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Nitrite Assessment of Processed Meat Products Commonly Consumed in Ghana

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Abstract Sodium and potassium salts of nitrite are used in meat products as preservative, antioxidant and colour fixative. However, high levels of these chemicals pose risks such as toxicity due to the formation of nitroso compounds originated from nitrite sources. The present study was conducted to assess the nitrite residual levels and to evaluate the health risk of consumers from exposure of nitrite in commonly consumed processed meat products (sausage, corned beef and bacon) on the Ghanaian market. A total of 300 questionnaires were administered to various age groups in the study population in the Ayawaso West Sub Metro, Accra. Participants were made to fill out the 24 h food frequency questionnaire. Fifty (50) different types and brands of processed meat products were randomly selected and analyzed for nitrite residues by spectrophotometric method. The effect of boiling, frying and grilling as cooking methods commonly employed were investigated. The mean nitrite content in the samples was 139.85 mg/kg and the mean daily intake estimated at 114.89 mg/kg/day. Significantly, the nitrite levels found in this study was higher than the WHO/EU recommended levels of 125 mg/kg in processed meat. First order Monte Carlo simulation at 10,000 iterations estimated chronic daily intake of nitrite as 5.05 mg/kg/day. Subsequently, the average risk of consumption of processed meat within the limits of reference dose of nitrite (0.33 mg/Bw/day) for the studied population was estimated at 15.65 (>>1) indicating of adverse health effect to the consumers. Overall, boiling was safer cooking method for cured meat and sausages found on the Ghanaian market.

Keywords: *sausage, cooking method, residual nitrite, exposure and risk*

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1. Introduction

Nitrite is used as food additive due to its antimicrobial properties in cured meat products and its desirable effects on colour, flavour and texture. High exposure to nitrite is considered a health risk by its ability to form carcinogenic nitrosamines in foods and in humans. Due to globalisation and population growth, there is a need to minimise spoilage of meat by preserving and, maintaining the nutritional value, texture and flavour. Energy-intensive freezing operations are the greatest way to preserve meat and meat products for a longer time which inhibits bacterial growth, except the psychrophilic bacteria and the spores, thus these psychrophilic bacteria and the spores survive freezing and grow during thawing [1,2].

Traditional methods for preservation of meat by salting and pickling are well-accepted procedures. Other chemicals have been used as food additives for the preservation of meat, but every country has drawn its rules and regulations and established limits for prevention of harmful effects to human [3]. The meat industry over the years uses salts of nitrate and nitrite to prevent the growth of *Clostridium botulinum*, provide the products with a pinkish colour, cured meat typical flavour, and antioxidant effect. The major dietary resource to nitrite and nitrosamine is associated with cancer [4]. According to epidemiology and clinical studies, high intake of dietary nitrate and nitrite has been associated with aetiology of human gastric cancers [5]. A report by World Cancer Research Fund and American Institute of Cancer Research indicates that a high intake of red or processed meats is a convincing and probable cause of colorectal cancer. The risk of colorectal cancer has been estimated to increase by 29% for every 100 g/day increase in red meat and by 21% for every 50 g/day increase in processed meat consumption [6].

In the oral cavity and stomach, nitrates are converted to nitrites that react with amines and amides to form a group of carcinogens called N-nitroso compounds. The low pH in stomach promotes nitrosation [5]. Nitrite combines with secondary and tertiary amines present in the meat, creating nitrosamines that are highly carcinogenic substances. They can also combine with haemoglobin creating methaemoglobin, which damages the blood oxygen transport.

