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Estimation of nitrite level and effect of processing on residual nitrite level in sausages marketed in Dharan, Nepal

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The study was conducted to examine nitrite levels in processed meat products (sausage, salami, bacon, and ham) marketed in Dharan City and to determine the effect of common methods of processing on residual nitrite levels in sausages. Preliminary survey was conducted to know the popular processed meat products. Total of 44 samples were analyzed for nitrite levels and 10 sausage samples were subjected to two different heat processing methods: Frying and boiling. The nitrite contents in all samples were found to fall within the mandatory limits by the Nepal government with a wide range of variation (3.18-101.26 ppm). Both frying and boiling reduced the residual nitrite level in sausages with greater reduction by boiling at 100°C for 15 min. As long as the measured nitrite level in the sausages produced continues, the level of nitrite is not considered to pose a major risk to human health or safety.

Key words: Nitrite level, processed meat, sausages, heat processing, Dharan.

INTRODUCTION

Nitrites and nitrates are food additives (preservatives) with wide applications in the meat industry. They improve the quality, durability and safety of products, and, above all, inhibit the growth and reproduction of bacteria *Staphylococcus aureus* and *Clostridium botulinum*. On combining with myoglobin nitrites give the stable nitrosomyoglobin, a heat stable bright red color compound, which is most desirable in the meat products (Heinz and Hautzinger, 2007). Nitrates are relatively non-toxic, but nitrites and nitrite metabolites such as nitric oxide and N-nitrosocompounds have potential adverse health effects. According to the International Agency for Research on Cancer (IARC), nitrates or nitrites consumed are likely to be human carcinogens under conditions favouring endogenous nitrosation. Legal limits for the addition of nitrates and nitrites have been set by several countries (Govari and Pexara, 2018). Nitrite has an acceptable daily intake (ADI) of 0.07 mg kg^{-1} body weight and is dependent upon harmful effects in rodents on the lungs and cardiovascular system (Merino et al., 2016). The government of Nepal has prescribed the legal limit of nitrite in food to be 200 ppm (DFTQC, 2017).

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However, Codex Alimentarius has limited the residual nitrite content as 80 mg/kg in processed comminuted meat, poultry and game products (Codex Alimentarius, 2017). Despite such regulations, the chance of a high amount of nitrate and nitrate in cured meat cannot be ruled out. It should be kept at a minimum level as possible to care the health of consumers. There have been numerous attempts to reduce the use of nitrites in the meat industry due to their proven carcinogenic effects of nitrosamines (Lee et al., 2018).

The intake of per capita meat in Dharan was 13 kg while national meat consumption per capita was just 9 kg (Ekantipur, 2014). Dharan is thus one of the largest consumers of meat and meat products in the Nation, yet detailed study in the preferences on processed meat products is not available. As the consumption of meat has been growing rapidly among the urban population, and the country has been importing processed meat products. However, strict regulation and monitoring for processed meat are still lacking. The ignorance along with the haphazard use of additives might cause adverse effects on consumer's health.

Sausages like meat products are generally processed before consumption. The amount of residual nitrite in sausages decreases with storage time and preprocessing before consumption. More than 80% of the nitrite is already degraded within two days (Qadar, 2013). The greater the temperature and the duration of cooking, the more is the reduction (Wang et al., 2012) and leaching of nitrite on boiling water also reduces the nitrite level (Mckenzie, 2017). Research shows that the amount of residual nitrite decreases with several cooking conditions, but there has been less research in Nepal on residual nitrite levels of sausages after treatment. This study is conducted to examine nitrite levels in processed meat products (sausage, salami, bacon and ham) marketed in Dharan City and to determine the effect of the common method of processing on residual nitrite levels.

MATERIALS AND METHODS

Study area and time

The study area is a Dharan Sub metropolitan city of Province-1, Nepal. It is situated in the foothills of the Mahabharat range in the north and the Terai region in the south at an altitude of 349 m. It is a commercial center between the hilly region and the Terai plains of the eastern development region. The study was conducted from October 2018 to January 2019.

Preliminary market survey/observation

A preliminary observation/survey was carried out before sample collection to identify the popular processed meat product, traded places, way of consumption and methods of processing. A total of 110 meat lovers and street vendors were asked for their favorite processed meat products and processing method preferred before consumption.

Sample collection

A total of 44 processed meat samples (sausages, salami, bacon and ham) were collected from different parts of Dharan city. Samples were sent to the laboratory by maintaining the cold chain and stored in a refrigerator at 4°C. If the analysis could not be completed within two days, the leftover samples were frozen in polyethylene bags at -18°C in unopened condition before the analysis could begin.

