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RESEARCH ARTICLE

Determination of toxic elements in meat products from Serbia packaged in tinplate cans

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Abstract

This work aimed to examine the influence of the storage period on the content of toxic elements (As, Cd, Hg, and Pb) in five types of canned meat products regularly used in the Serbian Armed Forces. Cans of beef goulash (BG), pork ragout (PR), spam (SP), liver pate (LP), and meatballs in tomato sauce (MB), produced according to military standards and stored under regular conditions, were analyzed. Meat products were packed in tin cans made according to special requirements in terms of tin and varnish application and stored for up to 6 years. The content of toxic elements varied depending on the analyzed product. The highest average content of arsenic was in BG (10.00 μg/kg), cadmium in LP (35.91 μg/kg), and mercury and lead in PR (15.04 and 8.00 μg/kg, respectively). The average concentrations of As, Cd, Hg, and Pb in all types of canned meat products were significantly lower than the maximum permitted levels in food currently in force by local and EU legislation. The storage period did not significantly affect the level of toxic elements, although higher concentrations were found in samples stored for more than 2 years. Examination of raw materials, spices, and additives showed that the highest Cd and Pb concentrations, which can affect the total level of these elements in meat products, were found in red ground pepper (Cd above 150 μg/kg) and dish supplement (Pb of 250 μg/kg). The assessment of the weekly intake of toxic elements through canned meat showed that it is significantly lower than the values that affect adversely to human health, as determined by the FAO/WHO and EFSA. However, as there is a constant possibility of contamination of raw materials and food additives, primarily due to environmental pollution, it is recommended to monitor the content of heavy metals in food permanently and assess their risk to human health.

Keywords Canned meat · Ingredients · Storage · Tinplate packaging · Toxic elements

Introduction

Canned meat products occupy an important place in the diet of members of the Serbian Armed Forces. They are largely specific compared to products of the same type intended for civilian use in Serbia. The specificity is reflected in the quality of packaging material, application of tin and varnish, quality of basic and added ingredients, and production process, which keep pace with international food safety standards. Thanks to their specifics, they are high-quality products with preserved nutritional and energy values, sensory properties, and shelf life of at least 4 years.

Electrolytic tinplate is used for the production of metal packaging (cans) for the Serbian Armed Forces. The tinplate base is steel that provides good mechanical properties, while the tin coating gives a glossy appearance and protects the steel from corrosion (Arcelor Mittal 2013; Nikčević-Grassino et al. 2010). For the Serbian Armed Forces' needs, the application of tin is a minimum of 5.6 g/m^2 on external and internal can surfaces (E–5.6/5.6), which is twice as high as for civilian use in Serbia (Stojanović et al. 2019, 2020). Thanks to numerous advantages over other packaging types, tinplate packaging is most often used for the canned meat products. An organic

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epoxy-phenolic coating (varnish) is applied to protect the tinplate from external and internal corrosion. The basic property that each coating must show is stability at the sterilization temperature and according to the packaged food ingredients. The can coating must not change the food's sensory properties and should be elastic, continuous, of uniform thickness, and without porosity. An appropriate coating is of primary importance for maintaining the safety and quality of canned products. Monitoring the quality of the coating is extremely important to reduce the risk of loss of desirable product properties and the risk of contamination and negative impact on consumer health. For the needs of the Serbian Armed Forces, the application of varnish on the inner surfaces of the can is 6 g/m^2, while that on external surfaces is 5 g/m^2 (Stojanović et al. 2019, 2020).

Contamination of food with toxic elements is a global problem, and there are justifiably growing concerns about its safety. The World Health Organization (WHO), through the Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme (GEMS/Food), encourages countries to conduct studies for assessing exposure to chemical pollutants, including toxic elements, through diet. Representative data on food consumption can be combined with contaminant concentration data to derive dietary exposure (FAO/WHO 2007). Severe numerous health problems can arise due to excessive uptake of heavy elements through the food. It is well established that more than 90–95% of the total daily exposure to toxic heavy elements comes from the diet (Bocio et al. 2005; Martí-Cid et al. 2009). Canned food can be contaminated with toxic elements from raw materials, additives, and spices during the production process or by migration from packaging material. In real circumstances, consumers are often and significantly exposed to toxic elements through canned food. Arsenic is the only carcinogen in humans with registered evidence of carcinogenic risk by inhalation and ingestion, which belongs to Group 1 of carcinogens according to the International Agency for Research on Cancer (IARC 2012, 2016).

Arsenic can cause tumors of the skin, kidneys, bladder, and lungs. According to new modeling approaches, based on 0.5% increased incidence of lung cancer in humans, EFSA set the reference point, i.e., the Benchmark dose lower confidence limit (BDML05) for As at 21.0 μg/kg bw/week (JECFA 2011). Cadmium is also classified as a "human carcinogen" (Group 1) and is a highly toxic metal that occurs naturally in soil (IARC 2012, 2016). Tolerable weakly intake (TWI) for Cd is 2.5 μg/kg bw/week (EFSA 2011). Similarly to cadmium, lead has no benefits in human metabolism, showing progressive toxicity (Zhu et al. 2011). Inorganic lead is classified as a probable human carcinogen (Group 2A) (IARC 2006, 2016). The lower benchmark dose ($BDML_{10}$) for nephrotoxic effects of Pb was set at 4.4 μg/kg bw/week (EFSA 2012). Mercury occurs in several forms: elemental, inorganic, and organic. It is widely distributed in food at very low levels, mainly as the divalent inorganic (Hg^{2+}) form and organic methylmercury (CH_3Hg). Elemental Hg and inorganic Hg compounds are not classified as carcinogenic to humans (Group 3), while methylmercury compounds are classified as possibly carcinogenic (Group 2B) (IARC 2016), with TWI of 1.3 μg/kg bw/week (EFSA 2015).

