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ASSESSMENT OF RESIDUAL NITRITE LEVELS IN COOKED SAUSAGES: COMPLIANCE, THERMAL PROCESSING EFFECTS, AND CONSUMER SAFETY

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Abstract: The increasing global consumption of processed meat, which often contains nitrite as a preservative, raises health concerns due to potential adverse effects from its metabolites, such as nitric oxide and N-nitroso compounds. The study sought to evaluate the food safety of processed meat products within the Serbian market, specifically in the Belgrade region. Nitrite levels were analysed in cooked sausages, both in their raw state and after undergoing the manufacturer's recommended thermal processing prior to consumption, if applicable. Additionally, thus far, there has been a lack of research exploring the potential influence of residual nitrite levels in the meat products prepared as per manufacturer recommendations prior to the consumption of meat products, as well as their contribution to acceptable daily intake (ADI), which provides crucial insights into the overall dietary safety of processed meats. During a three-year period, the study performed analysis on a total of 77 cooked sausages, following the standard ISO methodology. Boiling the cooked sausages led to a major reduction in this meat-product additive, whereas frying led to a comparatively smaller decrease in nitrite concentration. Additionally, the greatest exposure to nitrite compounds occurs when consuming meat products without prior preparation, i.e., without thermal treatment by consumers before ingestion. In summary, the assessment of the ADI for nitrites revealed a high level of food safety, with all values noticeably below the maximum permitted levels specified by national legislation (150 mg/kg).

Key words: residual nitrite, thermal processing, cooked sausages, ADI

Introduction

Early human consumption of meat, beginning over 2 million years ago, likely contributed to cerebral evolution due to its rich energy and nutrients. Initially, humans consumed freshly hunted meat due to the lack of long-term storage methods, later developing techniques for prolonged preservation. Today, meat processing not only extends shelf life but also facilitates the creation of diverse products, enhancing the value of less preferred meat types or its by-products commonly used in products like sausages (Rodrigues et al., 2022). Meat curing, an age-old technique for food preservation, continues to be widely practiced even today. It entails adding salt and spices to fresh meat, typically at different levels of comminution and different stages of processing (Shakil et al., 2021). Synthetic nitrite, typically in the form of nitrite salt combined with common salt (NaCl), is commonly used in the meat processing industry due to its cost-effectiveness and ease of application. Nitrite serves as a multifunctional agent in meat products, such as colour preservation, flavour enhancement, antioxidant properties, and antimicrobial function, especially *Clostridium botulinum*, whose toxins could lead to food poisoning (Dragoev et al., 2014; Hospital et al., 2016).

The colour of meat products exhibits considerable variation and is influenced by numerous factors. Upon the introduction of nitrite to meat, it undergoes several chemical reactions, ultimately converting into nitric oxide (NO). NO subsequently binds with the iron found in both myoglobin (Fe^{2+}) and metmyoglobin (Fe^{3+}), resulting in the development of a cured pink hue in the meat products (Alahakoon et al., 2015). Myoglobin, a sarcoplasmic protein responsible for the occurrence of the red colour in meat, experiences oxidation to form metmyoglobin, which gives the products a brownish hue. The interaction of nitric oxide with myoglobin results to the formation of a vibrant red nitrosyl-myoglobin complex, which forms the basis for the characteristic colour of cured meat. However, this complex is highly unstable and transforms into a stable, visually appealing reddish-pink pigment known as nitroso-hemochrome during the heat treatment process (Shakil et al., 2021). As the hematin and heme proteins, such as myoglobin, hemoglobin, cytochrome oxidase, and peroxidases, possess the ability to trigger lipid peroxidation processes (Chabi et al., 2008), added nitrites, and consequentially, formed nitric oxide, exerts antioxidant effects through various mechanisms: (1) It reduces the formation of Fe^{2+} ions, preforming as a radical acceptors, thereby minimizing oxidative stress by inhibiting Fenton's reaction (d'Ischia et al., 2011); (2) NO suppresses the activities of enzymes which play roles in lipid oxidation processes (Dragoev, 2023); (3) During the smoking of meat products, nitrogen oxides formed outside the membranes stabilize unsaturated lipids, contributing to lipid stability (Zhang et al., 2023). In general, the antioxidant effect of nitric oxide is diverse, enhancing the oxidative stability of meat products not just during processing but also during storage.

