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ORIGINAL ARTICLE

Detection and distribution of residues and additives in meat and meat products in Zenica-Doboj canton, Bosnia and Herzegovina

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Abstract

Objective: Many additives are of essential importance for the taste and quality of products, however, many of them pose a danger to human health, and are subject to daily routine quality control of products intended for human consumption. In addition to additives, residues represent a major challenge as possible contaminants of products from the field to the dining table.

Methods: Through the research, 85 samples of meat products (fresh meat, salami, sausages, dried meat products) were processed and all were analyzed for the presence of aflatoxin B1 (AFL B1), as well as for chlorides, nitrites and heavy metals (cadmium and lead).

Results: The average content of AFL B1 in the meat samples was 0.048 µg/kg (0.019 to 0.105 µg/kg) which is significantly lower than the recommended value in some European countries (1 µg/kg). Chloride content was detected in the range from 0.000 to 9.955 %m/m (average concentration 2.377 %m/m). The average nitrite content was 8.330 mg/kg (min. 0.550 – max. 45.705 mg/kg), and maximum permitted levels in meat products is 150 mg/kg. Among the 85 processed samples, lead and cadmium were detected in 79 and 29 products (92.94% and 34.11%), with an average of 0.136 and 0.042 mg/kg, respectively. Lead was detected in the range from 0.000 to 3.474 mg/kg, and cadmium from 0.000 to 2.544 mg/kg. Maximum permitted level for lead si 0.1 mg/kg, and for cadmium is 0.05 mg/kg, so this results are very concerning

Conclusion: The results show the need for continuous monitoring of the amount of additives in meet products on the market, and regular monitoring of residues in products intended for human consumption. It is necessary to stick to proper hygienic practices during the preparation of the product at all stages from the field to the dining table.

Keywords: Aflatoxin B1, Nitrite, Chloride, Lead, Cadmium, Meat Products, Health Riscs

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INTRODUCTION

Since time immemorial, it has been recognized that food serves as the cornerstone of human health. Dietary habits and human nutritional requirements have evolved in tandem with technological and industrial advancements throughout history. The journey from field to table was considerably shorter, as food was prepared and consumed at home within a shorter time frame. However, this trajectory has substantially elongated, amplifying the number of risks that impact the safety of our food.[1]

One such risk is the potential contamination by various types of toxins, including mycotoxins, heavy metals, pesticides, etc. Such contamination can occur during the production, storage, and distribution of food products, with the source of these toxins emanating from the environment, raw materials, or arising during the food processing itself.[2] Another risk associated with the extension of the field-to-table journey is the use of food additives. In some instances, their use remains unregulated and may have adverse implications for human health.

Mycotoxins are secondary metabolites synthesized by molds during their growth on substrates of both plant and animal origin. According to Food and Agriculture Organization (FAO) statistics, mycotoxin-producing fungal species damage one-fourth (25%) of all agricultural products worldwide. [3] The occurrence of mycotoxins is contingent upon factors such as the specific mold species, climatic conditions, as well as various physicochemical elements including temperature, moisture content in food, gas concentrations in the atmosphere, and others. Among the aflatoxins identified, AFL B1 is the most widely distributed and most harmful substance.[4] This mycotoxin is of significant concern in the field of food safety and public health. AFL B1 is classified by the International Agency of Research on Cancer (IARC) as a Group 1 carcinogen, with high risks for hepatocellular carcinoma (HCC) in individuals exposed to aflatoxins.[5]

Heavy metals are defined as metals with a density greater than 5 g/cm^3. They gradually accumulate in the food chain and have adverse effects on human health. Their toxicity depends on several factors, including dosage, exposure route, chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals.[6] Due to their high degree of toxicity, cadmium, lead, arsenic, chromium, and mercury are classified as priority metals of public health concern. Several acute and chronic toxic effects of heavy metals affect different body organs. Gastrointestinal and kidney dysfunction, nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects, and cancer are examples of the complications of heavy metals toxic effects. Simultaneous exposure to two or more metals may have cumulative effects .[7] Lead affects the function and structure of the kidneys, bones, the central nervous system, and results in harmful biochemical, histopathological, neuropsychological, fetotoxic, and reproductive effects. Heavy metal cadmium is a Group 1 carcinogen and currently ranked 7th on the 2022 Agency for Toxic Substances and Disease Registry (ATSDR) and EPA list of hazardous substances.[8] Recent epidemiological data indicate that Cd exposure may be associated with prostate cancer, bladder cancer, pancreatic cancer, and kidney cancer[9]

