



# Volatile N-nitrosamine, residual nitrite, and ascorbic acid levels in sausages during storage

Houra Ramezani, Khadijeh Abhari<sup>1</sup>, Zahra Pilevar<sup>1</sup>, Hedayat Hosseini\*<sup>1</sup>, Abdorreza Mohammadi\*\*

Food Sciences and Technology Department, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

\* e-mail: [hedayat@sbmu.ac.ir](mailto:hedayat@sbmu.ac.ir)

\*\* e-mail: [ab.mohammadi@sbmu.ac.ir](mailto:ab.mohammadi@sbmu.ac.ir)

Received September 01, 2019; Accepted in revised form November 13, 2019; Published February 25, 2020

## Abstract:

**Introduction.** The increasing global consumption of processed meat products has led to certain concerns. For instance, processed meat products are known to contain carcinogen precursor compounds, thus creating the risk of chronic diseases. The present study was performed to estimate the food safety status of processed meat products available in Iran and evaluate the related effective factors.

**Study objects and methods.** 140 samples of seven most popular commercial types of cooked sausages were obtained from four major meat factories (A, B, C and D) in 140 samples were collected from seven most popular commercial types of cooked sausages as follows: beef salami 90%, chicken salami 90%, dry cured sausage 70%, dry cured salami 60%, beef sausages 55%, chicken sausages 55% and Frankfurt sausage 40% (n = 5) from four major meat factories (A, B, C and D) in Tehran. The samples were screened for residual nitrite, ascorbic acid, and nitrosamine contents on days 0, 7, 14, 21, and 28. The results indicated that products from meat factory B had lower residual nitrite content in the samples with high content of meat. Beef salami (90% of meat) and Frankfurt sausage (40% of meat) contained the lowest and highest amounts of residual nitrite on day 0 – 73.99 and 177.42 mg of nitrite per 1 kg of meat, respectively.

**Results and discussion.** Beef salami contained 90% of meat, chicken salami – 90%, dry cured sausage – 70%, dry cured salami – 60%, beef sausages – 55%, chicken sausages – 55%, and Frankfurt sausage – 40% (n = 5). Nitrite reduction rates in sausages with a smaller diameter, e.g. Frankfurt sausage, were significantly lower ( $P < 0.05$ ), compared to salami samples. The difference can be explained by the shorter cooking time. Nitrosamine formation increased during refrigerated storage; however, it was not significant in all samples. During refrigerated storage, nitrosamine formation depended on the level of added nitrite, the amount of residual nitrite, ascorbic acid, pH, and cooking temperature. Ascorbic acid content decreased significantly ( $P < 0.05$ ) during refrigerated storage.

**Conclusion.** The findings demonstrate significant correlation between the meat content, cooking time, nitrite content, and nitrosamine formation.

**Keywords:** Meat industry, processed meat, meat products safety, carcinogenic agents, preservation

**Funding:** This research was financially supported by National Nutrition and Food Technology Research Institute (NNFTRI) of Iran.

**Please cite this article in press as:** Ramezani H, Abhari K, Pilevar Z, Hosseini H, Mohammadi A. Volatile N-nitrosamine, residual nitrite, and ascorbic acid levels in sausages during storage. *Foods and Raw Materials*. 2020;8(1):107–114. DOI: <http://doi.org/10.21603/2308-4057-2020-1-107-114>.

## INTRODUCTION

To reduce the adverse effects of red meat and artificial additives on human health, one can reduce the consumption of red meat or processed meat products. However, studying possible ways to reduce the harmful effects might be as effective as limiting consumption levels. Nitrite/nitrate salts are usually added to meat products to guarantee their safety, since these salts inhibit the growth of *Clostridium botulinum* and prevent

heat-resistant spores from producing toxins. Moreover, nitrite/nitrate salts improve the color, flavor, and aroma of the finished product and postpone lipid oxidation processes [1].

In spite of the numerous advantages, an excessive intake of nitrite can produce adverse effects on human health. Nitrite can be transformed to a nitrosating agent (NO<sup>+</sup>), which reacts with biogenic amines and creates carcinogenic N-nitrosamines. Bacterial and meat

enzymes cause decarboxylation of free amino acids, which leads to the formation of biogenic amines. Several intrinsic and extrinsic factors, e.g. salt content, can also affect the biogenic amine formation level [2–4].

In meat and meat products, carcinogenic N-nitrosamines are usually formed under acidic conditions caused by the electrophilic reaction between a nitrosating agent, e.g. nitrite or nitrous acid, and secondary/tertiary amines that result from protein and lipid degradation [5]. The mechanism of nitrosamine formation in meat products has been thoroughly studied. Nitrosamines with generic chemical structure of  $R_2NN = O$  are produced under certain conditions of low pH, high temperature, and presence of some reducing agents associated with processing and composition of a particular meat product [6]. There are several hurdle technologies that can reduce the concentration of nitrite required to inhibit bacterial growth, e.g. ascorbic/erythorbic (isoascorbic) acid and essential oils, certain processing conditions, or various non-thermal methods [1, 7]. Exposure level of N-nitrosodimethylamine (NDMA) compound via consumption of food and beverages was estimated to be 0.09 and 0.1  $\mu\text{g}/\text{day}$  in the Netherlands and Germany, respectively [8, 9]. The level of these compounds in nitrite-preserved meat products varies significantly. It depends on the ingoing volume of nitrite, meat quality, and fat content, as well as on processing, ripening, and storing conditions. There have been many reports on nitrosamines detected in processed meat products [10, 11].

The research objective was to examine the safety of emulsion-type cooked sausages available on the Iran markets by assessing the contents of residual nitrite, ascorbic acid, and nitrosamine. In addition, we also studied the effects of processing conditions and related factors on these compounds to provide information for health professionals and food manufacturers.