Exposure to toxic elements and their harmful effects on human health has been the subject of intensive scientific research worldwide. However, data on the occurrence of toxic elements in canned food available on the Serbian market are minimal (Milenkovic et al. 2019; Novakov et al. 2017; Popovic et al. 2018; Škrbić et al. 2013), unlike numerous studies published in other countries. Also, the largest number of papers published worldwide relates to toxic elements in canned fish (Ashraf et al. 2006; Emami Khansari et al. 2005; Hosseini et al. 2013, 2015a; Hosseini et al. 2015b; Kim et al. 2020; Lourenço et al. 2004; Norhazirah et al. 2020; Okyere et al. 2015; Pappalardo et al. 2017; Rodriguez-Mendivil et al. 2019; Russo et al. 2013; Shiber 2011; Sobhanardakani 2017; Sobhanardakani et al. 2018), while a relatively small number include canned meat testing (Ainerua et al. 2020; Buculei et al. 2014; Khalafalla et al. 2016; Korfali and Hamdan 2013; Kowalska et al. 2020; Massadeh et al. 2018). Maximum levels of toxic elements in food are determined by the EU regulation (2006) and the regulation of the Republic of Serbia (2019).

The urgent need to study the toxic elements in canned food from the Serbian market and periodically monitor and assess such products' health safety has initiated this research. Five different products (beef goulash [BG], pork ragout [PR], spam [SP], liver pate [LP], and meatballs in tomato sauce [MB]), which consumers of the Serbian Armed Forces regularly use, were analyzed for the content of four toxic elements (As, Cd, Hg, and Pb). Monitoring the quality of canned meat is very important since it is a strategic food. The influence of the length of the storage period on the metal content was examined. The obtained concentrations were compared with the maximum levels of toxic elements in food currently in force. The influence of raw materials, spices, and additives on the final metal content in BG and MB samples was also examined. Finally, since it is well known that dietary habits significantly affect the exposure of the population to toxic elements, the potential health risk of consumers associated with the intake of As, Cd, Hg, and Pb through the examined canned meat was assessed.

Experimental

Samples

Empty tinplate cans Empty two-piece and three-piece cans were made of electrolytic tinplate and produced in the can factory in Serbia. Two-piece and three-piece cans, cylindrical in shape, with the following dimensions, were used in the production of canned meat products: (1) two-piece cans, Ø 73 × 29.5, for liver pate of 100 g; (2) three-piece cans, Ø 73/70 × 43, for spam of 150 g; and 3) three-piece cans, Ø 99/96 × 63, for beef goulash, pork ragout, and meatballs in tomato sauce of 400 g. The tinplate quality corresponded to the Standards (European standard 2003, 2016) with additional requirements related to the thickness of the sheet, the tin's application, and the application and quality of the varnish. The tin application was at least 5.6 g/m^2 on the inner and outer surfaces (E–5.6/5.6), which is twice the value compared to cans of the same type for civilian use, where the tin application is usually 2.8 g/m$^{/2}$. A layer of varnish was applied to the tin layer. All inner surfaces of the cans are lacquered with epoxy-phenolic aluminum pigmented varnish with a minimum of 6 g/m^2, while all outer surfaces are lacquered with transparent gold varnish with a minimum of 5 g/m^2.

Canned meat products Five types of canned meat products were used for testing: BG, PR, SP, LP, and MB, produced according to military requirements, in industrial plants of six different producers who had a contract with the Serbian Army in the year of production. After filling and sealing, the cans were thermally treated at the sterilization temperature by heating to 120 °C/30 min (LP); 118 °C/50 min (SP); 120°/70 min (BG and PR); or 118 °C/105 min (MB). The examined cans were undamaged, stored for up to 6 years in typical military facilities that provide appropriate conditions (temperature up to max 25 °C and relative humidity up to max 75%). Descriptive analysis of the packaging condition, i.e., the most significant deviations in the properties of cans, which were observed during the storage period, was performed before the analysis.

Measurements

ICP-MS The analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS), using iCapQ mass spectrometer (Thermo Scientific, Bremen, Germany). All the samples were analyzed in duplicate, and metal content was presented as an average. The cans' contents were homogenized, from which about 0.5 g was taken for microwave digestion, and 5 ml of HNO_3 and 1.5 ml of H_2O_2 were added. The digested samples were filtered through nylon filters into polypropylene volumetric flasks, diluted to 100 ml with deionized water, and used for As, Cd, Pb, and Hg determination. Simultaneously with the samples, an internal standard was introduced into the ICP-MS device. Before each reading of the element concentration, the system's parameters are automatically adjusted with the basic calibration solution. The differences between duplicates were ≤ 5.8%. Instrumental limits of detection (LODs) and quantification LOQs were calculated as the concentration of the element that produced a signal three (LOD) and ten (LOQ) times higher than those of the averaged blanks. Analytical method parameters are shown in Table 1.

By analyzing certified reference materials NIST 1577c (bovine liver from the National Institute of Standards and Technology, USA) and ERM-CD281 (rye grass from the Sigma-Aldrich) in each series of analyzed samples, quality control was performed. Solvents and spiked samples were included in each batch of digestion and analysis. Average recoveries from spiked meat samples were 96.5%, 98.8%, 96.6%, and 91.1% for As, Pb, Cd, and Hg, respectively. Average recoveries from spiked dry onion were 96.5%, 97.6%, 98.1%, and 94.3% for As, Pb, Cd, and Hg, respectively. The most abundant isotopes were used for quantification. The concentrations were within the range of the certified values for all isotopes. As no information was given regarding Hg content in the reference material NIST 1577c, analytical recoveries of 93.5–106.0% were determined using spiked samples (Hg = 10 mg/kg; n = 10).

