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## **ORIGINAL ARTICLE**

# **Evaluation of Nitrite Exposure from Meat Products Supplied in Tehran, Iran**

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### ABSTRACT: Nowadays, due to the increased consumption of meat products, investigation of their additives **KEYWORDS** such as nitrite seems necessary. Nitrate in combination with salt has a synergistic characteristic against pathogenic microorganisms. Nitrite combines with protein, fats, and volatile and non-volatile compounds in Meat products; Nitrite; meat and plays an important role in flavoring meat products. Excessive use of this substance leads to a more Red meat; dangerous compound called nitrosamine. The present study aimed to investigate the nitrate content of meat Chicken; products with different levels of meat. Health risk assessment toward nitrite was also calculated. A total of 108 Exposure assessment samples of meat products in various commercial brands were collected and analyzed from retail markets of Tehran to detect the amount of nitrite in them. Meat products in terms of their meat percentage were divided into three categories under 50%, 50% to 70%, and more than 70%. Estimated daily intake (EDI), based on per capita consumption rate and hazard quotient (HQ) for adults and children were also estimated. The mean concentration of nitrite in meat products with >70% meat, 50-70%, and <50% were 28.04, 30.07, and 27.02 mg $kg^{-1}$ , respectively that did not resemble any significant difference (p>0.05). The results indicated that the levels of nitrite were lower than permissible levels. The calculated HQ was less than 1 for meat products. However, this value was more for children. So is necessary to take precautions any over-consumption of this type of product in children.

#### INTRODUCTION

Food additives are added to food products for a variety of reasons, including color, flavor, consistency, and increased shelf life [1]. In the meat products industry, nitrite is used to stabilize the product's color and prevent the growth of

\*Corresponding author: ta.zeinaly@gmail.com (T. Zeinali) DOI: 10.22034/jchr.2022.1939656.1403 spoilage microorganisms. Nitrite in combination with salt has a synergistic characteristic against pathogenic microorganisms [2]. They inhibit the growth of *Clostridium botulinum* [3]. Nitrite combines with protein, fats, and volatile and non-volatile compounds in meat and plays an important role in flavoring meat products [4]. Excessive use of this substance leads to a more dangerous compound called nitrosamine. Under acidic conditions in the stomach, nitrite is converted to nitrosamines. Nitrosamine is a cause of bladder, stomach, and esophagus cancers [5]. Assessing and determining the amount of nitrite in food due to the formation of nitrosamine in the stomach is essential. Nitrite also reacts with the heme of myoglobin to form nitrosomyoglobin [6].

Most human exposure to nitrite is through consuming water and food including meat products which in some cases, too much nitrate is added. Nitrite poisoning due to consumption of meat products containing excessive nitrite has been reported in many countries [7, 8]. In the previous study, the amount of nitrite in meat products collected from the Tehran markets was reported in the remarkable amounts and in the range of 2.93–13.9 mg/100 g [9]. In recent years, quantification of nitrite and associated health risks studies has become popular [6-8, 10]. Therefore, the amount of nitrite in meat products and dietary health risks should be evaluated regularly. The main purpose of this study was determination of nitrite in different types of meat products and assesses human exposure to nitrite due to consumption of these products.

#### MATERIAL AND METHODS

#### Sample collection

A total of 108 samples of meat products were collected from the Tehran retail market with different meat percentages. Meat products were divided into three categories in terms of meat percentage: under 50%, 50% to 70%, and more than 70%. All samples had specific production and expiration date. Samples were sent to the laboratory at cooled transport, immediately.

#### Preparation of the samples

10 g of each sample was poured to a 250 ml volumetric flask and then 100 ml water at 70 °C and 5 ml of saturated borax solution (Merck KGaA, Darmstadt, Germany) (50 g of sodium hydrate tetra borate dissolved in some water and then reached to a volume of one liter) was added respectively. This was left for 15 minutes inside the boiling water, then, cooled to laboratory temperature and 2 ml of protein precipitate solution1 (containing 106 g of hydrated potassium Ferro cyanide dissolved in water to a volume of 1 liter) (Merck KGaA, Darmstadt, Germany) was added. After stirring, 2 ml of protein precipitate solution 2 (containing 220 g of zinc acetate and 30 ml of concentrated acetic acid dissolved in water to a volume of one liter) (Merck KGaA, Darmstadt, Germany) was added. After maintaining for 30 minutes at room temperature, the flask was filled with distilled water to the marked line (250 ml). The certain volumes (20, 15, 10, 5 ml) of the filtered solution were added to 100 ml balloons and about 50 ml of water was added. Then, 10 ml of sulfanilamide solution (Merck KGaA, Darmstadt, Germany) and 6 ml of 5 N hydrochloric acid solutions (Merck KGaA, Darmstadt, Germany) were added to each of them and after mixing, the solutions were left in dark for 5 minutes and then 2 ml of N-(1-Naphthyl) ethylene diamine dihydrochloride solution (Merck KGaA, Darmstadt, Germany) was added and placed in the dark for 5 minutes and then the absorbance was recorded at wavelengths of 538 nm (UV-Vis Spectrophotometer, Hach, England).