## STUDY OBJECTS AND METHODS

**Samples.** The samples were collected from four major meat factories in Tehran (A, B, C, and D) out of the total of 189 meat factories in Iran. Seven most popular commercial types of cooked sausages were randomly purchased. Seven most popular commercial types of cooked sausages were randomly purchased as follows: beef salami 90%, chicken salami 90%, dry cured sausage 70%, dry cured salami 60%, beef sausages 55%, chicken sausages 55% and Frankfurt sausage 40%.

Five samples (2 kg) from each type were examined on days 0, 7, 14, 21, and 28 of storage. The samples contained beef (15% fat) or chicken (10% fat), water, oil, sodium caseinate, sodium polyphosphate, garlic, salt, wheat flour starch, spices, gluten, natural flavorings (paprika, curcumin, ginger, and cinnamon), ascorbic acid, and sodium nitrite. The samples were immediately transferred to the laboratory and kept refrigerated until tested.

**Residual nitrite determination.** The nitrite content was evaluated during 28 days on days 0, 7, 14, 21, and 28 using slightly modified calorimetric method of AOAC method no. 973.31 [12]. The samples were cut into pieces and homogenized. An aliquot of about 2.5 g of minced sausage was added to 5 mL of saturated borax and 25 mL of deionized water ( $> 70^\circ\text{C}$ ) in Falcon tubes. After stirring and cooling in room temperature, 1 mL of each Carrez solution (I and II) was added to each sample and adjusted to the volume of 50 mL. Carrez solution I was prepared by dissolving 10.6 g of ferrocyanide in distilled water (100 mL) according to the method introduced by Ramezani *et al.* [13]. Carrez solution II was also made by mixing 21.9 g of zinc acetate with acetic acid (3 mL) and adjusted to the volume of 100 mL using distilled water. After centrifugation at 4000 rpm for 5 min (Hettich Rotorfix 32A), 25 mL of supernatant was transferred to a 100 mL tube. Ten mL of sulfanilamide reagent and 6 mL of dilute HCl were added to the supernatants and kept in the dark for 5 min. Then, 2 mL of naphthyl-ethylenediamine solution was added to obtain high intensity azo dyes. The samples were kept in the dark for 10 min to achieve complete reactions. Absorbance was measured at 538 nm.

**Ascorbic acid determination.** To determine the ascorbic acid content, 2 g of minced sausage was mixed with 10 mL of meta-phosphoric acid solution (3%), tertiary butylhydroquinone (TBHQ) (0.1%), and acetic acid (8%). After that, they were centrifuged at 4000 rpm for 10 min. After filtration, 20  $\mu\text{L}$  of supernatant solution was injected to a Cecil CE-4900 high-performance liquid chromatograph coupled to a UV-vis detector (HPLC-UV/VIS, Cambridge, England). The analytical HPLC was equipped with two CE-4100 pumps, vacuum degasser, six port valves (Rheodyne, USA), mixing chamber, multiple solvent delivery unit, and an ODS column (250 mm  $\times$  4 I.D., 5  $\mu\text{m}$ ). The mobile phase consisted of acetonitrile and sodium phosphate buffer solution (50:50). The flow rate was 1.2  $\text{mL}\cdot\text{min}^{-1}$  at room temperature [14].

**N-nitrosamine determination.** The experiment evaluated seven volatile nitrosamines in the popular cooked sausages from four major Iran meat factories. The nitrosamines included N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomorpholine (NMOR), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosodi-n-butylamine (NDBA), and N-nitrosodiphenylamine (NDPheA). The presence of nitrosamine was determined and quantified according to the method described in our previous study [13]. The method presupposed using microwave-assisted extraction coupled with dispersive liquid-liquid micro extraction (DLLME) followed by gas chromatography–mass spectrometry (GC-MS).

**Statistical analysis.** All experiments were carried out in triplicate. One-way ANOVA was performed to determine significant differences. Duncan's multiple

range test was used to define the differences of mean value. Data analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA);  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

The present research featured samples from seven most popular types of sausages in Iran. The selected sausages differed in the amount of nitrite, ascorbic acid, and nitrosamine. This difference is associated with the variations in concentration of added nitrate and nitrite salts (sodium and potassium), storage conditions, and different pH values [15, 16]. Following the EU legislations, 2006/52/EC directive limited the usage of nitrite to 150 mg per 1 kg of meat. However, the national laws in Iran are more restricting. Iranian provisions allow for maximum 120 mg of nitrite per 1 kg of meat (ppm) in sausages [17]. Table 1 displays the residual nitrite, ascorbic acid, and nitrosamine contents in the meat samples under study during storage.

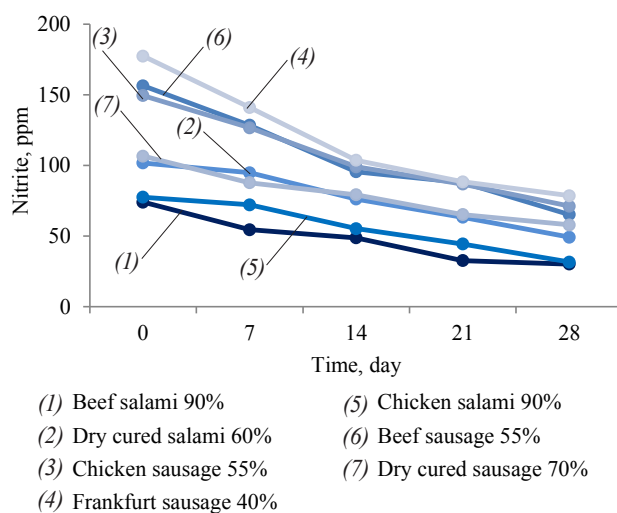
Figure 1 illustrates the results of nitrite content changes (mg/kg meat) in the meat samples obtained from factory B as representative of all the four meat factories.

