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Status of Sodium Nitrite in Meat and Meat Products Available in the Market of Kathmandu, Nepal.

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Sodium nitrite was determined in 64 meat and meat products available in National Food and Feed Reference Laboratory from July 2017 to June 2018 following the AOAC (2016). None of the samples exceeded the Government of Nepal and India standard (200 ppm) and approximately five percent of the total samples had crossed the European Union standard (150ppm). Highest range (1.49-165.72 ppm) of sodium nitrite was found in chicken sausages and lowest (Not detected-55.83 ppm) in miscellaneous products (meat pickle, mo:mo, kebab, dried meat, and claws).Chicken and buff sausages were spiked at 50, 100 and 200 ppm level and the recovery were found to be 84.32, 94.97, 89.97 and 99.84, 104.36, 105.99% respectively. Overall recovery were significantly higher (p=0.000) in buff sausage (103.40 ± 3.57%) *than in chicken sausage* (89.75 ± 4.79 %) *at 5% level of significance. Sodium nitrite in the quality control sample was found to be 162.5±1.08 ppm which was within the range (138-226ppm) given by the supplier.*

Keywords: Sodium nitrite, ppm, recovery, limit of detection, limit of quantification

Introduction

Meat and meat products are one of the major food products for human. Consumption of meat and meat products in Nepal has been increasing day by day. From July 2014 to June 2015, 879501 metric tons of meat was consumed in Nepal (CBS, 2015). The reasons behind the increased in consumption are increase in per-capita income, fast growing urbanization, and readily availability of meat and meat products. Besides consumption of meat itself, consumption of different meat products is also increasing day by day. Different kinds of meat products such as: sausages, ham, bacon, dried meat, meat pickles, *kebab*, meat balls, meat fingers, patty etc. are now readily available in the Nepali market.

Among the different ingredients added in meat products, sodium nitrite is used relatively in small quantity as a nitrite curing salt during the production of meat products to produce the desired pickling red color (Belitz, Grosch, & Schiebierle, 2009). Nitrite combines with myoglobin to give stable nitrosomyoglobin, a bright red color compound, which is heat stable (Heinz & Hautzinger, 2007). Mechanism of development of characteristic red color in meat after addition of nitrite is shown in the Figure 1 (Belitz, Grosch, & Schiebierle, 2009).

In addition to this effect, nitrite has certain inhibitory effect on growth of bacteria, attributes to desire curing flavor and stabilizing action against fat (Heinz & Hautzinger, 2007). 5-20mg of nitrite per kg is considered sufficient for red color development whereas, 50 mg per kg is required to develop the characteristics taste and 100mg per kg to have antimicrobial effect (Belitz, Grosch, & Schiebierle, 2009). However, nitrite is considered as a precursor of carcinogenic compounds like nitrosamines and has also been related to increased risks of gastric, esophageal, nasopharyngeal and bladder cancers (Zhang, Sun, Han, Zhang, & Hou, 2017). Because of this food safety aspect, usage of nitrite in meat products is regulated by law. Mandatory Food Standards of Nepal (DFTQC, 2016) and Food Safety Standard of India (FSSAI, 2011) both have allowed a maximum of 200 ppm of sodium nitrite in meat and meat products. Similarly, European Union has set the maximum limit of 150 mg per kg of nitrites in non-heat-treated and heat-treated (Except sterilized meat products) meat products (European Commission , 2011). However, Codex Alimentarius has limited the residual nitrite content as 80 mg/kg in processed comminuted meat, poultry and game products (Codex Alimentarius, 2017).

Different methods have been used to determine nitrites and nitrates in food products. However, extraction of nitrites and nitrates from the food matrix is vital for their determination. Siddiqui, Wabaidur, Khan, Alothman, Rafique, & Alqadami, (2018) and Hsu, Arcot, & Lee, (2009) had extracted nitrite from the sample by subsequent procedures of homogenization, heat treatment, centrifugation, filtration and injection in high pressure liquid chromatography for the estimation. However, Adam, Mustafa, & Rietjens, (2016) and Leth, Fagt, Nielsen, & Anderson, (2008) had heated the mixed sample, precipitated the protein by adding Carrez I and II solutions, developed a color using sulphanilamide and N- (1-napthyl)-ethylene-diammonium chloride and measured spectrophotometrically at 540 nm. Other researchers have used cyclic voltammetric (Yildiz, Oztekin, Orbay, & Senkal, 2014), capillary electrophoresis (Oztekin, Nutku, & Erim, 2002) and ion chromatography with UV detector (Siu and Henshall, 1998) method for detection of nitrite and nitrate in meat, meat and vegetable products.

