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Distribution of Seven N-Nitrosamines in Food

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N-nitrosamines, which are classified as carcinogens by IARC and US EPA, can be easily found in various foods. They are reaction products between nitrogen oxide and secondary amines, but can also be generated during fermentation. Ever since the 1960s, when nitrite, used as a preservative in processed meats, was suspected to generate N-nitrosamines, the usage of the food additive has been debated. However, the benefit of nitrite in food supply could not be ignored and the risk-benefit analysis has become a key issue in the use of the additive. For a risk analysis, an accurate estimation of the hazardous material is necessary; therefore, analytical methods for nitrosamines have continuously evolved from the 1950s. Solid supported liquid-liquid extraction and solid phase extractions have replaced the distillation for the clean-up steps, and tandem mass spectrometry is employed for higher selectivity and sensitivity. In the present study, for a better estimation of N-nitrosamine intake, the total diet study samples were prepared for the N-nitrosamines analysis. In order to obtain the most sensitive results, a partial preparation procedure was developed and modified for different food matrices. Among seven N-nitrosamines (N-nitrosodimethylamine, N-nitrosomethylethylamine, N-nitrosodiethylamine, N-nitrosodibutylamine, N-nitrosopiperidine, N-nitrosopyrrolidine, and N-nitrosomorpholine) analyzed in the present study, N-nitrosodiethylamine has shown the highest detection rate in agricultural foods, while N-nitrosodimethylamine has appeared most frequently in livestock and fishery food products. The concentration of N-nitrosodimethylamine was the highest in seasoning.

***Key words*:** N-nitrosamine, Total Diet Study

INTRODUCTION

N-nitrosamines have been found in air, water, foods, cosmetics, tobacco, and packing materials (1). N-nitrosamines have been an issue due to nitrite which was added to prevent the growth of *Clostridium botulinum* in processed meats. For this reason, high concentrations of nitrosamines have been reported in bacon, sausage, and ham in high rate (2), while meats that did not go through processing showed low, if any, amount of nitrosamines. N-nitrosamines were also reported in salted fish, beer, and water (3).

N-nitrosamines are formed by reactions of organic amines and their derivatives with nitrosating compounds; however, most stable nitrosamines are formed from secondary amines (4). Since these precursors can, in turn, be generated from pesticides and herbicide, as well as nitrogen fertilizers, N-nitrosamines can be found as contaminants in various foods. The chemical reactions in which N-nitrosamines are formed from various sources are well reviewed by Rostkowska *et al.* (4). In foods, nitrosamines are formed by reactions of nitrogen oxide with amines. Nitrite in food, whether reduced from nitrate fertilizer or added as a preservative, is hydrogenated to hydronitrogenoxide ($H_2NO_2^+$) in acidic condition (Fig. 1) (4). The resulting hydronitrogenoxide ($H_2NO_2^+$) reacts with another molecule of nitrite to form nitrogen anhydride after dehydration. Nitrogen anhydride donates nitroso group to the amines in food to produce N-

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Abbreviation: NDMA, N-nitrosodimethylamine; NMEA, N-nitrosomethylethylamine; NDEA, N-nitrosodiethylamine; NDBA, N-nitrosodibutylamine; NPIP, N-nitrosopiperidine; NPYR, N-nitrosopyrrolidine; NMOR, N-nitrosomorpholine; NDPhA, N-nitrosodiphenylalanine; NPRO, N-nitrosoproline; NSAR, N-nitrososarcosine.