Long-term nitrite intake can cause methemoglobinemia, mainly in children, and nitrite intoxication causes vasodilatation, smooth muscle relaxation, face redness, gastrointestinal discomfort, headache, cyanosis and vomiting. This can be fatal, particularly in newborn infants in which the methaemoglobin-reducing capacity is low, leading to so-called `blue baby syndrome' [7]. The N-nitroso compounds formed from the nitrite reactions with the amines are mutagenic and teratogenic. The risk of colorectal and stomach cancer has been suggested to increase with intake of red and processed (nitrite-preserved red) meat according to epidemiologic studies with processed meat showing higher risk estimates per gram of intake than red meat [8]. Nitroso compounds can be formed via nitrosation of organic compounds in meat by nitrite. Endogenous N-nitrosation of amines forms nitrosamines. Several N-nitroso compounds are known carcinogens and can alkylate DNA leading to G-to-A mutations. The Heme iron, abundantly present in red meat, also has a catalytic effect on the endogenous formation of N-nitroso compounds [6].

In Ghana, research suggests that 34,000 deaths from cancer every year could be due to diets high in processed meat [9]. It is estimated that 16,600 cases of cancer occur annually in Ghana with an occurrence rate of about 109.5 cases per 100, 000 persons according to Cancer Control Division of Ghana Health Service [10]. The report stated that most of the cases seen in Ghana and other West African countries are identified with young people, which is a contrast of what has been reported in the developed world [10]. Studies indicate that breast cancer cases among Ghanaian women are on the rise. This assertion could be due to high levels of curing agents in Ghanaian diets from processed meat [10].

The World Health Organisation/European Union rules, specify a residual amount of 120 mg/kg of sodium nitrite and an indicative maximum ingoing amount of 200 mg/kg [11]. Also in Ghana, the Ghana Standards Authority stipulate a maximum ingoing amount of 200 mg/kg and a residual amount of 120 mg/kg sodium nitrite [12]. The maximum ingoing amount of nitrite for processed meat products is normally up to 200mg/kg of product, and the residual nitrite in a finished product should not exceed 125 mg/kg [13].

Current regulations on the use of nitrite and nitrate vary depending on the curing method and the type of product. For comminute products, the maximum ingoing nitrite is limited is 156 mg/kg. For immersion-cured and massaged or pumped products, the limit is 200 mg/kg. The ADI for nitrites, as nitrite ion, is 0.07 mg/kg body weight [5,10]. Denmark permits 60 mg/kg maximum ingoing level of sodium nitrite for most products with some speciality products allowed to have up to 150 mg/kg. The results of the study conducted in Denmark from 1998 to 2006, indicated that the residual nitrite level was between 6-20 mg/kg [13]. In a similar time frame (2000-2004), the mean nitrite concentrations of Estonian cooked sausages, smoked sausage and ham were in the ranges of 22-38, 14-30, and 8-29 mg/kg, respectively and estimated a mean intake of 105% of the ADI value of 0.06 mg nitrite kg^{-1} bw day^{-1} for children 1 to 6 year age group [20]. In Australia, nitrites (sodium or potassium salts) are allowed at a maximum level of 125 mg/kg in cured, dried and slow-dried cured meat and 50 mg/kg in sterile and canned meat [9].

Kim and others [6] reported that nitrite content of bacon in the UK was 24 mg/kg, while the level in ham was 26 mg/kg. A multi-year survey of Canadian products indicated that the overall mean residual nitrite levels in cured meats had declined over the past 20-25 years averaging 28 mg/kg in 1972, 44 mg/kg in 1983-1985, 31 mg/kg in 1993-1995, and 28 mg/kg in 1996 [13].

Finnish cured meat products have been observed to range from 2.3-31.6 mg/kg for nitrite content [14]. Based on this brief overview of the nitrite concentration in cured meat products from other countries, Ghanaian products often contain relatively high levels of nitrite. Some factors contributing to high residual nitrite level could be the excessive use of sodium or potassium nitrite, poor storage and other factors. Owing to the growing concern of N-nitroso compounds, it has become necessary for long-term monitoring of nitrate and nitrite concentrations in foods for susceptible populations. This study, therefore, seeks to determine the levels of residual nitrite in some processed meat products in Ghana and to assess the health risk of consumption of nitrite preserved meat in a bid to raise awareness regarding the correct use of this additive in Ghana.