Statistics

In order to apply any statistical treatment, it is necessary to determine the distribution of data in the appropriate set. The Shapiro-Wilk test was used to verify the normality of the data distribution. According to the Shapiro-Wilk test, data sets that were found not to be subject to normal distribution were not further treated by statistical methods due to various influences that could have led to deviations. For data below the LOQ but

Table 1 Limits of detection (LODs) and quantification (LOQs), assigned and measured concentrations of the reference materials (n = 10)

Element	LOD, µg/kg	LOQ, µg/kg	Method repeatability (precision), %	Certified value,# µg/kg	Analyzed value, µg/kg	Recovery, %	Certified value,# µg/kg	Analyzed value, µg/kg	Recovery, %
				NIST 1577c (bovine liver)			ERM-CD281 (rye grass)		
As	1.2	4.0	3.57	19.3 ± 1.4	20.5 ± 1.1	106.2	42 ± 10	39 ± 10	92.9
Cd	0.4	1.0	8.99	97.0 ± 1.4	97.9 ± 2.6	100.9	120 ± 7	113 ± 8	94.2
Pb	2.0	4.0	3.65	62.8 ± 1.0	63.3 ± 2.6	100.8	1670 ± 110	1620 ± 130	97.0
Hg	0.3	1.0	6.90	-	-	-	16.4 ± 2.2	15.0 ± 2.0	91.5

The data are presented as means ± standard deviation

Certified values, given by the producer

above the LOD, a value between those two limits was expressed as LOQ/2. Data sets that are normally distributed are presented in the form MV ± SD (mean ± standard deviation) with minimum and maximum value in a given group. The mean concentrations of toxic elements were compared with Serbian permissible levels using a one-sample *t*-test. Probabilities less than 0.01 were considered statistically significant ($p < 0.01$). All analyses were performed using the IBM SPSS Statistics 19 software package.

Results and discussion

Canned meat products analyzed in this work (BG, PR, SP, LP, and MB) were produced for the needs of the Serbian Armed Forces, according to military standards, in industrial facilities of the various manufacturers. Since representing strategic food, their quality is very important, starting from the cans to the final products. The content of As, Cd, Hg, and Pb was tested depending on the length of storage — from 1 month to 6 years. The level of the same elements was checked in raw materials, spices, and additives. The impact of toxic elements ingested from canned food on consumer health was investigated by comparing their weekly intake with reference values.

External appearance of cans during storage

Cans of the same quality were used for the production of meat products. Meat products were produced according to the same recipe by different producers and, after production, were stored under the same, regular conditions. The changes on the outer surface of the cans observed during the 6 years of storage are shown in Table 2. The changes on the LP and MB cans were noticed in the third year of storage, i.e., during the defined shelf life of 4 years. On BG cans, marbling and corrosion were determined in the first half of the fourth year of storage, while corrosion of PR cans was observed after 5 years. It is interesting to note that no changes were observed on BG, PR, and LP cans stored for 6 years, indicating that factors other than storage length could cause corrosion.

Concentration of toxic elements in the content of cans

Toxic elements can enter the human body through canned food, although humans can also be exposed due to polluted water, air, and soil. There is a large amount of literature data on the adverse influences of toxic elements on human health (Azeh Engwa et al. 2019; Fu and Xi 2020; Jaishankar et al. 2014; Järup 2003; Morais et al. 2012; Olmedo et al. 2013; Rehman et al. 2017). Some of the harmful effects include impaired kidney (Pb, Cd, Hg) and liver (Pb and Cd) function, impaired reproductive system (Cd, Pb), decreased cognitive function (Pb, Hg), hypertension (Cd), neurological problems (Hg, Pb), teratogenic (Hg), and carcinogenic effects (Cd).

So far, toxic elements have been analyzed in a minimal number of products from the Serbian market (Milenkovic et al. 2019; Novakov et al. 2017; Popovic et al. 2018; Škrbić et al. 2013). It is also very important to note that none of the published papers presented the results related to canned food produced in Serbia. In this work, the level of As, Cd, Hg, and Pb in five types of canned meat was determined during the storage period of up to 6 years. Although the shelf life of the products stored for more than 4 years has expired, these cans have also been tested to determine the extent of toxic metal concentration changes. Monitoring changes in these elements' concentration was particularly interesting as the food was packaged in cans where tin and varnish application was twice as high as in cans for civilian use in Serbia. It was expected that the migration of metals from such cans into the packaged product would be minimal. The results showed that toxic elements were present in the contents of all types of canned meat but not in significant concentrations in each storage period (Table 3).

Table 2 Changes on cans during storage

Period of storage (year/month = y/m)		Type of meat can				
		BG	PR	SP	LP	MB
0 y /1 m–2 y /6 m	While shelf life	nd	nd	nd	nd	nd
2 y /6 m–3 y /0 m		nd	nd	nd	Corrosion	Corrosion
3 y /0 m–3 y /6 m		Marbling, corrosion	Insignificant damages	Insignificant damages	-	-
3 y /6 m–4 y /0 m		-	nd	-	-	Corrosion
4 y /0 m–4 y /6 m	Expired shelf life	Marbling, corrosion	nd	Corrosion	-	-
4 y /6 m–5 y /0 m		-	-	Corrosion	Corrosion	-
5 y /0 m–5 y /6 m		nd	Corrosion	Corrosion	Corrosion	-
5 y /6 m–6 y /0 m		nd	nd	Insignificant damages	nd	-

nd= changes not detected

In BG cans, the Pb value exceeded LOQ (4 μg/kg) only in the sample that was stored for the most prolonged period (5 y/5 m) and amounted 6 μg/kg, while Cd values were at the LOQ limit or lower (< 1 μg/kg) in all storage periods. Because of that, the Pb and Cd data were not statistically processed, unlike the values of As and Hg. For similar reasons, only the Hg values in PR samples were processed by statistical analysis and Cd and Hg data in SP samples. The data of Cd and Hg were also statistically processed for LP samples. For the MB samples, only the values of Cd were above LOQ (> 1 μg/kg) and therefore statistically processed. Heavy metals were not detected (nd) in about 20% of cans (26/128; Table 3).