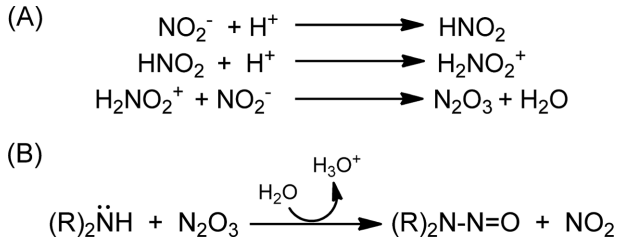

Fig. 1. N-nitrosamine formation (modified from Rostkowska *et al.* (4)). (A) Formation of a nitrous anhydride from a nitrite (B) nitrosation from a nitrous anhydride and an amine.

nitrosamines (5). Secondary amine can form stable nitrosamine, while nitrosamines derived from primary amine break down quickly; it is also known that tertiary amine can hardly form nitrosamine (6). In spinach, cabbage, and other vegetables, nitrate has been reported to be reduced to nitrite by microorganisms (3). The nitrosating reaction may also occur in the stomach by the reaction of nitric oxide from nitrite or nitrate with amines in acidic condition. The optimum pH of the reaction is 3 to 4 and synthesis of the nitrosamines in the rabbit, cat, and human stomach from the precursors has been reported (7,8).

N-nitrosodimethylamine in beer has been an issue in Germany (9). It was shown that during the kilning (drying of malt) procedure of beer production, nitrogen oxide from combustion would react with amines like gramine and hordenine in the malt to form NDMA. To reduce nitrosation during kilning, sulfur dioxide or indirect-fire kilns were used in the malting industry (9). The possibility of nitrosamine formation and contents of nitrosamine were high in the past; however, N-nitrosamines in beer have gradually decreased these days because of the efforts poured in to inhibit nitrosation by controlling the brewing procedures (10). It has also been suggested that bacterial biochemical pathway may contribute to the nitrosamine formation during fermentation. Microorganisms reduce nitrate to nitrite, degrade proteins to secondary amines, and create an appropriate environment (slightly acidic). Microorganism in the wild, including bacteria and yeasts, utilized nitrite and nitrate for growth and generate the nitrosating compound in the process. Decarboxylation of amino acids by a microbial enzyme is a well-known reaction in fermentation and an important pathway to generate precursors of nitrosamines. Microbial polyamine synthesis following decarboxylation of amino acids provides amine precursors for cyclic N-nitrosamines (N-nitrosopiperidine and N-nitrosopyrrolidine) (11).

Nitrosamine detected in water has been considered to be a disinfection by-product; afterwards, it has been suggested that the reaction between dimethylamine, a contaminant from wastewater, and monochloramine may form N-nitrosodimethylamine (NDMA). Monochloramine is used instead of chlorine to regulate halogenated by-products (12). In the 1960s, it was revealed that nitrite which was used to preserve Pacific herring was related to ruminants' liver disease. As N-nitrosodimethylamine (NDMA) was separated from the Pacific herring, the concerns about the nitrosamines in foods have been increased (12). Nitrosamines that have been found in foods are NDMA (N-nitrosodimethyamine), NDEA (N-nitrosodiethylamine), NDBA (N-nitrosodibutylamine), NPIP (N-nitrosopiperidine), NPYR (N-nitrosopyrrolidine), NMOR (N-nitrosomorpholine), NDPhA (N-nitrosodiphenylamine), NPRO (N-nitrosoproline), and NSAR (N-nitrososarcosine) (13). In the 1950s, carcinogenicity studies disclosed that nitrosamine could induce cancer in rats. NDMA and NDEA were classified as 2A (probably carcinogenic to human), NMEA, NDBA, NPIP, NPYR, NMOR, and NSAR were classified as 2B (possibly carcinogenic to human) by the International Agency for Research on Caner (IARC) (14). However, NPRO and N-nitrosodiphenylalnine (NDPhA) were classified as 3 (not classifiable as to its carcinogenicity to humans).