So far in our knowledge, there hasn't been any research on the nitrites in the meat and meat products. The aim of this study is to find the status of nitrites in meat and meat products available in the market of Kathmandu, Nepal.

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Materials and methods

Sample collection

Samples of meat products brought by customers in National Food and Feed Reference Laboratory from July 2017 to June 2018 were used for analysis. 64 samples available in the laboratory were analyzed among which, 62 products were from Nepal and two products were from the People's Republic of China. The samples were classified into five categories namely buff sausages, chicken sausages, others (pork/fish/mutton) sausages, ham/bacon/salami/cold cut, and miscellaneous products (meat pickles, dried meat, *mo:mo*, *kebab*). Sample distribution is shown in the Table 1.

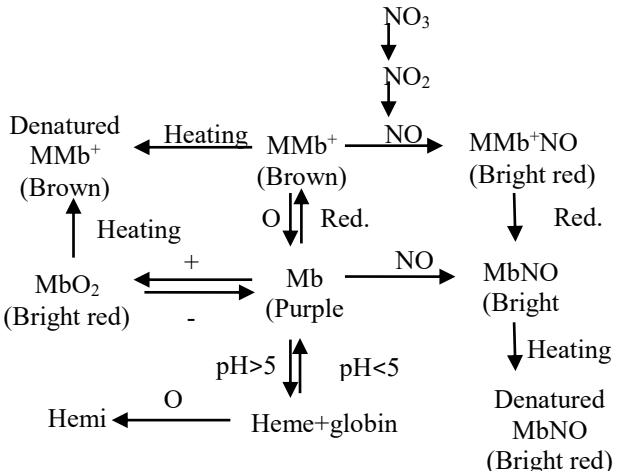

Figure 1. Myoglobin reactions (Mb: myoglobin, MMb^+: metmyoglobin, MbO_2: oxymyoglobin, MbNO: nitrosylmyoglobin, MMb^+NO: nitrosylmetmyoglobin) (Belitz, Grosch, & Schiebierle, 2009).

Materials UV-Vis spectrophotometer (Thermo, Genesys 10UV, USA), centrifuge (MPW-260, MPW MED. Instruments, Poland), water bath, centrifuge tubes, sodium nitrite standard (Sigma-Aldrich, purity: 99.999%), N-(1-Napthyl) ethylenediamine.2HCl (NED, AR grade), sulphanilamide (AR grade), acetic acid (AR), distilled water were used for the analysis.

Method Determination of nitrites in meat samples were performed following AOAC (2016), 20th edition (Method no. 973.31) with slight modification. Briefly, approximately 5g of minced sample was mixed with hot water and transferred into 100 ml volumetric flask, which was then heated in water bath (80ºC) for two hours with occasional shaking. The sample was cooled, volume was made up and was centrifuged at 6000 rpm for 10 minutes. 5-10 ml aliquot was taken in 50 ml volumetric flask and sulphanilamide and NED reagents were added to develop the color. Absorbance was measured at 540nm after 15 minutes against reagent blank using UV-Vis spectrophotometer. Calibration curves (0.0-1.0 ppm) were drawn for every lot of analyzed samples as shown in Figure 2. A regression coefficient (R^2) value of 0.997 to 1.0 was achieved. Each sample was analyzed in triplicate.

Results were expressed as $NaNO_2$ in ppm. Values are shown in term of mean, estimated standard error of the estimated mean, minimum and maximum value.

Data analysis

MS Excel and *SPSS Statistics 21* were used for data analysis. Descriptive analysis and analysis of variance, two-sample t-test were performed for statistical analysis. Statistical analysis was performed at 5% level of significance.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ of the spectrophotometer were calculated from calibration curve following the International Conference on Harmonization guideline (ICH Guideline, 1994). Formulas for calculation are given below.

a. Limit of Detection (LOD)

$$LOD = \frac{3.3 * \text{Sa}}{\text{b}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots I$$

b. Limit of Quantification (LOQ)

$$\text{LOQ} = \frac{10 * \text{Sa}}{\text{b}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots II$$

where, **Sa** is the standard deviation of the response and **b** is the slope of the calibration curve. The standard deviation of the response was estimated by the standard deviation of *y*-residuals of regression lines.

