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The effect of cooking methods on the formation of volatile N-nitrosamine in sausages with different contents

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ABSTRACT

The study's main aim is to assess the levels of volatile N-nitrosamine (VNA) in sausages based on different brands, ingredients, and cooking methods. The effects of sausage composition and cooking method on VNA formation, which caused this difference, were studied. For this, sausages from 17 different brands and 3 different ingredients (beef, chicken, and turkey) were bought from the market, cooked using 3 different techniques (frying, boiling, and microwave), and their VNA levels were determined by GC-MS (Gas chromatography-mass spectrometry). The study found different levels of VNA in all sausages. NDMA, NDEA, NDPA, NPYR, and NPIP were found in more than 70% of the samples, according to the study's findings. In the samples, the most NDMA, NPYR, and NPIP formation were observed. Total VNA levels in the study ranged from 0.18 to 109.28 ppb.

Keywords: Volatile nitrosamines, GC-MS, Sausage, Carcinogen

Introduction

Cured, heat-treated, or emulsified meat products are essential food industry products that serve consumers. Emulsified meat products like sausage and salami are popular because they are delicious and convenient. These products, in addition to their benefits, pose a risk due to the carcinogenic nitrosamines they contain. The fact that nitrite gives color (Horsch, 2013) to meat products and is an effective antimicrobial against *C. Botulinum* (Archer, 2002) and both gram-positive and gram-negative microorganisms (Horsch, 2013) makes it an essential additive for meat products. However, in spite of these advantages of nitrite, it is also known that nitrosamines formed in meat products can pose a risk and danger to consumer health according to the International Cancer Agency (IARC) (IARC, 2010). It is known that high temperature, long processing time and protein oxidation affect VNA formation in products. (Lu et al., 2022).

In sausages, Jo et al.(2003), found nitrosamines in concentrations ranging from 10 to 40 µg/kg. Cintya et al., (2019) on the other hand, found no NDEA in any of the meat products they purchased from the market in their current study of 5 different brands of sausage, smoked meat, burger, and canned meat samples. NDMA and NPYR were found in approximately 90% of fried, grilled, and smoked sausages purchased in Estonian supermarkets. Fried poultry meat with red pepper contains 24.42 µg/kg Nitrosamine, according to the same study (Yurchenko and Mölder, 20007). In a different study, NDMA levels in market-provided products such as dry-cured pork, cooked pork, mortadella (an Italian sausage), and bresaola (Italian pastrami) were all found to be between 0.3 and 1.1 g/kg (Sannino and Bolzoni, 2013). Yuan et al. (2015) investigated the nitrosamine levels of 28 different meat products purchased from the market, based on the cooking methods followed, and found that the highest nitrosamine levels were found in grilled sausages, with nitrosamine levels ranging from 0.42 to 51.018 ppb. The researcher claimed in another study examining the maturation process in terms of nitrosamine formation that three volatile N-nitrosamine (VNA) derivatives (NDMA, NDEA, and NPYR) were formed and the amount increased in the process. The chemical and microbiological reactions that occur during the ripening process are the reason for this situation (Xiao et al., 2018). According to research, cured, salted, or emulsified meat products are risky in VNA, especially when fried or grilled (Lee, 2019). While heat treatment applications (cooking time, method, and temperature) greatly affect the process, nitrate, nitrite, primary, secondary and tertiary amines, amides, proteins, peptides, amino acids, different precursors, and microbial activity are required for nitrosamine formation (Yurchenko and Mölder, 20007). In this context, it is of great significance for public health that the production of this type of processed food is carried out carefully in terms of the amount of additives and processing time (Özbay et al., 2019).

VNA are potential carcinogens whose exposure should also be evaluated. The tolerable intake dose of NDMA has been determined to be 96 ng/day (Anon, 2019a). Another volatile toxic N-nitrosamine, NDBA, has been related to the development of tumors in the liver and esophagus. The urinary bladder has been identified as the location where NDBA has the strongest cancer effect. While NDEA causes tumors in the liver and esophagus (EPA, 2016), 26.5 ng/day has been reported as a tolerable intake amount for NDEA (Anon, 2019a). In terms of acute toxicity, a single oral dose of NMEA with an LD50 of 90 mg/kg in mice has been reported. The genotoxic and mutagenic effects of NPYR and its causation of liver tumors have been detected in in vivo and in vitro studies (EPA, 2016). The European Union, on the other hand, recently suggested that the use of nitrates and nitrites causes the formation of nitrosamines, and in this respect, their use with ascorbic acid will reduce the amount (Anon, 2019b). In addition, it is important to use natural alternative nitrite and nitrate sources or to reduce the amount of nitrite-nitrate used in meat products. (Flores and Toldra, 2021).