Additives are substances or mixtures of substances added to meat and other food products during production, processing, packaging, storage, or transportation to enhance their qualitative characteristics, including appearance, taste, smell, color, texture, and shelf life.[10] Additives are categorized into various groups, including preservatives, antioxidants and synergists of antioxidants, flavor enhancers, emulsifiers, thickeners, binding agents, gelling agents, colorants, sweeteners, acid regulators, enzyme preparations, and other additives.[11] It's important to distinguish additives from spices, such as common table salt (NaCl) and herbs, which are not considered additives. In the meat industry, nitrites have found widespread application as additives, while salt is utilized as a seasoning or an ingredient in brine mixtures for curing. Nitritesin meat and meat products belong to the group of preservatives. They improve the quality, shelf life, and safety of products, primarily by inhibiting the growth and reproduction of bacteria like *Staphylococcus aureus* and *Clostridium botulinum.* Nitrites also influence the color, smell, taste, and texture of meat products. Despite their beneficial characteristics as preservatives, their use has a detrimental impact on human health, and efforts are made to minimize their usage.[12] Additionally, kitchen salt, aside from enhancing meat flavor, inhibits the growth and reproduction of bacteria, reduces water activity in meat, and affects water-binding capacity.[13]

The aim of the research is to examine the quality of meat in terms of mycotoxins, nitrites, chlorides, lead, and cadmium, and compare it with existing regulatory limits. Since there are no regulatory limits for mycotoxins or salts, the objective of the study is also to emphasize the importance of establishing regulatory limits for all parameters, thereby reducing the potential for manipulation by producers. In Bosnia and Herzegovina, producers are not legally required to undergo mandatory testing of meat and meat products for these parameters. Inspection officials at all levels of administration conduct official product controls; however, their numbers are limited and often do not include the mentioned parameters.

METHODS

The research was conducted at the Chemical department in Institute for Health and Food Safety in Zenica, under the auspices of the Ministry of Agriculture, Water Management, and Forestry of the Zenica-Doboj Canton. The study encompassed 85 different samples of meat and meat products available in the largest retail chain in Bosnia and Herzegovina, aiming to assess the market status accessible to every consumer. The study included products from a large number of manufacturers, both domestic and imported. After collecting the products, they were sorted in the laboratory, homogenized, and prepared for analysis. Nitrite analysis was conducted first due to methodological requirements, and then followed by the other analyses. All samples were stored in plastic packaging, frozen, and kept until the end of the testing.

Aflatoxin B1

Determination of aflatoxin B1 was performed using the immunoenzymatic method on an ELISA device (LABTRONE LMPR-A20) in accordance with the manufacturer's specification. The homogenized meat sample (2±0.05 g) was put into a 50 ml centrifuge tube, then added 8 ml of ethyl acetate, oscilated for 5 min, centrifuged at room temperature (4000xg, 10 min). After that 2 ml supernatant was taken, dried with water bath at 50° C. The residual was dissolved with 2 ml of N-hexane, oscilated for 1 min, added 1 ml of Sample Diluent A, and centrifuged at room temperature (4000xg, 5 min). After that 50µl of the lower liquid was taken, and the content of AFL B1 was determined by ELISA kit (Art. No. E-TO-E017, Elabscience Biotechnology).

Lead and Cadmium

The determination of lead and cadmium was carried out using a standard method.[14] The homogenized meat sample (0.5 g) was put into a 70 ml vessel, then added 1 ml of deionized water, 8 ml of nitric acid (not less than 65 % mass fraction) and 1 ml of hydrochloric acid. The vesseles were sealed and samles were digested as prescribed by manufacturer (Anton Paar Multiwave ECO). The graphite technique method was used for determination, and examples of wavelength, gas mixture/temperature programmes and other instrumental parameters appropriate for each metal are found in manuals provided with the instrument (GBC SavantAA Z enduro).

Nitrite

The nitrite content determined according to the procedure described in International standard[15] and expressed as milligrams of sodium nitrite per kilogram. The method's principle is extraction of test portion with hot water, precipitation of the proteins and filtration, in the presence of nitrite development of a red colour by addition of sulphanilamide and N-1- naphthyl ethylenediamine dihydrochloride to the filtrate and photometric measurement at wavelength of 538 nm. For the photometric measurement was used Shimadzu spectrofotometer UV-2600.

