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To cite this article: D Vranic *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012106

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Nitrite content in meat products from the Serbian market and estimated intake

D Vranic¹, V Koricanac¹, D Milicevic¹, J Djinovic-Stojanovic¹, T Geric¹, S Lilic¹ and Z Petrovic¹

¹Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade, Republic of Serbia

E-mail: [danijela.vranic@inmes.rs](mailto:danjela.vranic@inmes.rs)

Abstract: The aim of this study was to determine levels of nitrites in some meat products sold on the Serbian market over a period of 3 years (2018-2020) and to compare results with maximum residue levels as well to discuss dietary exposure of the Serbian adult population to nitrites. A total of 1291 meat product samples, produced by the Serbian meat industry or imported (509 dry fermented sausages, 37 semi-dry fermented sausages, 451 finely minced cooked sausages and 294 coarsely minced cooked sausages), were obtained from the Serbian retail market during 2018-2020. Higher mean levels of nitrite content, expressed as NaNO₂, were found in cooked sausages (40.35 mg kg⁻¹, finely minced and 33.75 mg kg⁻¹, coarsely minced) compared to fermented sausages (1.86 mg kg⁻¹ dry fermented and 1.83 mg kg⁻¹, semi-dry fermented). The average dietary exposure to nitrites, expressed as nitrite ion, for the Serbian adult population varies from 0.001 to 0.015 mg kg⁻¹body weight (BW) day⁻¹ and was far below the European acceptable daily intake (0.07 mg kg⁻¹ BW day⁻¹). In conclusion, the concentrations of nitrite in all meat products were below established maximum permitted levels (national and European), indicating that the use of nitrite as a food additive in Serbia is generally in line with existing regulations.

1. Introduction

Protecting consumer health through improving food safety and quality has been an increased focus for both food processors and researchers. Consumers today are much more demanding in terms of food quality and safety, and product labelling, while producers are determined to implement necessary standards in food production, etc. [1]. The main goal of the meat industry is reducing economic losses and increasing the shelf life and storage stability of meat products while maintaining consumer health.

The use of curing agents (nitrite, nitrate) in meat processing has been a controversial issue since the 1970s. The mechanism of curing colour formation is well known and has been summarised by Honikel [2]. Nitrates and nitrites, in the form of the sodium and potassium salts, are widely used as preservatives in meat production. From the technological point of view, the main reason for adding nitrites and/or nitrates in the processing of meat products is to improve the quality (formation the unique pink colour, texture and flavour), [2, 3, 4], durability, due to its antioxidant action against lipid oxidation [5] and increase the safety of products [6, 7]. Taken together, because of their antimicrobial properties, they inhibit the growth and reproduction of bacteria *Staphylococcus aureus* and *Clostridium botulinum*. As an unstable ion, nitrite undergoes a series of reactions as soon as it is added to food. In an acid environment, nitrite is converted into nitric acid, which decomposes into nitric oxide. Nitric oxide, being



an important product from the standpoint of colour fixation in cured meat, reacts with myoglobin to produce a red pigment-nitrosomyoglobin [8]. The intake of nitrite is normally low and not an acutely toxic dose, but nitrite in food is considered primarily to cause health problems because its presence both in food and in the body could lead to the formation of carcinogenic nitrosamines [9, 10] and the clinical symptom of methemoglobinaemia [11, 12]. Time, temperature, pH and additives have an important effect on the depletion of nitrite in cured meat [8]. The contribution of dietary intake is given by exogenous nitrite via consumption of vegetables, fruits, water and meat. Cured meat products are the major source of the nitrites and N-nitrosamines in human dietary intake. Many studies have suggested that high dietary nitrate and nitrite intake is an aetiological factor in the development of certain cancers [13]. It is now well understood that endogenously acquired nitrate/nitrite can be converted to carcinogenic N-nitroso compounds upon digestion [14, 15]. Large amounts of these compounds in the body contribute to the development of cancer in gastrointestinal tract organs, the most commonly affected being colon, rectum, liver, thyroid and stomach [16]. The use of nitrite could lead to the formation some acute and chronic toxicity such as methemoglobinaemia, thyroid disorders and increased risks of gastric, oesophageal, nasopharyngeal and bladder cancers [17, 18, 19]. Nevertheless, some authors revised this risk downwards and claimed that nitrate and nitrite could have potential beneficial effects on human health by reducing hypertension and cardiovascular diseases [20, 21]. Another aspect to consider is the fact that there is no direct link between the in-going and the residual amount of nitrite, but the degradation of nitrite added to meat is influenced by several factors like pH, storage temperature, heat treatment of meat and the presence of reducing substances. Added ascorbate especially will increase the rate of degradation of nitrite [22].

