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Determination of dietary exposure and extraction efficiency of nitrosamine from cooked meat

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ABSTRACT

Meat products are claimed to be a source of carcinogenic nitrosamines (NAs) exposure in food. In this study, dietary exposure of six nitrosamines: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA) were estimated by Gas chromatography method. Four types of processed beef products were collected from different restaurants of Dhaka city, Bangladesh and analyzed by Gas chromatography-Mass spectrometry (GC-MS) after extracting under different methods. Nitrosamines were extracted by three different methods: i) Ultrasonic, ii) Autoclave for 10 min, iii) Autoclave for 20 min, and mean recoveries were 73%, 85% and 62% respectively. The LOD (limit of detection) and the LOQ (limit of quantification) for the six nitrosamines were in the range of 0.05–0.3 μg/kg and 0.85–1.5 μg/kg, respectively. The total nitrosamine content in beef products were Shik kabab (20.87 μg/kg) > Burger patty (20.44 μg/kg) > Steak (15.84 μg/kg) >Chap (14.95 μg/kg). The daily dietary exposure for commonly consumed beef products ranged from 0.029 to 0.056 μg/kg body weight which was less than the limit set by World Health Organization (WHO). Simultaneous determination of six nitrosamines by Gas chromatography can be used for monitoring the content of nitrosamines in meat products to ensure food safety.

1. Introduction

Dietary exposure refers to ingesting food chemicals (like-contaminant, pesticide, drug) that are unintentionally present in food, or added to food for a processing purpose. Chronic exposure happens when a person is exposed to a substance (like-acrylamide, mycotoxins, Nitrosamine) continuously or repeatedly over a longer period. N-Nitrosamines (NAs), a subcategory of the N-nitroso compounds, have been found in all sorts of foodstuff. There are two categories of NAs-volatile and nonvolatile. Among those nitrosamine's- N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA) are remarkable and frequently studied in various samples (Scanlan, 2003).

The formation of those NAs in meat is a complex cycle and a large variety of substances could influence nitrosation reaction. Sodium nitrite (by curing) and gaseous nitrogen oxides (by smoking) are the main sources of nitrosyl donors (Hotchkiss and Parker, 1990). Those nitrosyl donors react with secondary amines and tertiary amine yield to N-nitrosamines. Secondary amines may be introduced into the food products by different routes. In meat products, biogenic amines and other protein degradation products are assumed as important sources of amine precursors (Eveline et al., 2015). Moreover, it is reported that microorganisms can reduce nitrates to nitrites and contribute to degrade proteins to form amines and amino acids (Tricker and Preussmann, 1991). The occurrence of NDEA, NDBA is often related to the migration of amine precursors from packaging materials (Sen and Baddoo, 1986; Sen et al., 1993; Kataoka et al., 1997; Domanska and Kowalski, 2003). Use of some spices like black pepper and paprika may cause the formation of NPIP and NPYR (Nakamura et al., 1976). Those nitrosamines were relatively stable compounds and difficult to destroy once formed. It is stable in neutral and basic media but becomes degrades in the presence of UV light (IKEDA et al., 1990).

Most volatile *N*-nitrosamines are strong mutagens, and their intake can lead to organ-specific tumors (Lijinsky, 1999). Based on epidemiological studies and evidence of animal experiments, the International

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Agency for Research on Cancer (IARC) recognized *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) as probably carcinogenic for humans. Other *N*-nitrosamines commonly found in meat products, e.g., *N* nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) are classified as possibly carcinogenic to humans (IARC, 1998). They were claimed to be related to gastric cancer, esophageal cancer, colon cancer, and other tumors (Park et al., 2015; Bedale et al., 2016). According to WHO (World health organization), the daily limit of total nitrosamine was 10 μg/kg body weight (Cintya et al., 2018). To ensure food safety, the level of harmful compounds in food products must be monitored by appropriate methods and techniques.

NAs detected by different methods and techniques including – fluorometric method (Pokrovskii et al., 1978), gas chromatography-mass spectrometry (Stephany et al., 1978), liquid chromatography (Zhukova et al., 1999; Mottram et al., 1977), micellar electrokinetic chromatography (Sanches et al., 2003). GC-MS has the highest sensitivity than other methods and this technique was used in this study for the estimation of different NAs.