To ensure food safety, the European Commission (2006) has established maximum acceptable residual levels for toxic elements, except for As, where no limits have yet been set. Serbian regulation (2019) has set maximum Cd, Pb, and Hg levels in fresh meat, meat products, offal, kidneys, fish, and seafood products, which are in line with EU regulation (Table 4). Serbian law set maximum values for arsenic as well, unlike European legislation. Together with the legal levels, the mean values of As, Cd, and Hg, found in the meat products of this work, are shown in Table 4. The comparison of the mean concentrations of different toxic elements can be seen in Fig. 1. It was found that there were no statistically significant changes in

Table 3 Content of toxic elements in canned meat foods

Sample	Producer	Period of storage (y/m)[a]	Toxic elements, μg/kg[b,c]			
			As	Cd	Hg	Pb
BG	A	0/3	4.00	1.00	nd	nd
		0/11	< LOQ	nd	nd	nd
		2/2	10.00	1.00	< LOQ	< LOQ
		3/2	5.39	< LOQ	6.71	< LOQ
	B	4/0	5.22	1.55	4.58	nd
		5/1	5.13	< LOQ	9.48	< LOQ
		5/9	5.19	< LOQ	8.48	6.00
PR	A	0/3	nd	1.00	< LOQ	nd
		0/6	nd	nd	nd	nd
		2/2	< LOQ	nd	< LOQ	nd
		3/2	< LOQ	< LOQ	6.49	< LOQ
	B	4/0	< LOQ	< LOQ	11.72	< LOQ
		5/1	< LOQ	2.18	15.04	8.00
		5/9	9.33	2.03	8.29	< LOQ
SP	C	0/8	< LOQ	< LOQ	< LOQ	nd
		1/1	5.66	3.50	5.10	< LOQ
		2/1	< LOQ	1.16	2.12	6.00
	B	3/1	4.58	13.28	11.44	5.00
	D	4/4	nd	1.55	nd	< LOQ
	B	5/0	< LOQ	1.00	< LOQ	nd
	E	6/0	4.96	1.49	4.56	< LOQ
LP	C	0/7	< LOQ	6.00	< LOQ	< LOQ
		1/1	5.47	2.48	1.72	nd
		2/6	< LOQ	10.20	7.01	< LOQ
	B	3/0	4.70	35.91	6.63	4.00
	D	4/4	nd	11.13	< LOQ	nd
	B	5/0	nd	8.00	< LOQ	< LOQ
	E	6/0	< LOQ	6.71	3.55	< LOQ
MB	F	0/1	< LOQ	3.00	nd	nd
		1/10	< LOQ	7.00	nd	< LOQ
		2/8	5.00	4.00	< LOQ	nd
		3/9	< LOQ	2.00	< LOQ	< LOQ

[a] y/m = years/months

[b] nd = not detected

[c] LOQ (As) = 4 mg/kg; LOQ (Cd) = 1 mg/kg; LOQ (Hg) = 1 mg/kg; LOQ (Pb) = 4 mg/kg

Table 4 Mean values with standard deviation (MV ± SD) and maximum acceptable residual levels for toxic elements, established by Serbian regulation

Toxic element, μg/kg	MV ± SD, μg/kg					Serbian regulation (2019), μg/kg
	BG	PR	SP	LP	MB	
As	5.28 ± 2.41	-	3.53 ± 1.72	-	-	100 (fresh meat) 300 (meat products) 500 (offal) 3000 (fishery products)
Cd	-	1.24 ± 0.82	3.21 ± 3.10	11.49 ± 11.14	4.00 ± 2.16	50 (fresh meat) 500 (offal) 1000 (kidneys) 1000 (tuna fish) 2500 (sardines)
Hg	5.95 ± 3.57	7.09 ± 5.89	4.04 ± 4.12	2.92 ± 2.88	-	30 (fresh meat) 100 (offal) 1000 (fishery products)
Pb	-	-	-	-	-	100 (fresh meat) 500 (offal) 300 (fish) 500 (crabs) 1500 (mussels)

the concentration of toxic elements concerning the mean values in all samples ($p > 0.01$). The obtained levels of all toxic elements in analyzed canned meat produced for the Serbian Armed Forces were far below both national and EU limits (European Commission 2006; Serbian regulation 2019).

The mean concentrations of the toxic elements (As, Cd, and Hg) were statistically compared with the maximum acceptable residual levels for fresh meat, given in Table 4. The results of the one-sample *t*-test, presented in Table 5, revealed significant differences (*p* below 0.01) between the values of toxic elements found in analyzed meat products and the levels prescribed by Serbian regulation (2019). The higher the absolute value of *t*, the lower the value of *p*, and thus, the evidence

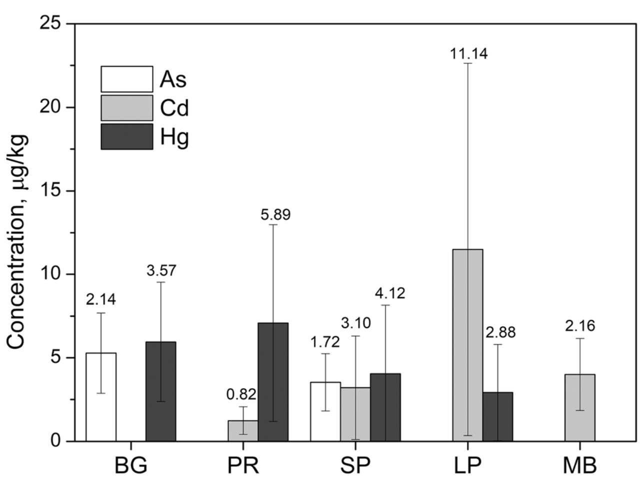

Fig. 1 Comparative levels of As, Cd, and Hg in different meat products. Data are presented as mean ± SD

that the mean value of the sample will be below the allowed limit is more convincing.