The current Serbian legislation has restricted the concentration of residual NaNO_2 in processed meat to 100 and 150 mg kg^{-1} depending of the type of product [23, 24] whereas regulation in Europe permits concentrations up to 100 mg kg^{-1} [25]. The Joint Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) for nitrite of 0-0.07 mg kg^{-1} body weight, expressed as nitrite ion on the basis of a no effect level (NOEL) of 6.7 mg kg^{-1} body weight per day for effects on heart and lung in a 2-year study in rats and a safety factor of 100 [19, 26, 27].

The aims of this study were to determine the levels of nitrites in some meat products sold on the Serbian market over a period of 3 years (2018-2020) and to compare the results obtained with maximum permitted levels (MPL) [23], as well as to estimate and discuss dietary exposure of the Serbian adult population to nitrites.

2. Materials and Methods

2.1. Meat products and sample preparation

A total of 1291 meat product samples, produced by the Serbian meat industry or imported (509 dry fermented sausages, 37 semi-dry fermented sausages, 451 finely minced cooked sausages and 294 coarsely minced cooked sausages) were obtained from different regions from the Serbian retail market during 2018-2020. In the most of the meat products, all parameters of quality defined by the legislation were examined, and in a smaller number, analyses were carried out as per client's request. The content of nitrite in examined meat products was determined according to the standard ISO procedure [28].

2.2. Exposure Assessment and risk characterization

The estimated daily intake (EDI) of nitrite from processed meat by consumers was calculated based on the individual food consumption data [29], body weight [30] and the analytical results obtained in the present study for the collected meat samples.

2.3. Statistical analysis

For statistical evaluation on data, Minitab 17 Ink statistical software was used ((Minitab Ink., Coventry, UK).

3. Results and discussion

The results of the determination of nitrite content in each type of processed meat products, expressed as minimum, maximum, mean and median as well as number of tested samples and MPL are presented in Table 1 and Figure 1. The data of the EDI for nitrite in the examined processed meat products are shown in Table 2. None of the analysed meat products exceeded the maximum permitted nitrite level of 150 mg kg⁻¹, according to the Serbian legislation on food additives [23].

Table 1. Mean levels and ranges of nitrite content expressed as NaNO₂ (mg kg⁻¹) in examined processed meat products; limit of quantification =0.03 mg kg⁻¹

Meat product	N	n (%)	Mean ±SD (mgkg ⁻¹) ^a	Mean ±SD (mgkg ⁻¹) ^b	Median (mgkg ⁻¹) ^b	Min–Max (mgkg ⁻¹)	MPL (mgkg ⁻¹)
Dryfermented sausages	509	332 (65.2)	1.86±2.68	2.85±2.87	1.905	0.05-24.88	
Semi-dryfermented sausages	37	28 (75.6)	1.83±2.48	2.42±2.60	1.36	0.1-10.02	150
Finely mincedcooked sausages	451	451 (100)	40.35±19.93	40.35±19.93	40.30	0.09-94.57	
Coarsely minced cooked sausages	294	292 (99)	33.75±23.40	33.75±23.40	31.66	0.05-113.51	
Total	1291	1103 (85.4)	22.57±24.22	26.41±24.18	23.26	0.05-113.51	

N–total number of analysed samples; n–number of samples that contained nitrite (%); ^a mean nitrite content in the total number of analysed samples; ^b mean/median nitrite content in the number of samples that contained nitrite; MPL–maximum permitted level [23].

Table 2. Estimated dietary intake and risk characterization of nitrite ion (NO₂⁻) intake.