With the flourishing economic development (World bank, 2019) and the improvement of living standards, the food habits are changing among citizens of Bangladesh. In recent times, beef-based baked foods become very popular in Bangladesh. Consumers like to take out or have these items in restaurants as evening snacks or dinner. Due to the attractive taste and flavor consumption of these products is increasing. So far, no reports have been published on these products regarding risk exposure of nitrosamines. The purpose of this present study was to evaluate dietary exposure to volatile NAs through the consumption of meat products and the concentration of added nitrite salt in the formation of NAs. An efficient extraction method for these compounds from the meat matrix for Gas chromatography was also investigated.

2. Materials and methods

2.1. Sample collection

All meat products-Beef shik kabab(n = 11), Beef chap(n = 13), Beef steak(n = 9) and Beef burger patty(n = 12) were collected from different restaurants located Dhaka City of Bangladesh. A single composite sample of a homogeneous mix of units of the same type and source of food item was followed (Shaheen et al., 2013). All the samples were stored at −20 °C until analysis.

Beef shik kabab is a special type of meat product and popular in Southeast Asia. Mince beef is mixed with an appropriate amount of chapped onion, chili, a paste of bread, coriander, ginger paste, yogurt, and oil. A small cylinder shape was given by hand and baked on the grill (charcoal).

Beef chap is another popular form of meat product in Bangladesh. A boneless thin flat piece of meat is used for the preparation of this meat product. Other ingredients are red chili paste, ginger paste, cumin powder, black pepper powder, cinnamon powder, salt, onion, mustard oil, lentil powder. Meat is marinated for 7–8 h in the refrigerator. Then pieces are fried in hot oil until deep brown.

Beefsteak is also known as steak, is a flat cut of beef and cut perpendicular to the muscle fibers. They are about 2 cm thick (T-bone steaks or porterhouse, rib eye, sirloin) or about 2.5 cm (tenderloin steaks). Those beef pieces are mixed with salt and pepper followed by heating on a grill (charcoal or gas) for 13–20 min. The beef burger patty is prepared from minced beef, onion, egg, bread crumb, and pepper. They are also baked on the grill for 7–8 min.

2.2. Chemicals and reagents

Sodium hydroxide (NaOH), Sodium Chloride (NaCl), Sodium nitrite ($NaNO_2$), sodium sulfate($NaSO_4$), and dichloromethane(CH_2Cl_2) were purchased from Merck (Merck KGaA, Germany). EPA 521 nitrosamine mix standard (2000 μg/ml) was supplied by Sigma-Aldrick (Laramie, USA). This solution contained six nitrosamines: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA). All glassware was washed with 3.65 g/L HCL (Merck, Germany) solution and deionized water for minimizing contamination.

2.3. Apparatus

Heat stable Screw cap glass test tube (pyrex) and glass column (30cmX1.5 cm) were used. Water was purified (18 MΩ cm-1 quality) by a Milli-Q system (Millipore, Bedford, MA, USA). Autoclave (Sturdy SA-300VF, Taiwan), Concentrator (Techne DB-3, UK), sonicator (Branson 2510, Mexico), and Rotary evaporator (IKA RV 10, USA) were used for this study.

2.4. Sample extraction

2.4.1. Ultrasonic extraction

The sample was extracted according to Yuan et al. (2015) with slight modification. Approximate 10g of homogenized sample was mixed with 50 ml of dichloromethane in a conical flask and sonicated for 15 min. After sonication, the liquid portion was filtrated through sodium sulfate. The filtrate was mixed with 50 ml of dichloromethane and repeated the process twice. Extracted liquid was concentrated to about 1 ml by rotary evaporator at 35 °C and concentrator. Then, the concentrated liquid was filtrated using a 0.45 μm syringe filter before analysis in GC-MS/MS.

2.4.2. Autoclave extraction (10 min)

1g of sample was taken in a Screwcap glass test tube. After adding 10 ml of 1N NaOH, this tube was autoclaved at 121 °C for 10 min. Then the autoclaved solution was transferred to a 50 ml separatory funnel and was repeated twice. The funnel was shaken after adding 10 ml of 10% aqueous sodium chloride. After 15 min dichloromethane layer was collected and passed through Sodium sulfate and silica gel column. This solution was subsequently concentrated to about 1 ml by a rotatory evaporator (35 °C) and concentrator. The final solution was filtrated through (0.45 μm) filter for GC-MS/MS analysis.