A detailed discussion of the concentration of each toxic element in an individual meat product is given below. A comparison of obtained data in this work with other authors' results is presented in Table 6.

Arsenic

It is well known that arsenic enters the human body through water, which is the most important source of exposure, followed by food and air (Brandon et al. 2014; Kim et al. 2015). Our results showed that concentration of As varied significantly in BG samples, from below LOQ (first year of storage) to 10.00 μg/kg (more than 2 years of storage). Arsenic was not detected, or the values were below the LOQ in all PR cans, except in the sample with the most prolonged storage period (5 y/9 m), when it was 9.33 μg/kg. In the SP and LP samples, the highest values were detected at the beginning of the second year of storage — 5.66 and 5.47 μg/kg, respectively. The highest value in MB samples was 5.00 μg/kg in the third year of storage. In a significant number of samples, in all five types of canned meat, As values were not detected or were below the LOQ, as shown in Table 3. Based on the obtained results, no correlation can be established between the storage period and As concentrations. Arsenic levels in the products from this work were significantly lower than the permitted values for meat products (300 μg/kg) and offal (500 μg/kg), prescribed by the Serbian regulation (2019) and given in Table 4. The results of other authors, presented in Table 6, show that canned fish generally contain significantly higher

Table 5 Results of one-sample *t*-test analysis comparing the mean concentration of toxic elements with the maximum acceptable residual levels

Sample	As		Cd		Hg	
	t	*P*	*t*	*P*	*t*	*P*
BG	− 104.18	< 0.00001			− 15.06	< 0.00006
PR					− 9.53	< 0.00011
SP	− 137.77	< 0.00001	− 27.27	< 0.00001	− 15.43	0.00001
LP			− 9.15	0.00005	− 24.85	< 0.00001
MB			− 42.59	0.00001		

levels of As than canned meat, except for the products analyzed by Massadeh et al. (2018), where 2900 (± 1400) μg/kg was found in canned beef. According to Serbian regulation (2019), the value of 2900 μg/kg exceeds almost six times the maximum allowed value. The lowest As levels of 2–3 μg/kg were found in canned meat products analyzed by Kowalska et al. (2020). In the work of Škrbić et al. (2013), the concentrations of arsenic below 30 μg/kg were found in fresh beef

Table 6 Comparison of data obtained in this work with the results of other authors (chronological order)

Reference	Canned food	Area	Toxic elements,[a] μg/kg			
			As	Cd	Hg	Pb
	Canned meat products					
Present study	Beef goulash, pork ragout, spam, liver pate, meatballs in tomato sauce	Serbia	nd–10.00	nd–35.91	nd–15.04	nd–8.00
Korfali and Hamdan (2013)	Luncheon meat	Lebanon	-	25–75	8–30	1–32
Škrbić et al. (2013)	Beef [b]	Serbia	< 30	< 0.3	-	38
Buculei et al. (2014)	Pork meat in own juice	Romania	-	610–800	-	770–1370
Khalafalla et al. (2016)	Canned beef	Egypt		nd–110	nd–1220	nd–830
Massadeh et al. (2018)	Canned beef[c]	Jordan	2900 (± 1400)	510 (± 20)	-	2970 (± 600)
Ainerua et al. (2020)	Canned meat	Nigeria	nd	nd–400	-	nd–400
Kowalska et al. (2020)	Canned meat	Poland	2–3	< LOQ–27.31	0.01–0.07	13–395
	Canned fishery products					
Lourenço et al. (2004)	Canned fish and seafood	Portugal	-	10–1100	nd–1000	nd–300
Emami Khansari et al. (2005)	Canned tuna	Iran	36.9–261.8	4.6–72	43–253	16.2–72.6
Ashraf et al. (2006)	Canned salmon, sardine and tuna fish	Saudi Arabia	-	10–690	-	30–1970
Shiber (2011)	Canned sardines	USA	490–1870	< 10–70	nd–90	60–270
Hosseini et al. (2013)	Canned fish	Iran	-	-	0.3–408	-
Korfali and Hamdan (2013)	Canned fish	Lebanon		21–645	25–395	7–187
Russo et al. (2013)	Canned tuna	Italy	-	< LOQ–65	< LOQ–205	< LOQ–513
Škrbić et al. (2013)	Canned fish	Serbia	430	29	-	11
Hosseini et al. (2015b)	Canned tuna	Iran	-	nd–270	-	20–5500
Okyere et al. (2015)	Canned fish	Ghana		< 10–450	< 10–200	< 10–1440
Pappalardo et al. (2017)	Canned tuna	Italy	-	< 3–60	50–480	< 1–20
Sobhanardakani et al. (2018)	Canned fish	Iran	-	60–150	90–180	190–1590
Novakov et al. (2017)	Canned tuna, sardines	Serbia	170–3730	3–80	7–640	10–280
Popovic et al. (2018)	Canned tuna, sardines, mackerel	Serbia	620–1700	14–26	36–68	42–48
Rodriguez-Mendivil et al. (2019)	Canned tuna	Mexico	-	nd–7	5–1170	70–320
Milenkovic et al. (2019)	Fresh and canned fish, shrimps and mussels	Serbia	-	10–810	10–1470	20–6560

[a] Range (minimal and maximal values)

[b] Fresh meat

[c] Mean values are given

samples. Arsenic compounds are often used as bactericides, herbicides, fungicides, and admixtures to animal feed. Consequently, arsenic can later be found in food, but the debate over its concentration is difficult due to the lack of regulation in the EU.