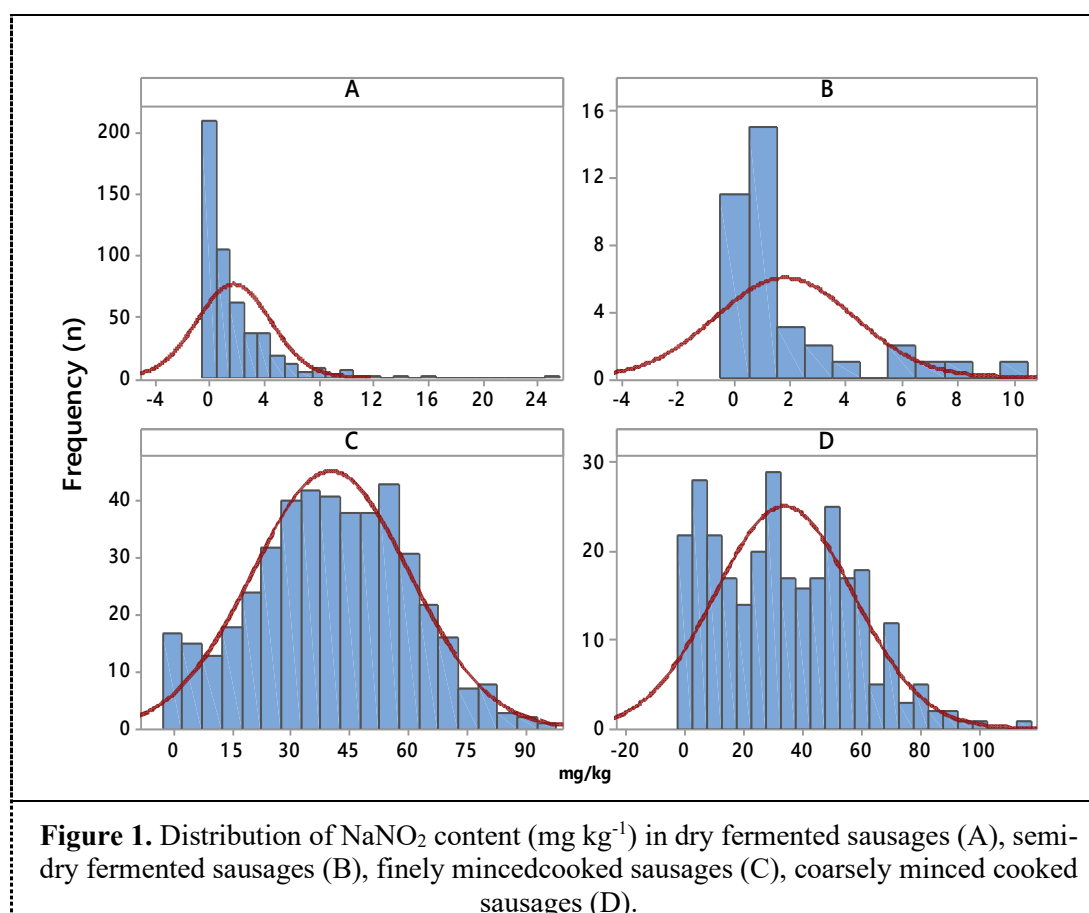
	Mean ±SD (mgkg ⁻¹)	ADC (gday ⁻¹)	EDI (mgkg ⁻¹ BWday ⁻¹)	Contribution to ADI (%)	ADI (mgkg ⁻¹ BWday ⁻¹)
Dryfermented sausages	1.24±1.80		0.001	1.42	
Semi-dry fermented sausages	1.22±1.65	38.3	0.001	1.42	0.07
Finely mincedcooked sausages	26.90±13.29		0.015	21.42	
Coarsely minced cooked sausages	22.50±15.60		0.012	17.58	
Average	12.96±20.50		0.007	10.46	

Nitrite ion content (66.65% of NaNO₂); ADC–average daily consumption of meat products [29]; EDI–estimated daily intake; BW –default body weight value for adults was 70 kg [30]; ADI–acceptable daily intake [19, 26].

