Dedicated to Professor Florin Dan Irimie on the Occasion of His 65th Anniversary

SPECTROPHOTOMETRIC DETERMINATION AND ASSESSMENT OF POTENTIAL HEALTH RISK OF NITRITE FROM MEAT AND PROCESSED MEAT PRODUCTS

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ABSTRACT. Nitrite content was determined in fresh meat of chicken and pork and in different processed meat products such as sausages, salami, ham and pate with declared and not declared nitrite addition. The spectrophotometric method based on diazo-coupling reaction was used. Under the optimum working conditions, the method showed a limit of detection of 0.4 mg kg^{-1} and a limit of quantification of 1.2 mg kg^{-1}. The method proved to be accurate with nitrite recovery of 98±14% from spiked samples. In the analyzed samples the nitrite content was in the range 1.1±0.1 to 26.4±2.5 mg Kg^{-1}, values that are below the maximum admitted level of nitrite in meat products (150 mg Kg^{-1}). The lowest nitrite concentration was found in chicken meat products (between 1.1±0.2 and 1.8±0.1 mg kg^{-1}) while in salami, ham and sausages products the content was high (26.4±2.5; 19.0±0.7 and 13.4±1.1 respectively). The health risk exposure parameters were between 4% and 94 % and between 2% and 50% from established Acceptable Daily Intake (ADI of 0.07 mg/kg body weight/day) in case of adults and children respectively. Higher values correspond to pork meat products as salami, sausages and ham. The risk exposure to nitrite estimated for occasional consumption of meat products proved to be much more informative than the nitrite concentration. For these products consumption should be limited to 150-300 g per week. Non-carcinogenic health effects, evaluated based on Target Hazard Quotient (THQ) were revealed for the investigated meat products by occasional consuming of 3 serving/week.

Keywords: nitrite, meat products, UV/Vis spectrophotometry, risk exposure assessment

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INTRODUCTION

The salts of nitrite and nitrate are commonly added to perishable food products especially for their preservation and also to help hinder the growth of harmful microorganisms as in particular Clostridium botulinum (the bacterium responsible for botulism) [1-3]. The safety of their use has been contested especially because of toxicological aspects related to oxidation of hemoglobin to methemoglobin a compound incapable of transporting oxygen in the blood [4]. Moreover, dietary nitrites can form carcinogenic nitrosamines under acidic conditions or during food processing procedures [5-9]. As concern in this field, the Joint Expert Committee of the Food and Agriculture Organization (JECFA) / World Health Organization (WHO) established an Acceptable Daily Intake (ADI) of 0.07 mg nitrite kg^{-1} of body weight that appears to be safe for healthy neonates, children and adults [10].

It is well known that water and leafy vegetables are natural sources of dietary nitrate whereas cured meats are the major sources of dietary nitrite [11-13]. The use of nitrite in certain foodstuffs and meat products has been periodically regulated by European Comission (EC) and European Food Safety Authority (EFSA) [14-18]. In meat industry potassium nitrite (E249) and sodium nitrite (E250) are permitted additives with anti-microbial action, preservation effect, red color fixation and beneficial effect on flavour [19,20]. According to the European Comission Directive 95/2/EC their maximum amount in manufactured meat products is 150 mg Kg^{-1} [21]. Methods based on different analytical techniques, such as spectrophotometric, electrochemical, chromatographic, chemiluminescent, capillary electrophoresis and electrochemiluminescent methods have been reported for detection and determination of nitrite [22]. Though new methods have been published in the last years [23-25], the spectrophotometric ones are by far the most widely used for nitrite determination due to its simplicity and inexpensive analytical feasibility. These methods are mainly based on the reaction (diazotization or nitrosation) of nitrite with some detecting reagents and determination of nitrite concentration based on the absorbance measurement of the reaction product.

Based on sufficient evidence in humans that the consumption of processed meat causes colorectal cancer, the WHO's International Agency for Research on Cancer (IARC) classified in 2015 the consumption of processed meat as carcinogenic to humans (Group 1) [26]. However, the packaging information table of meat products does not contain relevant data on nitrate/nitrite content. In the best case the consumers are informed if the nitrite was added or not in certain meat products. As concern in the field, there is an emergent need to investigate the nitrite level in commercially available fresh meat as well as in processed meat products. The health risk exposure associated with their consumption should be evaluated especially for those products that are frequent consumed in the usually diet.