2.4.3. Autoclave extraction (20 min)

This method was the same as section 2.4.2 with an autoclave time of 20 min.

2.5. GC-MS system

This analysis was performed in a GC-MS (Model: TRACE 1310, Thermo Fisher Scientific, USA) equipped with a Thermo Scientific™ TG-WAX MS Column (30m × 0.25 mm X 0.5 μm) and coupled with mass spectrometry (Model: TSQ DUO, Thermos Scientific, USA). The injector temperature was set at 250 °C. The column temperature gradient was as follows: initial temperature 45 °C, holding time 3 min, then increasing to 130 °C at 25 °C/min and 230 °C at 12 °C/min, holding time 1 min. Other conditions such as injection mode, injection volume, carrier gas, flow rate, transfer line temperature, and electron ionization (EI) source temperature are given in Table 1. Chromeleon software (Version 7.20) was used for the data acquisition, peak integration, and calibration curve preparation.

2.6. Standard preparation

Six different concentrations (1 μg/L, 5 μg/L, 10 μg/L, 20 μg/L, 40 μg/L and 80 μg/L) of nitrosamines were prepared by EPA 521 nitrosamine mix standard (2000 mg/L). The solutions were kept at −20 °C for further analysis.

Table 1
Chromatographic conditions.

Injector module	:	Split/splitless injector
Injector temperature	:	250 °C
Injection mode	:	Splitless
Splitless time	:	1.0 min
Analytical column	:	TG-WAX MS, (30m × 0.25 mm× 0.5μm)
Carrier gas	:	He(99.999% purity)
Flow rate	:	1.0 ml/min, constant flow
Injection volume	:	1 μL
Total analysis time	:	12 min

2.7. Peak characterization and quantification

Nitrosamines were identified by comparing against standard peaks using the retention time and the intensity of spectrum profile. Data were reported as means ± standard deviations of triplicate independent analyses.

2.8. Validation procedure

The validation process includes the following parameters: linearity, precision, repeatability, limits of detection (LOD), the limit of quantification (LOQ), and trueness. These parameters are suggested by European analytical chemistry (Eurachem, 2014). A calibration curve was prepared within a linear range of 1–80 μg/L for simultaneous detection six nitrosamines. Three replicate injections (n = 3) were performed in duplicate and least-square linear regression was applied to prepare the calibration curve. The Linearity was calculated by the evaluation of regression coefficient and considering 85–115% deviations of the mean calculated levels over three runs for nominal non-zero calibration (Heine et al., 2008). The percent relative standard deviation (%RSD) of repeatability (intra-day precision) and the intermediate precision (inter-day precision) were used to check the precision of this method. The LOD and the LOQ were established using spiked samples. The sample was fortified with appropriate volumes of standard solutions in dichloromethane to get recovery at the level of 0.5 μg/kg. The LOD is the quantity below which accurate identification is uncertain. It's also expressed as the analyte concentration which gives S/N (signal to noise ratio) of 3. The value of the LOD was calculated as follows: $LOD = L_{bl} + K.\ SD_{bl}$; where L_{bl} is the mean of the blank measures and SD_{bl} is the standard deviation of the blank measures, and K is a numerical factor chosen according to the confidence level desired. If the confidence level is 95%, the K is 3.36. The LOQ is then 3.3 times the LOD (The Nordic Committee of Food Analysis, 1996). The trueness was measured from recovery evaluation, by spiking meat samples at 20 μg/kg, 40 μg/kg, and 60 μg/kg). The recovery was then calculated as the average of three independent iterations using the following equation: $\%R = 100 \times ((S_{sample\ spike} - S_{sample})/S_{spike})$ where $S_{sample\ spike}$ is the concentration of analyte in the spiked sample, S_{sample} is the concentration of analyte in blank sample and S_{spike} is the spiked concentration of analyte.

Finally, the matrix effect was measured to find out any suppression or enhancement of analyte ion in a real sample. For this, one curve was generated by analyzing standard solutions in meat extract and the other one was prepared by standard solutions in dichloromethane (Lopez et al., 2016). Both calibration curves were then created by plotting the peak intensity ratios against the corresponding concentrations. Matrix effects (MEs) were calculated by comparing the slopes of the two calibration curves using the formula: $ME\ [\%] = 100\% \times (s_m/s_s - 1)$, where s_m is the slope of the calibration curve prepared in blank meat product extract (matrix), and s_s is the slope of the calibration curve prepared in dichloromethane.