It has been reported that the contents of nitrosamines changed with respect to cooking methods, temperature, time, moisture of food, or fat composition (15). Nitrosamine formation in bacon was tested with several cooking conditions, such as fried, baked, broil, and microwave cooking. In fried and baked bacons, up to 35 ppb of NPYR was detected, while none was detected in raw bacon. Microwave cooking generates only 3 ppb of NPYR. According to the results, cooking temperature of 99~185°C was most effective in accelerating nitrosation. At temperatures below 100°C, no NPYR or NPRO was detected (16). Furthermore, it was revealed that fat in bacon also had an effect on NPYR and NDMA contents. Bacon rashers were separated with lean and fat components to test the impact of fat. When fried separately using a thermostatically controlled electric frying pan at 178 ± 3°C for 12 min, fried whole rasher bacon showed 15.6 ppb (mean) of NPYR and 6.7 ppb (mean) of NDMA. Fat component of bacon showed 3.7 ppb of NPYR and 14.9 ppb of NDMA mean, while lean bacon showed 3 ppb of NPYR and 2.6 ppb of NDMA. Higher amounts of both nitrosamines were detected in the fat portion of fried bacon, as compared to the lean portion (17). Also, in the case of frankfurter sausage, NDMA increased during cooking (18).

The present study focused on seven most frequently reported N-nitrosamines in food, namely, NDMA, NMEA, NDEA, NDBA, NPIP, NPYR and NMOR. To obtain a better estimation of nitrosamine exposures, a total of 387 total diet study samples which reflects ready to fork foods were employed. To cover most of the food items in the Korean diet, an analytical procedure was developed to cover variety of food matrices. Finally, for a better understanding of nitrosamine sources within various foods, the results are presented in comparison with the reported values.

MATERIALS AND METHODS

Reagents. All reagents used in the experiments were GC or pesticide residue grade. C-18 cartridge (500 mg, 6 mL) and Florisil (1 g, 6 mL) were purchased from Waters (Milford, USA). Aluminum oxide (basic) was purchased from Sigma Aldrich (St Louis, USA). Extrelut NT was purchased from Merck (Darmstadt, Germany). EPA nitrosamine mixture was purchased from Sigma Aldrich. Another set of standard and deuterated internal standards were purchased from Chiron**.** Calibration curve was obtained from the standard solution which went through the experimental procedures.

Samples. A total of 387 diet study samples were used in this study. The foods were chosen based on a national nutrition survey over a 3 year period. Seven largest cities in Korea were selected based on population and samples purchased from these seven cities were pooled. Thus, in this study, only one composite sample was subject to analysis for each food item. The samples were homogenized for the experiments if needed. All samples were provided by Korea Heath Industry Development Institute.

Partial purification of food samples. The partial purification procedure was based on the method reported elsewhere (19,20), with modifications depending on food matrices (Table 1). The basic procedure is as follows: Deuterated internal standards and 5 mL of 0.1 N sodium hydroxide solutions were added to 5 g of sample in polypropylene conical tube. After vortexing, the solid sample was mixed with 6 g of prewashed Extrelut and loaded on to a column (3.7 cm × 20 cm). In the case of liquid samples, the sample was directly loaded onto the column preloaded with 6 g of Extrelut. The sample tube was washed twice with 5 mL of Dichloromethane (DCM) : n-hexane (9 : 1 v/v) and the washing solvents were poured to the column. The fraction containing nitrosamines was eluted with 40 mL of DCM : n-hexane (9 : 1 v/v). After the concentration under the reduced pressure, the eluate was loaded onto Florisil cartridge prewashed with 6 mL of n-hexane. The vial was washed three times with 1 mL of n-hexane and added to the cartridge. Lipophilic substances were washed out of the cartridge with 3 mL of n-hexane and the relatively polar nitrosamine fraction was eluted with 6 mL of DCM : MeOH (95 : 5 v/v). Following the concentration step, the volume of the eluate was adjusted to 1 mL with DCM.