2. Materials and Methods

2.1. Dietary and Lifestyle Questionnaires

Participants were made to complete a 24-hour self-administered food frequency questionnaire upon explaining the content of the questionnaire. The participants were required to describe the types and amounts of the under listed processed meat products; *sausages, salami, hotdogs, bacon, ham and corned beef* which they had consumed within 24 h and over the period from the time the questionnaire was administered to them. They were also supposed to provide the number of portions of specific processed meat they chose as well as the number of times they consumed per day. Real food models of standardised portions were used to help participants to better describe amounts weight. The questionnaire also required participants to provide socio-demographic data on the level of education, body weight and height and their ages. The actual weights and heights of participants were measured. The database of the data obtained from 300 participants was imported into Epi-info 7 (2013) and the consumption was analysed using frequencies and percentages. The products with the highest percentages were taken to be the most consumed processed meat by respondents.

2.2. Sampling

The most commonly consumed processed meat products as reported by the study population were obtained for laboratory analysis. Based on availability, fifty (50) different types and brands of the following processed meat products; Chicken Sausage, Pork Sausage, Beef Sausage, Corned Beef and Bacon were randomly purchased at supermarkets, minimarkets, and cold stores in the Ayawaso West Sub Metro, Accra and transported in a cold chain stored in plastic bags and then kept in a refrigerator prior to analysis.

2.3. Cooking Methods

Six different commercially commonly available sausages were used in this study and were cooked using three (3) different mostly used method of cooking in homes and outside homes, namely, boiling, pan-frying and oven grilling. One kilogram of sausage per brand was taken from the already acquired products and used for analysis. The samples were divided into four (4) portion of equal quantity, one portion for each cooking method and the last portion serving as the control for the analysis.

Boiling was done by the addition of water to the sausage in a sauce pan and cooked for about 7 min. The sausage was then drained of all the water and made to cool. Pan-frying was also done in frytol cooking oil for about 10 min, then removed and made to drain of all the oil and cooled. The third portion of the mother samples was grilled in an oven for 10 min and made to cool. The same procedure was applied for the other 5 brands of the sausage for completion for the analysis. Throughout the whole cooking process, moderate heat was applied to obtained optimize cooking throughout all the three cooking methods. The raw (uncooked) sausage serving as the control and all the cooked sausages was homogenized for analysis.

2.4. Nitrite Assessment

To evaluate the exposure estimates, all samples were analysed for nitrite using a spectrophotometric method based on the sensitive and widely used diazotisation-coupling Griess reaction [15]. Nitrite was determined by diazotising with sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye that was measured at 540 nm. Duplicate analyses were carried out to estimate the within and between-sample precision of the results.

2.5. Exposure Assessment

Mean and maximum nitrite intake exposure of the meat products in the study population were calculated using the formula:

$$CDI = \frac{C \times CR \times EFD}{BW} + \frac{1}{AT} \quad (1)$$

where: CDI is Chronic daily intake; the amount of chemical at the exchange boundary (mg/kg day). C is average exposure concentration over the period. CR is contact rate, the amount of contaminated medium contacted per unit time. BW is Average body mass over the exposure period (kg). AT is averaging time; the period over which the exposure is averaged (days). EFD is exposure frequency and duration, where it is divided into EF is exposure frequency (days/year), and ED is exposure duration (years).

2.6. Risk Estimation

In calculating the risk, distribution characteristics of each exposure parameter were tested to help in determining the probabilistic distribution of the exposure dose after simulating result using Monte Carlo simulations. The Monte Carlo simulations in risk assessment provide an understanding of the degree of uncertainty and variability around a risk estimate that single-point estimates of risk cannot provide [16].

After the CDI is obtained, a comparison between the CDI and the RfD (reference dose) should be made; if the CDI is below the RfD, it is assumed that the risk is negligible for almost all members of an exposed population, i.e. Risk $(HQ) = \frac{CDI}{R_f D}$.

