Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2023; 8(1): 102-107 Website: jnfs.ssu.ac.ir

Nitrite and Nitrate Residue in Non-Heat Treated Meat Products Marketed in East Azerbaijan Province

Marzie Hejazy; DVM. PhD*[1], Roghayeh Norouzi; PhD [2] & Farzin Javid; DVM, PhD [2]

[1] Basic Science Department, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran; [2] Pathobiology Department, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran; [2] Faculty of Veterinary Medicine, Islamic Azad University of Tabriz, Tabriz, Iran.

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 7 Sep 2021
Revised: 10 Nov 2021
Accepted: 15 Dec 2021

Corresponding author:
mhejazy@ut.ac.ir
Toxicology and pharmacology division, Basic Science Department, School of Veterinary Medicine, University of Tabriz, Iran.

Postal code: 5166616471
Tel: +98 912 2131435

ABSTRACT

Background: Nitrite and nitrate salts are used as preservatives in meat products to prevent the growth of pathogen organisms such as *Clostridium botulinum.* Nitrogen salts additives create the desired color in meat products. However, the use of these salts accelerates nitrosamine production and increases the risk of carcinogenesis. Hence, nitrate and nitrite consumption rate should be according to the approved standards. This analytical study is designed to determine nitrate and nitrite residue of non-heat-treated meat products including hamburger and kebab in East Azerbaijan Province, Iran during 2015-2020. **Methods**: 120 samples of non-heat-treated meat products from different brands were randomly collected. They consisted of 60 products manufactured in East Azerbaijan province and 60 products imported from other provinces. Nitrate and Nitrite residues in non-heat-treated meat products including hamburger and kebab were determined by spectrophotometric method. **Results:** Results showed that the residue levels of nitrate and nitrite content regarding non-heat- treated meat products were less than maximum residual limits (MRLs) of national standards. The study also indicated that frozen non-heated meat products did not have any nitrogenous additive. **Conclusion:** The results of this research revealed that the frozen non-heat treated meat products including hamburger and kebab, produced or consumed in East Azerbaijan province during 2015-2020 did not have nitrate and nitrite additives. Continuous monitoring to ensure the compliance of meat products with the standards is necessary.

Keywords: *Non-heat treated meat products; Hamburger; Kebab; Nitrate; Nitrite; East Azarbaijan*

Introduction

Among the meat products available in Iran, non-heat-treated meat products are the most consumed ones (Rezaei *et al.*, 2013). To enhance shelf life and maintain properties such as color and odor in heated meat products such as sausages, additives such as polyphosphate, nitrite and nitrate, and ascorbate are used. Sodium nitrite and sodium nitrate stabilize tissue color, contribute to meat's taste, and prevent from the activity of spoilage microorganisms and dangerous microbes such as

This paper should be cited as: Hejazy M, Norouzi R, Javid F. ***Nitrite and Nitrate Residue in Non-Heat Treated Meat Products Marketed in East Azerbaijan Province.*** *Journal of Nutrition and Food Security (JNFS)*, 2023; 8 (1): 102-107.

[DOI: 10.18502/jnfs.v8i1.11774]

clostridium botulinum in heated meat products (Kamkar *et al.*, 2004).

Carcinogenic effects of nitrite and nitrate additives are due to the reaction of nitric acid produced by the breakdown of nitrite with the second type of amines produces nitrosamine. Nitrosamines are transformed into unstable hydroxyl compounds, and eventually, produce activated carbon hydroxyalkyl ions. This causes alkylation of DNA and mutation, and eventually, cancer and teratogenicity. Nitrous amines have been detected in different parts of body including liver, lungs, kidney, bladder, and gastrointestinal. Nitrite also facilitates the process of carcinogenesis (Waltz *et al.*, 2015). If nitrite enters body through digestive tract, it will be absorbed into the bloodstream. It combines with the iron present in hemoglobin to produce methemoglobinemia. Methemoglobin is not capable of carrying oxygen and may cause cyanosis, especially in infants, known as baby blue syndrome. Symptoms may include tiredness; dizziness; vomiting; diarrhea; headache; dyspnea; cyanosis; and pale eyes, mouth, and lips, and extremities (Carter *et al.*, 2020). Maximum residual limits (MRLs) of nitrite and nitrate in meat products are in accordance with Iranian national standards of 2303, with a maximum of 120 ppm of nitrite and nitrate for non-heat treated meat products. According to the Food and Agriculture Organization (FAO) standards, the acceptable daily intake (ADI) of sodium or potassium and sodium nitrite is 5 and 0.4 mg/kg of human body weight per day, respectively. Therefore, monitoring meat products to assess compliance of these additives with international and national standards seems necessary (Müller, 1991).