As it was expected, the amount of nitrite residue in meat products of all four meat factories decreased during 28 days of refrigerated storage. The increase in meat content increased the reduction rate of sodium nitrite in the products. In other words, lower residual nitrite content was detected in high content meat samples. The highest reduction rate of nitrite content was observed in chicken and beef salami (90% of meat). On the first day of refrigerated storage, the Frankfurt sausage (40%) and both beef and chicken sausages (55%) contained 80 µg/kg of nitrite.

The detected amount exceeded the level permitted by Iranian laws as defined by the Institute of Standard and Industrial Research of Iran (ISIRI). The maximum permissible burden of nitrite is 66 and 63 µg/kg for processed meat products with 40% and 55% of meat content, respectively<sup>1</sup>. The concentration of nitrite was higher than the expected levels even after 28 days of storage. However, the residual nitrite content was not exactly equal to the initial added nitrite. First, it was partly degraded by heating process. Second, it decreased when ascorbic acid was applied to the meat product during the heating process to accelerate conversion of nitrite to nitric oxide.

The nitrite content varied due to interaction with heme-containing components, non-heme proteins, and fat tissues, conversion to nitrate, production of such gases as  $N_2$ ,  $CO_2$ , and NO, and nitrosamines [18]. Therefore, residual nitrite was associated with meat content due to different contents of myoglobin [19]. As a reactive agent, nitrite converts to nitrite oxide and forms

<sup>1</sup> ISIRI 932. Test method for determination of nitrite in meat and meat products (reference method). Iran: Iran Institute of Standards and Industrial Research; 2014.



**Figure 1** Residual nitrite content (mg/kg meat) in sausages with different meat content from factory B on days 0, 7, 14, 21, and 28

nitrite-heme-nitrosomyoglobin complex with myoglobin, thus producing nitrosomyochromogen. The latter is responsible for the characteristic bright pink color of cured meat products [20].

Hence, higher content of myoglobin results in lower nitrite content in the final meat product. The results obtained by statistical analysis indicate that residual nitrite in Frankfurt sausage (40%) was significantly higher ( $P < 0.05$ ), whereas in beef salami (90%) it was at its lowest. No significant differences were observed in other sausage and salami products ( $P > 0.05$ ).

Ascorbic acid, or ascorbate, is applied to meat products as an additive with high water solubility for three major reasons. First, the nitrosomyoglobin-forming reduction of nitrite to nitric oxide produces the required color. Second, the antioxidative activity of ascorbic acid slows down oxidation of pigments and lipids, which results in color and flavor stability. Third, residual nitrite decreases due to binding to nitrite in heated samples [21].

Ascorbate proved effective in nitrosamine inhibition. This quality is associated with rapid reactions of ascorbate with nitric oxide compared to nitrosating agents, e.g. amines. Therefore, nitrosamines are formed when the reaction rate constant of ascorbate is not much larger than amines [16]. Figure 2 and Table 1 demonstrate that ascorbic acid content of samples decreased significantly ( $P < 0.05$ ) during refrigerated storage.

Meat products with higher meat content exhibited lower ascorbic acid reduction rate, which was due to lower residual nitrite content. Ascorbic acid degradation in meat products could be related to oxygen content, temperature, light, water activity, presence of metal ions, e.g. copper and ferric iron, and storage time [22–24]. Degradation of ascorbic acid is increased in acidic conditions (pH = 3.3–5.5). In the current study,

**Table 1.** Residual nitrite, ascorbic acid and nitrosamine contents in sausages produced by four major meat factories during storage