# Dietary survey and risk assessment for nitrite in meat products

It is estimated that Iranians use about 6 kg of meat products per year. Therefore, the daily meat product consumption is about 0.016 kg. The average weight of an adult is 70 kg. Estimated daily intake (EDI) was obtained using equation [1].

$$EDI \text{ or al} = Ci \times Cc / BW \tag{1}$$

Ci: the mean concentration of nitrite  $(mg kg^{-1})$ 

Cc: the daily average consumption of meat product (kg person<sup>-1</sup> day<sup>-1</sup>)

#### BW: (body weight)

The average weight of adults and children was considered 70 kg and 15 kg, respectively. JECFA calculated 0  $0-0.07 \text{ mg kg}^{-1}$ .

BW per day as ADI for nitrite. The dietary risk assessment is performed by using the following equations [2].

Hazard Quotient (HQoral) = EDI/ADI (2)

The HQ displayed possible health hazards from the ingestion of contaminated food.

#### Statistical analysis

Statistical analysis was performed by SPSS version 21. The

Mean±SD was calculated for each group of samples. Kolomogorov-Smirnov test was used to determine the distribution of the data. All data had a normal distribution. Therefore, an independent T-test and one-way analysis of variance (ANOVA) were used to compare means.

#### RESULTS

#### Nitrite in meat products based on the percentage of meat

Residual sodium nitrite was measured as  $27.02\pm12.3$  mgkg<sup>-1</sup> in products containing less than 50% meat;  $30.07\pm16.2$  mg kg<sup>-1</sup> in products containing 50 to 70% meat, and  $28.04\pm11.5$  mg kg<sup>-1</sup> in products containing more than 70% meat (Table 1). There was no significant difference in the amount of nitrite between these groups (p>0.05). Figure 1 shows the average nitrite in red meat and chicken. There was no significant difference between groups (p=0.25).

Table 1. Different products based on the percentage of red meat or chicken

Product type Percent of meat/chicken	Mean ± SD (mg kg <sup>-1</sup> )	Min –Max _	Adult		Children	
			EDI	HQ	EDI	HQ
Under 50%		5-53	0.0061	0.087	0.028	
N=25	27.02±12.3					0.4
50 to 70%	20.07.16.2	0.32-102	0.068	0.97	0.032	0.45
N=50	30.07±16.2					0.45
Over 70%	20.04.11.5	11.61	0.0064	0.001	0.020	0.41
N=33	28.04±11.5	11-61	0.0064	0.091	0.029	0.41



Figure 1. The concentration of nitrite in meat products based on the type of meat (red meat or chicken).

### Dietary Exposure and risk assessment for nitrite in adult and children

Risk assessment was also performed in the present study for adults and children. The risk assessment of hazards is an effective way to manage the additive residues in foods. Daily dietary intake data of meat products were obtained from meat products traders' union survey. Children are very interested in consuming these types of products; therefore, the EDI of different products for adults and children was also calculated. The EDI was measured in the range of 0.068-0.0061 and 0.028-0.032 mg kg<sup>-1</sup> bw day<sup>-1</sup> for adults and children, respectively (Table 1). Subsequently, the calculated HQ was less than one (Table 1) for adults and children.

#### DISCUSSION

In present study, the amount of residual nitrite in all samples was less than international and national standards. Similar results were observed in the samples collected in Belgium. Most of the samples were within the permissible limit and below 20 mg/kg [4]. Chetty et al. reported nitrite content in meat product samples in the range of 10.07 to 164 mg kg<sup>-1</sup> in Fiji, which was more than the present study [6]. A total of 85% of meat products in China contained nitrite with the range of 0 to 2808.2 mg kg<sup>-1</sup> [11]. Nitrite was detected in all 90 analyzed meat samples with an average amount of  $46.2\pm10.1$  mg kg<sup>-1</sup> [12] that was not exceeded the maximum residue level of the European Union.