Processing methods applied to the sausages

After raw sample analysis, sausage samples having nitrite levels greater than 10 ppm were randomly selected and coded as A1, A2…, and A10. Then samples were subjected to two different thermal processing methods; frying and boiling. Each treatment was carried out in triplicate manner for all 10 samples. The samples used for the treatments were of dimension 21 to 22 mm diameter and 120 to 150 in length.

Frying

The samples were fried at 149±2°C for 4 min (2 min on each side) on a frying pan.

Boiling

The sausages were boiled with potable water at 100°C for 15 min in a stainless-steel bowl of capacity 2 L. The ratio between sausages to the amount of water used was 1:10 by weight. After heat processing, samples were cooled to room temperature and again analyzed for the residual nitrite level.

Determination of nitrite in meat samples

Nitrites contents in the samples were determined by the method described in AOAC (2016), with slight modification. The sample was extracted with hot distilled water and the extract was filtered. Nitrite contained in the filtrate produces on reaction with sulphanilamide and N-1-napthylethylene diamine dihydrochloride a red color and its absorbance was measured at 540 nm in a UV-vis spectrophotometer. In brief, 5 g of crushed sample was mixed with distilled water in a 100 ml volumetric flask. The extraction was carried out by placing the flask in a shaker water bath at 80°C. After 2 h of extraction, solution was cooled and filtered. 10 ml of filtrate was then taken for color development in 50 ml volumetric flask followed by recording the absorbance for each sample. The concentration for the corresponding absorbance was found from the standard curve obtained by plotting absorbance vs. different concentrations of nitrite. Each sample was analyzed in triplicate. Results were expressed as $NaNO_2$ in ppm. Values are shown in terms of mean, estimated standard error of the estimated mean, minimum and maximum value.

Data analysis

MS Excel and *GenStat Release 12.1* were used for data analysis. Descriptive analysis and analysis of variance, were performed for statistical analysis. Fischer's Least Significance Difference (LSD) was used further to compare sample means. Statistical analysis was performed at 5% level of significance.

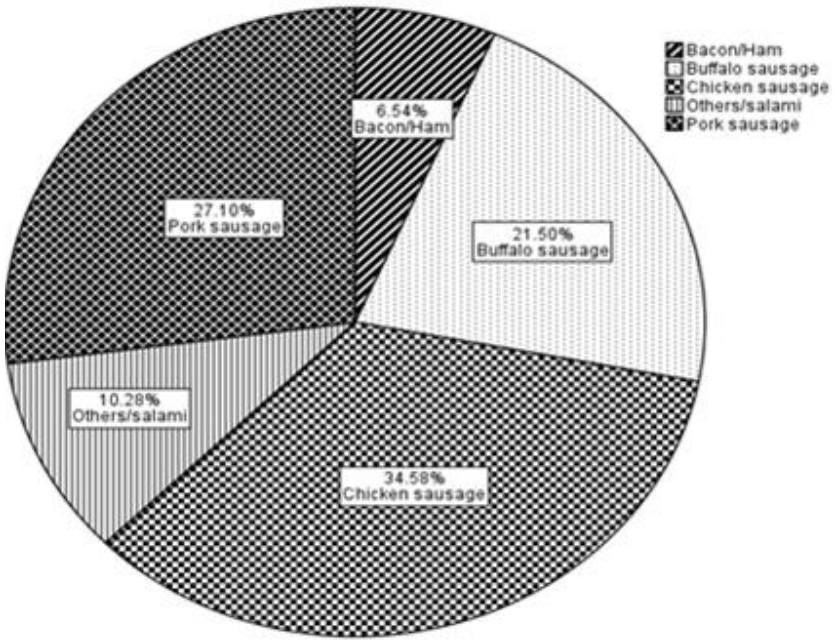

Figure 1. Consumer preferences for processed meat products in Dharan (N=107).

Table 1. Amount of nitrite in raw sausages samples.

Product type	Sample size (n)	Mean nitrite content (ppm)
Chicken sausage	13	29.08±22.10 (3.18-62.20)
Buffalo sausage	11	49.96±28.14 (15.26-83.12)
Pork sausage	9	64.83± 31.15 (26.01-101.29)
Others/salami	7	49.64±27.40 (17.27-83.12)

Values in the parenthesis are minimum-maximum amount of that product type.

RESULTS AND DISCUSSION

Survey results

One hundred and seven respondents have responded positively towards the survey. Their preferences on processed meat products are shown in Figure 1. Sausages, ham, bacon and salami were the popular products available. Sausages are more popular among the consumers while other meat products are still in the preliminary phase of introduction in the Dharan market. These processed meat products were found to be manufactured by 11 different manufacturers. On observation, cured meat products were found to be sold at departmental stores in refrigerated conditions. Besides these, sausages could be found in some street food stalls and major food outlets as well.