Nevertheless, despite its benefits, the use of sodium nitrite in meat and its link to N-nitrosamine (NAs) formation raised health concerns among consumers as early as the 1970s (Hur et al., 2015; Lee et al., 2021). NAs can form in meat products during production processes, as previously described, during at-home preparation, and even in the digestive tract after consumption (Shakil et al., 2021). Currently, it is understood that there is a positive relationship between the amount of nitrite added and the production of NAs, although this relationship does not follow a straightforward pattern. The phenomenon of "endogenous nitrosation" primarily occurs within the digestive system, particularly in organs such as the stomach (at lower pH), rectum, colon, and urinary bladder, and is typically considered organ-specific (Said Abasse et al., 2021). Overconsumption of nitrite can cause respiratory center paralysis and hypoxia symptoms by reducing hemoglobin's oxygen-carrying capacity (Chen et al., 2022). Methemoglobinemia, or "blue baby syndrome," is a life-threatening condition resulting from high nitrite intake, causing reduction of oxygen supply to tissues, leading to cyanosis. This condition is most severe in infants under six months, although it has been reported in children and adults (Sorour et al., 2023).

In the Republic of Serbia, nitrites in processed meats are listed among permitted additives in meat products (in form of potassium and sodium salts, E 249 and E 250, respectively), and the maximum permitted level (MPL) is defined by the Rulebook on Food Additives (2018), which is harmonized with European Directive (EFSA, 2011), setting the maximum permissible amount that can be added during the production process expressed as $NaNO_2$ or $NaNO_3$ to 150 mg/kg of the product, whereas the residual amount of nitrites in meat products is not set, leaving it with quite unclear boundaries. However, according to the latest European Food Safety Authority Panel on Contaminants in the Food Chain opinion (EFSA, 2023), meat and meat products are identified as the primary source of public exposure to carcinogenic nitrosamines, prompting recent legislative changes by the EU Commission to establish lower limits for nitrites as food additives. This aims to reduce consumer exposure to carcinogenic NOs while ensuring food safety. Specifically, heat-treated meat products like cooked sausages now have a maximum permissible nitrite concentration during production of 80 mg/kg under the latest EU Commission legislation (EFSA, 2023). Additionally, there's a prescribed maximum residual nitrite amount for products ready for market and throughout their shelf life, capped at 45 mg/kg as NO_2^-. Moreover, there is a set value for the maximum acceptable daily intake (ADI) of synthetic nitrite at 70 μg/kg body weight/day, established by both The Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2002) and the European Food Safety Authority (EFSA, 2003).

Cooked sausages are meat products processed before consumption - cured, often lightly smoked, and precooked. The residual nitrite content in sausages decreases over time during storage and thermal treatment during preparation before consumption (Paudel et al., 2021). This research aims to investigate the residual nitrite levels in selected categories of cooked sausages available in the market of the Republic of Serbia. It seeks to provide insights from the consumers' perspective, considering how the products are consumed as marketed by the manufacturers—whether raw, boiled, or fried. Our research aimed to address several key questions: What is the amount of nitrite ingested by consumers, allowing us to assess the estimated daily intake (EDI)? How much of these nitrites originate from meat products in comparison to the allowable daily intake (ADI)? Do all products comply with national legislative standards regarding the maximum permitted levels (MPLs) of nitrites, as well as the proposed new regulations by the EU Commission (EFSA, 2023) on MPLs and residual nitrite levels? Furthermore, we explored the impact of frying and boiling processes on residual nitrite levels in sausages.

Materials and Methods

Over a three-year period (2021-2024), nitrite analysis was conducted on a total of 77 products sourced from various market-chains in the Belgrade region. The analysis followed random selection method and aimed to determine the presence of residual nitrite in cooked sausage samples. Samples were sent to the laboratory while maintaining the cold chain, vacuumed if needed, and stored at 4°C in a refrigerator prior to analysis. The selected samples are categorized into three groups of products: finely ground cooked sausages (small- and large-diameter) with 41 samples, coarsely ground cooked sausages with 33 samples, and cooked sausages with meat chunks (Šunkarica sausage) with 3 samples. The nitrite concentration was determined following the spectrofotometric reference ISO method (SRPS ISO 2918:1999). It is noteworthy to mention that all of the analysed products exclusively listed E 250 (sodium nitrite) additive on their food labels. Each sample underwent triplicate analysis to determine its residual nitrite content both before and after thermal processing, following the manufacturer's guidelines specified on the packaging. Approximately 250 g of each sample was selected for analysis. Samples intended for consumption in their raw state, such as Šunkarica sausage, Extra sausage, Tirolska sausage, Toast sausage, and Novosadska sausage, were solely analysed in their raw form, with the entire 250 g homogenized. For small- and large-diameter finely ground cooked sausages, including Hot dogs, Hot dog-type sausages, and Debreciner sausage, a portion of approximately 120 g was analysed in its raw state, while the remaining portion was analysed after boiling for

5 minutes in water maintained at 100 ± 5°C, as recommended by the manufacturer. The same division of the sample was applied to coarsely ground sausages, such as Grill sausage, Srpska sausage, Domaća sausage, and Beef sausage, with a portion analysed in its raw state and another portion analysed after frying at 149 ± 2°C for 5 minutes (2.5 minutes on each side) in a frying pan. Following the heat processing, the samples were cooled to room temperature and thoroughly homogenized in a food processor before analysis for residual nitrite levels.