Based on these considerations, it was proposed to evaluate the nitrite risk exposure parameters in order to find which is more suggestive for the consumers. Thus, the aim of this paper was determination of nitrite content in meat based products and evaluation of the health risk exposure arising from usual consumption. For this study, spectrophotometric method based on Griess assay procedure was used. Fresh meat of chicken and pork and related sausages, salami, ham and pate processed meat products were taken into consideration. The health risk exposure arising from their usual consumption was evaluated both for adults and children for individual categories of meat products based on Acceptable Daily Intake (% ADI) and target hazard quotient (THQ) determination.

RESULTS AND DISCUSSION

Method performance and validation characteristics

Under the optimal working conditions, the calibration curve generated over the range 0-0.80 mg L^{-1} nitrite in the spectrophotometric assay had the correlation coefficient of 0.9999. The limit of detection and limit of quantification for nitrite were 0.4 mg kg^{-1} and 1.2 mg kg^{-1} respectively. Thus the proposed method is suitable for nitrite determination in fresh meat and processed meat products since the detection limit is 405 times lower than the current maximum concentration of 150 mg kg^{-1} admitted in meat products [16, 17], while the quantification capabilities are 135 fold lower.

The results for spiked samples of processed meat products, analyzed to check method accuracy, are presented in Table 1.

The developed methodology for nitrite determination provided good accuracy with an average recovery in the range 98±14%, consistent with the AOAC recommendations [27], namely 75-120% for 95% confidence level.

Table 1. Nitrite recovery in spiked samples

Sample name	*Nitrite (mg Kg^{-1})				Recovery (R±CI) (%)	Average recovery (R±CI) (%)
	Determined in original sample (C±CI)	Added (C$_a$)	Determined in spiked sample (C$_s$±CI)	Found C$_a$ (C$_{af}$±CI)		
Salami	3.45±0.40	5.30	8.60±0.60	5.16±0.72	97±14	
Sausage	12.54±0.56	5.30	17.60±0.50	5.06±0.75	95±15	98±14
Pork ham	22.10±0.47	5.30	27.47±0.44	5.37±0.64	101±12	

* Mean values of five parallel samples; CI - is confidence interval for 95% confidence level (n=5)

Sample analysis and health risk exposure assessment

The results of nitrite determination in commonly consumed fresh meat and processed meat products are presented in Table 2.

Table 2. Nitrite concentration in meat and processed meat products

Sample no.	Sample Name / code	Declared content		Nitrite content (mg kg^{-1})	
		Kind of meat	Added nitrite	RSD (%)	Mean value (±CI)
1	Sausage (C)	pork	No	11.2	13.4±1.1
2	Sausage (D)	pork/beef	No	8.1	3.8±0.2
3	Sausage (D)	pork	No	5.7	2.8±0.1
4	Sausage (D)	pork/beef/horse	No	0.8	2.5±0.1
5	Salami (A)	pork	Yes	13.4	26.4±2.5
6	Salami (E)	pork	Yes	0.9	6.6±0.1
7	Salami (E)	pork	Yes	3.6	3.6±0.1
8	Salami (E)	pork	Yes	13.6	3.1±0.3
9	Pork ham (B)	pork	No	5.0	19.0±0.7
10	Pork meat (F)	pork	No	4.9	1.2±0.1
11	Chicken breast (G)	chicken	No	1.2	1.6±0.1
12	Chicken pulp (G)	chicken	No	3.6	1.1±0.1
13	Chicken pulp (G)	chicken	No	2.6	1.2±0.1
14	Chicken liver (G)	chicken	No	1.1	1.8±0.1
15	Chicken pate (G)	chicken	Yes	0.5	5.5±0.1
16	Chicken pate (G)	chicken	No	5.7	1.4±0.1
17	Goose pate (G)	goose/chicken	No	2.7	1.1±0.2
18	Pork pate (F)	pork	Yes	0.9	3.4±0.2
19	Pork pate (F)	pork	No	4.0	1.5±0.4
20	Pork pate (F)	pork /chicken	No	0.5	3.7±0.1

A, B, C – samples with high content of nitrite; D – samples of sausages with similar nitrite content; E – samples of salami with similar nitrite content; F – samples of pork meat and pork pate with similar nitrite content; G – samples of chicken meat (breast, pulp and liver) and chicken pate with similar nitrite content; RSD - Relative standard deviation (%); CI - Confidence Interval for 95% confidence level (n=5)