Factory A		Day 0	Day 7	Day 14	Day 21	Day 28
Beef salami (90% of meat)	Nitrite, mg/kg	67.91 ± 0.605 <sup>a</sup>	59.95 ± 0.360 <sup>b</sup>	46.06 ± 0.326 <sup>c</sup>	27.75 ± 0.119 <sup>d</sup>	16.07 ± 0.294 <sup>e</sup>
	Nitrosamine, ng/g	5.43 ± 0.004 <sup>a</sup>	6.71 ± 2.887 <sup>b</sup>	13.44 ± 0.033 <sup>c</sup>	17.25 ± 0.563 <sup>d</sup>	22.01 ± 0.038 <sup>e</sup>
	Ascorbic acid, ng/g	214.08 ± 0.262 <sup>a</sup>	216.81 ± 0.026 <sup>a</sup>	194.98 ± 0.018 <sup>b</sup>	171.95 ± 0.017 <sup>c</sup>	167.82 ± 0.026 <sup>c</sup>
Chicken salami (90% of meat)	Nitrite, mg/kg	73.20 ± 0.376 <sup>a</sup>	62.90 ± 0.134 <sup>b</sup>	51.72 ± 0.226 <sup>c</sup>	33.00 ± 0.156 <sup>d</sup>	17.75 ± 0.356 <sup>e</sup>
	Nitrosamine, ng/g	6.30 ± 0.135 <sup>a</sup>	10.91 ± 0.173 <sup>b</sup>	16.47 ± 0.610 <sup>c</sup>	18.68 ± 0.856 <sup>c</sup>	22.44 ± 0.616 <sup>d</sup>
	Ascorbic acid, ng/g	175.39 ± 0.039 <sup>a</sup>	168.53 ± 0.003 <sup>a</sup>	159.21 ± 0.027 <sup>b</sup>	140.84 ± 0.039 <sup>c</sup>	134.40 ± 0.021 <sup>d</sup>
Dry cured salami (60% of meat)	Nitrite, mg/kg	90.93 ± 1.036 <sup>a</sup>	85.00 ± 0.457 <sup>b</sup>	72.64 ± 0.678 <sup>c</sup>	61.36 ± 0.719 <sup>d</sup>	36.73 ± 0.381 <sup>e</sup>
	Nitrosamine, ng/g	6.93 ± 0.0561 <sup>a</sup>	14.77 ± 0.037 <sup>b</sup>	18.03 ± 0.060 <sup>c</sup>	21.82 ± 0.518 <sup>d</sup>	24.67 ± 0.091 <sup>e</sup>
	Ascorbic acid, ng/g	185.86 ± 0.021 <sup>a</sup>	180.79 ± 0.003 <sup>b</sup>	172.05 ± 0.062 <sup>c</sup>	168.86 ± 0.683 <sup>d</sup>	162.35 ± 0.706 <sup>d</sup>
Beef sausage (55% of meat)	Nitrite, mg/kg	181.80 ± 0.274 <sup>a</sup>	137.28 ± 0.760 <sup>b</sup>	108.10 ± 0.716 <sup>c</sup>	83.97 ± 0.080 <sup>d</sup>	70.78 ± 0.387 <sup>e</sup>
	Nitrosamine, ng/g	14.47 ± 0.399 <sup>a</sup>	24.80 ± 1.06 <sup>b</sup>	32.31 ± 0.389 <sup>c</sup>	33.60 ± 0.566 <sup>c</sup>	42.52 ± 1.077 <sup>d</sup>
	Ascorbic acid, ng/g	198.22 ± 0.149 <sup>a</sup>	203.91 ± 0.057 <sup>a</sup>	196.78 ± 0.031 <sup>a</sup>	182.49 ± 0.033 <sup>b</sup>	175.88 ± 0.018 <sup>c</sup>
Chicken sausage (55% of meat)	Nitrite, mg/kg	187.42 ± 0.315 <sup>a</sup>	145.40 ± 0.447 <sup>b</sup>	114.00 ± 0.151 <sup>c</sup>	91.21 ± 1.279 <sup>d</sup>	76.29 ± 0.390 <sup>e</sup>
	Nitrosamine, ng/g	7.71 ± 0.075 <sup>a</sup>	25.96 ± 0.1063 <sup>b</sup>	33.32 ± 0.053 <sup>c</sup>	34.29 ± 0.067 <sup>c</sup>	41.91 ± 0.0167 <sup>d</sup>
	Ascorbic acid, ng/g	165.27 ± 0.009 <sup>a</sup>	163.34 ± 0.009 <sup>a</sup>	161.99 ± 0.024 <sup>a</sup>	158.26 ± 0.072 <sup>b</sup>	149.46 ± 0.031 <sup>c</sup>
Dry cured sausage (70% of meat)	Nitrite, mg/kg	82.11 ± 0.757 <sup>a</sup>	69.78 ± 0.758 <sup>b</sup>	54.94 ± 0.115 <sup>c</sup>	46.35 ± 0.314 <sup>d</sup>	39.79 ± 1.206 <sup>e</sup>
	Nitrosamine, ng/g	14.32 ± 0.052 <sup>a</sup>	15.36 ± 0.012 <sup>a</sup>	17.86 ± 0.083 <sup>ab</sup>	20.81 ± 0.765 <sup>b</sup>	21.55 ± 0.045 <sup>b</sup>
	Ascorbic acid, ng/g	129.67 ± 0.416 <sup>a</sup>	120.55 ± 0.007 <sup>ab</sup>	117.99 ± 0.033 <sup>b</sup>	106.79 ± 0.039 <sup>c</sup>	105.82 ± 0.705 <sup>c</sup>