The average daily consumption of processed meat products was calculated using the latest data available from the Statistical Office of the Republic of Serbia in 2022 (SZS, 2023). Estimated daily intake (EDI) was determined following the method outlined by Temme et al., 2011. The EU Commission Authority (EFSA, 2023) established an acceptable daily intake (ADI) for nitrites at 0.07 mg/kg body weight per day. Thus, the contribution of nitrite (expressed as $NaNO_2$) to ADI was evaluated. The EFSA Scientific Committee (EFSA, 2012) recommended using an estimated average body weight of 70 kg as the default for the European adult population (aged 18 years and above).

For data analysis, the MS Excel was used.

Results and Discussion

According to the latest data from the Statistical Office of the Republic of Serbia in 2022 (SZS, 2023), the average number of household members is 2.67, and the average consumption of processed meat items per household per year is 36.1 kg. Therefore, the estimated average consumption of processed meat, including cooked sausages, is approximately 37.04 g/day/person. This allows for further calculation of the average EDI (mg/kg bw/day) and its contribution to ADI (%) of nitrites for individuals above 18 years of age. The nitrite levels considered for this calculation are residual nitrite levels, which vary based on the preparation method recommended by the manufacturer (whether the product is intended to be consumed raw, boiled, or fried). The results for the finely ground cooked sausages and cooked sausages with meat chunks are presented in Table 1, while the results for the coarsely ground cooked sausages are shown in Table 2.

Table 1. Nitrite concentrations in finely ground cooked sausages (small- and large-diameter) and cooked sausages with meat chunks samples analysed raw and after boiling (where needed, according to manufacturers' label), percentage loss from thermal processing, and contribution to final residual nitrite levels in relation to EDI and ADI

Product type (sausage type)	Average nitrite levels (mg/kg) (min – max)		Average percentage decrease of nitrites after thermal process (%) (min – max)	EDI (mg/kg bw/day) (min – max)	Average contribution to ADI (%) (min – max)
	Raw	Boiled			
Small-diameter finely ground cooked sausages, n=33					
Hot dog sausage, n=6	33.89 (4.40 - 47.29)	13.53 (0.70 - 22.67)	50.70 (40.76 - 58.49)	0.007 (0.000 - 0.012)	10.23 (0.53 - 17.14)
Hot dog-style sausage, n=27	31.86 (5.57 - 68.22)	14.24 (1.40 - 35.29)	48.59 (30.03 - 69.30)	0.008 (0.001 - 0.035)	10.75 (1.06 - 26.68)
Large-diameter finely ground cooked sausages, n=8					
Debreciner sausage, n=3	44.59 (33.78 - 60.64)	23.27 (18.42 - 29.95)	47.33 (45.48 - 50.61)	0.012 (0.010 - 0.016)	17.59 (13.93 - 22.64)
Extra sausage, n=5	42.10 (29.30 - 58.82)	-	-	0.022 (0.016 - 0.031)	31.83 (22.15 – 44.47)
Finely ground cooked sausages with meat chunks, n=3					
Šunkarica sausage, n=3	33.56 (14.42 - 51.22)	-	-	0.018 (0.008 - 0.027)	25.37 (10.90 - 38.72)

EDI - estimated daily intake; ADI - acceptable daily intake; n – number of samples;

Table 2. Nitrite concentrations in coarsely ground cooked sausages samples analysed raw and after frying (where needed, according to manufacturers' label), percentage loss from thermal processing, and contribution to final residual nitrite levels in relation to EDI and ADI