Chloride

Determination of chloride content was according to standard procedure based on Volhard method.[16] The method's principle is extraction of test portion with hot water and precipitation of the proteins, after filtration and acidification, addition of an exces of silver nitrate solution to the extract, and titration of this excess with potassium thiocyanate solutin.

RESULTS

The average content of AFL B1 in the samples was 0.048 µg/kg (with a range of 0.019 to 0.105 µg/kg). Results of AFL B1 residue levels ranged from 0.019 to 0.105 with an average value of 0.051 µg/kg for beef products; 0.019 to 0.090 with an average value of 0.047 µg/kg for chicken products; 0.032 to 0.105 with an average value of 0.048 µg/kg for Türkiye products; and 0.034 to 0.042 with an average value of 0.038 µg/kg for pork product (Table 1).

Table 1. Concentration of AFL B1 in different kinds of meat product

Meat products	Mean value	Highest value	Lowest value	Maximum permitted levels
	Aflatoxin B1 (µg/kg)			
Beef products	0.051	0.105	0.019	*
Chicken products	0.047	0.090	0.019	*
Türkiye products	0.048	0.105	0.032	*
Pork products	0.038	0.042	0.034	*

* The Regulation does not define the content of aflatoxin B1 in meat products

The legislative framework in the EU establishes maximum levels (MLs) for mycotoxins in various types of food through Commission Regulation No. 1881/2006 and additionally through Commission Regulation No. 165/2010, specifically for AFB1. However, these regulations do not prescribe MLs for these mycotoxins in meat and meat products. Nonetheless, some EU countries such as Italy and Denmark have set MLs of 1 μg/kg for this group of products (meat, meat products, and offal) under their national legislation. In Bosnia and Herzegovina, national legislation in this area has not been established, but producers should be aware of the potential for contamination and should implement systematic mycotoxin control measures.

Among the 85 processed samples, lead was detected in 79 and cadmium was detected in 29 products (92.94% and 34.11%), with an average of 0.136 and 0.042 mg/kg. Lead was detected in the range from 0.000 to 3.474 mg/kg, and cadmium from 0.000 to 2.544 mg/kg. According to all results, the lead content exceeded the maximum permitted limit (MPL) for meat (0.1 mg/kg) in 24% of samples,while the cadmium content exceeded the MPL for meat (0.05 mg/kg) in 6% of samples. Concentracions of lead and cadmium in different kinds of meat products are shown in Table 2.

Table 2. Concentracions of lead and cadmium in different kinds of meat products

Meat products	Mean value	Highest value	Lowest value	Maximum permitted level
		Lead (mg/kg)		
Beef products	0.234	3.474	0.000	0.10
Chicken products	0.089	1.416	0.000	0.10
Türkiye products	0.023	0.117	0.000	0.10
Pork products	0.068	0.088	0.049	0.10
		Cadmium (mg/kg)		
Beef products	0.09585	2.544	0.000	0.050
Chicken products	0.00705	0.091	0.000	0.050
Türkiye products	0.00756	0.037	0.000	0.050
Pork products	0.00000	0.000	0.000	0.050

The average nitrite content was 8.330 mg/kg (min. 0.550 – max. 45.705 mg/kg). Table 3. shows average concentracion of nitrites in different meat categories, depending on their thermal proccesing. The average nitrite concentrations were 11.396 mg/kg in smoked meat, 10.721 mg/kg in sausages, 6.145 mg/kg in bacon, 4.712 mg/kg in dried meat, and 8.861 mg/kg in fresh meat.

According to the Regulation on Food Additives (Official Gazette of BiH, No. 33/18), the maximum permitted level of nitrites in the tested types of meat products is set at 150 mg/kg, and therefore, all samples are below the prescribed limit. However, this Regulation does not regulate the content of nitrites in fresh meat. The Regulation on Minced Meat, Semi-Processed, and Meat Products (Official Gazette of BiH, No. 82/13) defines fresh meat as all edible parts of the animal that have not undergone any preservation process except chilling or freezing. Based on this definition, it can be concluded that none of the tested samples of fresh meat meet the specified criteria.

Table 3. Concentracion of nitrites in different meat categories

Meat categories	Mean value	Highest value	Lowest value	Maximum permitted level
			Nitrite (mg/kg)	
Smoked meat	11.396	23.290	1.320	150
Sausages	10.721	45.705	0.985	150
Bacon	6.145	11.184	2.413	150
Dried meat	4.712	12.110	1.180	150
Fresh meat	8.861	24.205	1.085	0

Chloride content was detected in the range from 0.000 to 9.955%m/m (average concentration 2.377%m/m). The average chloride concentrations were 1.85% in smoked meat, 2.04% in sausages, 9.96% in bacon, 3.92% in dried meat, and 1.04% in fresh meat. Bacon have the highest concentracion of chlorides (Table 4).