In this study, seven different nitrosamines among the nitrosamines, which have nearly three hundred types in matrices such as air, water, soil, and food, were studied together with seven different nitrosamines, which are considered to be possible carcinogens by the International Agency for Research on Cancer (IARC), and whose carcinogenic effects are emphasized by the United States Environmental Protection Agency (EPA). These nitrosamine derivatives are N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodine-butylamine (NDBA), N-nitrosopiperidine (NPIP) N-nitrosopyrrolidine (NPYR) N-nitrosodine propylamine (NDPA) and N-nitrosomethylethylamine (NMEA) and their levels were determined in sausage samples with different contents and cooking methods of different brands by GC-MS (Gas Chromatography-Mass Spectrometer) device.

With the study, it was found what level of VNA was formed in sausage consumption depending on different cooking and content preferences. Depending on this situation, a viewpoint can be developed to improve consumption preferences.

Materials and Methods

Chemicals and Standards

All chemicals used in the analysis were of analytical purity. A nitrosamine standard mixture in dichloromethane (2000

mg/L) containing *N*-Nitrosodiethylamine (NDEA), *N*-Nitrosopiperidine (NPIP), *N*-Nitrosomethylethylamine (NMEA), Nitrosopyrrolidine (NPYR), *N*-Nitrosodi-*n*-propylamine (NDPA), *N*-Nitrosodimethylamine (NDMA), *N*-Nitrosodibutylamine (NDBA), was obtained from Sigma Aldrich (EPA 521 Nitrosamine Mix, 2000 ppm, Sigma-Aldrich, St Louis, USA). Different concentrations N-nitrosamine standards in were stored at -18 ºC. N-nitrosamines are potential carcinogens, so studies have been done carefully.

Materials

Sausages determined as the sample of analysis has been taken from the markets in Aksaray province. For this purpose, samples of 17 different samples has been purchased in order to examine the effect of different cooking methods on the formation of N-nitrosamine, these sausages were subjected to different cooking processes. Uncooked sausage samples were analyzed as a control group. Thus, sausage samples belonging to 17 different brands were cut into approximate sizes and analyzed by cooking with 1 control (uncooked) and 3 cooking methods (microwave, frying, boiling). Thus, 68 samples (17x4) belonging to 3 different cooking groups and a control group of 17 brands of sausages were analyzed. The study was completed in two parallels. (n=136)

The cooking process parameters were determined by preliminary tests. Accordingly, the parameters in which the sausages were cooked without burning were recorded. Sausage cooking parameters are shown in Table 1.

Table 1. Susage cooking parameters

Cooking Type	Time (min)	Ambient	Temperature
Boiling	10	Boiling water in a steel pot	Water, 100°C
Frying	4	Hot sunflower oil in a teflon pan	Sunflower oil, 200°C
Microwave	3	600 W with microwave energy in porcelain bowl	-

Methods

Determination of N-nitrosamines

Extraction of N-nitrosamines was done using method of Özbay and Şireli (2021a). Sausage samples were broken and homogenized with a blender (Philips, HR1316/00, Istanbul, Türkiye), after 20 g sample was weighed with a precision scale (KERN, ABJ 220-4NM, Ballingen, Germany). After 40 ml DClM (Dichloromethane) (Merck, Dichlotomethane for gas chromatography, Darmstadt, Germany) was added to it. The samples were then kept in an ultrasonic water bath (VWR, Ultrasonic Cleaner - USC-TH, Leicestershire, England) for 15 minutes. At the end of the period, the samples were filtered with filter paper (S & H Labware, Ø125 mm, Ankara, Türkiye) and the filtrate was collected. The samples were then treated with DClM (40 ml) again in the water bath for 15 minutes. The collected filtrate was transferred to the rotary evaporator (Heidolph, Hei-VAP Advantage, Schwabach, Germany) and the solvent (DClM) in it was removed. The extract was collected with 1 ml of methanol (Merck, Methanol for chromatography, Darmstadt, Germany), filtered (0.45 µm, Sartorius Stedim, Göttingen, Germany) and taken into glass vials. The samples taken into the vial were homogenized by vortexing (VELP Scientifica, Velata, İtaly). Samples kept in vials were kept closed and parafilm wrapped in refrigerator until analysis.