Product type (sausage type)	Average nitrite levels (mg/kg) (min – max)		Average percentage decrease of nitrites after thermal process (%) (min – max)	EDI (mg/kg bw/day) (min – max)	Average contribution to ADI (%) (min – max)
	Raw	Fried			
Srpska sausage, n=4	40.81 (34.18 - 52.68)	26.21 (20.29 - 31.91)	35.54 (23.86 – 43.31)	0.014 (0.011 - 0.017)	19.81 (15.34 - 24.12)
Grill sausage, n=6	40.69 (18.99 - 54.92)	33.32 (17.67 - 43.17)	16.32 (6.95 - 24.11)	0.018 (0.009 - 0.023)	25.19 (13.36 - 32.64)
Domaća sausage, n=4	39.52 (36.37 - 42.58)	33.42 (25.24 - 40.11)	16.37 (7.90 - 32.22)	0.018 (0.013 - 0.021)	25.26 (19.08 – 30.32)
Beef sausage, n=4	48.94 (24.34 - 59.30)	42.57 (20.81 - 54.38)	14.16 (8.31 - 17.58)	0.023 (0.011 - 0.029)	32.18 (15.73 - 41.11)

Product type (sausage type)	Average nitrite levels (mg/kg) (min – max)		Average percentage decrease of nitrites after thermal process (%) (min – max)	EDI (mg/kg bw/day) (min – max)	Average contribution to ADI (%) (min – max)
	Raw	Fried			
Tirolska sausage, n=7	41.53 (18.91 - 67.49)	-	-	0.022 (0.010 - 0.036)	31.40 (14.30 – 51.02)
Toast sausage, n=4	29.74 (17.53 - 49.21)	-	-	0.016 (0.009 - 0.026)	22.48 (13.25 – 37.20)
Novosadska sausage, n=4	46.81 (34.91 - 52.60)	-	-	0.025 (0.018 - 0.028)	35.39 (26.39 – 39.76)

EDI - estimated daily intake; ADI - acceptable daily intake; n – number of samples;

For heat-treated processed meat like cooked sausages, Denmark reported a consumption of 5.6 mg/kg, while the mean reported value for other EU countries (excluding Denmark) was 11.6 mg/kg (EFSA, 2017), indicating significantly lower exposure to nitrites from meat products compared to the population of the Republic of Serbia. Moreover, the EFSA's research (EFSA, 2017) reveals that the population across European countries is exposed to levels of nitrites in meat products, ranging from 0.01 to 0.04 mg/kg body weight/day, or 14.3% to 57.1% of the ADI. Similarly, our study found that exposure to residual nitrites from cooked sausages varied from 0.00 (from hot dog sausages) to 0.036 mg/kg body weight/day (Tirolska sausage), representing a wide range of 0.56% to 51.02% of the ADI, with an average of 22.91%.

All products, both raw and post-heat treatment, complied with the national legislation, with a set value of 150 mg/kg $NaNO_2$ (Rulebook on Food Additives, 2018), and remained below the maximum permissible nitrite concentration of 80 mg/kg under the latest EU Commission legislation (EFSA, 2023).

The results of nitrite concentrations in raw sausages varied widely, with the small-diameter finely ground cooked sausage exhibiting the highest range, ranging from 4.40 mg/kg for hot dog samples to 68.22 mg/kg for chicken hot dog-style sausage samples. The variation of nitrite values in the samples may result from nitrite depletion during product storage, from manufacturing to analysis. It is typical to use 50-100 mg/kg $NaNO_2$ during the manufacturing process in cooked sausages products to inhibit *Clostridium botulinum* growth (Paudel et al., 2021). Nitrite loss over time depends on factors such as heat processing, product pH, storage temperature, and the presence of reducing agents (Paudel et al., 2021). Approximately one-fourth (20.78%) of all the raw analysed samples had nitrite content less than 20 mg/kg, while the majority of the samples (74.04%) fell within the range of 20-60 mg/kg. Only 6.50% of the samples had nitrite contents in the range of 60 mg/kg and higher, but remained well below 80 mg/kg. However, according to the latest EU Commission legislation (EFSA, 2023), which sets the maximum residual nitrite amount for meat products at 45 mg/kg, 39% of the cooked sausage samples intended for consumption without prior preparation exceed this threshold. Among these, the majority (6 out of 23) are coarsely ground cooked sausages, with three out of four product samples from Novosadska sausages surpassing this limit.