In all the analyzed samples the nitrite content was below the maximum admitted concentrations established by European Commission Regulations in meat products [17, 21]. The obtained results were evaluated considering as individual group each of the meat products category. For some of the meat products categories, the samples were divided into sub-groups based on

t-test results for 95% confidence level used to compare the mean nitrite content of the group with the individual values in the same group. Samples with significantly high content of nitrite ($p < 0.05$) such as kind of salami, pork ham and kind of sausages were considered separately and noted in the Table 2 with (A), (B) and (C) respectively. The pork meat products as sausages and salami in which the nitrite content was not significantly different from mean value were considered as groups and noted with (D) and (E) respectively. Using the same principle, pork meat and pork meat pate was considered as one group and noted with (F) while chicken meat (breast, pulp and liver) and chicken pate the group noted with (G).

The highest nitrite concentration was observed in salami (A), pork ham (B) and sausage (C) with values of 26.4 ± 2.5 mg Kg^{-1}, 19.0 ± 0.7 mg Kg^{-1} and 13.4 ± 1.1 mg Kg^{-1} nitrite respectively (Figure 1). In other processed pork meat products such as salami (E), sausage (D) and pate (F) the nitrite content was lower with values in the range 3.1 ± 0.3 and 6.6 ± 0.1, 2.5 ± 0.1 and 3.8 ± 0.2, and 1.5 ± 0.4 and 3.7 ± 0.1 respectively.
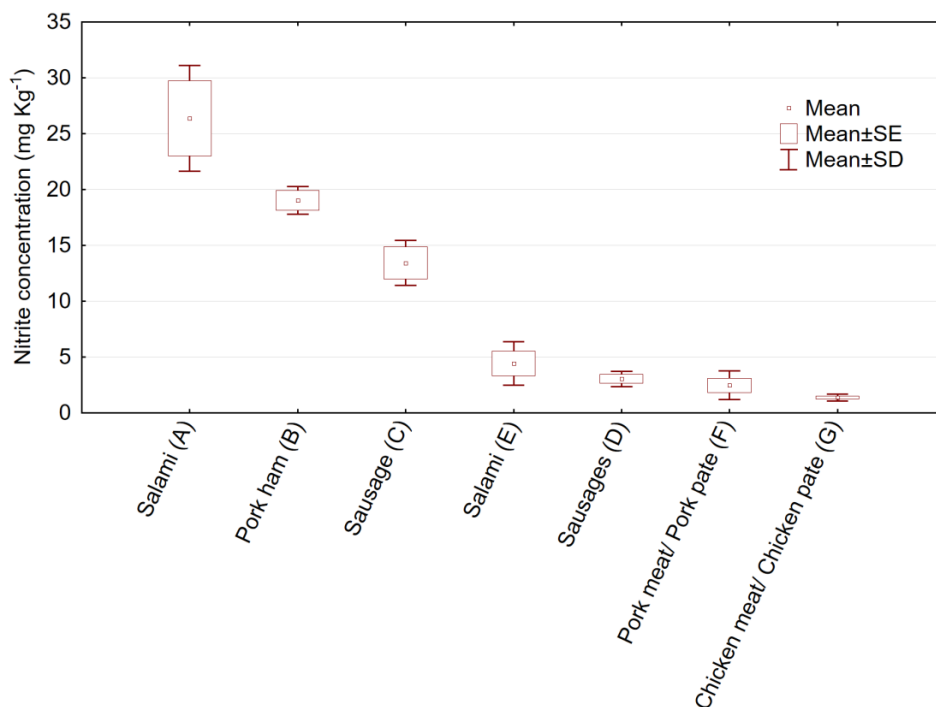

Figure 1. Nitrite concentration in categories of meat products
(mg kg^{-1}, mean values / category of products)

The lowest nitrite concentrations were observed in chicken meat samples where the nitrite ranged from 1.1±0.1 to 1.8±0.1 mg Kg^{-1} with exception of one type of pate sample (5.5±0.1 mg Kg^{-1} nitrite) with declared added nitrite.

Thus, it can be concluded that the highest amount of nitrite was found in processed pork meat products, mainly salami, sausages and ham, while the lowest amount was found in fresh meat and pate of pork and chicken. Similar results were reported also in literature [25, 28].

