Original Article

Nitrite assessment in highly used processed meat products in growing age group in Isfahan city

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ABSTRACT

Aims: The aim of the study is to assess nitrite residual in highly used processed meat products and investigate risk assessment nitrite in the products in growing age group in Isfahan in 2014.

Materials and Methods: The study is descriptive and analytical that 180 highly used processed meat product samples for its nitrite residue has been evaluated by Association of Official Analytical Chemists. In this study, the amount of highly used processed meat product consumption in growing age group has been provided by the questionnaire that has been confirmed by reliability and validity.

Results: The amount of nitrite residue in 180 samples that has been evaluated is below the standard of Iran (120 ppm). Amount of nitrite intake from meat products that has been consumed by the growing age group was 0.0058 mg/kg that is 8.28% of daily intake of nitrite.

Conclusion: The amount of nitrite intake from highly used processed meat products in growing age group is not in risk range for health human.

Key words: Meat products, nitrite, risk assessment

INTRODUCTION

Nowadays, with the progress of industrialization, consumption of the fast foods particularly, those containing meat and its processed derivatives become prevalent.[1,2] As the processed meat products now is an important part of daily diet, and also is considered as one of the alternative protein sources.[3] Annual consumption of processed meat products for Iranian people is estimated 1.5 kg for a person.[3] In order to reach some technologic, organoleptic purposes and also to prolong the storage time of processed meat products, some food additives are applied in the formulation process.[4,5] In this regard, sodium nitrite is a commonly used additive in processed meat products which is applied for some purposes such as; stabilizing color, leaving a suitable taste and creating an antioxidant as well as, antimicrobial effects particularly, against infectious bacteria such as clostridiums.[6-8] On the other hand, deteriorative effects of nitrite against human health, has raised some concerns about excessive uptake of nitrite through consumption of processed meat products.[9] Some studies showed that the reaction between nitrite and secondary amines in stomach leads to nitrosamines compounds that their carcinogenicity is observed in some neoplasia like; gastric cancer, pediatric leukemia, and bone tumors. Furthermore, nitrite induces the oxidation of hemoglobin to methemoglobin (MetHb), which is safe in its physiologic level in blood (1-3% of total MetHb), however when increased to 20% and 50%, may result in cyanosis

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and suffocation, respectively.[9-12] Food and Agriculture Organization and World Health Organization determined an acceptable daily intake of 0.07 mg/Kg of human body weight for nitrite.[9] The acceptable maximum level for sodium nitrite in processed meat products is 120 ppm in Iran.[13] In recent years, several investigations conducted concerning with decreasing the concentration of nitrite in processed meat products and replacing it with other chemically safer compounds. In some cases, this kind of researches have been of successful results; For instance, it is shown that a part of functional activities of nitrite in processed meat products could alternatively be achieved through replacing it by sodium ascorbate. Although this replacement reduces the amount of nitrite in the final product, however, it does not compensate for the lack of strong inductive influence of nitrite in the prevention of botulism infection. Therefore, it may be claimed that the protective effect of nitrite in prevention from botulism in processed meat products is not still comparable.[14] Results from recent researches investigating the dietary patterns in teenagers and young people reveal the progressive development of processed meat consumption in these age groups. Quality control studies conducted on processed meat products in our country are limited only to the measurement of remaining nitrite in experimented samples and comparison of their mean with the standard ones, and no study investigated the biohazards of the nitrite consumption in the currently used patterns.

Risk assessment is a process that estimates the probability of harmful effects that contact with chemical agents could leave on human health in present or future. This process is carried out in four steps including; hazard identification (the assessment of potential risk for human), dose-response (the assessment of relationship between contact and effect), exposure assessment (assessment of the human contact level with the risk factor), and risk characterization (the comparison between contact level and the range of risk).[15] The present study designed to investigate the risk assessment concerning the remaining nitrite of highly used processed meat products in growing age groups at Isfahan city.

