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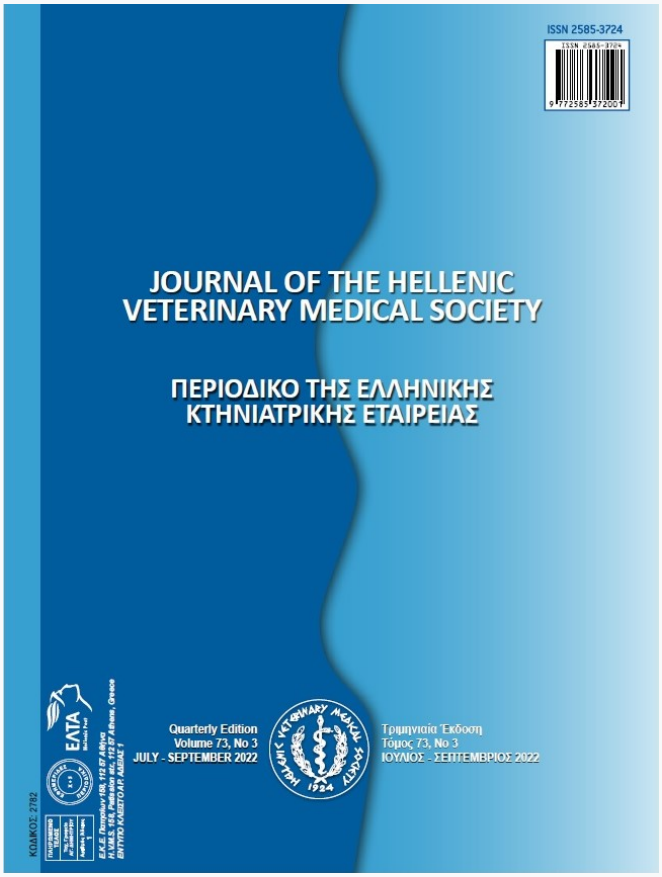

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Research article
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Evaluation of the residual nitrite concentrations of locally produced and imported meat products in Kosovo

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ABSTRACT: The use of food additive nitrite as curing agent is common in meat products, but its concentration in these products has raised the interest of researchers, because of the possible toxicity to humans. The aim of this study is to assess the nitrite concentration in meat products, which is highly used by all population groups in Kosovo. Forty-four samples of meat products purchased in Kosovo markets were analyzed for residual nitrites using the spectrophotometric method that uses absorption in visible part of spectra. The amount of residual nitrites was detected in 19 (43%) of the samples, which included beef and chicken sausages, chicken & beef salami as well as beef prosciutto samples. The nitrite residue ranged between 0.1 and 11.5 mg/kg and was below the limits on the concentration of nitrites in meat products established by EU regulation 1333/2008. Although these findings show that, the nitrite residue in the analyzed meat products is within the permitted limits, the highest presence of residual nitrite in industrial and low-cost meat products indicates a need for further assessments of consumers' exposure to nitrites.

Keywords: Residual nitrite; UV/VIS spectrophotometry; meat products; Kosovo; imported products

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INTRODUCTION

Nitrates and nitrites are commonly added as additives to meat during curing meat process with the aim of enhancing shelf life, colour and flavor.According to Commission Regulation (EU) No. 1129/2011 (Commission regulation, 1985), nitrates (sodium nitrate, E251; potassium nitrate, E252) and nitrites (potassium nitrite, E249; sodium nitrite, E250) are listed as official permitted food additives.

The EU Regulation No. 1169/2011, determines that all products with nitrite added must list this on the ingredient list, explicitly as either sodium/potassium nitrite or stating the E number (E250/E249).Meat processors use nitrates and nitrite salts for several reasons. They typically use these additives to prevent possible growth of dangerous anaerobic bacteria *Clostridium botulinum*(Mortensen et al., 2017), which produces botulinum toxin responsible for botulism that can lead to serious respiratory problems and death. Nitrates and nitrites are also added to influences the colour of meat and to give flavor. Nitric oxide (NO) deriving from nitrite combines with myoglobin to form NO myoglobin contributing an increasing pink colour to the meat, which is a desirable trait for consumers. (Shiva et al., 2007), (Farouk, 2011). Nitrates and nitrites delay rancidity of meat products and have also antioxidant characteristics (Park, 2008), (Gassara et al., 2016).

Nitrates are relatively non-toxic, but nitrites, which are the highly active curing compounds and nitrites metabolic compounds such as nitric oxide and N-nitroso compounds (NOCs), have raised concern over potential adverse health effects because some of NOCs are known to be carcinogenic (IARC, 2010), (Gassara et al., 2016).