This study was designed to monitor nitrate and nitrite content in non-heat treated meat products marketed in East Azerbaijan province, Iran, during 2015-2020.

Materials and Methods

Study design: 120 samples of meat products of various brands were randomly selected. They were based on the national standard No. 690 on sampling of meat products, including those produced in East Azerbaijan province, as well as products imported from other provinces with different percentages of meat (30-50, 51-70, and 71-90 percent) (Institute of Standards and Industrial Research of Iran, (2000)). The samples were collected during 2015-2020 and transferred to the toxicology laboratory of the faculty of veterinary medicine, in Tabriz University. Measurements then were taken up to the fourth day after manufacturing products, and up to 30 days after production of the province's imported items. Nitrate and nitrite levels were measured using the standard methods 923 and 988 in the Iranian Institute of Standards and Industrial Research (Institute of Standards and Industrial Research of Iran, 2020a, b). The principles of this method are sample extraction with hot water, precipitation of proteins and dyes in the samples to minimize the effect of dyes and proteins in the samples on their light spectrum in spectrophotometry, filtering, adding sulfanilamide, N-(1-Naphthyl) ethylenediamine dihydrochloride, and photometrically measuring the intensity of the dye produced in the presence of nitrate and nitrite at a wavelength of 538 nm.

Sample preparation: Each sample weighed 200 g, and was equally mixed twice using a meat grinder. After the authors weighed 10 g of completely uniform samples, they were placed in a 250 ml beaker, followed by 100 ml of water at 70 °C and 5 ml of saturated borax solution (50 g of hydrated sodium tetraborate in some water, which was then reduced to one liter) added to it. The beaker was placed on a baking sheet for 15 minutes, being stirred once every few minutes. After cooling, 2 ml of precipitating protein solution number one (106 g of hydrated potassium ferrocyanide dissolved in water to a volume of one liter), which was stirred, was added. This was followed by 2 ml of precipitating protein solution number two (220 g of hydrated zinc acetate and 30 ml of concentrated citric acid dissolved in water to a volume of one liter). The contents of the beaker

[DOI: 10.18502/jnfs.v8i1.11774]

were moved to a 200 ml volumetric flask, reducing the capacity to one liter. The solution was placed at ambient temperature for 30 minutes. It was then strained to obtain a clear solution. 10 ml of sulfanilamide solution and 6 ml of 5 N hydrochloric acid solutions were added to each sample. After mixing the solutions, they were placed in the dark for 5 minutes before receiving 2 ml of alpha-naphthyl ethylenediamine hydrochloride solution and being left in the dark for 10 minutes. Finally, the absorbance was read at a 538 nm spectrophotometer. Based on the different amounts of light absorption measured by spectrophotometry against different concentrations of sodium nitrite (ppm) in standard solutions, a standard curve was prepared. The amount of nitrite in each sample was calculated after comparison with the standard curve and using Excel software. Nitrite content was measured according to the national standard No. 923 (Institute of Standards and Industrial Research of Iran, 2020a). Nitrate content was measured according to the national standard (Institute of Standards and Industrial Research of Iran, 2020b).

Data analysis: Statistical analysis was performed using graph pad software (prism), t-test and one way ANOVA methods. P-value < 0.05 was considered significant.

Results

Table 1 reports the mean and standard error of nitrite and nitrate content of meat products by meat percentage. The results of statistical analysis based on one-way ANOVA showed that there was no significant relationship between meat percentage and nitrite/nitrate content.

Table 2 shows the mean and standard error of nitrite and nitrate content by the location of manufacture of the product and its production date. The results suggested no significant difference in nitrite and nitrate content during storage. According to the national Iranian standard, the use of nitrate and nitrite are not allowed in hamburger or frozen meat products such as kebab. However, the use of nitrate and nitrite are allowed in heated-meat products such as sausages. The national standard for maximum residual limit in heated-meat products is 120 ppm for nitrite and 300 ppm for nitrate. Low level of detected nitrite and nitrate in hamburger and kebab is because of presence of nitrogenous compound of raw meat and expected error during the analysis.

Table 1. Mean (±SD) of nitrite and nitrate content for non-heat-treated meat products presented in Tabriz in 2015-2020 based on meat percentage.

Number	Nitrate (ppm)	Nitrite (ppm)	Meat (%)
42	4.50 ±1.47	0.33±0.12	30-50
43	5.71 ±1.62	0.46±0.21	51-70
35	6.70 ±2.03	0.58±0.34	71-90

Table 2. Mean (±SD) of nitrite and nitrate content of meat products marketed in Tabriz in 2015-2020 based on the production location and production date.