GC (Agilent Technologies, 7890A, Santa Clara, United States) device and integrated mass spectrometer - MS (Agilent Technologies, 5975C, Santa Clara, United States) detector were used for volatile N-nitrosamine analysis. DB624 (Agilent, Santa Clara, USA) capillary column (30 m, 0.25 mm I.D and 1.40 µm) was used as the GC-MS column. Chromatographic conditions were as follows: inlet temperature, 180°C; inlet mode, pulsed split-less, septum purge flow: 3 mL/min, using helium (purity ≥ 99.999%) as carrier gaz. The oven temperature was programmed as follows: start temperature of 60°Cfor 2 min, then increased to 120°Cfor 2 min, at a rate of 20°Cper minute followed by a further increase to 220°Cat a rate of 20 °C per minute. Finally isothermally at 220°Cfor 2 min. The conditions set for the mass spectrometer were as follows: transfer line temperature, 180°C, electron impact ionization mode at 70 eV; scan range from m/z 40 to 200. The time for solvent delay was set to 5 min. The retention times and qualifier ions are shown in Table 2. A representative chromatogram from GC analysis is shown in Figure 1.

Table 2. Retention times, molecular weight, qualifier ions for the detection in MS SIM mode applied method for the 7 VNAs using GC-MS

Compound	Weight (M/W)	Rt (min)	Qualifier ions (m/z)
NDMA	74	5.441	74.1 / 84.0 / 86.0
NMEA	88	6.82	88.1 / 71.1 / 73.1
NDEA	102	7.839	102.1 / 71.1 / 73.1
NDPA	130	10.071	70.1 / 113.1 / 130.1
NPYR	100	10.426	100.1 / 71.1 / 85.1
NPIP	114	10.909	114.1 / 71.1 / 85.1
NDBA	158	12.664	84.1 / 99.1 / 116.1