Cadmium

The cadmium level in food is influenced by factors such as type of food, growing plant conditions, anthropogenic contamination of air, soil, and water systems (Tchounwou et al. 2012). Our work results show that the highest Cd value in BG samples was 1.55 μg/kg after four storage years. In PR samples, Cd concentration was 2.18 μg/kg after 5 years, while in SP samples, the Cd value was 13.28 μg/kg after 3 years of storage. In LP and MB samples, Cd values were above the LOQ level in all storage periods. In the case of LP samples, the values ranged from 2.48 μg/kg, after 1 year, to 35.91 μg/kg, after 3 years of storage. In MB samples, cadmium values ranged from 2.00 μg/kg, after 3 years, up to 7.00 μg/kg, after 1 year of storage. Mean Cd values for all types of cans were significantly below the permitted values for meat (50 μg/kg), liver (500 μg/kg), and kidneys (1000 μg/kg), prescribed by Serbian regulation (2019) (Fig. 1). The highest value was detected in LP samples (35.91 μg/kg), which is not surprising considering that the liver is cadmium's target organ. This finding was in accordance with the work of Akan et al. (2010), where Cd concentrations in the liver and kidneys ranged from 160 to 760 μg/kg. Based on the obtained results, it can be concluded that there was no strong correlation between the detected Cd values and the length of the storage period. However, in more extended periods of storage, higher concentrations were found. The cadmium concentration in the samples from this work was low compared to other authors' findings (Table 6). A review of the results of other studies shows that the values differ significantly in both fish and meat products and range from immeasurable to about 1000 μg/kg. The highest cadmium levels in meat products (610–800 μg/kg) were found in the work of Buculei et al. (2014). The authors noted that Cd concentrations increased with a storage time of 0 to 36 months. It was also found that migration is more intense in cans with polyester varnish than in those with epoxy phenolic. A type of varnish significantly influenced cadmium migration into food ($p < 0.05$). The cans tested in our work were coated with twice as thick epoxy-phenolic varnish as the cans for the Serbian civil market, which was also a significant reason for the low cadmium content due to the prevented migration from the metal base.

Mercury

According to the literature data, the most significant mercury intake is through food, of which about 90% comes from sea fish and fish products. Small part of the intake is through cereals, flour, milk, fruits, and vegetables, while the intake through meat and offal is about 9% (Tchounwou et al. 2012). In our study, in one-half of the samples, Hg was below LOQ or not detected. The highest Hg values in BG and PR samples were after 5 years of storage (9.48 and 15.04 μg/kg, respectively). In SP samples, the highest Hg value was after 3 years of storage (11.44 μg/kg), while in LP samples, the highest value was detected after more than 2 years (7.01 μg/kg). In all MB samples, the values of Hg were below LOQ. The mean values of Hg were significantly lower in all types of canned meat, related to the official maximum values: 30 μg/kg for fresh meat and 100 μg/kg for offal and meat products, prescribed by the Serbian regulation (2019). It can be concluded that there is no strong correlation between the storage period and the obtained mercury values, although in more extended storage periods, increased Hg concentration in almost all meat products can be observed. Samples of meat products analyzed in the reviewed literature, in many cases, were not tested for the presence of mercury (Table 6). In the work of Korfali and Hamdan (2013), mercury values for canned meat were similar to those in our study (8–30 μg/kg), while in Kowalska et al. (2020), concentrations were significantly lower (0.01–0.07 μg/kg). By contrast, high maximum values up to 1220 μg/kg were found in the work of Khalafalla et al. (2016), which is atypical, given that fish and seafood are the primary source of mercury and dietary intake of Hg through other food is relatively low. The concentration of mercury was significantly higher in samples of canned fish and seafood when it ranged up to 1470 μg/kg in the work of Milenkovic et al. (2019). This value was determined in the sample of predator fish — shark. It was above the maximum allowed level of mercury (1000 μg/kg; Table 4) in fish and other seafood according to the valid Serbian regulation (2019). This result is not surprising because fish have a high ability to bioaccumulate environmental Hg in their tissues and keep it elevated. In the works of other authors from Serbia, lower mercury values were found — below 640 μg/kg in Novakov et al. (2017) and even below 68 μg/kg in Popovic et al. (2018).

Lead

The primary sources of lead to which the general population is exposed are beer, cereals, flour, potatoes, fruits, and vegetables (Tchounwou et al. 2012). Pb values in all BG cans were below LOQ or not detected in our work except for the sample with the most prolonged storage period of 6 years (6.00 μg/kg). In all PR samples, Pb values were similarly below LOQ, except for the sample stored for more than 5 years (8.00 μg/kg). The Pb values were below LOQ for the SP samples stored for less than 1 year, while for the samples stored for more than 2 years, the Pb level was above 5 μg/kg. For the samples stored for more than 4 years, the level of Pb again dropped below LOQ. Pb values in all LP samples were below LOQ or not detected, except for the sample stored for 3 years (4.00 μg/kg). In MB cans, the value of Pb in all samples was below LOQ. A significant number of samples with nd values or values below LOQ indicated that the storage period's length did not affect Pb concentration. Detected Pb values are significantly lower than the maximum values of 100 μg/kg for fresh meat and 500 μg/kg for offal, prescribed by the Serbian regulation (2019). Lead values in the samples from our work were among the lower ones obtained for canned meat (Table 6). The highest Pb values of 2970 (± 600) μg/kg for the canned meat were obtained in the work of Massadeh et al. (2018). Popovic et al. (2018) found that the values of lead in canned fish from the Serbian market ranged from 42 to 48 μg/kg, which was far below the legal limits for Pb. In contrast to these results, in the work of Novakov et al. (2017), the maximum value of lead in canned sardines (280 μg/kg) was slightly below the maximum allowed value for fish according to Serbian regulation (300 μg/kg; Table 4). Finally, a level of lead more than 20-fold higher (6560 μg/kg) than the allowable one was detected in a blue sea fish on the Serbian market, in the work of Milenkovic et al. (2019).