It has also been established that an HQ value more than 1 (HQ >1) shows a significant risk level, while an HQ <1 shows no health risk for consumers [17].

2.7. Statistical Analysis

The questionnaires filled by subjects and the gathered data about nitrite residual and amount of consumed meat products by the study population went under statistical analysis by statistical software SPSS version 16 at a significance level of 5%. ANOVA test, Tukey HSD option were applied. To calculate the health risk of a populace that are exposed to a chemical hazard, the probability of getting the endpoint disease (not the probability of dying of the endpoint disease) and the associated dose, consist of an average taken over an assumed 70-year human lifetime if dose is carcinogenic and 30 years if non- carcinogenic. To correct all uncertainties, exposure to nitrites were calculated considering 10,000 iterations using the Monte Carlo simulation technique which is based on @RISK software (Palisade, US-version 6) carried out using Microsoft Office Excel.

3. Result and Discussion

3.1. Nitrite Concentrations in Some Selected Meat Products

Nitrites are used as antimicrobials agents which provide protection against toxic microorganisms such as *Clostridium botulinum* and give cosmetic effect and enhance the flavour of meat products. Data from the questionnaires showed that bacon, corned beef and sausages were commonly consumed meat products on the market; however, beef, chicken and pork sausages were more consumed than the other products. The mean concentrations of the meat products groups analysed in this study as presented in Table 1 indicate that residual nitrite in all samples tested were below the recommended permissible level of 120 mg/kg by WHO/EU except sausages (beef, pork and chicken) which contained higher levels. However, at a significance level of 0.05, the total mean nitrite content in all the processed meat analysed was 139.85 mg/kg. Studies from other parts of the world have shown that nitrite content in different types of meat product demonstrates great variability in concentrations [18].

Table 1. Levels of Nitrite in Selected Processed Meat

Meat product	Mean (mg/Kg)
Bacon	43.08±5.08
Corned beef	79.05±7.24
Beef sausage	191.54±21.70
Chicken sausage	131.94±16.08
Pork sausage	163. 48±18.34

Values are Mean ±SE.

3.2. Nitrite Levels in Commonly Consumed Sausage Samples

Figure 1 shows the uncooked (raw) mean nitrite values of the six selected brands of sausage compared to residual nitrite values obtained from three (3) cooking methods applied to same raw sausages.

The residual nitrite concentration of uncooked German veinna sausage was 164 mg/kg which was not within the allowable residual nitrite level of 125 mg/kg for sausage [12]. The results showed that all cooking methods had a decreasing effect on the residual nitrite level of uncooked samples. Boiling method had better reduction effect (78 mg/kg) in comparison with grilling (80 mg/kg) and pan frying (81 mg/kg) methods, respectively.

The residual nitrite concentration of uncooked Nempa sausage was 248 mg/kg that was much higher than the maximum acceptable limit (125mg/kg) for residual nitrite in cured meat product especially in sausages. It was determined that boiling (218 mg/kg) was the only cooking method which had a reduction effect on the nitrite level of raw sausage. Pan frying (269 mg/kg) and grilling (387 mg/kg) both showed an increasing effect on the residual nitrite level of samples (Figure 1).

The mean nitrite level of uncooked Adom sausage (108 mg/kg) was within the recommended acceptable maximum limit for cured meat products such as sausage. From the point of view of cooking effect on residual nitrite level, boiling (104 mg/kg) was within the acceptable limit and the only method which had a reduction effect on the nitrite level of raw sausage. Pan frying (217 mg/kg) and grilling (198 mg/kg) all resulted in an increase in the content of residual nitrite (Figure 1).

The results indicated that the nitrite level of uncooked Bino sausage brand (187 mg/kg) was higher than the recommended acceptable maximum limit. Although the boiling (154 mg/kg) and grilling (150 mg/kg) methods had a reduction effect on the nitrite level of raw sausage but this level was not within the acceptable range.. On the other hand, pan frying (255 mg/kg) had an increasing effect on the residual nitrite level which were higher than the acceptable limit.