Number	Nitrate (ppm)	Nitrite (ppm)	Nitrite remaining Place of production
60	5.63±1.18	0.49±0.17	Meat products manufactured in the province (Up to 4 days after production)
60	5.10±0.89	0.47±0.09	Meat products imported from other provinces (Up to 30 days after production)

[DOI: 10.18502/jnfs.v8i1.11774]

Discussion

According to the results of this study, the average nitrite and nitrate in all samples of non-heated-meat product, including hamburger and kebab, was lower than the standard limit. The highest detected levels were < 6.7 ± 2.03 ppm for nitrate, and < 0.58 ± 0.34 ppm for nitrite. The lowest detected levels were (< 4.50 ± 1.47) for nitrate, and (< 0.33 ± 0.12) for nitrite. It seems that the permissible amount of nitrite-and nitrate-based additives in factories is under control. Non-heated-meat products are kept frozen and nitrite and nitrate are not added as additive or preservative to them. Thus, the higher levels of nitrite or nitrate in such products are considered fraud in food production.

Mirzaei *et al* showed that the amount of nitrate and nitrite added to the heat-treated meat products decreased during storage. However, the results of this study did not show significant difference in nitrite and nitrate levels during storage (Mirzaei *et al.*, 2007). There was no significant difference between the nitrite and nitrate content of the products and different percentages of meat. Another factor affecting the amount of nitrite was adding other additives to meat products. For example, when using ascorbic acid or erythrobic acid to release nitric oxide from nitrite, the bulk of nitrite is converted to nitric oxide and combined with myoglobin, sulfhydryl groups, lipids and proteins, leaving only a small fraction of it in the form of nitrite. Moreover, when nitrate is used as a preservative, the nitrate added to the product is gradually recovered under the influence of enzymatic agents and microorganisms and converted to nitrite (Babaei *et al.*, 2012). In a study carried out by Kamkar *et al.* on meat products supplied in Iranian stores in 2003 in terms of residual nitrite content, 4.4% of the tested samples contained levels of nitrite residual which were higher than the standard level (Kamkar *et al.*, 2004). Also, in another study in 2003, which examined the residual nitrite content in non-heat-treated meat products supplied in Tehran, there was no significant difference between the percentage of processed meat and its nitrate content (RezaieE *et al.*, 2004). In a 2007 study in Semnan province, which lasted for a maximum of one to two days after production, the meat products were in accordance with the Iranian national standard. The calculated mean level of nitrite was below the standard limit in the range of 7.79-55.16 mg/kg. Nitrite levels have also been shown to decrease over time (Nassehi Nia *et al.*, 2008). In this study, nitrite levels have been reduced over time, but other studies have reported an increase in N nitrosamines in parallel with a decrease in nitrite (Özbay and Şireli, 2021). In Babai's study of sodium nitrite residues in meat products produced in some cities of Mazandaran province in 2008, the mean level of nitrite residues in sausages and non-heat treated-meat products were 39.61, 9.29 and 20.50 mg/kg (Babaei *et al.*, 2012). Nitrite measurements in 180 samples of non-heat-treated meat products manufactured in 5 factories in Ahvaz in 2010 showed their compliance with the national standard permissible values (Fazlara *et al.*, 2012). Nitrate and nitrate levels in 34% and 30% of samples in Kermanshah province, respectively, were higher than the standard level and in the range of 96 to 168 mg/ kg, respectively (Samadbeik *et al.*, 2014). Another study showed that the residual concentrations of nitrite were between 3.3-94.9 ppm in heated- meat products in Tabriz, which was less than the approved national maximum residual limit (Hejazy, 2018).

In order to reduce nitrosamines concurrently with sodium nitrite use, additives such as sodium ascorbate, potassium sorbate, alpha-tocopherol, vitamin C, cysteine, and gallic acid have been recommended (Abd Hamid *et al.*, 2020, RezaieE *et al.*, 2004).Various studies have demonstrated that nitrate and nitrite content decreases with increasing of shelf life. However, it increases N nitrosamine levels (Nassehi Nia *et al.*, 2008, Refai and Sebaei, 2020).