Concentration of toxic elements in raw materials, spices, and additives

Although the storage period did not significantly affect the toxic elements' values, higher concentrations were usually found in samples stored for more than 2 years. The highest value of As was in the BG sample stored for more than 2 years (10.00 μg/kg). The highest Cd value was detected in the LP sample stored for 3 years (35.91 μg/kg), while in the PR sample stored for 5 years, the highest concentrations of mercury and lead were found (15.04 and 8.00 μg/kg, respectively). Based on these results, it can conclude that the migration of toxic elements from metal packaging into the food was most likely influenced by the quality and continuity of protective coatings (tin and varnish) on the inner surfaces of cans, which decreased during storage. However, the toxic elements' concentration was also affected by the natural pollution of raw materials, spices, additives, and secondary pollution associated with the production process. Similar observations were made by other authors (Buculei et al. 2014; Khalafalla et al. 2016), who, in addition to the storage period, listed many other factors that affect the toxic elements content in canned food. Khalafalla et al. (2016) examined the presence of Pb, Cd, Hg, and Sn in canned chicken and beef products, with an emphasis on sources of contamination.

Therefore, the content of toxic elements in raw materials, spices, and additives for the production of BG and MB (samples stored 3 and 1 month, respectively) was examined in order to roughly assess their contribution to the concentration of As, Cd, Hg, and Pb in the tested food. In the case of BG cans, beef and beef tallow (raw materials), dry onion and red ground pepper (spices), and kitchen salt (additive) were tested. Levels of all toxic elements in raw materials and additives were below the LOQ, while Cd and Pb were detected in spices in more significant concentrations. A very high Cd concentration in red ground pepper (163 μg/kg) was particularly noteworthy (Table 7).

In the case of MB cans, beef and pork meat and tomato sauce (starting raw materials), dry onion, red ground pepper, flour, sugar, dish supplement, food additive, and kitchen salt were tested. Results are presented in Table 8. Concentrations of all toxic elements were below LOQ in beef and pork, while Pb and Cd were detected in tomato sauce (20.0 and 10.0 μg/kg, respectively). Higher concentrations of As, Cd, and Pb were found in spices and additives, with especially

Table 7 Toxic elements in raw materials, spices, and additives for the production of beef goulash

Raw materials, spices and additives	Toxic elements, μg/kg[a]			
	As	Cd	Hg	Pb
Beef meat	< LOQ	< LOQ	< LOQ	< LOQ
Beef tallow	< LOQ	< LOQ	< LOQ	< LOQ
Dry onion	< LOQ	19.0	< LOQ	18.0
Red ground pepper	< LOQ	163.0	< LOQ	24.0
Kitchen salt	< LOQ	< LOQ	< LOQ	< LOQ

[a] LOQ (As) = 4 mg/kg; LOQ (Cd) = 1 mg/kg; LOQ (Hg) = 1 mg/kg; LOQ (Pb) = 4 mg/kg

Table 8 Values of toxic elements in raw materials, spices, and additives for the production of meatballs in tomato sauce

Raw materials, spices and additives	Toxic elements, μg/kg[a]			
	As	Cd	Hg	Pb
Beef meat	< LOQ	< LOQ	< LOQ	< LOQ
Pork meat	< LOQ	< LOQ	< LOQ	< LOQ
Tomato sauce	< LOQ	10.0	< LOQ	20.0
Dry onion	40.0	20.0	< LOQ	20.0
Red ground pepper	40.0	150.0	< LOQ	20.0
Minced onion	10.0	< LOQ	< LOQ	20.0
Minced pepper	< LOQ	< LOQ	4.0	30.0
Dish supplement	10.0	40.0	< LOQ	250.0
Additive	10.0	70.0	< LOQ	< LOQ
Flour	10.0	10.0	< LOQ	50.0
Sugar	< LOQ	< LOQ	< LOQ	20.0
Kitchen salt	< LOQ	< LOQ	< LOQ	< LOQ

[a] LOQ (As) = 4 mg/kg; LOQ (Cd) = 1 mg/kg; LOQ (Hg) = 1 mg/kg; LOQ (Pb) = 4 mg/kg

Table 9 Intake of toxic elements calculated from the mean concentration data of this work and combined with dietary information and the average body weight of 1000 individual consumers

Type of food	Mean value, µg/kg	Monthly consumption, kg		Intake of toxic elements through canned meat products			
				MI[a] µg/kg bw/month		WI[b] µg/kg bw/week	
		Regular	Emergency	Regular	Emergency	Regular	Emergency
As							
BG	4.99	0.160	0.320	0.0113	0.0226	0.0028	0.0056
PR	2.00	0.400	0.800	0.0113	0.0226	0.0028	0.0056
SP	2.00	0.450	0.900	0.0127	0.0254	0.0032	0.0064
LP	2.00	0.300	0.600	0.0085	0.0170	0.0021	0.0042
MB	2.00	0.400	0.800	0.0113	0.0226	0.0028	0.0056
Total				0.0551	0.1102	0.0139	0.0274
PTWI[c]						15.0 µg/kg bw/week	
$BMDL_{05}$						21.0 µg/kg bw/week	
% PTWI or % $BMDL_{05}$						0.09 0.07	0.18 0.13
Pb							
BG	2.00	0.160	0.320	0.0045	0.0090	0.0011	0.0022
PR	2.00	0.400	0.800	0.0113	0.0226	0.0028	0.0056
SP	2.00	0.450	0.900	0.0127	0.0254	0.0032	0.0064
LP	2.00	0.300	0.600	0.0085	0.0170	0.0021	0.0042
MB	2.00	0.400	0.800	0.0113	0.0226	0.0028	0.0056
Total				0.0483	0.0966	0.0120	0.0240
PTWI[c]						25.0 µg/kg bw/week	
$BMDL_{10}$						4.4 µg/kg bw/week	
% PTWI or % $BMDL_{10}$						0.05 0.27	0.10 0.54
Cd							
BG	0.50	0.160	0.320	0.0011	0.0022	0.0003	0.0006
PR	0.50	0.400	0.800	0.0028	0.0056	0.0007	0.0014
SP	3.21	0.450	0.900	0.0205	0.0410	0.0051	0.0102
LP	11.49	0.300	0.600	0.0488	0.0976	0.0122	0.0244
MB	4.00	0.400	0.800	0.0227	0.0454	0.0057	0.0114
Total				0.0959	0.1918	0.0240	0.0480
TWI[d]						2.5 µg/kg bw/week	
% TWI						0.10	0.20
Hg							
BG	4.39	0.160	0.320	0.0099	0.0198	0.0025	0.0050
PR	6.15	0.400	0.800	0,0348	0.0696	0.0087	0.0174
SP	3.53	0.450	0.900	0.0225	0.0450	0.0056	0.0112
LP	2.23	0.300	0.600	0.0095	0.0190	0.0024	0.0048
MB	0.50	0.400	0.800	0.0028	0.0056	0.0007	0.0014
Total				0.0795	0.1590	0.0199	0.0398
TWI[d]						1.3 µg/kg bw/week	
% TWI						1.53	3.06