2.9. Conversion of nitrite to nitrosamines

An experiment was set for finding whether nitrite was converted to nitrosamine or not. This study was according to Yurchenko and Molder, 2005 with slight modification. The homogenized meat was mixed with sodium nitrite (75 mg/kg, 150 mg/kg and 300 mg/kg). Then, thick portions of homogenized spiked meat were baked in an electric oven at 200 °C for 30 min according to the modified method of Yurchenko and Molder, 2005. After that, the amounts of nitrosamines were assessed in GC-MS.

2.10. Recovery of nitrosamines for different methods

This experiment was designed to find out a suitable Methods for the extraction of nitrosamine. Nitrosamines were recovered by comparing various methods such as ultrasonic extraction (section 2.4.1) and autoclave extraction (section 2.4.2).The duration of the autoclave method for sample extraction were 10min and 20 min. Meat products were spiked with nitrosamines (10 μg/kg and 20 μg/kg). Recovery was calculated by using this equation: $\%R = 100 \times ((T_{sample\ spike} - T_{sample})/S_{spike})$ where T_{sample} spike is the analyte concentration in the spiked sample, T_{sample} is the analyte concentration in the blank sample and T_{spike} is the spiked concentration of analyte.

2.11. Dietary exposure

Evaluation of dietary exposure involves the food consumption information as well as the records on the concentration of a chemical in food. Then, the calculated exposure level is judged against the health-based guidance level for the specific chemical or contaminants of concern. In this study, dietary exposure was assessed according to International Programme on Chemical Safety (IPCS, 2009).

Dietary Exposure (DE) = CXM/W, Where C = concentration of nitrosamine in meat product; M = consumption of meat product; W= Body weight (kg).

In this study, the concentration of nitrosamine in meat product (C) = sum of all six volatile nitrosamines; Serving size of beef chap, beef steak, beef shik kabab and beef burger patty were 0.115, 0.15, 0.12 and 0.14 kg/person/day, respectively. Bodyweight (W) of adult males and females of Bangladesh were 57.7 kg and 50.5 kg respectively (Worlddatainfo, 2021).

3. Results and discussion

3.1. Chromatographic separation

Fig. 1a shows a typical chromatogram of mixed nitrosamine standard solution (5 μg/L). The repeatability was established by using a standard solution of mixed nitrosamine injected every 6–8 samples and calculating the mean of the observed retention times: 6.714–6.821 min for NDMA, 7.412–7.561 min for NDEA, 8.251–8.422 min for NDPA, 9.755–9.792 min for NDBA, 9.915–9.951 min for NPIP and 10.521–10.572 min for NPYR. There was an absence of interferences in the elution window for the retention time of six nitrosamines in spiked meat and standard mixture (Fig. 1a).The chromatograms of a mixed standard solution, spiked sample (1 μg/kg of mixed nitrosamine) and homogenized meat products are visible in plots (a) and (b) of Fig. 1, respectively.

3.2. Method validation

Table 2 shows the quality parameters which were obtained during the validation study, such as linearity, precision and trueness, LOD and LOQ.

Method linearity was determined by making injections of nitrosamine mix standard at the 1 μg/L, 5 μg/L, 10 μg/L, 20 μg/L, 40 μg/L and 80 μg/L levels. The correlation coefficient was found more than 0.997. Intra-day and inter-day %RSD variation was less than 1% and within limit of the accepted reference value (15%) (ICH 2005), thus confirming