While observing the processing methods used by street food stalls, it was found that sausages were generally fried for 4-5 min around 125-150°C and if boiled sausages were asked for, sausages were boiled at 100-105°C for 12-15 min. The time-temperature profile for processing of sausages in Dharan market showed close resemblance with Hill et al. (1973) and Li et al. (2016) for frying and boiling respectively.

Nitrite level on raw samples

The amount of nitrite in the sausage samples ranged from 3.18 ppm (chicken sausages) to 101.29 ppm (pork sausage) (Table 1). Similarly amount of nitrite on bacon/ham found in the range of 15.26-37.82 ppm (Table 2). Less than one third (27.27%) of the analyzed samples had nitrite content less than 20 ppm. Nearly half (43.18%) samples had the nitrite content falling between 20-50 ppm and 29.55% had the nitrite contents above 50 ppm. The average nitrite content (64.83) of pork sausages was found slightly higher than other types of products.

However, this variation did not appear to be related to the type of cured meat product and all the samples had nitrite contents within the limits specified by mandatory

Table 2. Amount of nitrite in bacon/ham.

Product type	Sample size (n)	Mean nitrite content (ppm)
Bacon/Ham	4	30.52±10.52 (15.26-37.82)

Values in the parenthesis are minimum-maximum amount of that product type.

Table 3. Residual nitrite level in sausages after frying and boiling.

Product type	Sample code	Raw (ppm)	Fried (ppm)	Boiled (ppm)	Percentage decrease	
					Frying (%)	Boiling (%)
Sausage	A1	13.2^{a}±1.72	10.50^{b}±1.37	6.66^{c}±0.87	20.45	49.55
	A2	33.07^{a}±1.06	28.99^{b}±1.02	23.02^{c}±0.38	12.34	30.39
	A3	55.15^{a}±0.50	49.42^{b}±0.45	41.89^{c}±0.38	10.39	24.04
	A4	63.20^{a} ±0.99	51.83^{b}±0.95	15.23^{c}±0.21	17.99	75.23
	A5	72.9^{a}±0.50	56.67^{a}±0.38	38.42^{b}±0.93	22.28	47.31
	A6	81.69^{a} ±1.24	60.07^{b}±0.91	60.87^{c}±0.93	26.47	25.49
	A7	17.27^{a}±2.01	15.56^{a}±1.81	7.36^{b}±0.86	9.90	57.38
	A8	35.61^{a}±2.21	33.07^{ab}±2.05	32.82^{b}±2.04	7.13	7.83
	A9	28.10^{a}±2.08	25.18^{a}±2.05	16.84^{b}±1.25	10.39	40.07
	A10	99.63^{a}±1.54	57.92^{b}±0.90	50.97^{b}±0.79	41.86	48.84

The same letters in the superscript, row-wise, signify no significant difference ($p>0.05$) between the means of triplicate determination.

standards of Nepal Government (DFTQC, 2017). While about 18% (N=8) of the samples had crossed the codex standard of 80 ppm. From the result, we can conclude that most of the cured meat products had added nitrite/nitrate. However, the level is within that prescribed by government regulation.

The comparatively lower value of nitrite in the samples might be due to the depletion of nitrite during storage of the product from the time of manufacture to the analysis. It is a common practice to use 50-100 mg kg^{-1} sodium nitrite in cooked cured type meat products against growth and botulism toxin by *C. botulinum* (Bardhi et al., 2014). Oxidation-reduction reactions converting nitrite to nitrous oxide could be the reason for the observed decrease in residual nitrite level in the products (Hill et al., 1973). The decrease in nitrite level from 116 to 9 ppm residual nitrite after 30 days of storage was observed by Bardhi et al. (2014). The loss of nitrite in a product with time is dependent on several factors including the heat process, pH of the product, storage temperature and addition of ascorbic acid or other reducing agents (Bardhi et al., 2014).

Effect of frying and boiling on sausage residual nitrite

All the sausages samples showed reduced nitrite level by both boiling and frying treatments, both the treatments significantly reduced the nitrite level with wide variation in percentage decrease values (Table 3). There was a maximum of 41.86% reduction on nitrite level was observed by frying with a minimum reduction of 7.13% in sample A8. Similarly, for boiling treatment maximum reduction of up to 75.23% was observed while the lowest reduction observed was 7.83%. The greater range of variability in percentage reduction in sausage nitrite levels upon both types of heat processing is in agreement with the results obtained by Hill et al. (1973). The decrease in nitrite caused by cooking ranged from 3.4 to 41.0% which did not appear to be related to a common parameter such as emulsion type, product size, or quantity of nitrite added initially (Hill et al., 1973).