Conclusion

This study suggests that frozen non-heat-treated meat products including hamburger and kebab produced or consumed in East Azerbaijan province did not have nitrate and nitrite additives during

[DOI: 10.18502/jnfs.v8i1.11774]

2015-2020. Continuous monitoring according to national standards are essential. Particularly in the case of food additives, it is necessary for keeping quality of in high level. Exceeding of food additives from the approved limits causes a threat to consumer's health. Continuous monitoring to ensure compliance of these products with the standards of meat products is necessary. This requires strict oversight of food hygiene authorities on manufacturing plants to ensure the quality of food products. The number of studies on nitrite and nitrate residues in the country should be increased. It is necessary to conduct similar studies to identify nitrite, nitrate and nitrosamine reducing agents.

Acknowledgments:

This study was carried out with financial and material support of University of Tabriz.

Author contributions

Hejazy M and Javid F designed the study and carried it and wrote the draft. Norouzi R wrote the manuscript and revised it. All authors read and approved the manuscript.

Conflicts of interest

There is no conflict of interest.

Reference

Abd Hamid NFH, Khan MM & Lim LH 2020. Assessment of nitrate, nitrite and chloride in selected cured meat products and their exposure to school children in Brunei Darussalam. *Journal of food composition and analysis.* **91**: 103520.

Babaei Z, Bagheri G, Salehifar E, Javadian B & Karimzadeh L 2012. Determination of residual nitrate and nitrite in meat products that were produced in some cities of Mazandaran province from autumn 2008 until winter 2009. . *Journal of Mazandaran University of medical science.* **21 (1)**: 228-233

Carter SJ, Gruber AH, Raglin JS, Baranauskas MN & Coggan AR 2020. Potential health effects of dietary nitrate supplementation in aging and chronic degenerative disease. *Medical hypotheses.* **141**: 109732.

Fazlara AS, Zand Moghaddam A, Loveimi M & Taheri M 2012. Evaluation of ash, sodium chloride and nitrite residues in sausages and hot sausages produced in Ahwaz and comparison with national standards of Iran. . *Journal of veterinary laboratory research.* **4 (2)**: 297-308.

Hejazy M 2018. Determination of nitrite residues in meat products marketed in Tabriz by spectrophotometric method. *Journal of food reearch (University of Tabriz)* **28 (1)**: 41-48.

Institute of Standards and Industrial Research of Iran 2020a. Determination of Nitrite in Meat and Meat Products.: Iran.

Institute of Standards and Industrial Research of Iran 2020b. Meat and meat products: Determination of Nitrate content (Reference method). . Iran.

Institute of Standards and Industrial Research of Iran (2000). Meat and meat products – sampling method. . Iran.

Kamkar A, Hosseini H, Alavi S & Bahonar A 2004. The study of nitrite residue in meat products consumed in Tehran in 2003. *Pajouhesh-va-Sazandegi.* **17 (2)**: 60-65.

Mirzaei H, Hosseini H & Rokni N 2007. Study of the Nitrite Reduction Curve in basins containing 90 and 40.60% meat during storage. *Iranian journal of food science and technology.* **4 (3)**: 41-45.

Müller W 1991. Curing nas smoking: are they healthier processes today than used to be *Fleischwirtschaft.* **71 (1)**: 61-65.

Nassehi Nia H, Mehdi Nia M, Ghorbani R & Norisepehr M 2008. Nitrite concentration in distributed sausage in Semnan province. Payesh. *Journal of The Iranian institute for health sciences research.* **7 (3)**: 202.

Özbay S & Şireli UT 2021. Volatile N-nitrosamines in processed meat products and salami from Turkey. *Food additives & contaminants: Part B.* **14 (2)**: 110-114.

Refai HM & Sebaei AS 2020. Daily intake of nitrate and nitrite via meat and poultry in Egypt. *Food additives & contaminants: Part B.* **13 (4)**: 292-297.

Rezaei M, Shariatifar N, Jahed Khaniki G & Javadzadeh M 2013. Nitrite in hamburgers in

[DOI: 10.18502/jnfs.v8i1.11774]

Arak, Iran. *Food additives & contaminants: Part B.* **6 (4)**: 285-288.

RezaieE M, et al. 2004. Determination of nitrite residues in meat products marketed in Iran by spectrophotometric method. *Journal of veterinary research.* **59 (2)**: 179-182.

Samadbeik M, Ahmadi M, Mohammadi A & Saravi BM 2014. Health information on internet: quality, importance, and popularity of persian health websites. *Iranian Red Crescent medical journal.* **16 (4)**.

Waltz P, Escobar D, Botero AM & Zuckerbraun BS 2015. Nitrate/nitrite as critical mediators to limit oxidative injury and inflammation. *Antioxidants & redox signaling.* **23 (4)**: 328-339.

[DOI: 10.18502/jnfs.v8i1.11774]