The residual nitrite concentration level of uncooked Frankfurter was 188 mg/kg which was higher than the maximum recommended level of 125 mg/kg. It was seen that, boiling (54mg/kg), and pan frying (45 mg/kg) and grilling (mg/kg) all had a reduction effect on the nitrite level of uncooked sausage. In addition these residual nitrite levels of all samples were within the recommended acceptable limit for nitrite in cured meat including sausages. Although uncooked Imperial sausage had the highest residual nitrite level (347 mg/kg) among the sausage brands boiling (190 mg/kg), grilling (205 mg/kg) and pan frying (218 mg/kg) all had a reduction effect on the residual nitrite , unless, all the nitrite levels were higher than the acceptable level.

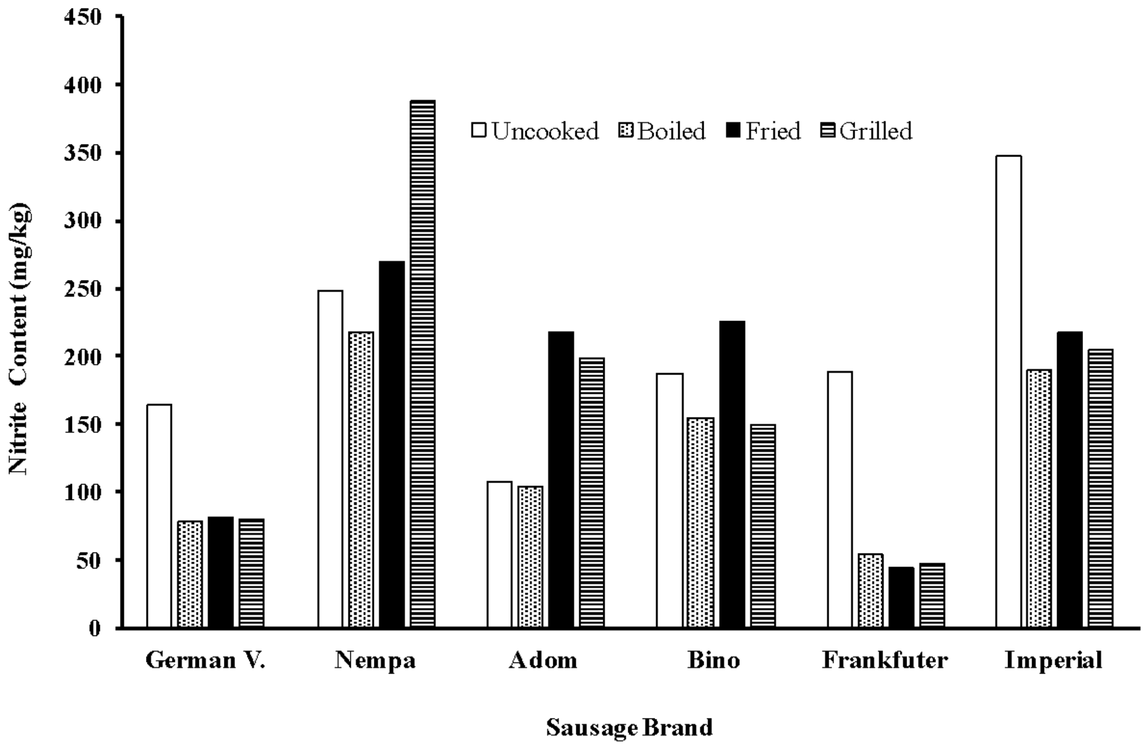

Figure 1. Mean nitrite content (mg/Kg) of uncooked and cooked sausage brands

As shown in Table 2, the mean residual nitrite values of uncooked sample (206.97 mg/kg) as control group and all the cooked samples such as boiled (132.89 mg/kg), pan fried (177.93 mg/kg) and griled (181.57 mg/kg had higher values than the acceptable limits (125 mg/kg) recommended by the Ghana Standard Authority [12]. In another study carried out in Denmark [13], the most processed meat including sausage products were evaluated and the authors indicated that the residual nitrite level was below the limit for nitrite in cured meat product (60 mg/kg for most product and 150 mg/kg for certain special products) recommended by Danish Veterinary and Food Administration.

