Determination of toxic elements in meat products from Serbia packaged in tinfoil 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, Pb, and Hg), in five types of canned meat products, which are 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 (temperature up to max 25 °C and relative humidity up to max 75%), were analyzed in this research. Meat products were made according to the special military requirements, packed in tinfoil cans, and stored for up to 6 years. The highest average contents of toxic elements were found to be 10.00 µg/kg for arsenic in BG, 35.91 µg/kg for cadmium in LP, 15.04 µg/kg for mercury in PR, and 8.00 µg/kg for lead in PR. The storage period did not significantly affect the level of toxic elements, although higher concentrations were found in samples stored for more than two years. The influence of raw materials, spices, and additives on the level of toxic elements in some meat products was also examined. None of the samples contained toxic elements at levels exceeding the currently maximum permitted levels. The consumption of this type of food represents a small risk to human health because the exposure of soldiers to toxic elements, calculated as weekly intake, is far below legal PTWI/TWI limits, established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA).

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 four years.

Electrolytic tinfoil is used for the production of metal packaging (cans) for the Serbian Armed Forces. The tinfoil 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/m² 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, tinfoil packaging is most often used for the canned meat products. An organic epoxy-phenolic coating (varnish) is applied to protect the tinfoil 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/m², while on external surfaces is 5 g/m² (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 weekly 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 (BDML₁₀) 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 (Hg²⁺) form and organic methylmercury (CH₃Hg). Elemental Hg and inorganic Hg compounds are not classified as carcinogenic to humans (Group 3) while methylmercury compounds 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. The largest number of papers published worldwide relate to toxic elements in canned fish (Ashraf et al. 2006; Emami Khansari et al. 2005; Hosseini et al. 2015; Kim et al. 2020; Lourenço et al. 2004; Norhazirah et al. 2020; Okyere et al. 2015; Pappalardo et al. 2015; Rodriguez-Mendivil et al. 2019; Russo et al. 2013; Shiber 2011; Sobhanardakani et al. 2018), while a relatively small number include canned meat testing (Ainerua et al. 2020; Buculei et al. 2014; European Commission 2006; Khalafalla et al. 2016; Korfali and Hamdan 2013;

Kowalska et al. 2020; Massadeh et al. 2018; Serbian Regulation 2019; Tuzen and Soylak 2007).

Numerous studies show that eating habits greatly influence the exposure of the population to toxic elements, precisely through increased consumption of canned food. Maximum levels of As, Cd, Pb, and Hg in food are determined by the EU Regulation (2006) and the Regulation of the Republic of Serbia (2019).

The main goal of this research was to examine the influence of the length of the storage period on the content of four toxic elements (As, Cd, Pb, Hg) in canned meat, produced according to Serbian military standards. Five different products (BG, PR, SP, LP, and MB), stored in regular conditions, were used for the research. Concentrations of toxic elements were statistically processed by determining measures of variation, levels of significance, and correlation in relation to the length of the storage period. Further, the aim was to determine which meat product contains the highest concentration of a particular element and to determine whether the concentrations of toxic elements exceed the maximum levels currently in force. The influence of raw materials, spices, and additives on the content of toxic elements in BG and MB was also examined. Finally, the potential risk to human health associated with the intake of toxic As, Cd, Pb, and Hg that arises from canned meat consumption has been 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/m² 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/m². 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/m², while all outer surfaces are lacquered with transparent gold varnish with a minimum of 5 g/m².

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/ 0 min (SP); 120°/90 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 HNO₃ and 1.5 ml of H₂O₂ 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 LODs and 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.

Table 1. Limits of detection (LOD) and quantification (LOQ), assigned and measured concentrations of the BCR-185R reference material (n=10)

Element	LOD, µg/kg	LOQ, µg/kg	Method repeatability/precision, %	Certified value,# µg/kg	Analyzed value, µg/kg	Recovery, %
As	1.2	4.0	3.57	19.3 ± 1.4	20.5 ± 1,1	106.2
Cd	0.4	1.0	8.99	97.0 ± 1.4	97.9 ± 2,6	100.9
Pb	2.0	4.0	3.65	62.8 ± 1.0	63.3 ± 2,6	100.8
Hg	0.3	1.0	6.90	-	-	-

The data are presented as means ± standard deviation.

#Certified values, given by the producer.

By analyzing certified reference material NIST 1577c (bovine liver) in each series of analyzed samples, quality control was performed. Solvents and spiked samples were included in each batch of digestion and analysis. 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, 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 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 $MV \pm SD$ (mean $\pm$ standard deviation) with minimum and maximum value in a given group. Statistical significance (p) was determined using the Student's t-test or ANOVA analysis, depending on the set's structure. All analyzes were performed using the *IBM SPSS Statistics 19* software package.