As concern in food safety investigations, the danger of food nitrite still gives rise to debate in the scientific community. Recently new analysis methods were developed and new parameters were proposed for the evaluation of risk exposure to nitrite [29-31]. Rather than the nitrite concentration, %ADI would be more informative, since ADI was established based meat products consumption and nitrite concentration. According to data in Figure 2, the daily consumption of a portion of 150 g/20 g of meat products by a 60 kg adult/15 kg child body weight respectively, pose no health risk of exposure since the %ADI value are not exceeded in any of the cases.
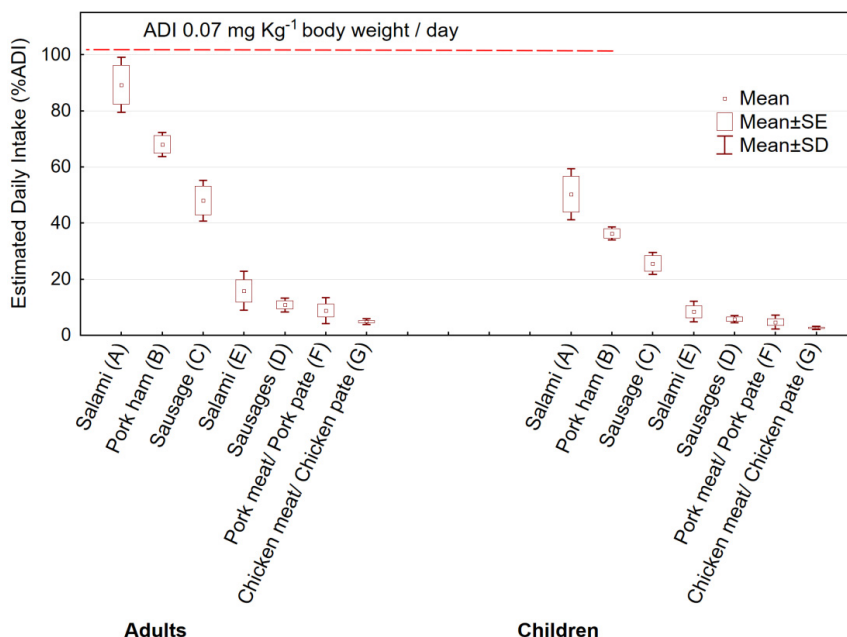

Figure 2. Daily risk exposure to nitrite via occasional consumption of a portion of 150 g meat product/day for a 60 kg body weight (bw) adult and 20 g meat product for a 15 kg body weight (bw) child, considering the current Acceptable Daily Intake (ADI) of 0.07 mg kg^{-1} body weight (bw)/day

It is important to note that processed pork meat products such as kind of salami (A), sausage (C) and pork ham (B) bring the highest values for %ADI being in the range 47-94% and 25-50% of nitrite ADI for adults and children respectively. These meat products with high nitrite content would be consumed with prudence. In these cases the weekly consumption should be limited to 150-300 g for adult which means 1-3 serving per week. The situation is also risk-free for children when consuming 40-150 g meat products, corresponding to 1-4 servings per week.

Other processed pork meat products such as salami (E) and susage (D) had lower %ADI values within the range 9-24% and 4-13% of nitrite ADI for adults and children respectively. For these products the weekly resk-free consumption is 600-900 g for adult and they could be daily consumed.

It is interesting to note that lowest %ADI were found for pork meat / pork pate (F) and chicken meat/chicken pate (G) products that have similar values within the range 4-13% and 2-7% for adults and children respectively. Thus for commercially available unprocessed meat and pate products from pork and chicken there is no health risk from exposure to nitrite and practically can be unlimited consumed.

From the carcinogenic point of view, the THQ parameter is more suggestive for nitrite risk exposure evaluation. According to data from Figure 3, for all of the investigated meat products non-carcinogenic effects are expected considering an exposure duration of 70 years with an average serving of 150 g for a 70 kg (bw) and an exposure frequency of 156 days (about 3 serving/week). It can be also observed that chicken meat and chicken products exhibits the lowest risk of exposure to nitrite.

CONCLUSIONS

The results of nitrite determination in commercially available fresh meat and processed meat products from pork and chicken revealed a nitrite concentration below the current maximum admitted limit established by European Comission Reglations at 150 mg kg^{-1}.

The highest nitrite concentration was found in pork processed meat products such as salami, sausages and ham, and some of these products should be consumed with prudence of 1-3 serving/week.

For unprocessed meat of chicken and pork there is no risk of exposure to nitrite and they could be practically unlimited consumed.

Non-carcinogenic health effects are expected by considering a consumption average of 3 serving/week.