Table 2. The mean residual nitrite levels in raw and cooked sausages

Sausage sample	Residual Nitrites Level (mg/Kg)
Raw sausage	206.97±33.57 [a]
Boiled sausage	132.90±26.60[b]
Fried sausage	177.93±49.16[a]
Grilled sausage	181.57±38.54[a]

Values are Mean ±SE. Values with the different letters denote significant difference ($p < 0.05$).

The effect of cooking methods on the content of residual nitrite in sausage is presented in Table 2. The results, expressed in mean values showed that all three cooking methods resulted in decreasing effect on the residual nitrite, additionally, boiling treatment of raw sausage showed a strong correlation at 67% and showing much significance at $p < 0.05$. This could be explained by the solvent nature of water compared to oil and grilling. The effect cooking methods on the residual nitrite level of the sausage was in agreement with some previous finding [19,20].

Among the cooking methods, none of the cooking method was able to effectively reduce the uncooked nitrite concentration level into the acceptable limit (125 mg/kg) set out by the Ghana Standards Authority [12].

The effects of brands by cooking method interaction on the residual nitrite levels were assessed using ANOVA (two-way) at the α-level of 0.05. The results indicated that the interaction of brands and cooking methods was significant ($p = 0.000$) and therefore a comparison analysis was required to further explain which levels of the interactions were significant. A Tukey pairwise comparison of the nitrite levels of brands by cooking method shows that fried Nempa had significantly higher nitrite concentrations than all the cooked forms of all the other brands except for raw Imperial, with which it was similar. On the other hand, Grilled Frankfurter had significantly lower nitrite concentrations than fried Nempa, raw Imperial, grilled Nempa, grilled Imperial, raw Nempa, grilled Bino, boiled Nempa, grilled Adom, fried Imperial, fried Adom, boiled Imperial, raw Frankfurter, raw Bino and raw German sausages whereas it had similar nitrite concentrations with boiled Bino, fried Bino, raw Adom, boiled Adom, grilled Germaine, fried Germaine, boiled Germaine, boiled Frankfurter and fried Frankfurter.

The results of residual nitrite levels in the brands by cooking effect, shows that it is important to know which cooking method is best for a particular brand in order to keep nitrite levels within the acceptable limits safe for human consumption. Different companies that manufacture sausage must therefore assess the nitrite levels of their products after subjecting them to different cooking levels so as to inform consumers on best cooking methods for their brands, and/or adopt measures in the production to keep the nitrite levels in their products safe even after cooking them. From the results, it was observed that the German and Frankfurter brands which had 188mg/kg and 164 mg/kg respectively as the mean nitrite value for the uncooked sausage, had significantly decreased ($p < 0.05$) nitrite levels after all the cooking methods was carried out on them, making them safe for any cooking method.

However, brands Nempa and Adom showed an increase in nitrite concentration from the uncooked state compared to the nitrite content after the cooking methods were done especially in fried Nempa and grilled Adom sausages showing sharp contradiction to the earlier two brands discussed. Additionally, these two brands were the only local brands in the brands surveyed indicating that the local sausage production companies need to be monitored strictly to abide by the internationally accepted standards for nitrite concentration and also, try to incorporate measures that can stabilize the nitrite levels in their products.