MATERIALS AND METHODS

The dietary pattern of processed meat products in middle school students

The present cross-sectional study is a descriptive-analytical research, which was carried out among 400 students (200 girls and 200 boys) between 13 and 17-year-old with an average weight of 57 kg at Isfahan city in 2014. Isfahan city was selected as the place where research carried out, and middle school students in the growing age group were selected as the statistical population. Sampling carried out in two steps through cluster sampling. In the first stage, some schools from the different areas of the city were randomly selected and in the second stage, classes were randomly selected from each school and a questionnaire was completed by students. Data on the dietary pattern were gathered by a self-administrated questionnaire that it measured the amount of meat products. The questionnaire was designed by a nutritionist, including some questions requesting for demographic information as well as information about types and amounts of processed meat consumption (that previously was heated). Answer choices were given on the basis of time (day, week, month and year), amount and the way of consumption (raw, roasted, fried and with food) for 9 prevalent processed meat products (German sausage, cocktail, hotdog, hamburger, lyoner, dry salami, ham chicken, ham meat and mortadella) in the market in the ninth row. The validity and reproducibility of the questionnaire were verified.

Sampling

Four highly used brands of sausage and salami manufactured at Isfahan companies used in the present study. For each brand of sausage and salami to measure nitrite in 3 times that at any time was 15 samples selected, the all of samples are 180 including 45 from each brand, carried at −4°C to laboratory to investigate.

Nitrite assessment

Processed meat products were evaluated using Association of Official Analytical Chemists in three intervals of 3, 10 and 17 days after production.[16] In this regards, 10 g of sample were homogenized and transferred to a 250 ml beaker and then 100 ml of 70°C tap water together with 5 ml saturated borax solution were added to the flask. The mixture was mixed for 1 min on Ben Mari and then cooled down to room temperature. Then after, 2 ml zinc acetate (219 g/L) was added to the mixture and after a 2 min mild mixing, 2 ml potassium ferrocyanide (106 g/L) also was added.

These contents were transferred to a 200 ml graduated volumetric flask and filled up with distilled water. The mixture left at room temperature for 30 min and then filtered through a conical 250 ml Erlenmeyer flask. After that, 20 ml of the filtered part was transferred to a 100 ml graduated volumetric flask and then 30 ml of distilled water, 6 ml hydrochloric acid 5 Mol and 10 ml sulfanilamide (2 g/l) were added in order. The flask put in a dark place for 5 min. Then 2 ml of alpha–naphthyl ethylene di amino hydro chloride (1 g/l) was added to the previous content of the flask and after 10 min in a dark place was filled with distilled water up to 100 ml and then its optical absorbance was measured at wavelength 538 nm with a spectrophotometer, model JENWAY 6105. A standard curve was developed using different concentrations of sodium nitrite (Merck, German, 99% $NaNO_2$).

Assessment of exposure

Mean and maximum nitrite intake exposure out of the meat products in the study population were calculated by the formula 1 and 2 respectively.[17]

$$MDI = \frac{C \times DI}{BW} \quad (1)$$

Where, MDI (mg/kg/day), C is nitrite levels in meat products (mg/kg), DI is an average daily intake rate of meat products (kg/day) and BW is study population body weight (kg).

$$M_X\ DI = \frac{C \times DI}{BW} \quad (2)$$

Where, M_x DI (mg/kg/day), C is the most amount of nitrite levels in meat products (Upper bound 95% confidence intervals) (mg/kg), DI is an average daily intake rate of meat products in highly used group (Upper bound 95% confidence intervals) (kg/day) and BW is study population body weight (kg).

Statistical analysis

In this study, 400 students were selected due to the fact that the differences in age groups and different sources of nitrite in various studies and similarity of current study to Reinik *et al*. study that in their study, the nitrite content of meat products were evaluated in 346 children.[18]

Subjects were informed and contributed to the research with consent. The questionnaires filled by subjects and the gathered data about nitrite residual and amount of consumed meat products in students went under statistical analysis by statistical software SPSS version 16 at a significance level of 5% . ANOVA test, Tukey HSD option were applied. The α and β coefficients of the error were determined 0.05 and 0.20, respectively. Variance for salami and sausage were set 11.17 and 9.88, respectively, and the sample size was determined 180 for both of them.[16]

RESULT

Data from the questionnaires showed that through nine commonly and highly consumed meat products on the market, German sausage and dry salami are more consumed than other types of consumption among a growing group. The results of the 180 samples of meat products in this study showed that residual nitrite in all samples tested below the standard for the products that standard is provided by the agency Industrial Research of Iran (120 ppm).

Table 1 presents the mean of nitrite residual in sausage and salami samples in 3 times (3rd, 10th and 17th days after production). The comparison of nitrite residual between two meat products showed nitrite residual in sausage was higher than salami. In addition, 95% confidence intervals for nitrite residual in sausage and salami were 67.65-75.63 and 42.25-44.09, respectively that has been measured at 3 times.