Factory B		Day 0	Day 7	Day 14	Day 21	Day 28
Beef salami (90% of meat)	Nitrite, mg/kg	73.99 ± 0.011 <sup>a</sup>	54.53 ± 0.130 <sup>b</sup>	48.76 ± 0.649 <sup>c</sup>	32.60 ± 0.626 <sup>d</sup>	30.20 ± 0.598 <sup>d</sup>
	Nitrosamine, ng/g	3.06 ± 0.058 <sup>a</sup>	5.13 ± 0.204 <sup>ab</sup>	7.05 ± 0.284 <sup>b</sup>	13.40 ± 0.122 <sup>c</sup>	14.18 ± 0.1054 <sup>c</sup>
	Ascorbic acid, ng/g	194.99 ± 0.016 <sup>a</sup>	192.30 ± 0.021 <sup>a</sup>	165.00 ± 0.699 <sup>b</sup>	144.62 ± 0.004 <sup>c</sup>	121.60 ± 0.060 <sup>d</sup>
Chicken salami (90% of meat)	Nitrite, mg/kg	77.55 ± 0.556 <sup>a</sup>	72.12 ± 0.505 <sup>a</sup>	55.24 ± 0.247 <sup>b</sup>	44.41 ± 0.830 <sup>c</sup>	31.62 ± 0.347 <sup>d</sup>
	Nitrosamine, ng/g	4.85 ± 0.045 <sup>a</sup>	4.83 ± 0.018 <sup>a</sup>	6.43 ± 0.09 <sup>a</sup>	11.43 ± 0.065 <sup>b</sup>	14.81 ± 0.031 <sup>b</sup>
	Ascorbic acid, ng/g	220.79 ± 0.022 <sup>a</sup>	192.84 ± 0.554 <sup>b</sup>	168.95 ± 0.134 <sup>c</sup>	164.89 ± 0.025 <sup>c</sup>	134.07 ± 0.492 <sup>d</sup>
Dry cured salami (60% of meat)	Nitrite, mg/kg	101.79 ± 0.290 <sup>a</sup>	94.85 ± 0.362 <sup>a</sup>	76.18 ± 0.359 <sup>b</sup>	63.30 ± 0.244 <sup>c</sup>	49.24 ± 0.396 <sup>d</sup>
	Nitrosamine, ng/g	11.93 ± 0.081 <sup>a</sup>	15.20 ± 0.256 <sup>ab</sup>	15.32 ± 0.298 <sup>ab</sup>	21.68 ± 0.307 <sup>b</sup>	28.38 ± 0.575 <sup>c</sup>
	Ascorbic acid, ng/g	201.24 ± 0.127 <sup>a</sup>	188.65 ± 0.038 <sup>b</sup>	172.34 ± 0.401 <sup>c</sup>	168.01 ± 0.002 <sup>d</sup>	145.69 ± 0.018 <sup>e</sup>
Beef sausage (55% of meat)	Nitrite, mg/kg	156.45 ± 0.774 <sup>a</sup>	128.46 ± 0.637 <sup>b</sup>	95.54 ± 0.323 <sup>c</sup>	87.82 ± 0.296 <sup>d</sup>	65.26 ± 0.971 <sup>e</sup>
	Nitrosamine, ng/g	13.21 ± 0.057 <sup>a</sup>	14.94 ± 0.04 <sup>a</sup>	23.64 ± 0.075 <sup>b</sup>	29.54 ± 0.94 <sup>c</sup>	37.73 ± 0.059 <sup>d</sup>
	Ascorbic acid, ng/g	200.90 ± 0.132 <sup>a</sup>	193.71 ± 0.015 <sup>ab</sup>	187.60 ± 0.345 <sup>b</sup>	183.60 ± 0.031 <sup>b</sup>	174.74 ± 0.297 <sup>c</sup>
Chicken sausage (55% of meat)	Nitrite, mg/kg	149.47 ± 0.197 <sup>a</sup>	126.70 ± 0.516 <sup>b</sup>	98.98 ± 0.708 <sup>c</sup>	86.83 ± 0.681 <sup>d</sup>	71.21 ± 0.192 <sup>e</sup>
	Nitrosamine, ng/g	7.85 ± 0.118 <sup>a</sup>	14.70 ± 0.077 <sup>b</sup>	24.73 ± 0.053 <sup>c</sup>	29.77 ± 2.93 <sup>d</sup>	37.99 ± 0.133 <sup>e</sup>
	Ascorbic acid, ng/g	181.71 ± 0.014 <sup>a</sup>	160.27 ± 0.020 <sup>b</sup>	138.63 ± 0.013 <sup>c</sup>	121.13 ± 0.003 <sup>d</sup>	104.42 ± 0.005 <sup>e</sup>
Dry cured sausage (70% of meat)	Nitrite, mg/kg	106.62 ± 0.277 <sup>a</sup>	87.71 ± 0.850 <sup>b</sup>	79.26 ± 0.211 <sup>c</sup>	65.21 ± 0.306 <sup>d</sup>	57.90 ± 0.226 <sup>e</sup>
	Nitrosamine, ng/g	8.18 ± 0.202 <sup>a</sup>	9.71 ± 0.469 <sup>a</sup>	15.22 ± 0.085 <sup>b</sup>	25.88 ± 0.1589 <sup>c</sup>	33.16 ± 0.622 <sup>d</sup>
	Ascorbic acid, ng/g	197.86 ± 0.215 <sup>a</sup>	156.10 ± 0.021 <sup>b</sup>	131.45 ± 0.073 <sup>c</sup>	127.63 ± 0.015 <sup>d</sup>	126.30 ± 0.007 <sup>d</sup>