The European Parliament through Council Directive has restricted the addition of sodium nitrite in processed meat to 150 mg/kg, and the residual amount must be below 100 mg/kg (EFSA, 2003). On the other hand, considering carcinogenicity and the development of methaemoglobin, the joint Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO) expert committee on food additives (JECFA) has agreed an acceptable daily intake (ADI) of nitrite to be 0.07 mg/ kg bodyweight(Mortensen et al., 2017).

According to the Scientific Committee for Food (SCF) (SCF (Scientific Committee on Food), 1997), the ADI mentioned is applicable to all sources of dietary exposure. Apart from meat and meat products, there are also high levels of nitrites in other foodstuffs than cured meat products (Larsson et al., 2011), (Iammarino et al., 2013).

Countries usually set legal limits for nitrates and nitrites in cured meat products. European Union set limits for nitrates and nitrites for meat products by regulating either the ingoing or the residual amounts of these salts in the products [Commission Regulation (EU) No. 601/2014](Paralament, 2014). The limits established in this regulation are presented in Table 1.

Table 1. Nitrates and nitrites legal limits reported in Regulation (EC) No. 601/2014

MEAT PRODUCTS	Legal Limit (mg/l or mg/kg as appropriate)		Limit application
	NITRATES[1]	NITRITES[2]	
Non-heat-treated processed meat	150	150	Maximum amount that may be added during manufacturing
Heat-treated processed meat Except sterilised meat products ($F_{\circ}$>3.00)[3]	…	150	Maximum amount that may be added during manufacturing
Only sterilised meat products ($F_{\circ}$>3.00)[3]	…	100	Maximum amount that may be added during manufacturing
Traditionally cured meat products with specific provisions concerning nitrites and nitrates	10-300	50-180	Depends on product: Maximum added amount or maximum residual amount, residue level at the end the production process

[1] E251 (sodium nitrate), E252 (potassium nitrite)
[2] E249 (potassium nitrite), E250 (sodium nitrite)
When labelled "for food use", nitrites may be sold only in a mixture with salt or a salt substitute
[3] $F_{\circ}$ - value 3 is equivalent to 3 minutes heating at 121 ˚ C for *C. botulinum*

Based on the evidence on the carcinogenicity of red and processed meats to humans, the International Agency for Research on Cancer (IARC), the cancer agency of the World Health Organization, has classified consumption of processed meat as probably carcinogenic to humans (World Health Organization, 2015).

The long debate about carcinogenic nitrosamines has contributed in a serious pressure to decrease the use of nitrite in meat curing (Sindelar & Milkowski, 2012). An example is Denmark that maintains maximum amount of 60 mg/kg instead of 150 mg/kg specified in the EU legislation, considering that preservative effect can be achieved at the lower maximum level of nitrites(Herrmann, 2014).

Since consumer concern is growing over the potential negative health effects of consumed foods with nitrates -nitrites additives, many assessments take place for the estimation of nitrates and nitrite levels in meat products. The aim of this study was to measure the residual nitrite concentration found in different locally produced and imported meat products such as beef sausage "sujuk"; chicken sausages;beef prosciutto and beef and chicken salami.These meat products are sold in the big supermarkets of Kosovo and are highly consumed by people of all aged groups including children. According to our best knowledge, there was no study in Kosovo determining the residual nitrite concentration of processed meat products sold in Kosovo's supermarkets.

MATERIALS AND METHODS

Samples

A total of 44 samples were purchased in the three large supermarkets chains in Kosovo (Viva Fresh; ETC and Interex). The collected samples were from meat products produced in Kosovo but also outside Kosovo, which were sold under twenty different trade names. Some of these different manufacturers produce several types of meat products. Commercially available meat product samples included local and imported beef homemade and industrial sausages called sujuk (*n=25*), local and imported chicken sausages (*n=8*), local and imported beef prosciutto (*n=5*) and local and imported beef and chicken salami (*n=6)*. After purchasing samples were stored in refrigeration at 4 ˚C. Samples were shipped in hand refrigerator to the laboratory of the Faculty of Agriculture and Food Sciences in Sarajevo, Bosnia and Herzegovina.

Determination of Residual Nitrite Levels

The Cary 1E UV/Visible Spectrophotometer was used in the laboratory to analyze compounds in the ultraviolet (UV) and visible (VIS) regions of the electromagnetic spectrum. The method uses absorption in visible part of spectra.