Excessive use of nitrite in meat will produce a significant amount of "nitrosamine" which is a carcinogen. Nitrosamine is produced due to the reaction of nitric oxide from nitrite with secondary amines [13]. Nitrites induce methaemoglobinaemia in children [7, 14].

The EDI in the current study was less than the amount approved by the JECFA (the Joint FAO/WHO Expert Committee on Food Additives) for nitrite. The JECFA committee considers a 0.07 mg kg<sup>-1</sup> bw day<sup>-1</sup> for nitrite. In a study conducted in New Zealand, the amount of nitrite in meat products was much higher than ADI [15], and HQ below one indicates no risk. The amount of HQ for children was less than one that is acceptable, but more than adults. It is necessary to suggest prudent measures in this regard. For example, these products should not be offered in school food stalls.

Elias calculated EDI for nitrite in meat products samples for children aged 3-10 years in Estonia as 0.016 mg kg<sup>-1</sup> bw day<sup>-1</sup> [16]. Furthermore, in a study conducted in Denmark, EDI for nitrite for the age of 15 to 75 years was calculated 0.003 mg kg<sup>-1</sup> bw day<sup>-1</sup> [15], which is lower than the current study. But in a study conducted in Sudan, EDI was calculated as 0.026-0.128 and 0.107-0.511 mg kg<sup>-1</sup> bw day<sup>-1</sup> for adults and children, respectively, which were more than the present study [12].

Nowadays, consumption of meat products is increasing due to the increase in the rate of women's employment and the speed and ease of preparation and consumption of meat products. The first effective item in the selection of a meat product by a customer is the color of the product [17]. The main role of nitrite in meat products is to give appropriate color and flavor to the product, preserve fat and protein from the oxidation process, and protect it against microbial hazards [18]. However, its addition leads to the conversion of carcinogenic compounds [19]. Nitrite causes the development of some cancers. The amount of 10 to 20 mg kg<sup>-1</sup> nitrite concentration is sufficient for the color stability of the product [4]. But, nitrite concentration for antimicrobial properties should be around 100 to 200 mg  $L^{-1}$ [6].

The maximum permissible limit for adding nitrite to the product is different from the maximum permissible for its residue in the product. According to EU regulations, the limit for potassium nitrite is 150 mg kg<sup>-1</sup>, while the limit for its residue in the product is 50 mg kg<sup>-1</sup> (Table 2). According to Iran's National Standard Organization, the permissible adding amount of nitrite is 120 mg kg<sup>-1</sup> in meat products while the residue amount is 60 mg kg<sup>-1</sup> (Table 2). According to the codex standard of food additives, the permissible limit of addition has not been announced and

the residue amount of nitrite in meat products has been announced as 80 mg kg<sup>-1</sup>. The current limit of nitrite in meat products is 150 mg/kg according to the European Union. Some countries are trying to reduce this amount, including Denmark, which has changed the limit to 60 mg  $kg^{-1}$  [20].

Standard	National standard ISIRI, Number; 2303	European union	Codex *	The australian food standard[5]	
Maximum adding level (mg kg <sup>-1</sup> )	120	150	-	125	
Maximum residual level (mg kg <sup>-1</sup> )	60	50	80	-	

Table 2. The maximum residue level (MRL) of nitrite (mg kg<sup>-1</sup>) in different countries

\*Codex standard on food additives

#### CONCLUSIONS

The nitrite concentration in 100% of the investigated products was within the permissible limit. Meat products with a lower percentage of meat are cheaper than other products. In this study, there was no significant difference between products with different percentages, so the price of products does not play an essential role in nitrite exposure. The amount of nitrite in these tested products is relatively low, so the possibility of nitrosamine formation is also low. Health risk assessment showed that adults and children are not at health risk of nitrite excess in meat products. Nitrite intake through meat products is less than the limits set by international agencies. Moreover, HQ showed no danger for adults and children. However, consumption of meat products in children must be accompanied with precautions.

#### Abbreviations

Bw: body weight; EDI: estimated daily intake; HQ: hazard quotient

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#### **Conflict of interests**

The authors didn't have any conflict of interest regarding the results of the study.

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