Fat-rich (food items containing over 10% of fat): The solid supported extraction using Extrelut was not applicable to the food items with large amount of fat. Since the nitrosamines are relatively polar organic compounds, a liq-

Table 1. Summary of analytical procedures for N-nitrosamine in various food matrices

	Food Matrices			
	Fatless (< 10% lipid)	Alcohol	Fat-rich (> 10% lipid)	
Procedures*	Milk, Apple juice, Porridge	Wine, soju	Beef	Corn oil
	Sample 5 g			
Deuterated Internal standards.‡	3	3	7	7
NaOH	○	○	×	×
Liquid Liquid Extraction	×	×	Acetone : ACN = 1 : 1 (40 mL)	Acetone : water = 3 : 1 (40 mL)
			Centrifugation (3500 rpm, 240 sec, 4°C), Store at −80°C for 35 min, Centrifugation	
Concentration	×	×	Reduced pressure	N_2
Solid Supported Extraction (Extrelut)	○	○	×	○
Concentration	Reduced pressure	Reduced pressure	×	Reduced pressure
SPE	Florisil	×	C-18 & alumina	×
Concentration	N_2	×	Reduced pressure	×
Final vol. and solvent	1 mL Dichloromethane (DCM)	1 mL DCM	1 mL Acetonitrile	0.5 mL DCM
Detection	GC-PCI-MS/MS			

* See text for details.
‡ 3 internal standards: NDEA-d10 for NDMA, NDEA, and NMEA; NPYR-d8 for NPIP, NPYR, and NMOR. NDBA-d18 for NDBA, 7 internal standards: deuterated counterparts for each N-nitrosamines.

Table 2. Instrument method of GC-PCI-MS/MS

Column	Pre: DB-5ms (5 m × 0.53 mm I.D., 0.25 μm df) Main: DB-wax (60 m × 0.25 mm I.D., 0.5 μm df)
Flow rate	He, 2.0 mL/min
Inject temp.	220°C
Inject volume	Fat less 2 μL/Fat-rich solid 4 μL
Oven	50°C (1 min, hold) → 20°C/min → 120°C (0 min) → 5°C/min → 200°C (0 min) → 20°C/min → 220°C (5 min, hold)
Transfer line temperature	240°C
Source temperature	150°C
Reagent gas	Ammonia (NH_3) gas (Flow: 2 mL/min)
Collision gas	Ar (cell pressure: 60 Pa)

uid-liquid extraction was employed for fat-rich food samples. Acetone-water (3 : 1) or acetone-acetonitrile mixture satisfied the terms. After the addition of internal standard, 5 g of fat containing samples were extracted with a 40 mL of acetone and acetonitrile mixture (50% v/v). For extraction, the tube containing solvent and sample was shaken thoroughly and centrifuged at 3000 rpm at 4°C for 240 sec to separate phases. The mixture was stored at −80°C for 30 min to help separate the phases. After following another centrifugation, supernatant (acetone-acetonitrile phase) was transferred to a glass vial. The remaining fat in the supernatant was removed by alumina-C18 resin. Two g of basic aluminum oxide aluminum oxide was overlaid on top of C-18 SPE cartridge and then prewashed with 25 mL of acetonitrile. The concentrated supernatant was passed through the alumina-C18 cartridge. A 25 mL of acetonitrile was used to wash the cartridge. Unbound and washing fractions were combined and concentrated. The final volume was adjusted to 1 mL with acetonitrile.

Alcoholic beverages: The method for alcoholic beverage followed the basic procedures except for the final Florisil - SPE step.

Oil: Relatively pure oil samples were extracted with a 40 mL of acetone-water (3 : 1) mixture. The acetone phase was removed after solidification of the oil phase at −80°C for 30 min, as in the fat-rich sample described above. After concentration, the extract was subjected to solid supported extraction with Extrelut, as described in the basic procedure. The eluate was concentrated under the nitrogen gas without further purification and the final volume was adjusted to 0.5 mL with the DCM.

Internal standards. For the fat-rich samples and oils, deuterated internal standards were used for each N-nitrosamine. However, for the fatless samples, for economical reason, NDEA-d10 was used for NDMA, NDEA, and NMEA; NPYR-d8 was used for NPIP, NPYR, and NMOR. NDBA-d18 was employed for NDBA, separately. All internal standards were used in this experiment at concentration of 5 μg/kg.