Frankfurt sausage (40% of meat)	Nitrite, mg/kg	177.42 ± 0.677 <sup>a</sup>	141.07 ± 0.075 <sup>b</sup>	103.64 ± 0.280 <sup>c</sup>	88.45 ± 0.662 <sup>d</sup>	78.61 ± 0.710 <sup>e</sup>
	Nitrosamine, ng/g	14.34 ± 0.05 <sup>a</sup>	16.12 ± 0.6746 <sup>b</sup>	27.20 ± 0.116 <sup>c</sup>	34.97 ± 0.202 <sup>c</sup>	45.06 ± 0.229 <sup>d</sup>
	Ascorbic acid, ng/g	192.57 ± 0.062 <sup>a</sup>	187.08 ± 0.032 <sup>b</sup>	169.47 ± 0.057 <sup>c</sup>	155.29 ± 0.008 <sup>d</sup>	137.55 ± 0.006 <sup>e</sup>
Factory C		Day 0	Day 7	Day 14	Day 21	Day 28
Beef salami (90% of meat)	Nitrite, mg/kg	59.08 ± 0.199 <sup>a</sup>	35.41 ± 0.347 <sup>b</sup>	25.26 ± 0.952 <sup>c</sup>	18.25 ± 0.297 <sup>d</sup>	8.42 ± 0.088 <sup>e</sup>
	Nitrosamine, ng/g	2.65 ± 0.0123 <sup>a</sup>	8.37 ± 0.058 <sup>b</sup>	15.51 ± 0.021 <sup>c</sup>	19.27 ± 0.057 <sup>c</sup>	33.83 ± 0.123 <sup>d</sup>
	Ascorbic acid, ng/g	232.27 ± 0.0321 <sup>a</sup>	187.13 ± 0.060 <sup>b</sup>	135.76 ± 0.025 <sup>c</sup>	122.71 ± 0.031 <sup>cd</sup>	108.52 ± 0.026 <sup>d</sup>
Chicken salami (90% of meat)	Nitrite, mg/kg	62.90 ± 0.220 <sup>a</sup>	50.30 ± 0.589 <sup>b</sup>	36.67 ± 0.347 <sup>c</sup>	19.66 ± 0.714 <sup>d</sup>	9.26 ± 0.076 <sup>d</sup>
	Nitrosamine, ng/g	2.78 ± 0.071 <sup>a</sup>	9.68 ± 0.078 <sup>b</sup>	14.47 ± 0.064 <sup>c</sup>	18.59 ± 0.083 <sup>d</sup>	25.34 ± 0.117 <sup>e</sup>
	Ascorbic acid, ng/g	162.54 ± 0.055 <sup>a</sup>	155.95 ± 0.086 <sup>b</sup>	121.19 ± 0.060 <sup>c</sup>	103.93 ± 0.112 <sup>d</sup>	92.68 ± 0.049 <sup>e</sup>
Dry cured salami (60% of meat)	Nitrite, mg/kg	69.96 ± 0.912 <sup>a</sup>	65.00 ± 0.721 <sup>a</sup>	50.22 ± 0.977 <sup>ab</sup>	36.57 ± 0.386 <sup>b</sup>	19.17 ± 0.448 <sup>c</sup>
	Nitrosamine, ng/g	3.51 ± 0.007 <sup>a</sup>	11.91 ± 0.042 <sup>b</sup>	19.61 ± 0.047 <sup>c</sup>	22.57 ± 0.052 <sup>d</sup>	23.76 ± 0.052 <sup>d</sup>
	Ascorbic acid, ng/g	248.29 ± 0.004 <sup>a</sup>	213.54 ± 0.630 <sup>b</sup>	198.32 ± 0.016 <sup>b</sup>	165.78 ± 0.032 <sup>c</sup>	119.79 ± 0.296 <sup>d</sup>
Beef sausage (55% of meat)	Nitrite, mg/kg	107.41 ± 0.682 <sup>a</sup>	92.93 ± 0.114 <sup>ab</sup>	85.60 ± 1.314 <sup>b</sup>	64.39 ± 0.142 <sup>c</sup>	31.88 ± 0.397 <sup>d</sup>
	Nitrosamine, ng/g	8.61 ± 0.157 <sup>a</sup>	17.01 ± 0.05 <sup>b</sup>	20.51 ± 0.547 <sup>b</sup>	34.51 ± 0.068 <sup>c</sup>	44.20 ± 0.051 <sup>d</sup>
	Ascorbic acid, ng/g	200.69 ± 0.45 <sup>a</sup>	196.17 ± 0.057 <sup>a</sup>	189.30 ± 0.047 <sup>ab</sup>	175.08 ± 0.107 <sup>b</sup>	142.58 ± 0.117 <sup>c</sup>
Chicken sausage (55% of meat)	Nitrite, mg/kg	116.20 ± 0.725 <sup>a</sup>	93.36 ± 0.354 <sup>ab</sup>	81.96 ± 0.279 <sup>b</sup>	55.90 ± 0.374 <sup>c</sup>	47.44 ± 0.658 <sup>d</sup>
	Nitrosamine, ng/g	9.85 ± 0.011 <sup>a</sup>	16.19 ± 0.145 <sup>ab</sup>	20.51 ± 0.547 <sup>b</sup>	35.51 ± 0.068 <sup>c</sup>	44.20 ± 0.051 <sup>d</sup>
	Ascorbic acid, ng/g	199.63 ± 0.588 <sup>a</sup>	184.59 ± 0.068 <sup>b</sup>	165.67 ± 1.013 <sup>c</sup>	102.61 ± 0.173 <sup>d</sup>	79.79 ± 0.033 <sup>e</sup>