Reagents

Acetic acid (CH3COOH, Puriss, p.a., ACS Reagent, Reag. ISO, Reag. Ph. Eur., ≥ 99.8% Glacial, Sigma-Aldrich Merck, Germany) solution: 15%/400ml.

N-(1-naphthyl)-ethylenediamine - NED (C12H14N2 •2HCl, Puriss, p.a., ACS Reagent, > 98%, Powder or Crystals, Sigma-Aldrich Merck, Germany): 0.2 g of NED was dissolved in 150 mL of acetic acid solution 15%.

Sulfanilamide ($C_6H_8N_2O_2S$, Reag. Ph Eur, ≥ 98%, Powder or Crystals, Merck, Germany solution: 0.5 g of sulfanilamide was dissolved in 150 mL of acetic acid solution 15%.

Sodium nitrite (NaNO2, ACS Reagent, ≥ 97.0%, Powder or Crystals, Sigma-Aldrich Merck, Germany) standard solution: 1 g of nitrite as sodium salt was dissolved in 1000 ml of distilled water (1000 ppm solution). 100 mL from 1000 ppm solution is further diluted in1000 ml (100 ppm solution). 10 ml from 100 ppm solution is diluted in 1000 ml in order to get 1 ppm solution.

Preparation of standard solutions

Four 50 mL volumetric flasks were used for preparation of standard solutions. 10, 20, 30 and 40 mL of 1ppm $NaNO_2$ solution was added in volumetric flasks. In each of these 2.5 ml of sulfanilamide solution was added, under stirring. After 5 min, 5 ml of NED were also added in each flask and after 15 min. followed the measurements for standardization of UV-VIS spectrophotometer. The suggested wavelength in literature was 540 nm, but we measured the maximum of absorption on instrument that we used, and it was 531-60 to 541.99 nm. Since our maximum of absorption was in correspondence with literature, we used 540 nm.

Preparation of samples for analyses

Sample portion was at first homogenized. 10 g of homogeneous samples were used to weight 2.5 g sample, which was put in a container. 40 mL of distilled water was added and heated at the temperature of 80

˚C, under stirring. After heating, the solution is gradually diluted (under stirring) with distilled water in e container of 250 mL and filtered. 10 mL was taken from the filtrate and place in the 50 mL container. For color development and measurement of absorbance, 2.5 ml of sulphanilamide solution was added to the volumetric flask, and after 5 minutes of waiting, 2 mL of NED solution was added. After 15 minutes, the solution was ready for spectrophotometric measurement. Results are calculated based on calibration curve which shows the content of nitrites in 2.5 g of the sample. Concentration is presented in µg/ml.

RESULTS

The amount of residual nitrites was detected in 19 (43%) of 44 samples, which included beef and chicken sausages, chicken & beef salami as well as beef prosciutto samples. The residual nitrite values ranged between 0.1 and 11.5 mg/kg. A more detailed overview of the results of nitrite concentration in different meat products is presented below according to the types of products analyzed.

The residual nitrite concentrations in beef sausage called "sujuk"

The analytical results obtained for residual nitrite concentration in beef sausage called "sujuk" are reported in Table 2.

Table 2. The amount of residual nitrite in beef sausage called "sujuk"

Sample type	All Samples (n)	Residual nitrite *mg/kg*	
		Positive samples (*n*)	Mean ± SD (min-max)
All samples	25	7	5.1±2.7 (1.8-8.8)
Local	17	2	3.3±2.2 (1.8-4.9)
Industrial	6	5	5.9±2.7 (1.9-8.8)

The residual nitrite presence was detected in seven(28 %) of the 25 analyzed beef sausage samples. The residual nitrite values (mg/kg)in the local and industrial assessed samples ranged between 1.8 and 8.8 mg/kg.Nitrite residues were not detected in the two samples of imported beef sausages.

The residual nitrite concentrations in chicken sausage called "virshlle"

Table 3 shows the residual nitrite levels in chicken sausage called "virshlle".

Table 3. The amount of residual nitrite in chicken sausage called "virshlle"

Sample type	Samples (n)	Residual nitrite *mg/kg*	
		Positive samples (*n*)	Mean ± SD (min-max)
All samples	8	6	4.0±1.8 (1.6-6.1)
Local	5	5	3.8±1.9 (1.6-6.1)
Imported	3	1	N.A.

The residual nitrite presence was detected in 6 (75 %) of the 8 analyzed chicken sausage samples. The residual nitrite values ranged between 1.6 - 6.1 mg/kg. The residual nitrite was not detected in two out of three samples of imported chicken sausages.