Table 4. Concentracion of chloride in different meat categories

Meat categories	Mean value	Highest value	Lowest value	Maximum permitted level
		Chloride (%)		
Smoked meat	1.854	2.975	1.330	*
Sausages	2.038	3.970	1.220	*
Bacon	9.955	13.654	4.781	*
Dried meat	3.917	5.950	3.090	*
Fresh meat	1.043	2.260	0.000	*

* The Regulation does not define the content of chloride in meats

The Regulation on Minced Meat, Semi-Processed, and Meat Products (Official Gazette of BiH, No. 82/13) does not define the chloride or salt content in any category. Through the discussion, we will reflect on the recommended values from previous studies in the region.

DISCUSSION

The highest concentration of AFL B1 is present in beef products (0.105 µg/kg) , while the lowest concentracion is in pork products (0.034 µg/kg), with very little difference between them.

Similar research was obtained by Algahtani from Egypt. Their results of Aflatoxin B1 residue levels ranged from ND to 13 µg/kg,[17] and are much higher than results in our study, which may be due to the use of meat additives previously contaminated with aflatoxins.

Although meat products in this study have lower concentationcs of Aflatoxin B1, long-term consumption may lead to public health hazard. The reason for this is the accumulation of mycotoxins in the organism and their resistance. Considering that a lifetime intake of 28 mg of AFB1 can lead to cancer, even extremely low concentrations (1 ppb) pose a significant health risk to the public.

To ensure hygienic conditions during processing, preparation, and handling, a concentrated effort is essential. This can be achieved by implementing hygienic procedures during the slaughtering of animals and meat preparation, selecting appropriate species and additives, properly packaging and cooling raw meat products, applying correct heat treatment to heat-treated products, and training meat factory workers effectively. Given that animal feed is one of the main sources of mycotoxins, more frequent inspections of their contamination by mycotoxins should be conducted. The results for heavy metals show a large variability of lead and cadmium concentrations in some of the meat and meat products groups. However, this variability in biological samples is considered to be normal since the sources of this metal are numerous. The main sources of heavy metal contamination are growing and are represented, especially, by pesticides, fertilizers, industrial processes and exhaust gases from automobiles, and they are deposited as residues in food, during processing.

Regarding the analysed meat product samples, both the lead and cadmium concentrations measured in this study were well above the values found in simmilar report from Spain.[18] Their average concetrations for lead and cadmium were: around 3 μg/kg and 4 μg/kg for ckicken products, 7 μg/kg and 5 μg/kg for beef products, 9 μg/kg and 6 μg/kg for Türkiye products, and 5 and 7 for pork products. Regarding the analysed pork meat product samples, lead concentrations measured in this study were similar with the values found in Romania, 58-96 μg/kg.[19]The results of heavy metal testing are alarming considering their impact on human health.

High lead concentrations can have adverse effects on humans. In adults, elevated lead levels can cause heart diseases, cancer, and infertility. In children, lead exposure can result in antisocial behavior, low intelligence, and hyperactivity.

Chronic exposure to Cadmium could cause nephrotoxicity in humans, mainly due to abnormalities of tubular re-absorption. The biological half life of Cadmium in the human kidney is long and has been estimated to be10 to 30 years. According to data from the World Health Organization, the permissible limit of cadmium intake for humans is 1 μg/kg/day, or 7 μg/kg/week.

According to reports from the Food Safety Agency of Bosnia and Herzegovina, the status regarding the contamination of meat and meat products by heavy metals has remained stable over the period from 2016 to 2023. Approximately 400 samples have been tested annually, with only about 0.5% of samples exceeding the maximum allowable limits. This suggests that a larger number of samples provides a more accurate representation of the situation and underscores the importance of continuing this monitoring practice with an even greater number of samples. Regarding the testing of nitrites, smoked meat samples have the highest average concentracion of nitrites (11.396 mg/kg), however, a surprisingly high concentration of nitrites was found in fresh meat (8.861 mg/kg). The higher concentration of nitrites in fresh meat can be explained by their effect on the characteristic color of the meat, inhibition of bacterial growth, and preservation of specific aroma. A similar study in Türkiye showed concentrations for nitrites in sausages around 103 mg/kg, and that is much higher than our study resullts.[20] In the fresh meat, Gozdecka from Poland measured nitrite content ranging from 1 to 5 mg/kg.[21] Simmilar repor from UK showed that the mean nitrite content in bacon was 24.0 mg/kg.[22] Thus, differences are more likely to be due to the manufacturing processes and different meat products.