The study highlighted that the risk assessment based on %ADI parameter is more suggestive. In order to better inform the consumer, %ADI values for a meal of 150/20 g for an adult/child would be provided in the table information on the packaging of the meat products.
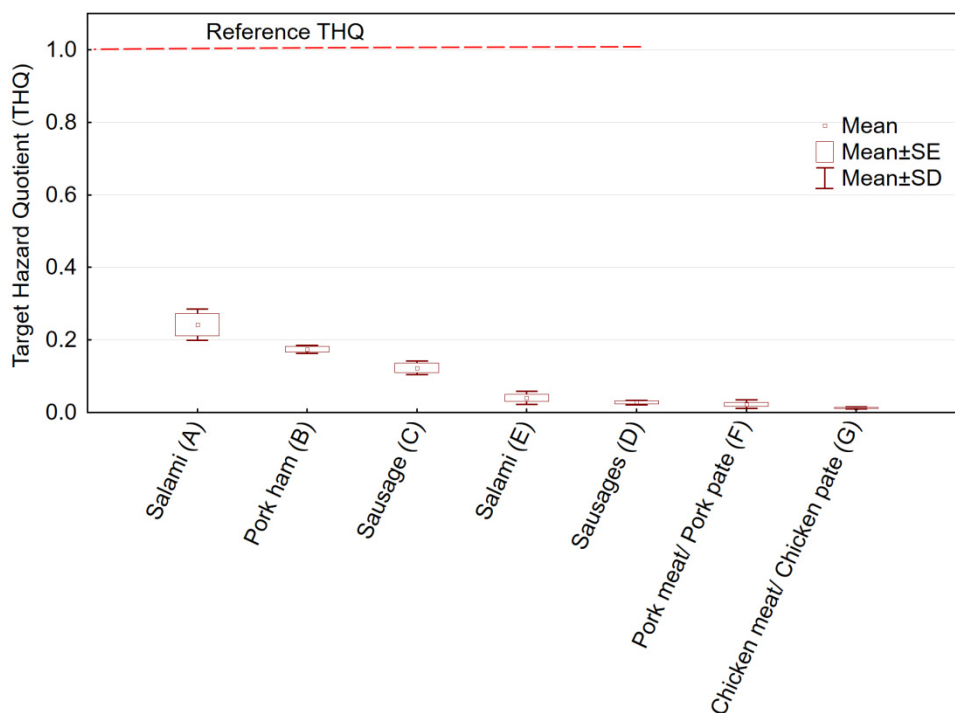
Figure 3. The health risk exposure to nitrite by occasional consumption of meat products (THQ - Target Hazard Quotient - calculated considering the exposure frequency to nitrite of 156 days, exposure duration of 70 years and an average serving of 150 g for a 70 kg (bw))

EXPERIMENTAL SECTION

Chemicals and solutions

All the chemicals were of analytical grade. Sodium nitrite ($NaNO_2$), sodium tetraborate (borax, $Na_2[B_4O_5(OH)_4] \cdot 10H_2O$), potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6]$, zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$), sulfanilic acid ($C_6H_7NO_3S$) , α-naphthylamine ($C_{10}H_9N$), glacial acetic acid ($CH_3COOH$) and sodium chloride (NaCl) were from Sigma-Aldrich (Steinheim, Germany).

For proteins precipitation solutions of potassium hexacyanoferrate II 10.6 % (m/V) in distilled water, zinc acetate 22% (m/V) in 3% acetic acid solution and borax 5% (m/V) in distilled water at 40-50°C were freshly prepared.

Griess reagent was freshly prepared as a mixture of equal volumes of sulfanilic acid solution with α-naphthylamine solution. For the preparation of sulfanilic acid solution 6.00 g of sulfanilic acid were dissolved in 200 mL

glacial acetic acid and 100 mL distilled water by heating on a water bath. After cooling, 200 mL of 10% (m/V) sodium chloride was added and the solution was diluted to 1000 mL with distilled water. The α-naphthylamine solution was prepared by dissolving 0.30 g of α-naphthylamine hydrochloride in 100 mL hot water. After cooling, 200 mL glacial acetic acid were added and the resulted solution was diluted to 1000 mL with distilled water.

Stock nitrite solution (4 mg/L) was obtained by dissolving appropriate quantity of sodium nitrite standard in distilled water.

Analytical procedure and equipment

For the spectrophotometric determination of nitrite in meat samples a modified version of Shinn method which is based on the Griess-Ilosvay diazo-coupling reaction was used [32]. The principle of the applied method is based on the ability of nitrite to react with sulfanilic acid in acidic medium to form a diazonium salt then coupling the diazotized sulfanilamide with 1-naphthylamine forming an intensely pink azo-dye [33] that can be determined by spectrophotometric measurements.