Results And Discussion

RESULTS AND DISCUSSION

The canned meat products used in this work (beef goulash-BG, pork ragout-PR, spam-SP, liver pate-LP, and the meatballs in tomato sauce-MB) were produced for the needs of the Serbian Armed Forces, according to the military standards, in industrial facilities of various manufacturers. Considering that they represent strategic foods, their quality is greatly important, starting from the cans to the finished product. On the other hand, it is crucial to monitor the migration of toxic elements into the contents of cans that have been produced according to special requirements and stored for a long time. The influence of the length of the storage period on the migration of toxic heavy elements - As, Cd, Pb, and Hg, from the tinplate packaging to the can's contents was investigated. In addition, the level of the same elements was checked in some raw materials, spices, and additives, to assess their contribution to the final content of As, Cd, Hg, and Pb in the canned products.

External appearance of cans during storage

Cans of the same quality are provided for the production of meat products. According to the same recipe, a specific meat product was produced with different producers and stored under the same regular conditions. Changes on the outer surface of the cans during six years of storage are shown in Table 2. Changes on the LP and MB cans had already been 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 noticed after five years. It is interesting to note that no changes were observed on the cans of BG, PR, and LP stored for six years, indicating that factors other than the length of storage could cause corrosion.

Table 2. Changes on cans during storage

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

nd= changes not detected

Concentration of toxic elements in the content of cans

One of the ways how toxic elements can enter the human body is through canned food, although people can also be exposed to them through contaminated 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 renal (Pb, Cd, Hg) and liver (Pb and Cd) function, decreased cognitive function (Pb, Hg), weakened reproductive system (Cd, Pb), hypertension (Cd), neurological problems (Hg, Pb), teratogenic (Hg), and cancerogenic effects (Cd).

In this work, the values of As, Cd, Hg, and Pb were determined in the content of five types of canned meat products during the storage period from 15 days to 6 years. Although the shelf life of canned meat products stored for more than four years has expired, these cans have also been taken for testing to determine the extent of changes in toxic metals concentration. The results show that toxic elements were present in the contents of all types of canned meat products but not in significant concentrations in each storage period (Table 3).

Table 3. Content of toxic elements in canned meat foods

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

	D	4/4	nd	11.13	< 1	nd
	B	5/0	nd	8.00	< 1	< 4
	E	6/0	< 4	6.71	3.55	< 4
MB		0/1	< 4	3.00	nd	nd
	F	1/10	< 4	7.00	nd	< 4
		2/8	5.00	4.00	< 1	nd
		3/9	< 4	2.00	< 1	< 4

a) y/m=years/months

b) nd=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 (5y/5m) 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). The mean values of As, Cd, and Hg, with standard deviations (SD) in different meat products, are shown in Fig 1. It was noticed that there were no statistically significant changes concerning the mean concentrations of toxic elements at the significance level of 99% ($p > 0.01$).

Data are presented as mean ± SD.

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, and kidneys, which are in line with EU Regulation (Table 4). Serbian Regulation gives maximum values for As as well (Table 4), unlike European legislation. The obtained levels of all toxic elements in analyzed canned meat products produced for the Serbian Armed Forces were below both legal national and EU limits (European Commission Regulation 2006; Serbian Regulation 2019).

Table 4. Maximum acceptable residual levels for toxic elements, established by Serbian Regulation

Toxic element	Serbian Regulation (2019), µg/kg
As	100 (fresh meat) 100 (meat products) 500 (offal)
Cd	50 (fresh meat) 500 (offal) 1000 (kidneys)
Hg	30 (fresh meat) 100 (offal)
Pb	100 (fresh meat) 500 (offal)

Arsenic

It is well known that arsenic enters the human body through water, which is the most important source of exposure, followed by air and food (Brandon et al. 2014; Kim et al. 2015). Our results showed that As varied significantly in BG samples, from below LOQ (first year of storage) to 10.00 µg/kg (more than two years of storage). The values of As were not detected or were below LOQ in all PR cans, except in the sample with the most prolonged storage period (less than six years), when it was 9.33 µg/kg. In SP and LP samples, the highest values were detected at the beginning of the second year of storage - 5.66 µg/kg and 5.47 µg/kg. 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, the values of As were not detected or were below LOQ, as shown in Table 3. Based on the obtained results, no correlation can be established between the length of the storage period and the concentration of As. The values of As found in canned meat products were significantly lower than the permitted values for meat products (100 µg/kg) and offal (500 µg/kg), prescribed by the Serbian Regulation (2019).