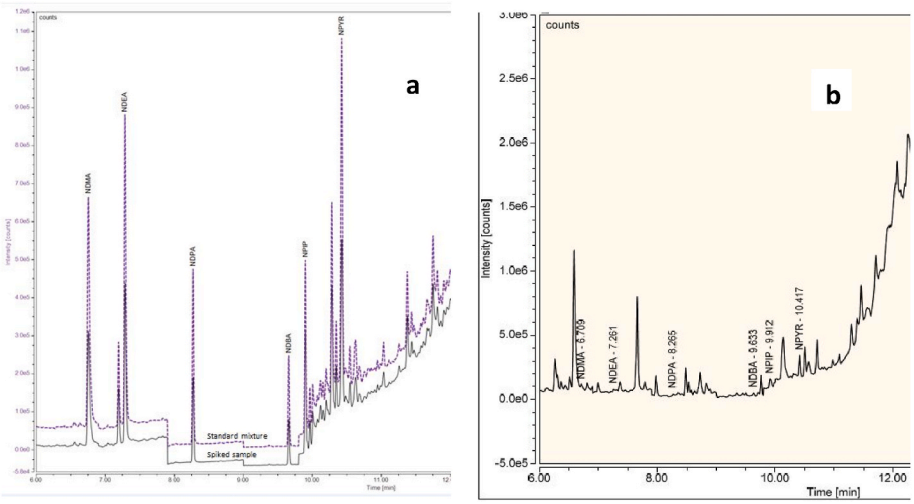

Fig. 1. Chromatogram of six nitrosamines-(a) Standard Solution (5 μg/L) and spiked sample (1 μg/kg),(b) Meat product.

Table 2
Quality parameters of the GC-MS method for six nitrosamines determination.

Quality Parameters		NDMA	NDEA	NDPA	NDBA	NPIP	NPYR
Linearity	Linear range (μg/L)	1–80	1–80	1–80	1–80	1–80	1–80
	Linearity Regression equation	y = 59935x-2362	y = 48047x+428	y = 30055x+71	y = 12227x-1748	y = 8439x+880	y = 47405x-4059
	Determination coefficient (R^2)	0.998	0.998	0.999	0.998	0.998	0.999
Repeatability Precision, %RSD (concentration level, μg/L)	Repeatability	0.10 (1)	0.11 (1)	0.2 (1)	0.8 (1)	0.10 (1)	0.1 (1)
		0.6 (5)	0.3 (5)	0.5 (5)	0.2 (5)	0.4 (5)	0.2 (5)
		0.7 (10)	0.9 (10)	0.7 (10)	0.9 (10)	0.4 (10)	0.5 (10)
	Intermediate precision	0.2 (1)	0.3 (1)	0.1 (1)	0.5 (1)	0.3 (1)	0.2 (1)
		0.1 (5)	0.1 (5)	0.1 (5)	0.1 (5)	0.1 (5)	0.2 (5)
		0.6 (10)	0.2 (10)	0.3 (10)	0.6 (10)	0.8 (10)	0.6 (10)
Trueness	%R (concentration, μg/L)	87% ±2 (20)	87% ±2 (20)	85% ±2 (20)	85% ±2 (20)	87% ±2 (20)	87% ± 2 (20)
		88% ±2 (40)	89% ±2 (40)	88% ±2 (40)	86% ±2 (40)	89% ±2 (40)	88% ±2 (40)
		88% ±1 (60)	87% ±2 (60)	88% ±2 (60)	86% ±1 (60)	87% ±2 (60)	88% ±1 (60)
	LOD (μg/kg)	0.25	0.05	0.1	0.25	0.5	0.3
	LOQ (μg/kg)	0.85	0.21	0.35	0.8	1.5	0.9

Repeatability = percent relative standard deviation (%RSD), trueness = percentage recovery (%R).

the suitability of this method for analyzing nitrosamines in spiked meat samples. In particular, the %RSD for repeatability was ranged between 0.1% and 0.7% for NDMA, 0.1%–0.9% for NDEA, 0.2%–0.7% for NDPA, 0.2%–0.9% for NDBA, 0.1%–0.4% for NPIP and 0.1%–0.5% for NPYR. These findings suggest that the GC-MS/MS method has strong repeatability as well as inter-day precision. The trueness was assessed as the percentage recovery and matrix effect. According to AOAC 2002, the recovery of the analytes was carried out using real samples spiked with three different concentrations of six nitrosamines. The recoveries were ranged from 85% to 89% for nitrosamines. (Table 2). The presence and concentration of interfering substances were determined by comparing the calibration curve generated by standard solutions in dichloromethane to that obtained using homogenized meat product samples (Fig. 2). Without matrix effects, the calibration curves were nearly parallel; the evaluated slope differences for NDMA, NDEA, NDPA, NDBA, NPIP, NPYR were 2.85%, 5.65%, 5.95%, 1.25%, 1.85% and 8.6%, plots (i), (ii), (iii), (iv), (v), and (vi) (Fig. 2), respectively, of a homogenized meat product sample. Fig. 2b shows that the curves of NDMA, NDEA, NDPA, NDBA, NPIP standard, and matrix sample were almost identical. In the case of NPYR, the values of the standards prepared with matrix were 9% lower than that of standards prepared with dichloromethane.