Recovery Test

Recovery test was performed in the chicken and buff sausages. Stock solution of 400 ppm $NaNO_2$ was used for spiking procedure. Spiking was performed at 50, 100 and 200 ppm level. Analysis was done following the procedure mentioned in the method section.

Percentage recovery was calculated as below.

% Recovery = (Observed – Neat) x 100/ Expected ..... *III*

where, Observed: Observed concentration in the spiked sample.
Neat: Observed concentration in the unspiked sample.
Expected: Expected concentration in the spiked sample (calculated based on assigned concentration of spiking stock and volume spiked into sample).

QA/QC sample analysis

Quality control sample of meat product was also analyzed to verify the results of the research work. The QC sample (Reference no. T15126QC) was bought from Fera Science Ltd (Fera) Sand Hutton, York, YO41 1LZ. Sample was kept under -18°C until analysis.

Results and Discussion

Sample analysis

Sodium nitrite content in 64 meat and meat products are shown in Table 1. None of the samples has exceeded the limit of $NaNO_2$ (200 ppm) set by Government of Nepal and India. However, approximately five percent (N=3) of the samples had crossed the European Union standard (150 ppm).

Highest range (1.49-165.72 ppm) of sodium nitrite was found in chicken sausages and lowest (Not detected-55.83 ppm) in miscellaneous products (meat pickle, *mo:mo, kebab*, dried meat, and claws).There is the possibility that the value of $NaNO_2$ of the products can also be supplemented by the

natural nitrites present in meat and other ingredients. Since nitrite can readily oxidize to nitrate due to presence of sequestering oxygen, nitrite content in the products can be affected due to conversion of nitrite into nitrate (Honikel, 2008). Merino, Darnerud, Toldra, & Ilback (2016) noted that nitrite was decrease faster for 24 hours after addition of nitrite during the storage of the meat products. No study was conducted so far on the presence of nitrite from natural sources in meat and meat products in the market of Nepal. So, there is no cut off value of $NaNO_2$ to consider whether the obtained value of $NaNO_2$ is residual. Different researchers had considered different residuals values for different types of meat and meat products.

Mean residual values of 0.1 to 12.2 ppm of nitrite in different category of conventional cured meat products were reported by (Gonzalez, et al., 2012). Iammarino & Taranto (2012) did not find nitrites in fresh meat (LOQ value of 4.5ppm) in their study. Similarly, Hsu, Arcot, & Lee, (2009) did not observed naturally presented nitrite in beef. Based on literature, this research considers 10 ppm of $NaNO_2$ as residual amount for data analysis.

Table 1 shows that all the buff sausages and ham/bacon/salami/cold-cut contained added $NaNO_2$. This indicates that the use of $NaNO_2$ is common in these kinds of products. Similarly, 90 % of chicken sausages and 67 % of other sausages (pork/fish/mutton) contained added $NaNO_2$. Meat (N=5) contain $NaNO_2$ lower than 10 ppm (Data not shown) indicating the use of $NaNO_2$ in this kind of miscellaneous meat products is still not common.

Table1
Sample distribution and $NaNO_2$ content in different meat products.

Type of Meat Product	No. of Sample	NaNO2, in ppm
		Average ± Standard error
Buff Sausage	19	72.42 ± 11.70 (14.83 - 159.70)
Chicken Sausage	19	56.70 ± 9.27 (1.49 -165.72)
Other sausage	6	44.17 ± 17.33 (2.55 - 114.97)
Bacon/Ham/ Salami	11	47.22 ± 7.36 (10.37 -87.59)
Miscellaneous	9	13.72 ± 6.24 (N.D.*- 55.83)
Total	64	

*N.D. indicates not detected, values in the parentheses are minimum and maximum value of $NaNO_2$.

Meat pickles and dried meat are the common Nepali products, which have comparatively higher self-life and do not need to add nitrite for color development. This might be the reason for low nitrite in these products. In other four miscellaneous products: chicken claw, chicken *mo:mo*, chicken Sheikh *Kebab* and prawn *achar*, contain higher than 10ppm of $NaNO_2$, which can be the outcome of added $NaNO_2$ or can be due to presence of nitrite in other ingredients.

