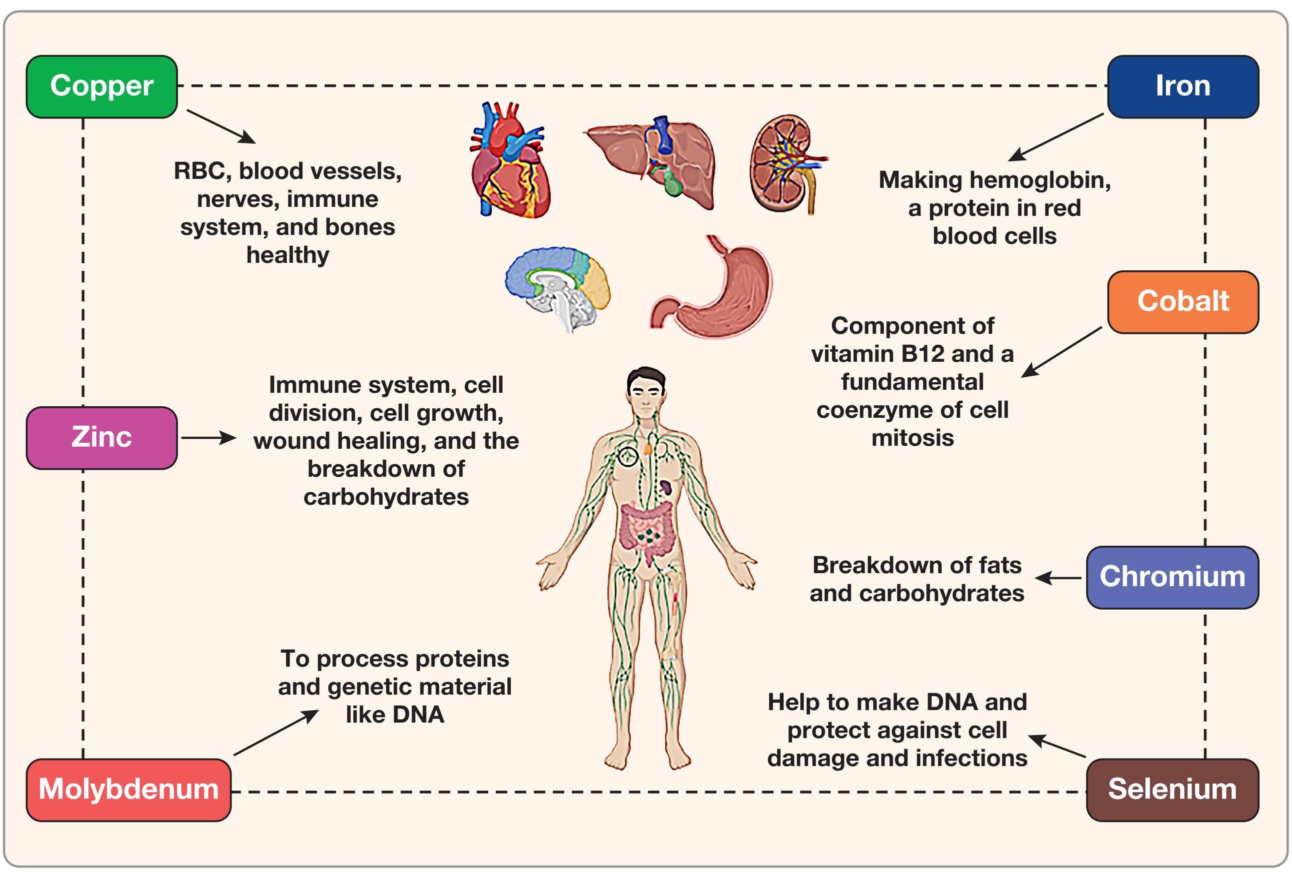

Figure 2. Role of microelements (trace elements) in human health (*Islam et al., 2023*).

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

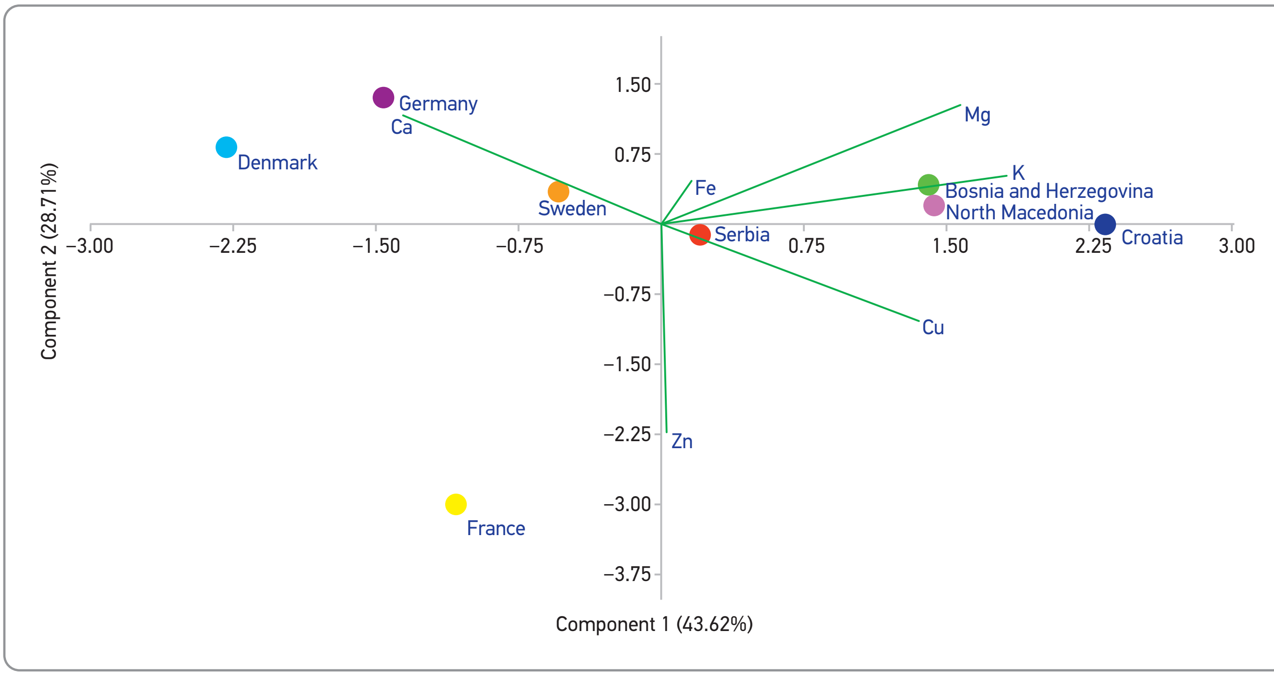

Figure 3. Bi-plot graphic of PCA of Ca, Mg, K, Fe, Cu and Zn levels in mechanically separated meats from different countries.

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.

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