Cadmium

The cadmium level in food is influenced by factors such as type of food, growing plant conditions, anthropogenic contamination of air, soil, water systems, etc (Tchounwou et al. 2012). The results of our study show that the highest Cd value in BG samples was 1.55 µg/kg, after four years of storage; in PR samples, it was 2.18 µg/kg, after five years, while in SP samples concentration of As was 13.28 µg/kg, after three 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 one year of storage, to 35.91 µg/kg, after three years of storage. In MB samples, the As value ranged from 2.00 µg/kg, after three years,

up to 7.00 µg/kg, after one year. 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 of Cd was detected in LP samples, which is not surprising considering that the liver is the target organ of cadmium. This was also confirmed in the work of Akan et al. (2010), where Cd concentrations in the liver and kidneys ranged from 70 to 760 µg/kg. Based on the obtained results, it can be concluded that there is no strong correlation between the detected Cd value and the length of the storage period, although, in longer periods of storage, higher concentrations are detected.

Mercury

According to the literature data, the largest intake of mercury in the human body is through food, of which about 90% comes from sea fish and fish products; 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 five years of storage (9.48 µg/kg and 15.04 µg/kg, respectively). In SP samples, the highest Hg value was after three years of storage (11.44 µg/kg), while in LP samples, the highest value was detected after more than two 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 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 can be observed increased Hg concentration in almost all meat products.

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 five years (8.00 µg/kg). The Pb values were below LOQ for the SP samples stored for less than one year, while for the samples stored for more than two years, the Pb level was above 5 µg/kg. For the samples stored for more than four 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 three 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 length of the storage period did not affect Pb concentration. Detected Pb values are significantly lower than the maximum values prescribed by the Serbian Regulation (2019): 100 µg/kg for fresh meat and 500 µg/kg for offal.

Concentration of toxic elements in raw materials, spices and additives

Although the storage period does not significantly affect the toxic elements' values, it can be observed that higher concentrations are usually found in samples stored for more than two years. The highest As value was found in BG stored more than two years (10.00 µg/kg), the highest Cd value in LP stored three years (35.91 µg/kg), while the highest Hg and Pb values were found in PR stored five years (15.04 and 8.00 µg/kg, respectively). The migration of toxic elements from metal packaging into the contents of cans is most likely influenced by the quality and continuity of protective coatings (tin and varnish) on cans' inner surfaces, which decrease during storage. However, the concentration of toxic elements is also affected by the natural pollution of raw materials, spices, additives, and secondary pollution related to the production process. Similar observations are also given by other authors (Buculei et al. 2014; Khalafalla et al. 2016), who, in addition to the storage period, list many other factors that affect the content of toxic elements in canned food. These facts are also confirmed by Khalafalla et al., who examined the presence of Pb, Cd, Hg, and Sn in canned products (chicken, beef, frankfurter, salted beef), with an emphasis on sources of contamination and limits (Khalafalla et al. 2016). 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), as well as kitchen salt (additive) were tested. Concentrations of all toxic elements in raw materials and additives were below the LOQ, while Cd and Pb were detected in spices. A very high Cd concentration in red ground pepper (163 µg/kg) was particularly noteworthy (Table 5).

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

Table 5. Toxic elements in raw materials, spices, and additives for the production of beef goulash

Raw materials, spices and additives	Toxic elements, µg/kg			
	As	Cd	Hg	Pb
Beef meat	< 4	< 1	< 1	< 4
Beef tallow	< 4	< 1	< 1	< 4
Dry onion	< 4	19.0	< 1	18.0
Red ground pepper	< 4	163.0	< 1	24.0
Kitchen salt	< 4	< 1	< 1	< 4

Based on the determined concentration values, it can be concluded that the raw material composition, and especially spices and additives, potentially can affect the concentration of toxic elements in the finished product. However, the concentrations of cadmium and lead in BG and MB 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 a tiny amount of spices and additives that are added to dishes. Nevertheless, the quality and safety of spices and additives in terms of toxic elements must be constantly monitored to prevent harmful effects on consumer health.