Among the five types of meat products, greater interference was found in buff sausages. During the triplicate analysis of buff samples following the same procedure at the same time, pink color did not developed in some of the triplicates. However, such interferences did not occur in other meat products. Wootton, Kok & Buckle (1985) had also observed similar matrix interference during the analysis of meat and meat products.

Limit of detection (LOD) and limit of quantification (LOQ)
LOD and LOQ of the spectrophotometer were calculated as 0.0076 and 0.023 ppm. This indicates that the instrument used was sensitive to determine $NaNO_2$ in the samples with the given mandatory standard of Nepal Government.

Recovery test
Percentage recoveries of $NaNO_2$ after spiking in chicken and buff sausage are shown in the Table 2. The recovery percentages were found in the range of 80-120%. However, overall recovery percentages were significantly higher in buff sausage (103.40 ± 3.57%) than in chicken sausage (89.75 ± 4.79 %) at 5% level of significance (p=0.000).

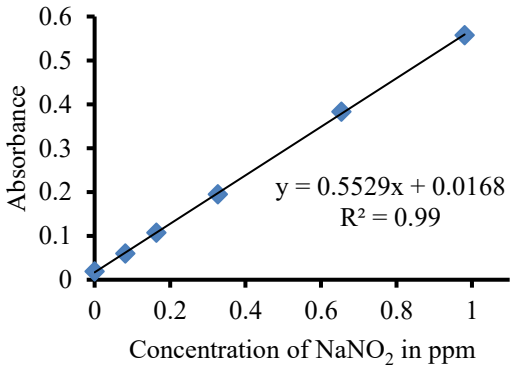

Figure 2. Calibration curve of sodium nitrite.

It was also found that recoveries were lowest when spiked at 50 ppm among the three concentrations in chicken sausage. Recoveries were significantly different at the three concentrations in case of chicken sausage while there was no significant difference in buff sausage. Yildiz, Oztekin, Orbay, & Senkal (2014) had found recovery of 98-103% in salami, sausage and bologna.

Table 2
Recovery test of $NaNO_2$ after spiking in chicken and buff sausage at different concentration.

Spiking concentration (ppm)	Recovery percentage ± standard deviation	
	Chicken sausage	Buff sausage
50	84.32 ± 1.91^{a}	99.84 ± 2.57^{x}
100	94.97 ± 0.89^{b}	104.36 ± 3.12^{x}
200	89.97 ± 1.57^{c}	105.99 ± 2.04^{x}
Overall recovery	89.75 ± 4.79^{p}	103.40 ± 3.57^{q}

Different superscripts for different spiking concentrations indicate difference at 5% level of significance

Analysis of variance was performed separately for chicken and buff sausage. Overall recovery was compared using two-sample t-test. Similarly, Hsu, Arcot, & Lee (2009) recovered 80 to 109% in cured and fresh meat. Type of meat source, magnesium, calcium and iron from other ingredients and reactive nature ofnitrite itself can be the reason for the variation in recoveries (Hsu, Arcot, & Lee, 2009).

Quality control sample analysis

In the QC sample, concentration of NaNO2 was 162.5±1.08 ppm, which was within the range given by the supplier (138-226 mg/kg) and was within the limit of ±2 z-scores. This indicates that the analysis preformed in the laboratory was satisfactory.

Conclusions

Consumption of meat and meat products are increasing day by day in Nepal. Added sodium nitrite in meat products for color development and antimicrobial purpose has several health effects in human. Sodium nitrite in meat and meat products available in Kathmandu valley was estimated in this study. Among the 64 samples analyzed, none of the samples exceeded the maximum value set by Government of Nepal and India. However, five percent of the total samples had crossed the European Union standard. The highest range of Sodium nitrite was found in chicken sausages (1.49-165.72 ppm) whereas the lowest in miscellaneous products (Not detected-55.83 ppm). It was found that all the buff sausages, ham, bacon, salami and cold-cut contained added $NaNO_2$. However, this was not the case in chicken and pork sausages and miscellaneous products. The recoveries in both chicken and buff sausages at 50, 100 and 200 ppm of spiking were 84.32, 94.97, 89.97% and 99.84, 104.36, 105.99% respectively. The overall recovery was significantly higher in buff sausage (103.40 ± 3.57%) than in chicken sausage (89.75 ± 4.79 %).

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