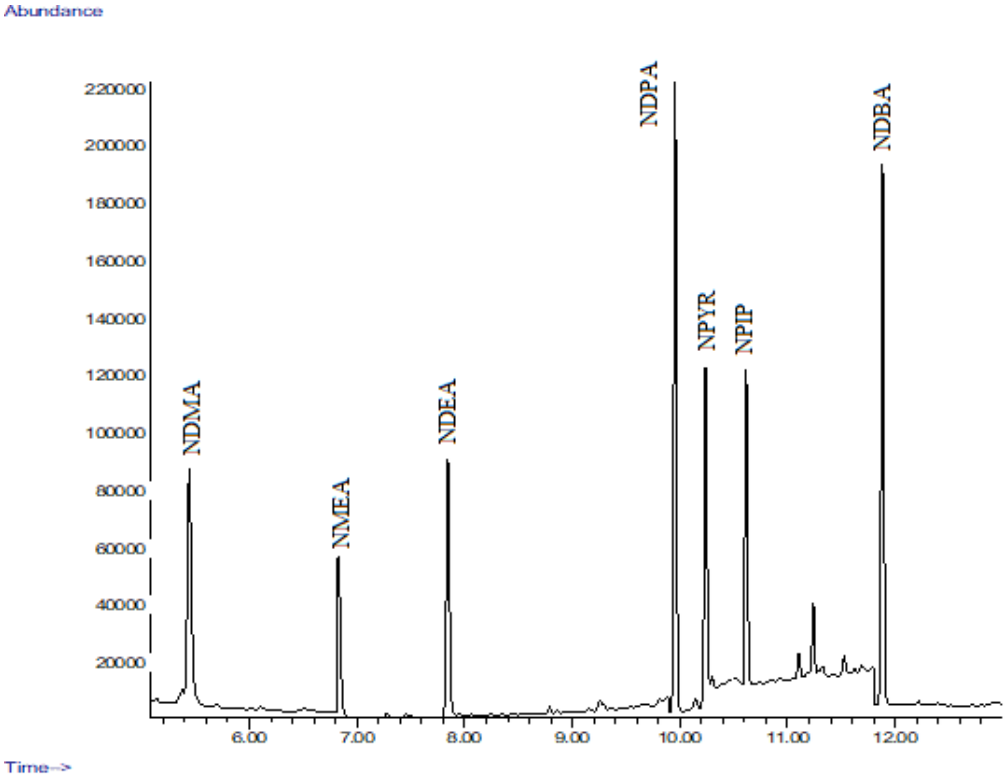

Fig. 1 GC-MS chromatography showing seperation of N-nitrosamines

To calibrate the gas chromatography mass spectrometry (GC-MS) chromatogram, 7 different standard solutions were prepared, which covered the concentration range 0.5 to 75 µg/mL. The lowest detectable concentrations for NAs were established between 0.06 and 0.17 μg/mL. The limit of quantification (LOQ) was calculated as 3 × LOD (between 0.21 and 0.56 μg/mL).

For the recovery experiment, a sample with low content of N-nitrosamines was chosen and fortified with 3 different levels of standard solutions. Recoveries were found to be between 87.70% and 95.82%. Validation of GC-MS method was carried out by following Eurochem method validation steps (Magnusson and Örnemark, 2014). Validation study results are shown in Table 3.

Table 3. Method validation results

Compounds	Calibration (µg/L)	Linearity (r^2)	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)	Reproducibility (%RSD)
NDMA	0.5 - 75	0.999225	0.11	0.36	87.70	3.5342
NMEA	0.5 - 75	0.989719	0.12	0.40	92.90	2.0331
NDEA	0.5 - 75	0.998683	0.11	0.37	90.02	8.1090
NDPA	0.5 - 75	0.979911	0.10	0.32	87.44	5.0704
NPYR	0.5 - 75	0.999627	0.06	0.21	94.5	3.4497
NPIP	0.5 - 75	0.995153	0.17	0.56	93.9	4.5262
NDBA	0.5 - 75	0.994560	0.08	0.26	95.82	5.0957

Statistical Analysis

One Way ANOVA was applied to the data by using SPSS (Statistical Package for the Social Science) 15.0-licensed program for the statistical analysis of all samples. Thus, 136 samples of 17 brands were analyzed in the study.

Results and Discussion

NDMA, NDEA, NDPA, NPYR, and NPIP were found in more than 70% of the samples, according to the study's findings. In the samples, the most NDMA, NPYR, and NPIP formation were observed. Kaban et al. (2021), found the most NDMA, NPYR and NPIP in heat-treated sausages similar to our study. They reported that NPIP occurs at the highest level. NDMA, NPIP, and NPYR are mainly formed in sausages, according to Lee et al. (2019). According to Campillo et al. (2011), the most common volatile N-nitrosamine derivatives in processed meat products are NDMA and NPIP, which is consistent with previous studies. While in another study, 7.86–29.11 ppb level of VNA was detected in salami collected from the market (Özbay and Şireli, 2021b), while in this study, a varying level of VNA was found at the level of 8.45-65.2 ppb. This situation was interpreted as a cooking relationship with VNA formation. Similarly, in a study examining VNA in raw meat products, NDPA, NDBA and NPIP were detected in almost all samples (Sun et al., 2020). The fact that VNA formation and levels are affected by very complex processes is thought to be the reason for these differences.

The VNA values of cooked sausages ranged from 0.9 to 109.28 ppb, according to the findings of the study. In their review analysis, Gushgari and Halden (2018) found that 118 different processed meat products included a variable amount of total VNA ranging from 0.1 to 121 ppb. Kaban et al. (2021) reported that they detected NPIP at the level of 5.19 – 16.40 ppb in heat-treated sausage, followed by the formation of NDMA and NPYR. Cintya et al. (2019) evaluated non-volatile N-nitroso-thiazolidine-4-carboxylic acid (NTCA) in 20 samples of ready-to-eat sausage, smoked meat, burger, and canned meat purchased from the market. N-nitrosamine derivatives such as N-nitroso-2-methyl thiazolidine-4-carboxylic acid were detected in high concentrations (NMTCA). The amount of NTCA in the samples varies between 500-4227 ppb, while NMTCA varies between 20-990 ppb.