According to the above table, nitrite residual and intensity of reduction in two meat products has been a decreasing trend. The nitrite average in sausage was significantly different among 3rd, 10th and 17th days ($P < 0.05$) and the same relationship was seen for salami, although was not significant between 10th and 17th days ($P > 0.05$).

The risk estimation to humans

The average consumption of sausage and salami were 6.19 and 3.88 g/person/day in study population and 7.15 and 4.59 g/person/day in high consumption group based on questionnaire results. Table 2 presents the results of nitrite intake through the use of two meat products at three times in growing age group. The average intake of nitrite through sausage consumption was significantly different at three times ($P < 0.05$) and the average intake of nitrite through consumption of salami was not significant between 10th and 17th days ($P > 0.05$).

DISCUSSION

The results of the present study were to determine the role of meat product as agent of nitrite, an indicator of the role of salami become less apparent than sausage. According to international studies, the overall percentage of the daily nitrite intake from meat products, vegetables (vegetables and fruits) and water were 19%, 15%, and 7%, respectively. Accumulation of nitrite in the tissues of animals is low by consuming food

Table 1: Comparison of nitrite residual average in sausage and salami samples in 3rd, 10th and 17th sampling days*

Days	Number of samples	Sausage	Salami	ANOVA test was repeated
3rd	30	$10.46^{Aa} \pm 92.93$	$4.04^{B} \pm 47.99$	$P<0.05$
10th	30	$9.81^{Ab} \pm 68.01$	$1.74^{B} \pm 41.10$	
17th	30	$10.11^{Ab} \pm 53.99$	$1.80^{B} \pm 40.42$	
Total	90	71.64 ± 19.04^{A}	43.17 ± 4.38^{B}	

*Information tables based on the mean and SD is reported (x ± SD). Large Latin letters in each row indicate significant differences between the mean values of result for each type of product in the sample ($P < 0.05$). Small Latin letters in each column indicate significant differences between the mean values of results for each type of product in the three samples in 3 times ($P < 0.05$). SD: Standard deviation

Table 2: Amount of nitrite intake (mg/kg) through sausage and salami in growing age group

Days	Sausage		Salami	
	x ± SD	95% CI	x ± SD	95% CI
3rd	0.0109 ± 0.0190	0.0090-0.0127	0.0035 ± 0.0070	0.0028-0.0042
10th	0.0080 ± 0.0139	0.0066-0.0093	0.0030 ± 0.0060	0.0024-0.0036
17th	0.0063 ± 0.0110	0.0052-0.0074	0.0030 ± 0.0059	0.0024-0.0036
Total	0.0084 ± 0.0151	0.0075-0.0092	0.0032 ± 0.0063	0.0028-0.0035

Based on the above table result highly used meat has assigned 8.28% of the daily intake of nitrite to itself. SD: Standard deviation, CI: Confidence interval

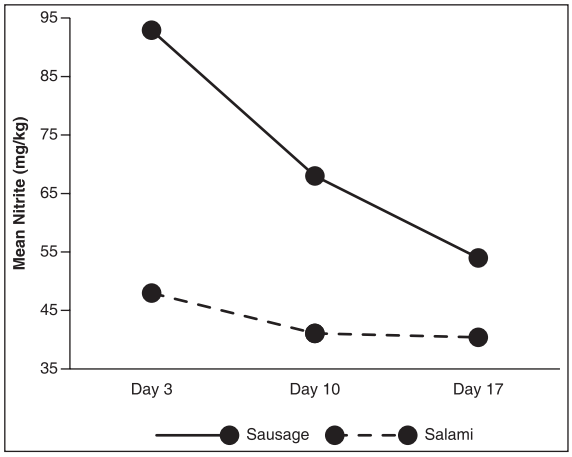

Figure 1: Amount of nitrite residual changes in 3rd, 10th and 17th days in sausage and salami