Table 6. 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			
	As	Cd	Hg	Pb
Beef meat	< 4	< 1	< 1	< 4
Pork meat	< 4	< 1	< 1	< 4
Tomato sauce	< 4	10.0	< 1	20.0
Dry onion	40.0	20.0	< 1	20.0
Red ground pepper	40.0	150.0	< 1	20.0
Minced onion	10.0	< 1	< 1	20.0
Minced pepper	< 4	< 1	4.0	30.0
Dish supplement	10.0	40.0	< 1	250.0
Additive	10.0	70.0	< 1	< 4
Flour	10.0	10.0	< 1	50.0
Sugar	< 4	< 1	< 1	20.0
Kitchen salt	< 4	< 1	< 1	< 4

Finally, meat cans were produced in six industrial facilities, which had a concluded contract with the Serbian Armed Forces in a certain year. The producers are marked with letters A, B, C, D, E, and F (Table 3). The meat cans of the same type were produced by the same recipes 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 dietary intakes of heavy elements are required. In order to estimate dietary exposures of the soldiers of Serbian Armed Forces to Cd, Pb, Hg, and As, through consumption of the canned meat food examined in this work, the mean concentration data were combined with dietary information and the average body weight of 1000 individual consumers. 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 grams of LP, and 400 g of MB, monthly. In emergency conditions, the amount of food consumed doubles.

Based on canned food consumption values, the exposure of soldiers to toxic elements was calculated and expressed as weekly intake per kg body weight. The results are shown in Table 7, together with provisional tolerable weakly 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 examined in this work and TWI or BDML for the particular toxic element. Weekly intake of a metal (µg/kg bw/week) was calculated in the following way:

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

Where TE_{conc} is the mean concentration of particular toxic element (µg/kg); MC is monthly consumption of specific canned food (kg); and bw is the mean soldgers body waight value (70.6 kg).

Table 7. 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, $\mu\text{g/kg}$	Monthly consumption, kg		Intake of toxic elements through canned meat products			
				MI ^{a)}		WI ^{b)}	
				$\mu\text{g (kg bw)}^{-1} \text{ month}^{-1}$		$\mu\text{g/kg bw/week}$	
		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 $\mu\text{g/kg bw/week}$	
BMDL ₀₅						21.0 $\mu\text{g/kg bw/week}$	
% PTWI or % BMDL ₀₅						0.09	0.18
						0.07	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 $\mu\text{g/kg bw/week}$	
BMDL ₁₀						4.4 $\mu\text{g/kg bw/week}$	
% PTWI or % BMDL ₁₀						0.05	0.10
						0.27	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 ^{e)}						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 ^{e)}						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)

e)Established by EFSA CONTAM Panel (2011, 2015)

It can be concluded from Table 7 that the intake of toxic elements through canned meat is significantly lower than the established values of TWI/BMDL. The highest %TDI value is only 1.53 for Hg, while in emergencies, it amounts and 3.06. However, it is essential to note that soldiers can also ingest toxic elements by consuming other types of food and other sources. Therefore, the daily intake of toxic elements is undoubtedly significantly higher. Since toxic elements cause many diseases, it is necessary and crucial 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 tested samples of canned meat. Moreover, there were no statistically significant changes concerning the mean concentrations at the significance level of 99% ($p > 0.01$). However, the level of individual elements increased in some samples, especially during longer storage times, such as: arsenic in BG samples (up to 10.00 $\mu\text{g/kg}$); cadmium in SP and LP samples (up to 35.91 $\mu\text{g/kg}$), and mercury in PR and SP samples (up to 15.04 $\mu\text{g/kg}$), indicating possible migration of toxic metals from tinplate packaging. The concentration of toxic elements did not exceed the maximum permitted levels currently in force by Serbian and EU legislation. The level of all toxic elements was below current limits in raw materials, as well. However, certain toxic elements were somewhat increased in some spices and additives, such as Cd and Pb. It can be concluded that the consumption of canned meat products represents a small health risk to the Serbian Armed Forces consumers because the weekly intake of toxic elements is far below legal PTWI/TWI limits. However, constant estimation of contaminants intake through food is necessary for risk evaluation. Such exposure evaluations are useful when deciding on the regulation of chemical contaminants and food products' safety. The intake of As, Cd, Hg, and Pb through the diet is of great public concern. It must be monitored continuously and rapidly updated to identify possible health risks of toxic elements in different countries. Based on the results obtained in this work, it can be concluded that the level of toxic elements in canned food that soldiers regularly have on the menu does not currently pose a risk to their health and that there is no reason to change their eating habits.

Declarations

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Consent for publication - Not applicable

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Authors' contributions

BS - Investigation

SJ – Formal analysis

VĐ – Methodology

SM – Validation

DV – Conceptualization

ZS – Resources

MB – Visualization

VA - Project administration, Supervision, Writing – original draft

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Authors' information (optional)

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Figures

Figure 1

The mean values of As, Cd and Hg in different meat products. Data are presented as mean $\pm$ SD.