Table 4 shows the statistical relationship between the VNA level in sausages and cooking, brand, and content. The table shows that there is a significant relationship between cooking methods and VNA formation (P<,005). In the same table, it can be seen that the brand has no significant effect on VNA formation (P>,005). According to the table, the sausage content only has an effect on the formation of NDPA. In addition to these findings, it was found that veal sausage contained higher VNA than other ingredients when the average VNA levels in Table 5 were evaluated. Turkey sausage and chicken sausage come after beef sausage. The study obtained results in parallel with the study of Moradi et al (2021). In both studies, meat sausages contained significantly higher VNA compared to chicken sausage.

In the Table 5, it is seen that frying causes higher VNA amount in terms of cooking methods. Next comes the boiling process. The fact that microwave cooking is the cooking method that remaining of the lowest level of VNA formation is observed. The effects of content and cooking method on the average VNA formation separately are shown in Figure 2 and Figure 3, respectively.

Table 4. Statistical analysis results

	NDMA	NMEA	NDEA	NDPA	NPYR	NPIP	NDBA	Total NAs
Cooking (Raw, fried, boiled, microwave)	.021	.002	.000	.002	.005	.001	.000	.000
Brand	.021	.272	.535	.017	.124	.004	.200	.040
Contents (Beef, turkey, chicken)	.158	.050	.038	.001	.030	.062	.024	.007

Means in the same row with different superscripts are significantly different (P<.005)

Table 5. Average volatile N-nitrosamine results by cooking and content (ppb)

Content	NDMA	NMEA	NDEA	NDPA	NPYR	NPIP	NDBA	Total NAs
Beef Sausage	2.6996	2.6279	3.3946	3.4283	2.6129	2.6450	2.5396	19.9479
Turkey Sausage	0.7015	0.5335	1.4315	2.0390	0.7195	1.7010	0.8100	7.9360
Chicken Sausage	1.3929	0.4087	1.2287	0.6258	1.0796	0.9317	1.0300	6.6975
Cooking Type								
Raw Sausage	0.4118	0.2659	0.3859	0.3700	0.4876	0.4371	0.2241	2.5824
Fried	3.5106	3.9765	4.7953	3.6082	3.1912	3.5594	3.6271	26.2682
Boiled	2.2571	0.6494	2.1188	2.5265	1.7876	2.0347	1.8594	13.2335
Microwave	0.4235	0.0229	0.9112	1.6176	0.5929	1.0194	0.2818	4.8694

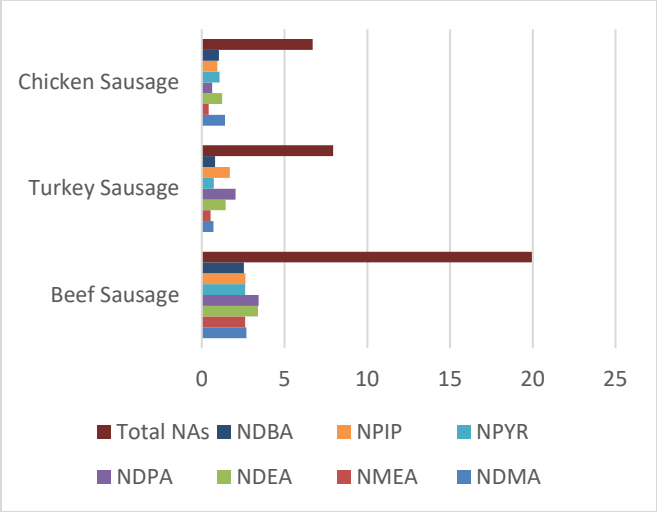

Figure 2. Average volatile N-nitrosamine levels (ppb) depending on sausage content