3.3. Nitrite Dietary Exposure Assessment

As recommended by international guidelines [21], the use of residue level mean when calculating dietary exposure is a realistic and appropriate estimation of chronic exposure to be compared with ADI, a toxicological reference value established over an entire lifetime. The mean dietary nitrite exposure per person in the study population was 5.05 mg/kg/bw. However, the dietary nitrite exposure for 50^{th} and 95th percentile consumers were 2.09 and 19.85 mg/kg/bw, respectively and the values determined were higher than the acceptable daily intake (ADI) of 0.07mg/kg/bw/day (Table 3). It can further be explained that, the concentration of nitrite that a person ingests per day to stand a probable risk of adverse health effect within 1 year is 5.05 mg/kg/day for the average consumer, and 19.85mg/kg/day for the 95th percentile consumer or the heaviest consumers and those who do not consume much are free of adverse effect. The dietary intake of nitrite obtained in this study is far higher than that estimated by [22,23] (0.0 - 0.07 mg/kg bw/day equivalent to 4.2 mg/ day for an individual weighing 60 kg) for the general population [24].

Although other studies in other countries have recorded higher nitrite exposure rates which revealed an average nitrite uptake through diet in students as 1.7 mg/kgbw [25], still the dietary intake (5.05mg/kg bw/day) in this study remains higher. For example, in Denmark, a study in children indicated nitrite intake of 0.014 mg/kg bw due to meat products consumption [26]. It has been reported that the nitrite uptake from the diet in Swedish children between 11 and 12-year-old was 0.007 mg/kg bw [27]. Similar study conducted in 346 children revealed that nitrite intake of 0.83 mg/day [28]. Their study concluded that the average nitrite intake (0.83 mg/kg/day) was far higher than the average nitrite intake from EU foods (0.06 mg/kg/day). In this regard, findings from the previous study show that nitrite intake among the study population is much higher which requires immediate measures. The high nitrite intake recorded in this study may be attributed to the percentage of meat in the processed meat, processing, and poor storage conditions among others.

Table 3. Mean and Percentile Dietary Exposure Estimates from Nitrite in the Processed Meat

Mean	5.05
50th Percentile	2.09
95th Percentile	19.85
Acceptable Daily Intake (ADI) mg/kg/bw	0.07

3.4. Nitrite Dietary Risk Estimation

The risk (hazard quotient) evaluated in the study after Monte Carlo simulation at 10,000 iterations resulted in a mean value of 15. 65 which mean the hazard quotient (HQ) is greater than 1. Table 3 indicates the mean, the 50th and the 95th percentiles of the risk estimates in the study population. Since cancer assessment is not assessed under the EPA IRIS program the emphasis was on their non-carcinogenic effect and the risk (hazard quotient) evaluated in the study resulted in a mean value of 15. 65 which means a hazard quotient (HQ) > 1 and therefore a potential risk of adverse ill effect on the population (Table 4). At 95 % confidence interval, the probability of a person to the risk of adverse health effect due to the eating of processed meat products is between 0 and 54.40. That is, fewer consumers of these products do not stand the risk of adverse effect in a year whiles about 55 out of 100 people who ingest the products stands a maximum risk of adverse health effect in a year.

Table 4. Mean and Percentile Risk Estimates Estimates from Nitrite in the Processed Meat

Mean	15.65
50th percentile	8.70
95th percentile	54.40
Risk (hazard Quotient)	≥ 1

4. Conclusion

The findings of this study show that commonly consumed processed meat products among Ghanaians in the Ayawaso West Sub Metro contained a higher amount of residual nitrite (139.85 mg/kg) than permitted by WHO/EU standard of 120 mg/kg of nitrite. The estimated risk value shows that consuming sausages, corned beef and bacon are at a greater disadvantage to a range of health risks. Thus, the probability of 50 % of the study population who consume processed meat especially sausages was about 9 out of every 100 people. The risk quotient or hazard quotient obtained was far greater than 1 indicating potential adverse health effect to the consumers. However, the results suggest boiling as a safer cooking method for cured meat and sausages studied.

Conflict of Interest

None.

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