All cooked sausage samples exhibited a reduction in nitrite levels following both boiling and frying treatments, with wide variations in the percentage decrease, as depicted in Tables 1 and 2. Thermal processing significantly decreased residual nitrite levels, particularly in products subjected to boiling, with reductions ranging from 30.03% to 69.30%, and an average reduction of approximately 50%. These findings are consistent with previous studies. Nurlailah et al. (2020) observed a 45% reduction in nitrite levels in sausages boiled for 5 minutes at 90°C. Paudel et al. (2021) reported reductions ranging from 7.83% to 75.23%, indicating varying effects not attributed to common parameters such as emulsion type, product size, or initial nitrite concentration. Additionally, Iammarino et al. (2023) found that boiling treatment significantly decreased residual nitrite levels ($p < 0.05$) in sausage samples, with reductions ranging from 25.8% to 43.5%. On the other hand, Merino et al. (2016) argue that the results of their pilot study indicated that the boiling process did not alter the residual nitrite level at all, whereas frying resulted in a decrease of around 50% from the initial level. Our results differed from these findings. While frying of the sausages has led to decrease in residual nitrite levels as well, this reduction was not as substantial as that observed with the boiling process. Abdel-Atty et al. (2022) reported a decrease of approximately 26% in sausages marketed in Egypt following the frying process, consistent with our findings, where nitrite levels were reduced by 20.21%. The next question is: how did thermal processing affect residual nitrite levels, and do they comply with the newest requirements from the EU Commission (EFSA, 2023)? Out of a total of 36 cooked sausages subjected to boiling, all of them had residual nitrite levels well below 45 mg/kg. Regarding sausages subjected to frying, all of which were coarsely ground cooked sausages and initially had higher nitrite levels when analysed raw, only 2 out of 18 exceeded the newly set values by the EU Commission (EFSA, 2023).

Conclusion

The use of synthetic nitrites in meat processing, particularly in the production of cooked sausages, serves various functions such as colour preservation, flavour enhancement, and antimicrobial action. However, concerns regarding the formation of carcinogenic N-nitrosamines and potential health risks associated with nitrite consumption have prompted regulatory measures to ensure food safety. Our investigation into residual nitrite levels in cooked sausages available in the Serbian market revealed several key findings. While there were variations in nitrite content among different sausage types, our findings indicate that all products met national legislative standards. However, there are areas for improvement, particularly concerning products intended for consumption without prior thermal preparation, as some exceeded the newest legislative threshold for residual nitrite set by the EU Commission. Thermal processing, especially boiling but also frying, resulted in substantial reductions in residual nitrite levels, thereby enhancing food safety.

However, conducting a survey on current consumption habits among the population of the Republic of Serbia is necessary to obtain more precise insights into the assessed exposure to nitrites from meat products, thereby enabling the accurate determination of nitrite EDI and, consequently, ADI.

Procena nivoa rezidualnih nitrita u barenim kobasicama: usklađenost sa regulativom, uticaj termičke obrade i bezbednost potrošača

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Rezime

Rastuća globalna potrošnja proizvoda od mesa, koji često sadrže nitrite kao konzervans, dovodi do povećane zabrinutosti za javno zdravlje usled postojanja potencijalno štetnih efekata njihovih metabolita, poput azot-oksida i N-nitrozo jedinjenja. Istraživanje je imalo za cilj da izvrši evaluaciju bezbednosti proizvoda od mesa na srpskom tržištu, fokusirajući se na region grada Beograda. Izvršena je analiza određivanja koncentracije nitrita u barenim kobasicama, kako u nativnom stanju, tako i nakon podvrgavanja preporučenoj termičkoj obradi preporučenoj od strane proizvođača (ukoliko je postojala). Pretragom literature, došlo je do shvatanja da dosadašnja istraživanja nisu obuhvatila analizu potencijalnog uticaja rezidualnih nitrata u mesnim proizvodima koji su termički obrađeni u skladu sa preporukama proizvođača pre njihovog konzumiranja, odnosno da nije ispitan njihov doprinos prihvatljivom dnevnom unosu (ADI) za nitrite. Ovakva istraživanja su od suštinskog značaja za dublje razumevanje ukupne bezbednosti ishrane proizvodima od mesa. Tokom trogdišnjeg perioda, u studiji je izvršena analiza na 77 uzoraka barenih kobasica, prema ISO standardu. Termički proces barenja kobasica doveo je do bitnog smanjenja ovog aditiva, dok je proces prženja doveo do nešto manjeg smanjenja koncentracije nitrita. Pored toga, najveća izloženost nitritnim jedinjenjima se javlja pri konzumiranju proizvoda od mesa koji

ne zahtevaju prethodnu pripremu, odnosno termičku obradu od strane potrošača pre konzumacije samog proizvoda. Procenom ADI za nitrite u odnosu na dostupne proizvode na tržištu mesa, utvrdili smo visok nivo bezbednosti barenih kobasica, pri čemu su vrednosti za sve proizvode primetno ispod maksimalno dozvoljenih nivoa propisanih nacionalnim zakonodavstvom (150 mg/kg).

Ključne reči: rezidualni nitriti, termička obrada, barene kobasice, ADI

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Conflict of interest
The authors declare that they have no conflict of interest.

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