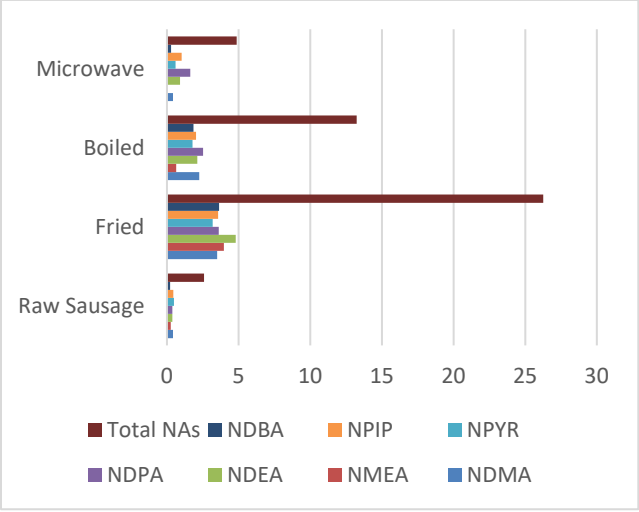

Figure 3. Average volatile N-nitrosamine levels (ppb) depending on cooking method

According to the study's findings, total VNA levels in fried sausage range from 8.24 to 109.28 ppb. The highest value was obtained from fried beef sausage, while the lowest level was obtained from turkey sausage. All of the fried samples revealed NPYR, NMEA, NDPA, NPIP, and NDBA. Similarly, Gloria et al (1997) found NDMA, NDEA, NDBA, NDEA, NPIP, and NPYR in the samples they examined in their study by frying 37 processed meat products from the market, such as bacon and its derivatives. They also found NPYR at levels ranging from 7 to 25 ppb in all of the products they examined. In another study, NPYR was found to be higher (5.02 ppb) than uncooked sausage (3.97 ppb) after frying dry cured sausages. In parallel, researchers reported that they detected NPYR in 90% of 386 (raw, fried, smoked, canned) processed meat products. In the same study, the highest volatile VNA level belonged to fried meat, then grilled meat and finally cured meat (Yurchenko ve Mölder, 2007). In general, the fact that frying processes contain high levels of volatile N-nitrosamines exhibits parallelism with this research.

Boiling is another cooking method used in the study. During the boiling process, a total of 3.67–58.24 ppb VNA was produced in the sausages. When compared to frying, boiling provides a smaller amount of total VNA. After boiling the sausages, Li et al. (2012) determined the formation of NDMA, NDEA, and NPYR volatile N-nitrosamine derivatives. Yurchenko and Mölder (2007) found similar results with the study. They analyzed the volatile N-nitrosamine levels of sausages purchased directly from the market, fried, grilled, smoked etc., and found NDMA and NPYR in approximately 90% of all samples.

Microwave cooking is another method that has been studied. The microwave cooking process yielded the lowest results of all the cooking methods. According to the findings, the average total VNA level in sausages cooked in the microwave ranged from 0.9 to 17.06 ppb. In comparison to frying and boiling, microwave cooking yields lower total VNA levels. In microwaved sausage samples, Li et al. (2012) found 1.02 ppb NDMA, 0.18 ppb NDEA, and 3.76 ppb NPYR. In the same study, lower levels of volatile N-nitrosamine formation in microwave cooking were found to be similar to those found in other heat treatments (boiling, frying).

Important data was recorded on volatile N-nitrosamine formation at various levels during cooking methods. Many parameters, including heat treatment time, temperature, and method, ambient temperature, nitrosamine precursor concentration, salt concentration, and pH, are found to affect the complex processes that change the formation of VNA in this process (Gençcelep, 2010; Herrmann et al., 2015; Honikel, 2008; Özçelik, 1982). The fact that chemical and physical factors have a big impact on VNA formation is regarded to be the main reason why the amount varies by method and application. Researchers have reported detecting volatile N-nitrosamine results on a wide range of scales in several studies (Lee, 2019; Gushgari and Halden, 2018).

Conclusion

In the research, sausage samples belonging to 17 different brands obtained from the market were evaluated in terms of possible carcinogenic VNA levels. The VNA values of the sausages varied widely, depending on the different ingredients, brands and cooking methods.