To avoid indiscriminate use of nitrite and nitrate in meat or meat products, the scope for their addition is rigorously restricted in most countries, including Bosnia and Herzegovina. However data from the literature, as well as the results of this study, indicate that nitrites have been added to raw meat materials. As ingredient or hazardous compound levels are not monitored by regulation in raw meat materials in some countries, and considering that nitrite overuse in processed meat products has occurred the application of nitrite in raw meat materials may be a supervisory blind spot that increases the risk of overuse. Therefore, it is vital to strengthen the sampling regime for meat and meat products, especially raw meat where it is not currently included. In support of this, reports from the Food Safety Agency of Bosnia and Herzegovina covering the period from 2016 to 2023 indicate that only 317 analyses of meat and meat products for additives were conducted over eight years. This number is notably low, especially considering the significant risks associated with nitrites, including the development of various types of cancers, diabetes, neurological disorders, and overall poisoning of the body.

Nitrites and chlorides are often used together in meat preparation or processing procedures, hence it has been beneficial to monitor the concentration of both.

Average concentracion of chloride was 2.377% in the range from 0-9.955. Simmilar reports from Denmark[23] showed the salt content in crushed meat products such as sausage and cooked hams of 2.19 % and 2.28 %, respectively and from Netherland[24] average salt content of 1.93 to 2.66%. All of these results are quite similar, likely due to the use of identical or comparable technological production processes.

The quantity of salt in meat products can be said to be defined through organoleptic properties, particularly taste. It is prescribed for meat products to have a distinctive taste. A meat product containing too little salt may be bland or insufficiently salty, while if it contains too much salt, it can be overly salty, extremely salty, or even bitter. In such cases, the product lacks its characteristic taste, which is a quality flaw. Based on experimental data and a review of literature, it can be observed that the quantity and method of adding table salt are specific to each group of meat products.

However, they cannot be justified in terms of the harmful effects of salt on raising blood pressure in humans and its impact on the kidneys. Excessive salt consumption is also not justified because there are technological processing methods to avoid this. The World Health Organization recommends a daily intake of sodium chloride less than 5 g for adults but this amount is exceeded in some European countries by more than twice.[25]

CONCLUSION

The results of a study conducted in the Zenica-Doboj Canton on the contamination of meat and meat products with mycotoxins, heavy metals, and additives have revealed several important findings. The content of aflatoxin B1 in all tested samples was quite low, with an average value of 0.048 μg/kg, which is significantly lower than the recommended value in some European countries (1 μg/kg). However, due to the very limited number of such studies in our country, continuous monitoring of aflatoxin B1 contamination in both meat and animal feed is necessary. It is also crucial to establish maximum allowable limits for aflatoxin B1 in meat and meat products to prevent potential manipulation by producers.

The results also indicated significant contamination of samples with heavy metals, cadmium and lead, which can have dangerous effects on human health. In this regard, it is essential to continue similar studies and to develop a long-term action plan, considering that heavy metals accumulate in the body and their effects may become evident over the next decade or more.

Regarding the testing of nitrites in meat products, the results were significantly below the prescribed maximum limits (150 mg/kg). However, a notable amount of nitrites was also found in raw meat (8.86 mg/kg), which is a concerning finding since raw meat should not contain any additives. Accordingly, more extensive testing of raw meat for nitrite content is necessary.

Testing for chlorides in meat and meat products yielded results similar to those obtained in numerous other studies conducted in different countries.

In the course of meat processing, it is paramount to take into account the prevailing conditions and potential sources of contamination. Vigilant and frequent monitoring of meat and meat products is indispensable to ensure their safety and quality. Animal feed, constituting a pivotal link within the food chain, necessitates meticulous scrutiny to prevent contamination. Regulatory frameworks, which oversee the quality of meat and the presence of contaminants, ought to define precise limit values for all pertinent parameters.

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Author Contrubition: Conceived the analysis: DžH, ĆK, AI; Collected the data: DžH, ĆK, AI; Performed the analysis: DžH, ĆK, AI; Wrote manuscript: DžH, ĆK, AI.

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