Continuation of the table 1

Dry cured sausage (70% of meat)	Nitrite, mg/kg	84.48 ± 0.188 <sup>a</sup>	71.59 ± 0.763 <sup>b</sup>	57.72 ± 0.274 <sup>c</sup>	50.18 ± 1.247 <sup>c</sup>	27.29 ± 0.242 <sup>d</sup>
	Nitrosamine, ng/g	10.05 ± 0.033 <sup>a</sup>	10.35 ± 0.048 <sup>a</sup>	17.68 ± 0.031 <sup>b</sup>	20.81 ± 0.080 <sup>c</sup>	23.44 ± 0.037 <sup>d</sup>
	Ascorbic acid, ng/g	149.83 ± 0.017 <sup>a</sup>	114.28 ± 0.177 <sup>b</sup>	103.91 ± 0.122 <sup>c</sup>	89.73 ± 0.145 <sup>d</sup>	71.32 ± 0.035 <sup>e</sup>
Frankfurt sausage (40% of meat)	Nitrite, mg/kg	135.79 ± 1.314 <sup>a</sup>	98.41 ± 1.405 <sup>b</sup>	82.62 ± 0.592 <sup>c</sup>	80.51 ± 0.754 <sup>c</sup>	52.84 ± 0.489 <sup>d</sup>
	Nitrosamine, ng/g	10.05 ± 0.087 <sup>a</sup>	17.95 ± 0.086 <sup>b</sup>	24.09 ± 0.105 <sup>c</sup>	37.80 ± 0.065 <sup>d</sup>	41.32 ± 0.090 <sup>e</sup>
	Ascorbic acid, ng/g	141.12 ± 0.059 <sup>a</sup>	138.09 ± 0.012 <sup>a</sup>	102.27 ± 0.027 <sup>b</sup>	91.43 ± 0.068 <sup>b</sup>	74.10 ± 0.707 <sup>c</sup>
Factory D		Day 0	Day 7	Day 14	Day 28	
Beef salami (90% of meat)	Nitrite, mg/kg	96.65 ± 0.646 <sup>a</sup>	75.59 ± 0.754 <sup>b</sup>	59.11 ± 1.413 <sup>c</sup>	52.95 ± 1.361 <sup>cd</sup>	47.94 ± 0.628 <sup>d</sup>
	Nitrosamine, ng/g	28.96 ± 0.026 <sup>a</sup>	34.52 ± 0.042 <sup>ab</sup>	39.02 ± 0.054 <sup>b</sup>	44.38 ± 0.048 <sup>bc</sup>	49.52 ± 0.077 <sup>c</sup>
	Ascorbic acid, ng/g	233.43 ± 0.202 <sup>a</sup>	195.42 ± 0.054 <sup>b</sup>	163.34 ± 0.095 <sup>c</sup>	157.90 ± 0.158 <sup>c</sup>	132.90 ± 0.167 <sup>d</sup>
Dry cured salami (60% of meat)	Nitrite, mg/kg	101.88 ± 0.611 <sup>a</sup>	90.53 ± 0.437 <sup>a</sup>	78.70 ± 0.650 <sup>b</sup>	66.24 ± 0.271 <sup>c</sup>	53.22 ± 0.917 <sup>d</sup>
	Nitrosamine, ng/g	31.53 ± 0.017 <sup>a</sup>	35.12 ± 0.051 <sup>ab</sup>	39.81 ± 0.029 <sup>b</sup>	44.46 ± 0.036 <sup>c</sup>	48.46 ± 0.036 <sup>c</sup>
	Ascorbic acid, ng/g	169.07 ± 0.115 <sup>a</sup>	157.47 ± 0.032 <sup>b</sup>	114.25 ± 0.174 <sup>c</sup>	103.32 ± 0.151 <sup>d</sup>	89.92 ± 0.341 <sup>e</sup>
Beef sausage (55% of meat)	Nitrite, mg/kg	114.24 ± 0.862 <sup>a</sup>	99.66 ± 0.920 <sup>ab</sup>	86.06 ± 0.526 <sup>b</sup>	73.10 ± 1.452 <sup>c</sup>	61.39 ± 0.122 <sup>d</sup>
	Nitrosamine, ng/g	38.02 ± 0.031 <sup>a</sup>	74.74 ± 0.136 <sup>b</sup>	94.90 ± 0.021 <sup>c</sup>	124.58 ± 0.033 <sup>d</sup>	131.558 ± 0.063 <sup>d</sup>
	Ascorbic acid, ng/g	198.59 ± 0.035 <sup>a</sup>	104.30 ± 0.240 <sup>b</sup>	54.95 ± 0.079 <sup>c</sup>	35.61 ± 0.162 <sup>d</sup>	29.11 ± 0.452 <sup>d</sup>
Chicken sausage (55% of meat)	Nitrite, mg/kg	143.09 ± 0.787 <sup>a</sup>	127.56 ± 0.488 <sup>b</sup>	98.24 ± 0.196 <sup>c</sup>	72.37 ± 0.808 <sup>d</sup>	55.95 ± 0.069 <sup>e</sup>
	Nitrosamine, ng/g	38.02 ± 0.562 <sup>a</sup>	68.88 ± 0.125 <sup>b</sup>	70.90 ± 0.152 <sup>b</sup>	85.94 ± 0.099 <sup>c</sup>	89.91 ± 0.059 <sup>c</sup>
	Ascorbic acid, ng/g	201.10 ± 0.351 <sup>a</sup>	173.31 ± 0.494 <sup>b</sup>	138.33 ± 0.442 <sup>c</sup>	90.33 ± 0.953 <sup>d</sup>	68.82 ± 0.721 <sup>d</sup>
Frankfurt sausage (40% of meat)	Nitrite, mg/kg	150.45 ± 1.003 <sup>a</sup>	135.95 ± 0.765 <sup>b</sup>	109.52 ± 1.216 <sup>c</sup>	97.55 ± 0.084 <sup>d</sup>	80.41 ± 0.784 <sup>e</sup>
	Nitrosamine, ng/g	12.91 ± 0.085 <sup>a</sup>	38.73 ± 0.039 <sup>b</sup>	100.42 ± 0.093 <sup>c</sup>	153.60 ± 0.253 <sup>d</sup>	178.60 ± 0.293 <sup>e</sup>
	Ascorbic acid, ng/g	282.14 ± 0.310 <sup>a</sup>	181.60 ± 0.025 <sup>b</sup>	170.03 ± 0.169 <sup>c</sup>	117.97 ± 0.037 <sup>d</sup>	75.22 ± 0.897 <sup>e</sup>

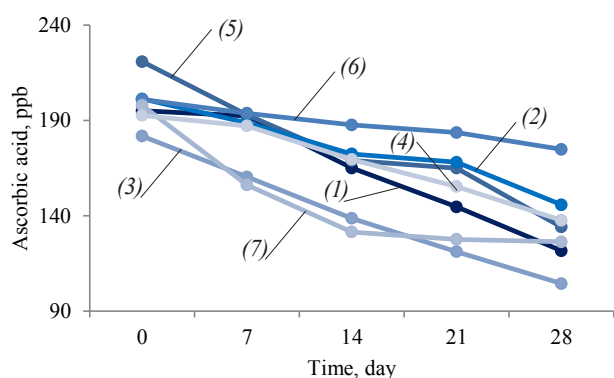
Different letters in the same row during storage within the same section (i.e. factories A-D) indicate a significant difference ( $P < 0.05$ )

pH values were in the range of 5–6 in all samples. Therefore, ascorbic acid degradation might have been due to the relative acidic condition of meat products and heating process.