As a crucial result of the study, it is possible to conclude that each cooking method increases the level of VNA in the sausage. As for cooking methods, frying has also been found to increase the risk of VNA formation. Microwave cooking might be considered a healthier option for sausage consumption as a cooking method. Furthermore, it is possible to assert that the sausage's content leads to the formation of VNA. While veal sausage has a higher overall VNA content, turkey and chicken sausage come in second and third, respectively. In this regard, the method of cooking and the composition of the sausage will help to limit the risks of sausage consumption. The formation of VNA in all sausages, albeit at varying levels, is also a significant result of the study.

In the future, it will be important to detect VNA derivatives by conducting similar studies for other foods that are considered as risk groups (ripened cheeses, pickles, alcoholic beverages, etc.). Furthermore, alternatives to these foods' consumption processes could have a positive impact on public health.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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Disclosure: -

References

Anon (2019a). WHO information note update on nitrosamine impurities. https://www.who.int/medicines/publications/drugalerts/informationnote_nitrosamine%20impurities/en/ Date of access: 27/12/2019.

Anon (2019b) Discussion paper on the use of nıtrates (ins 251, 252) and nıtrıtes. https://ec.europa.eu/food/sites/food/files/safety/docs/codex_ccfa_51_agenda_item-5c.pdf Date of access: 31/12/2019.

Archer, D.L. (2002). Evidence That İngested Nitrate And Nitrite Are Beneficial To Health. *Journal of Food Protection*, *65*(5), 872-875. https://doi.org/10.4315/0362-028X-65.5.872

Campillo, N., Vinas, P., Martínez-Castillo, N., Hernández-Córdoba, M. (2011). Determination of volatile nitrosamines in meat products by microwave-assisted extraction and dispersive liquid-liquid microextraction coupled to gas chromatography mass spectrometry. *Journal of Chromatography A*, 1218(14), 1815-1821. https://doi.org/10.1016/j.chroma.2011.02.010

Cintya, H., Silalahi, J., Putra, E.D.L., Siburian, R. (2019). Analysis of nitrosamines in processed meat products in medan city by liquid chromatography-mass spectrometry. *Open Access Macedonian Journal of Medical Sciences*, 7(8), 1382-1387. https://doi.org/10.3889/oamjms.2019.261

EPA (2016). Six-year review 3 technical support document for nitrosamines. Access address: https://www.epa.gov/sites/default/files/201612/documents/810r16009.pdf Date of access: 27/12/2019.

Flores, M., Toldrá, F. (2021). Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products-Invited review. *Meat Science*, 171, 108272. https://doi.org/10.1016/j.meatsci.2020.108272

Gençcelep, H. (2010). Sucuklarda Kalıntı Nitrit Miktarı ve N-Nitrozamin Oluşumu İle İlişkisi. Traditional Foods from Adriatic To Caucasus, April 15-17 2010, Tekirdağ, Türkiye. (In Turkish)

Gloria, M.B.A., Barbour, J.F., Scanla, R.A. (1997). Volatile nitrosamines in fried bacon. *Journal of Agricultural and Food Chemistry*, 45(5), 1816-1818. https://doi.org/10.1021/jf960973b

Gushgari, A.J., Halden, R.U. (2018). Critical review of major sources of human exposure to N-nitrosamines. *Chemosphere*, 210, 1124-1136. https://doi.org/10.1016/j.chemosphere.2018.07.098

Herrmann, S.S., Granby, K., Duedahl-Olesen, L. (2015). Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages. *Food Chemistry*, 174, 516-526. http://dx.doi.org/10.1016/j.foodchem.2014.11.101

Honikel, K. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, 78(1-2), 68-76. http://10.1016/j.meatsci.2007.05.030

Horsch, A.M. (2013). The effect of ph and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on listeria monocytogenes. Doctoral Dissertation, Iowa State University, USA.

IARC (2010). IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 94, Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins. International Agency for Research on Cancer, Lyon.