The LODs for NDMA, NDEA, NDPA, NDBA, NPIP, and NPYR using a 1 μL loop were 0.25, 0.05, 0.1, 0.25, 0.5 and 0.3 μg/kg, respectively and the LOQ values for NDMA, NDEA, NDPA, NDBA, NPIP, and NPYR were 0.85, 0.21, 0.35, 0.8, 1.5 and 0.9 μg/kg, respectively.

3.3. Effect of sodium nitrite on nitrosamine formation

This experiment revealed the influence of sodium nitrite on the growth of volatile NAs in meat products (Fig. 3). Sodium nitrite solutions with 75, 150, and 300 mg/kg concentrations were added to meat products.

Initially, nitrosamines were not detected in samples without sodium nitrite. At the level of 75 mg/kg of sodium nitrite, the amount for NDMA, NDEA, NDPA, NDBA, NPIP and NPYR were 0.95 μg/kg, 0.4 μg/kg, 0.31 μg/kg, 1.17 μg/kg, 6.65 μg/kg and 3.73 μg/kg, respectively. At the level of 300 mg/kg of sodium nitrite, the concentration for NDMA, NDEA, NDPA, NDBA, NPIP and NPYR were 4.55 μg/kg, 2.5 μg/kg, 1.42 μg/kg, 5.8 μg/kg, 26.7 μg/kg and 15.7 μg/kg, respectively. In the pearson Correlation analysis, a very strong positive correlation (r = 0.996) was found between the nitrite salt in meat products and NPIP formation. A previous study from Yurchenko and Molder (2005) also showed that these nitrosamines were getting higher with the addition of sodium nitrite.

3.4. Recovery of different methods

The recoveries were varied according to methods (Fig. 4). Ultrasonic extraction shows about 73% recoveries of nitrosamines where the

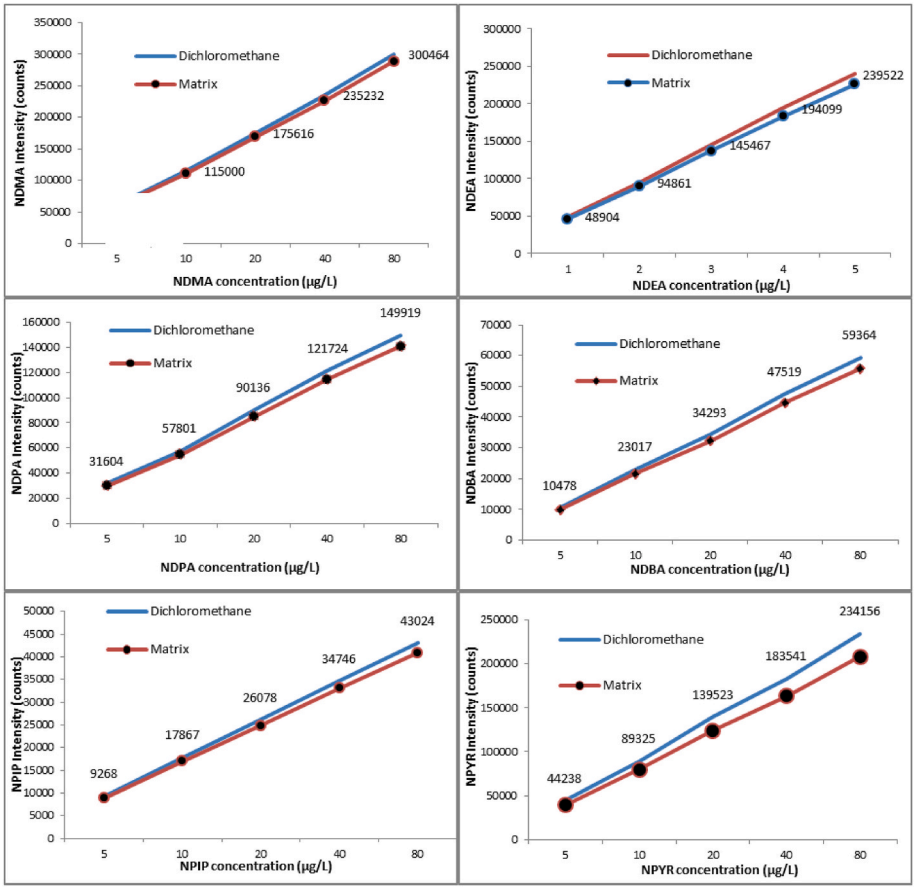

Fig. 2. Calibration curves of NDMA, NDEA, NDPA, NDBA, NPIP and NPYR.