The obtained results showed an increase in nitrosamine content of samples during refrigerated storage. However, it was not significant in all samples (Fig. 3).

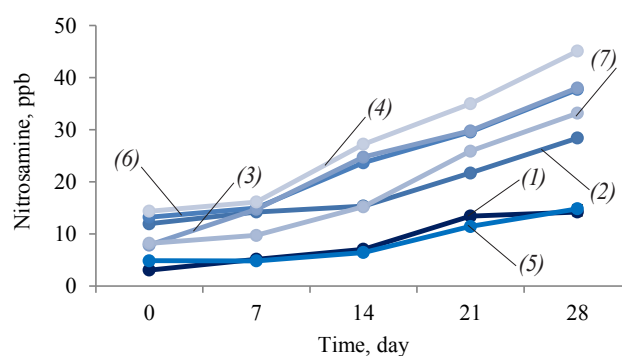
The nitrite reduction rates in the sausages with a smaller diameter, e.g. Frankfurt sausage, appeared significantly lower ( $P < 0.05$ ) than in the samples with a bigger diameter, e.g. salami. This difference can be

explained by the longer cooking time for salami (5–6 h) compared to Frankfurt sausages (3–4 h), which is associated with a higher reduction rate of residual nitrite. According to the results, the total volatile nitrosamine level of Iranian meat products with lower meat contents was generally higher than that of samples with high meat contents. The nitrosamine content in meat products was associated with the added nitrite and the residual nitrite [25]. The residual nitrite is a reactive agent that can be reduced by heating treatment or exposure to such meat components as proteins, lipids, and pigments [26, 27].



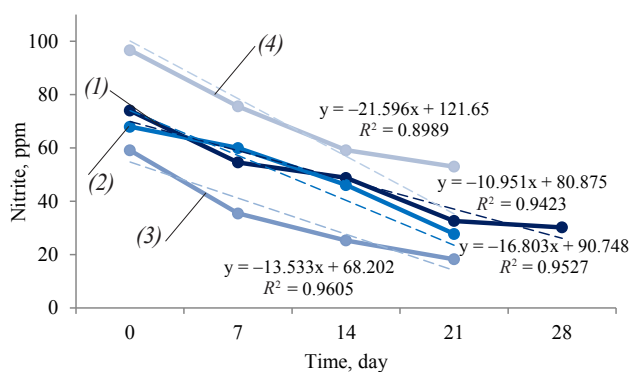
- (1) Beef salami 90%                      (5) Chicken salami 90%  
 (2) Dry cured salami 60%              (6) Beef sausage 55%  
 (3) Chicken sausage 55%                (7) Dry cured sausage 70%  
 (4) Frankfurt sausage 40%

**Figure 2** Ascorbic acid content (ng/kg meat) in sausages with different meat content from factory B on days 0, 7, 14, 21, and 28



- (1) Beef salami 90%                      (5) Chicken salami 90%  
 (2) Dry cured salami 60%              (6) Beef sausage 55%  
 (3) Chicken sausage 55%                (7) Dry cured sausage 70%  
 (4) Frankfurt sausage 40%

**Figure 3** Nitrosamine content (ng/kg of meat) in sausages with different meat content from factory B on days 0, 7, 14, 21, and 28



(1) Factory A (2) Factory B (3) Factory C (4) Factory D

**Figure 4** Correlation between residual nitrite content and nitrosamine in beef salami (90% meat) samples

Figure 4 shows that there was a significant correlation between the amounts of added nitrite and the nitrosamine contents in beef salami (90%) samples from all four meat factories.

In this study, the correlation factor was 0.9, which asserts the effects of added nitrite and also residual nitrite contents on nitrosamine formation in the processed meat product. Therefore, the products with lower meat content showed higher residual nitrite and, consequently, a greater nitrosamine formation. In meat products, presence of nitrosating agents increases the concentration of nitrosamine, whether grouped in  $\text{NO}_2$  related agents, e.g.  $\text{N}_2\text{O}_4$ , or grouped in nitrous acid derivatives, e.g.  $\text{N}_2\text{O}_3$  and  $\text{HNO}_2$  [28].

There are several suggested strategies to reduce nitrosamine formation in meat products to improve their healthy status and safety. The present research clarified that the levels of nitrosamines in the samples depended on the amount of residual nitrite, ascorbic acid, pH, and cooking temperature. Higher levels of residual nitrite

were detected in the samples with a lower amount of meat, compared to those with a higher amount of meat.

## CONCLUSION

In the current study, the residual nitrite, ascorbic acid, and nitrosamine contents of seven most popular Iranian processed meat products, namely sausages with different amounts of meat were evaluated to monitor the safety status of the meat industry. The samples contained various concentrations of nitrite and nitrosamine, which were above the permitted standard level. Several factors were found to affect the residual nitrite and nitrosamine contents, the meat content being a significant variable. Nitrite interacted with heme-containing components, non-heme proteins, and fat tissues, thus converted to nitrate and nitrosamine. Therefore, the contents of residual nitrite and, consequently, nitrosamine in products with lower meat content were significantly higher. A longer cooking time also decreased residual nitrite and nitrosamine concentration.

Further research is needed to identify new substances that could replace nitrites, as well as factors that reduce the required amount of nitrite in meat products.

## CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

## ACKNOWLEDGEMENTS

We would like to thank National Nutrition and Food Technology Research Institute for the financial support.

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**ORCID IDs**

Khadijeh Abhari  <https://orcid.org/0000-0001-9241-3563>

Zahra Pilevar  <https://orcid.org/0000-0001-7360-0478>

Hedayat Hosseini  <https://orcid.org/0000-0001-8301-4229>