[a] MI, monthly intake

[b] WI, weekly intake

[c] Former PTWI values withdrawn. Not possible to establish a new PTWI that would be considered safe for health (JECFA 2014)

[d] Established by EFSA CONTAM Panel (2011; 2015)

significant Pb values in dish supplement (250 μg/kg) and Cd in red ground pepper and additive (150 and 70 μg/kg, respectively).

Based on the determined concentrations, the raw material composition, especially spices and additives, can potentially affect the toxic elements' concentration in the finished product. However, the cadmium and lead concentrations in BG and MB samples were far below the values allowed by Serbian regulation (2019). It can be concluded that even a very high level of these toxic elements in spices and additives does not significantly affect their concentration in the final product. The reason for this is the tiny amount of spices and additives that are added to dishes. Nevertheless, spices and additives' quality and safety must be constantly monitored to prevent harmful consumer health effects.

Finally, meat cans were produced in six industrial facilities, which had a concluded contract with the Serbian Armed Forces in a particular year. The producers are marked with letters A, B, C, D, E, and F (Table 3). The particular product was produced by the same recipe in all facilities. However, the quality of the entire production process in a particular facility can also affect the final quality of the food and the level of toxic metals.

Influence of toxic elements from canned food on the health of consumers

Considering the health risks derived from toxic Cd, Pb, Hg, and As, regular monitoring and updates of heavy elements' intakes are required. The mean concentration data were combined with dietary information and the average body weight (bw) of 1000 individual consumers to estimate exposure to toxic elements through consumption of canned meat products from this work. The soldiers' average body weight was 70.6 kg, with minimal and maximal 50.0 and 94.0 kg values, respectively. According to the planned diet, in regular conditions, soldiers consume 160 g of GG, 400 g of PR, 450 g of SP, 300 g of LP, and 400 g of MB, monthly. In emergency conditions, the amount of food consumed doubles.

Based on canned food consumption values, soldiers' exposure to toxic elements was calculated and expressed as weekly intake per kg body weight. The results are shown in Table 8, together with provisional tolerable weekly intakes of toxic elements (PTWI), established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2014), and tolerable weekly intakes (TWI), established by EFSA (2011, 2015). Since EFSA and other food safety authorities no longer recommend the use of previously established PTWIs for As and Pb, the BMDL (benchmark dose lower confidence limit) values were used instead: BMDL05 of 21.0 μg/kg bw/week for lung cancer in human (As) and BMDL10 of 4.4 μg/kg bw/week for nephrotoxic effects (Pb) (EFSA 2012). The contribution to the TWI (%TWI) or BMDL (%BMDL) was calculated as the ratio of weekly intake (WI) of a specific metal through canned food and its TWI or BDML value. Weekly intake (μg/kg bw/week) was calculated in the following way:

$$WI\ [\mu g/kg\ bw/week] = \sum \frac{TE_{conc}(\mu g/kg) \times MC\ (kg/month)}{bw\ (kg) \times 4}$$

where TE_{conc} is the mean concentration of a specific element (μg/kg), MC is the monthly consumption of a particular canned food (kg), and bw is the mean body weight of a soldier (70.6 kg).

It can be concluded from Table 9 that the intake of toxic elements through canned meat is significantly lower than the established reference values. The highest contribution to the total body load with toxic elements is by the Hg — 1.53% of TDI during regular consumption and 3.06% in emergencies. Obviously, the contribution of canned meat to the total body load with toxic elements is almost insignificant. However, it is essential to note that soldiers can also ingest toxic elements by consuming other food and through other sources. Therefore, their daily intake is undoubtedly significantly higher. Since toxic elements cause many diseases, it is necessary to continually monitor canned food quality as a possible source of toxic elements.

Conclusions

No significant relationship was observed between the storage period and the concentration of toxic elements in the canned meat. Nevertheless, the level of individual elements increased in some samples during longer storage times. The highest level of arsenic was found in BG (10.00 μg/kg), cadmium in LP (35.91 μg/kg), and mercury and lead in PR (15.04 and 8.00 μg/kg, respectively). The concentrations of toxic elements in examined canned meat products and starting raw materials did not exceed the maximum permitted levels currently in force by Serbian and EU legislation and were significantly lower than in canned fish from the Serbian market and worldwide. However, the values of Cd and Pb were somewhat increased in some spices and additives. The contribution to the total body load with toxic elements from canned meat can be considered almost insignificant. Thus, canned meat products' consumption represents a negligible health risk to the Serbian Armed Forces members. However, the constant estimation of food products' safety is necessary, as the intake of toxic elements through the diet is of great public concern. It must be monitored regularly and updated rapidly to identify possible health risks in different countries. Currently, the level of As, Cd, Hg, and Pb in canned food does not pose a health risk, and there is no reason to change the consumers' eating habits.

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