The residual nitrite concentrations in beef and chicken salami

Table 4 presents the presence of nitrite residues in beef and chicken salami. All samples analyzed turned out to be positive. The highest concentration of nitrites was observed in beef sausage with a value of 11.5 mg/kg, while in the other samples the nitrite content was between 0.1 mg/kg (imported sausage) and 4.3 mg/kg (local sausage).

Table 4. The amount of residual nitrite in chicken & beef salami

Sample type	Samples (n)	Residual nitrite *mg/kg*	
		Positive samples (*n*)	Mean ± SD (min-max)
All samples	6	6	3.4±4.3 (0.1-11.5)
Local	4	4	5.0±4.5 (1.1-11.5)
Imported	2	2	0.15±0.07 (0.1-0.2)

The residual nitrite concentrations in dried beef prosciutto

Nitrite residue was not detected in any of the 5 samples of dried beef prosciutto (4 domestic and 1 imported).

DISCUSSION

Since this is the first study of nitrite in meat products in Kosovo, the focus was only to investigate the presence or absence of residual nitrites in certain final meat products, which are widely consumed in

Kosovo without considering other factors such as the production process, the amount of additives used, the storage time etc.

This assessment shows that in all analyzed samples, the nitrite residue was below the limits on the concentration of nitrites in meat products established by EU regulations (Commission Regulation, 2014), (Opinion, 2010).

Analyzes of 25 samples of beef sausages called "sujuk" have shown that only in 7 of them nitrite residues have been detected. The nitrite residue was detected in 5 out of 6 industrial beef sausages and in 2 out of 17 of local (homemade) beef sausages. The mean concentration of nitrites was higher in industrial sausages (5.9±2.7 mg/kg) in comparison to local (homemade) sausages (3.3±2.2 mg/kg). The residual nitrite was detected in 6 of the 8 analyzed chicken sausage samples called "virshlle". The mean concentration in local chicken sausage was 3.8±1.9 mg/kg. The presence of nitrite residues was observed in all 6 analyzed samples of chicken and beef sausage (local and imported). The highest concentration of nitrite residue (11.5 mg/kg) was detected in the beef sausage produced by a local producer. Nitrite residue was not detected in any of the 5 samples of dried beef prosciutto (4 domestic and 1 imported).

Our findings are in line with other survey data of residual nitrites level in cured meat products that comply with EU regulation. In 2003, EFSA (European Food Safety Authority)(EFSA, 2003) published data on residual nitrites level in meat products produced in different countries of EU. According to that survey, the levels of residual nitrites were below 20 mg/kg in the most of cured meat samples in France, Belgium and Germany.

Similar results are shown in a study conducted by De Mey et al. (De Mey et al., 2014), who examined the nitrates and residual nitrites levels in 101 cured meat samples. They found that the mean concentration of residual nitrates and nitrites was below 20 mg/kg.

According to Gavari & Pexara (Govari & Pexara, 2015) there is an indication of decreasing of nitrites content in meat products over last two decades that can be attributed to production technologies, less use of nitrites and use of different cure accelerators such as sodium or potassium ascorbate and erythorbate.

Our results present residual nitrite that is unreacted nitrite within the assessed meat products; however, scientists also raised objections on the limits of residual levels of nitrites since the measurement of the residual levels of nitrites in the final product may be of a limited value (EFSA, 2003). There is a study conducted by Merino et al. (Merino et al., 2016), which describes a step decrease of nitrite level from the point of addition until the recommended use-by date. They concluded that at the use-by date the residual level of nitrite in the products was 5-19% of the amount initially added, depending on the food product.

CONCLUSIONS

To conclude, this study examined residual level of nitrite in various meat products sold in Kosovo's market, which are popular among all people's groups including children. Our findings show that the nitrite residue in the analyzed meat products is within the permitted limits.

The obtained results from this research indicate that the nitrite residues are present in a different assessed meat products (beef & chicken sausage, beef and chicken salami), which are produced by certain producers. On the other hand, there are products produced by certain producers where nitrite residue was not detected. The highest concentration of residual nitrite is present in industrial and low-cost meat products.

Although these findings show that the nitrite residue in the analyzed meat products is within the permitted limits, some obtained data may be important indicators for further research in this regard.Production technology, the product recipe, the amount of nitrites and nitrates added, the way and time of storage as well as more update nitrite residue assessment method of meat products should be considered. Due to the potential health effects of nitrite, it is also of the importance to determine and calculate the acceptable daily intake (ADI) of nitrite from meat products but also to determine total exposure from other sources (vegetables and water).

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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