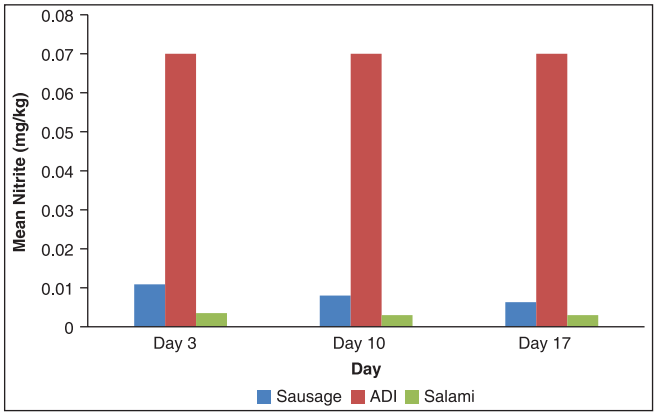

Figure 2: Comparison of nitrite intake average through sausage and salami with acceptable daily intake in growing age group

products such as meat, milk, eggs and fish due to the rapid excretion of nitrite and the percentage of the daily intake of food, 4%, 2%, 3% and 1% has been assessed.[9,19,20] There was concordance between the obtained result of this study and the result of Khodadady *et al*., comparing salami and sausage. In their study, the mean residual nitrite in sausage production was reported 38.4, 35.4, and 22.2 and in salami was reported 25.2, 24.2 and 18.8 (mg/kg) after 1, 7 and 14 days producing, respectively. However, the results of this study are not consistent with the results of their study due to the nitrite residues in the eyes of standard rules of the country so that 10% and 1.6% of sausage and salami samples contain more than the permitted limit were detected in the Khodadady' *et al*. study.[13] Nia *et al*., carried out a study at different times from the production of meat products from several factories that were examined for residual nitrite. Despite the concordance between result of this study and result of their study residual nitrite concentration decreased with elapsed time of maintenance period, the average concentration of nitrite residues was assessed close together in both product and 56.54-55.16 mg/kg, respectively.[16] In another study by Khaksar *et al*., they evaluated the concentration of nitrites in meat products. The concentration of nitrite in meat products by 40% the 1st day after baking was 66 mg/kg.[21] In the present study, 40% meat content in the samples, but when nitrite concentration are measured in samples in two studies was different. Therefore, the mean difference of concentration of nitrite in the two studies is justifiable. In all previous studies conducted in Iran, evaluation of the concentration of components residue is taken into consideration. This study examined daily intake in adolescents and calculated exposure even in the high-consuming individuals of this age group in this area provide more accurate data than any other national studies. Such information in similar international studies is offered in different parts of the world.

Results from the present study showed that the amount of nitrite uptake through consumption of sausage and salami in growing students were 0.0084 and 0.0032 mg/kg B.W, respectively and also overall nitrite uptake due to consumption of highly used processed meat products was 0.0057 mg/kg B.W. Results from our study is close to the previous studies in some parts; as one study in Denmark reported an average nitrite and nitrate uptake of 0.014 and 0.007 mg/kg B.W due to meat products consumption, respectively in boys and girls between 6 and 14-year-old.[22] Furthermore, Larsson *et al*. reported that the nitrite uptake from the diet in Swedish children between 11 and 12-year-old was 0.007 mg/kg B.W.[23] However, some other studies reported higher amounts of nitrite uptake from diet in their investigations; Wawrzyniak *et al*. indicated that average nitrite uptake through diet in student was 1.7 mg/kg B.W).[24]

In a study conducted by Reinik *et al*. at Stony amount of nitrate, nitrite, and nitrosamine in 346 children through the entire Estonian meat products were 1.7, 0.83 and 0.073 mg/day respectively.[18] Compared with the results of the present study that showed the amount of nitrite in meat products consumed by young adults is 8.28% of the daily permitted level was allocated. It could be said that Estonia children through the whole meat about 10 times lower than Iranian children are exposed to nitrite, but it be not generalized in highly consumed meat. Findings from Temme *et al*. was close to what was observed in the present study; they reported an overall average nitrite uptake of 6% from all meat products in the population older than 15 years old.[25] Other studies revealed a significant discrepancy with the results reported in the present study; a nitrite uptake from diet of 16% in adults,[26] 50-67% in children[27] and 28% in students.[24] It seems that the reason behind variation in results from different studies is associated with the difference in age groups studied, resources nitrite uptake form, and also the different permitted levels of nitrite for meat processed products in different regions of the globe.

CONCLUSION

According to the finding of this study, the amount of nitrite residue in meat products that produced in Isfahan city in the

production of meat products was lower than the permitted standards in Iran. Amount of nitrite in meat products consumed by the growing age group is not at a range of health risk. To estimate the total amount of nitrite from food in growing age group, it should be evaluated nitrite in food and other items, and it is emphasized in future studies.

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