The levels of residual nitrite and nitrate in processed meat products are variable depending on the time and temperature used during processing and storing, the initial addition of nitrite and nitrate, the composition of the meat, pH, addition of antioxidant components such as ascorbate and the presence of microorganisms [2, 31]. Honikel [2] estimated that the decline in nitrite levels due to heating during manufacturing is about 35% of the added level, and thereafter, there is a continuing decrease in nitrite levels during storage. Higher mean nitrite contents, expressed as NaNO₂, were found in cooked sausages (40.35 mg kg⁻¹, finely minced and 33.75 mg kg⁻¹, coarsely minced) compared to fermented sausages

(1.86 mg kg⁻¹, dry fermented and 1.83 mg kg⁻¹, semi-dry fermented). In 65.2% (332 samples) of examined dry fermented sausages and 75.6% (28 samples) of semi-dry fermented sausages, nitrites were detected, while in the rest of these samples, in 34.8% (177 samples) and in 24.4% (9 samples) respectively, nitrites were not detected, i.e. nitrite contents were under the limit of quantification (<0.03 mg kg⁻¹) (Table 1, Figure 1A and 1B). The results obtained show distributions of nitrites differed in the four product types (Figure 1), which is related to stability of the nitrite content in the different meat products, i.e. is the consequence of their differing preparation and processing methods [32]. In fermented sausages, the content of nitrite decreased during the ripening of sausages as a result of the process that takes place in the sausage, i.e. reduction of nitrite content is significant where main process is nitrite conversion into nitrates in the weak acid environment. In fermented sausages, the presence of nitrite becomes latent, because the process is reversible and nitrates, under certain conditions, can revert into nitrites [33].

Our results were similar to previously reported nitrite contents in dry fermented and cooked sausages, 0.65 mg kg⁻¹ and 36.60 mg kg⁻¹, respectively from the Serbian market in the period of 2016-2018 [32], and were lower than mean nitrite content in dry fermented sausages (7 mg kg⁻¹) from Croatia [34].



Many papers report the results of the analysis of the content of nitrite in different types of meat products, demonstrating a great variability in their concentrations [35]. According to the results of studies [36, 37, 38], the mean concentrations of nitrite in cooked sausages were 32 mg kg⁻¹; 26 mg kg⁻¹ and 30.5 mg kg⁻¹, respectively. In [39], sausages had a higher mean nitrite content 51.8±14.5 mg kg⁻¹, compared to our results. Yalcin [40] measured the residual nitrite content of dry fermented sausages and sausage samples from Istanbul and the reported average nitrite contents were 42.8 mg kg⁻¹ in dry

fermented sausage (n=65) and 102.8 mg kg⁻¹ in sausage (n=60), which were higher than our mean nitrite contents in these types of sausages.

The average dietary exposure to nitrites, expressed as nitrite ion, for the Serbian adult population varied from 0.001 to 0.015 mg kg⁻¹ body weight (BW) day⁻¹, according to the product type (Table 2) and was far below the European ADI (0.07 mg kg⁻¹ BW day⁻¹). Our results showed that cooked sausages, especially finely minced ones, were the main sources of nitrite intake, and they made up 21.42% of the ADI (Table 2). The lowest sources of nitrite intake among the examined meat product were dry fermented and semi- dry fermented sausages (both 1.42% of ADI).

The dietary exposure to nitrite was calculated in this study as accurately as possible. However, limitations were the impossibility of calculating relevant exposures for children and heavy consumers due to lack of data, and data paucity on consumer intake of nitrates, which contributes to the nitrite exposure because they are converted to nitrite during metabolism; nitrate intake was not included in the current intake estimates. Also, the consumption of processed meat per capita was estimated based on the family consumption divided by the number of family members, and assuming that the children consume the same amount as adults. Thus, the approach taken can be considered an initial approximation for the intake assessment.

4. Conclusion

In conclusion, the concentrations of nitrite in all 1291 processed meat samples in the period of three years were below established MPLs (national and European), indicating that the use of nitrite as a food additive in Serbia is generally in line with existing regulations. The overall situation is controllable and safe. However, if other sources of nitrite exposure (vegetables, cereals, dairy products and drinking water) were taken into account, the EDI would be higher. To provide adequate consumer protection, further research and additional dietary studies are needed: updating adult and child dietary habits; especially collecting data for children's exposure and; measuring the nitrite content in other food sources. All together, the results of this study suggest need to investigate the complex effects of various reduced levels of nitrite and potential alternative compounds and/or technologies that can substitute nitrite in meat products. Considering the toxicity of nitrites and the possibility of their transformation to carcinogenic N-nitrosamines, the importance of the information of daily intake by children and adults is clearly indicated.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, according to the provisions of the Contract on research financing in 2021 (No 451-03-9/2021-14/200050 dated 05.02.2021).

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