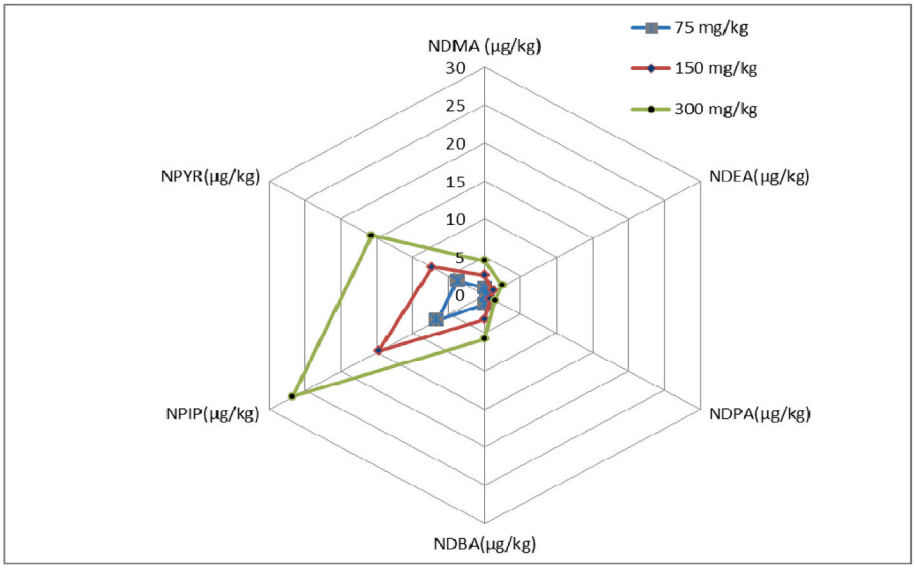

Fig. 3. Influence of $NaNO_2$ on nitrosamine formation.

highest recovery was for NDEA (78%) ($p = .03$). The autoclave method with 10 min incubation time had significantly highest recovery of nitrosamines (85%) than other two extraction method ($p = .01$). In this method, the highest recovery was for NPIP (89%) ($p = .02$). In another case, increase of incubation time to 20 min decreased the recovery to 62%. Kaseem et al. (2013) showed that, autoclave method had higher recovery than the simple extraction method.

3.5. Nitrosamines in meat products

The total amount of nitrosamine was varied according to samples. Fig. 5 shows that, Beef shik kabab contained the highest amount of nitrosamine (about 20.9 µg/kg) whereas beef chap had the lowest amount of nitrosamine (about 14.2 µg/kg). The processing technique of beef chap might be the foremost cause of less Nitrosamine formation

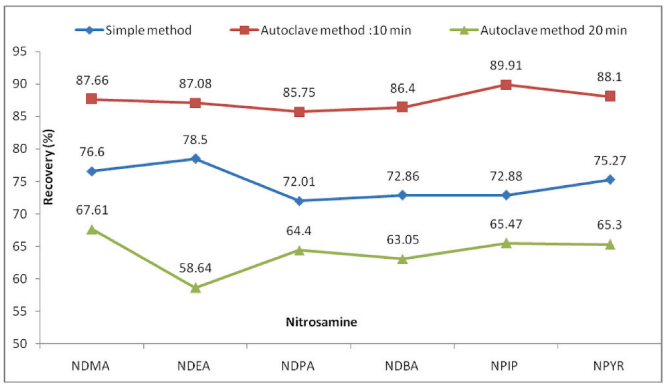

Fig. 4. Recovery of nitrosamine by different methods.

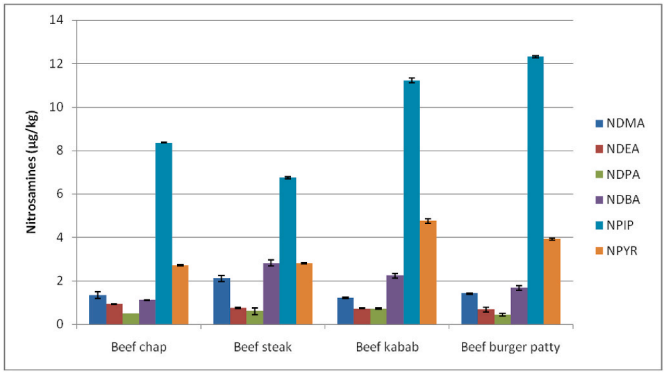

Fig. 5. Nitrosamines in meat products.