Regarding frying treatment, Soleimani et al. (2015) observed that initial nitrite content of 33.57 mg kg^{-1} in the sausage was decreased to 26.46 mg kg^{-1} after frying at 120°C for 5 min. Mean residual nitrite content was significantly different at other temperatures and cooking durations. The mean residual nitrite content reached to1.42 and nil after frying at 220°C for 5 and 10 min, respectively. With regards to this, the greater the temperature and duration of cooking, the more the reduction in residual nitrite content of the final product. This decreased nitrite upon frying can be attributed to the formation of nitrosamine (Wang et al., 2012).

Regarding boiling treatment, Li et al. (2016) have found boiling as method to remove the residual nitrite in sausage. And suggested that the optimal conditions for processing are 100°C for 15 min, and the solvent/ material ratio of 20, which resulted in the 19.47% removal

of nitrite in sausage. This is because of the solubility of nitrite in water. Since sodium nitrite is soluble in water it is easy to extract it from meat with hot water. Boiling and frying of the sausages are two common processing steps before consumption principally for the taste and safety concern. But at the same time the added advantages of such processing can be implicated though reduction in residual nitrite level in the products.

Conclusions

The level of nitrite in processed meat products marketed in Dharan is within the mandatory limits imposed by the Government of Nepal. As long as this level is maintained in the production of sausages, it will not pose health risks to a human. Furthermore, the processing methods used are also responsible for lowering the nitrite level significantly in the diet. Boiling of sausages before consumption is the best practice to be followed to reduce the nitrite level in the product.

Practical applications

Amount of nitrite in cured meat products is of great concern because of its potential carcinogenicity. The level of nitrite in commercially available meat products must be monitored regularly to safeguard the consumers' health. Results of the present study can serve as a useful parameter for regulatory agencies and producers. A similar study can be replicated to a different part of the country. The findings can be compared and used for policies formulation and action to be taken.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Association of Official Analytical Chemists (AOAC) (2016). Official Method of Analysis, Association of Official Analytical Chemists. 16th edition Washington DC, USA.

Bardhi G, Boci I, Laze, X (2014). Level of nitrite content in cured meat products produced and traded in Albania. European Conference of Food Science and Technology.

Codex Alimentarius Commission (2017). General Standard for Food Additives CODEX STAN 192-1995. Codex Alimentarius. Retrieve from http://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/.

Department of Food Technology and Quality Control (DFTQC) (2017). "Minimum Mandatory Standard in Food and Feed Products". Department of Food Technology and Quality Control (Ministry of Agriculture), Nepal. Retrieve from http://www.dftqc.gov.np/downloadfile/4_1590417369.pdf

Ekantipur (2014). Per capita meat consumption up 11 kg Kathmandu Post. Kantipur Publications Pvt. Ltd. Retrieved from http://kathmandupost.ekantipur.com/news/2014-05-16/per-capita-meat consumption-up-11-kg.html. [Accessed 29 March 2019].

Govari M, Pexara A (2018). Nitrates and nitrites in meat products. Journal of Hellenic Veterinary Medical Society 66(3):27.

Heinz G, Hautzinger P (2007). Meat processing technology for small to medium scale producers. http://www.fao.org/3/ai407e/ai407e.pdf

Hill LH, Webb NB, Mongol ND (1973). Changes in residual nitrite in sausage and luncheon meat products during storage. Journal of Milk Food Technology 36(10):515-519.

Lee H, Lee S, Kim S, Lee J, Ha J, Choi Y (2018). Microbiological safety of processed meat products formulated with low nitrite concentration-A review. Asian-Australas Journal of Animal Science 31(8):1073-1077.

Li Y, Gan CL, Cheng M, Chen W, Cao LR, Zhao T (2016). Effects of Cooking Process on the Content of Nitrite in Sausage. International Journal of Food Nutrition and Safety 7(1):52-60.

Mckenzie P (2017). Determination of Nitrite in Processed Meat. Retrieved from https://web.williams.edu/wpetc/chemistry/epeacock/EPL_AP_GREY/LABS/NITRITEs.pdf.

Merino L, Darnerud P, Toldra F, IIback N (2016). Time-dependent depletion of nitrite in pork/beef and chicken meat products and its effect on nitrite intake estimation. Food Additives and Contaminants 33(2):186-192.

Qadar H (2013). Spectrophotometric determination of nitrite in curing meat samples. Applied Science Reports 3(3):153-156.

Soleimani M, GhajarBeigi P, Javadi M, Jahed G, MohammadpourAsl A, Hajipour A, Hajhosseini A (2015). Effects of different cooking methods and temperatures on residual nitrite content in sausage. The Journal of Qazvin University of Medical Sciences 19(4):46-42.

Wang P, Xu X, Zhou G (2012). Influence of various cooking methods on the concentrations of volatile N-nitrosamines and biogenic amines in dry-cured sausages. Journal of Food Science 77(5).