Jo, C., Ahn, H.J., Son, J.H., Lee, J.W., Byun, M.W. (2003). Packaging and irradiation effect on lipid oxidation, color, residual nitrite content, and nitrosamine formation in cooked pork sausage. *Food Control*, 14(1),7-12. https://doi.org/10.1016/S0956-7135(02)00045-2

Kaban, G., Polat, Z., Sallan, S., Kaya, M. (2021). The occurrence of volatile N-nitrosamines in heat-treated sucuk in relation to pH, aw and residual nitrite. *Journal of Food Science and Technology*, 1-8. https://doi.org/10.1007/s13197-021-05186-2

Lee, H.S. (2019). Literature compilation of volatile N-nitrosamines in processed meat and poultry products-an update. *Food Additives & Contaminants: Part A*, 36(10), 1491-1500. https://doi.org/10.1080/19440049.2019.1649472

Li, L., Wang, P., Xu, X., Zhou, G. (2012). Influence of various cooking methods on the concentrations of volatile N-nitrosamines and biogenic amines in dry-cured sausages. *Journal of Food Science*, 77(5), C560-C565. https://doi.org/10.1111/ijfs.12069

Lu, J., Li, M., Huang, Y., Xie, J., Shen, M., Xie, M. (2021). A comprehensive review of advanced glycosylation endproducts and N-Nitrosamines in thermally processed meat products. *Food Control*, 108449. https://doi.org/10.1016/j.foodcont.2021.108449

Magnusson, B. Örnemark, U. (2014). Eurachem Guide: The Fitness For Purpose of Analytical Methods – A Laboratory Guide To Method Validation And Related Topics, (2nd Ed. 2014). ISBN: 978-91-87461-59-0

Moradi, S., Shariatifar, N., Akbari-adergani, B., Aghaee, E.M., Arbameri, M. (2021). Analysis and health risk assessment of nitrosamines in meat products collected from markets, Iran: with the approach of chemometric. *Journal of Environmental Health Science and Engineering*, 1-11. https://doi.org/10.1007/s40201-021-00692-z

Özbay, S., Sireli, U.T., Filazi, A (2019). Nitrosamines, their chemistries and effects on health. *International Journal of Scientific and Technological Research*, 5(4), 124-133. http://doi.org/10.7176/JSTR/5-4-13

Özbay, S., Sireli, U.T. (2021a). The effect of ascorbic acid, storage period and packaging material on the formation of volatile N-nitrosamine in sausages. *Journal of Food Science and Technology*, 1-8. http://doi.org/10.1007/s13197-021-05194-2

Özbay, S., Sireli, U.T. (2021b) Volatile N-nitrosamines in processed meat products and salami from Turkey, Food Additives & Contaminants: Part B, 14:2, 110-114. http://doi.org/10.1080/19393210.2021.1885502

Özçelik, S. (1982). Bazı gıdalarda nitrit ve nitrozaminlerin oluşumu ve sağlığa zararlı etkileri. *Gıda,* 7(4), 183-188.

Sannino, A., Bolzoni, L. (2013). GC/CI–MS/MS method for the identification and quantification of volatile n-nitrosamines in meat products. *Food Chemistry*, 141(4), 3925-3930. https://doi.org/10.1016/j.foodchem.2013.06.070

Sun, C., Wang, R., Wang, T., Li, Q. (2020). Primary evaluation of nine volatile N-nitrosamines in raw red meat from Tianjin, China, by HS-SPME-GC–MS. *Food Chemistry*, *310*, 125945. https://doi.org/10.1016/j.foodchem.2019.125945

Xiao, Y., Li Zhou, Y., Ma, F., Chen. C (2018). Effect of inoculating lactobacillus pentosus R3 on N-nitrosamines and bacterial communities in dry fermented sausages. *Food Control*, 87, 126-134. https://doi.org/10.1016/j.foodcont.2017.12.025

Yuan, Y., Meng, W., Yutian, M., Fang, C., Xiaosong, H. (2015). Determination of eight volatile nitrosamines in meat

products by ultrasonic solvent extraction and gas chromatography-mass spectrometry method. *International Journal of Food Properties*, 18(6), 1181-1190. https://doi.org/10.1080/10942912.2014.898652

Yurchenko, S., Mölder, U. (2007). The occurrence of volatile N-nitrosamines in Estonian meat products. *Food Chemistry*, 100(4), 1713-1721. https://doi.org/10.1016/j.foodchem.2005.10.017