(Yurchenko and Molder, 2005). Beef steak had a moderate level of nitrosamines (about 15.84 µg/kg).The level of NDPA in meat products was very low and that's about 0.5, 0.61, 0.73 and 0.45 µg/kg for beef chap, beef steak, beef shik kabab and beef burger patty, respectively. But, the amount of NPIP for beef chap (8.35 µg/kg), beef steak (6.75 µg/kg), beef kabab (11.21 µg/kg) and beef burger patty(12.31 µg/kg) was highest among six nitrosamines. Spices and different degrees of meat crumbling might cause variations in the concentration of NPIP content, resulting in varied spice penetration throughout the whole capacity of meat pieces (Domanska and Kowalski, 2003). The major source of NPIP might be black pepper, which includes piperidine. Cadaverine, which is formed during the thermal processing of beef by lysine decarboxylation, may also be a precursor to NPIP (Sen et al., 1974a; 1974b).The NPYR level was about 2.7, 2.8, 4.8 and 3.9 µg/kg for beef chap, beef steak, beef kabab and beef burger patty, respectively. The amounts of NDEA and NDPA in meat products were less than 1 µg/kg and NDMA was less than 2 µg/kg. A previous study in Sweden reported the low content of NDMA (0.8 µg/kg) and NDBA (0.3 µg/kg) in meat products (Österdahl, 1988). A survey in Estonia also reported the trace level of NDMA (0.8 µg/kg), NDBA(0.4 µg/kg) in cured meats (Yurchenko and Molder, 2005). Moreover, the levels of NAs in Bangladeshi meat products in the present study show good agreement with results from Poland (Domanska and Kowalski, 2003), France (Biaudet et al., 1994), Russia (Zhukova et al., 1999), Germany (Spiegelhalder et al., 1991), UK (Gough et al., 1978), and Japan (Yamamoto et al., 1984), Syria (Kaseem et al., 2013).

3.6. Dietary exposure

The dietary exposure of nitrosamine for both men and women is stated in Table 3 where daily exposure from beef burger patty (0.049 and 0.056 µg/kg body weight for men and women respectively) was highest among the meat products. Beef chap had the lowest daily exposure (0.029 and 0.034 µg/kg body weight for men and women respectively) than others. These results indicate that the daily dietary exposure of nitrosamine from commonly consumed four meat products was lower than 10 µg/kg body weight, the limit set by WHO (Cintya et al., 2018). In 2015, a study was performed in Denmark for the quantification of nitrosamine in meat products (Herrmann et al., 2015). This study showed a trace amount of nitrosamine exposure (0.001 µg/kg body weight). Tricker and Preussmann (1991) also showed the very low-level volatile nitrosamine exposure from 0.3 to 1 µg/kg body weight from various meat products.

Table 3
Nitrosamine exposure from meat products.

Meat products	Daily dietary exposure: Men (µg/kg body weight)	Daily dietary exposure: Women (µg/kg body weight)	Daily limit by WHO: (µg/kg body weight)
beef chap	0.029	0.034	10
beef steak	0.041	0.047	
beef shik kabab	0.043	0.049	
beef burger patty	0.049	0.056	

4. Conclusion

NPIP is the major compound formed from the nitrite salt in meat products processing and shows strong positive correlation. Autoclaving for 10 min can be a suitable option for the extraction of nitrosamine compounds from meat products. Although the daily dietary exposure was found lower than the recommended amount, awareness is needed on regular consumption of these meat products in aspect of food safety. Further investigation on other meat-based products and the effect of cooking methods on NAs production is required.

CRediT authorship contribution statement

Abu Tareq Mohammad Abdullah: Conceptualization, Methodology, Investigation, Project administration. **Tanzir Ahmed Khan:** Resources, Validation. **Miskat Sharif:** Investigation, Formal analysis, Writing – original draft. **Reaz Mohammad Mazumdar:** Resources, Investigation, Visualization. **Mohammad Mahfuzur Rahman:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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