The absorbance measurement was carried out at λ=520 nm using T80 UV-Vis double beam Spectrophotometer (PG Instruments Ltd., Lutterworth UK).

Sample preparation and method development

Working standard solutions with a concentration from 0.08 to 0.80 mg/L nitrite were freshly prepared by adding 10 mL of Griess reagent to appropriate volume of stock solution and diluted to 50 mL with distilled water. The reagent blank was prepared by dilution of 10 mL of Griess reactive to 50 mL with distilled water but in absence of nitrite.

The fresh and processed meat products samples were treated according to the AOAC method (AOAC, 1980, technique number 24.041) and ISO 2918:1975 procedure (reference procedure) recommended by the International Standard Organization reviewed and confirmed as current reference version in 2018. This method is based on extraction of sample in a hot water bath followed by reaction of nitrite with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to form a pink dye whose absorbance is measured. According to recommended procedure, portion of 10.00 g of each grounded sample have been subjected to extraction with 100 mL hot water at 80°C and 5 mL borax in a 200 mL volumetric flask for 15 minutes using a water bath. After the sample cooling, the precipitation of proteins was made by addition of 2 mL of potassium hexacyanoferrate (II) and 2 mL of zinc acetate stirring after each addition for 15 minutes. The sample was diluted to 200 mL volumetric flask and filtered. In each case 10 mL of Griess reagent was

added over a 30 mL portion of the filtrate and diluted to 50 mL volumetric flask with distilled water. Resulted solutions were well homogenized and let for 15 minutes in a dark place at room temperature for complete reaction and formation of red-pink dye complex. The absorbance was measured at 520 nm. Five parallel samples were subjected to extraction procedure and replicate portions of 30 mL were prepared for Griess reagent reaction.

Assessment of method performance

For the method accuracy evaluation, meat products samples with low, medium and high level of nitrite content were spiked by adding 5.30 mg kg^{-1} nitrite in each sample and run through the whole extraction process as described above.

The spectrophotometric method was characterized in terms of limit of detection (LOD), limit of quantification (LOQ), precision and accuracy under the optimal working conditions. The LOD was calculated according to the $3S_{y/x}/m$ criterion (eq. (1)):

$$LOD = \frac{3S_{y/x}}{m} \qquad (1)$$

where ($S_{y/x}$) is the residual standard deviation and (m) the slope of the calibration curve [34].

The LOD value was related to mass based on the sample preparation protocol while LOQ was calculated as 3xLOD.

The precision of the method as relative standard deviation (RSD) was estimated from confidence interval values for n=5 parallel samples.

Health risk assessment

The risk exposure to nitrite via occasional consumption of meat based products was evaluated accordingly with the Daily Intake (DI) estimation (eq. (2)):

$$DI = \frac{C \times m_s}{bw} \qquad (2)$$

were C is the concentration of nitrite in meat product (mg Kg^{-1}), m_s is the amount of consumed meat product/one serving considered 150 g for adult and 20 g for child and bw is body weight considered as 60 kg for adult and 15 kg for child respectively.

The corresponding daily risk exposure to nitrite (%ADI) [35] was calculated as percent of Acceptable Daily Intake of 0.07 mg kg^{-1} bw (recommended ADI) [10] (eq. 3):

$$\%ADI = \frac{100 \times DI}{0.07} \tag{3}$$

The non-carcinogenic health risk posed by exposure to nitrite was evaluated based on the Target Hazard Quotient (THQ) parameter defined as the ratio of exposure to a toxic compound and the reference dose which is the highest level at which no adverse health effects are expected (eq. (4)).

$$THQ = \frac{E_{FR} \times E_d \times F_{IR} \times C}{RfD \times BW_a \times AT_n} \times 10^{-3} \tag{4}$$

where E_{FR} is the exposure frequency to nitrite (considered 156 days equivalent with about 3 serving/week)), E_d is the exposure duration (70 years), F_{IR} is the meat product ingestion rate (an average serving of 150 g/day), C is the concentration of nitrite in meat product, RfD is the oral reference dose of nitrite (0.07 mg Kg^{-1} bw/day), BW_a is the reference body weight (70 kg bw), AT_n is the averaged exposure time (365 days x 70years) and 10^{-3} is the unit conversion factor [36].

ACKNOWLEDGMENTS

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