Table 3. Selected ions for seven N-nitrosamines

Compounds	Retention time (min)	Precursor ion (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)
NDMA	9.66	92	75	43
NMEA	10.45	106	89	61
NDEA	11.03	120	103	75
NDBA	17.41	176	159	103
NPIP	18.11	132	115	69
NPYR	18.64	118	101	55
NMOR	19.55	134	87	*

*Product ion 2 of NMOR could not be measured due to interference.

GC-PCI-MS/MS analysis. The extract was analyzed by gas chromatography-positive chemical ionization tandem mass spectrometer (GC-PCI-MS/MS) adopting ammonia gas as an ion source. DB-5ms (5 m × 0.53 mm I.D., 0.25 μm df) precolumn was used with DB-wax (60 m × 0.25 mm I.D., 0.5 μm df) main column. Injector temperature was 220°C and transfer line temperature was 240°C. Source temperature was 150°C. Oven temperature was increased from 50°C to 220°C and the total run time was 26.5 min for each sample (Table 2). The ions selected for seven N-nitrosamines are listed in Table 3.

RESULTS AND DISCUSSION

The method was validated according to suggestions of Myeong's report (21). Calibration curves, obtained by subjecting standard compounds to the analytical procedures, showed a correlation coefficient higher than 0.99 for all nitrosamines in all matrices. The method detection limit (MDL) values ranged from 0.10 to 0.30 μg/kg. These MDL values were similar to the instrumental LOD values reported

in the literature (28,34). Using three concentrations of standard solutions, recovery was measured in all matrices. The recoveries for all seven nitrosamines in different matrices ranged between 80 to 120%, i.e. within the acceptable range provided by CODEX (21). Relative standard deviation, measured with spiked samples in milk and rice soup, was below 14%, for both intraday and inter-day measurements in three concentration levels. The results were within the criteria of CODEX.

NDMA and NDEA were most frequently detected in agricultural food products (Table 4). Kim and Yoon reported a high concentration of nitrate in some of the fruits (22) and this nitrate may have been reduced to nitrite and contribute to the formation of NDMA. A small amount of nitrosamine was detected in tofu, mung-bean jelly, acorn jelly and buckwheat jelly. NMOR 0.15 μg/kg was the maximum detected content. 2.64 μg/kg of NDMA and 2.05 μg/kg of NDEA were detected separately in the kimchi group. Therefore, it can be concluded that NDMA was either formed during fermentation and/or by reactions between nitrite (from nitrate) and amines. The content of NDMA in kimchi was ca. 3 times lower than in Kim *et al.*'s results (23).

Less than 1 μg/kg was detected in the group of rice cake, flour, bread, doughnut, pickled vegetable, and croquette in all nitrosamines. NDMA ranged from ND to 1.71 μg/kg was detected in the cereal, potatoes, and beans group; other nitrosamines were detected in the quantities below 0.76 μg/kg. The maximum of 6.1 μg/kg and 4.9 μg/kg NDMA was detected in fresh vegetables and mushrooms, respectively, as well as the maximum of 6.11 μg/kg of NDBA in mushrooms. The study has demonstrated higher nitrosamine contents in vegetables than those previously reported in the literature (24). Since soil microorganisms are known to contribute to nitrosamine formation and given that nitrate contents in vegetables can also contribute (10), the fluctuation of the nitrosamine contents can be expected. It is notable that NDMA and NDEA were detected in the maximum of 2.95 and 2.22 μg/kg respectively, in snack samples. Other nitrosamines were relatively low.

NDMA, NDBA, and NMOR were detected in fishery products, but on a very low level (Table 5). Milk and milk products did not show remarkable contents of any of the tested nitrosamines. NDMA of cheese showed 0.72 μg/kg and the others, such as cake and ice cream, showed less than 0.56 μg/kg (Table 6). The exception was freshwater eel which showed 3.93 to 4.18 μg/kg of NDMA. Furthermore,

Table 4. N-nitrosamine contents in agricultural products (μg/kg)

Product	The number of samples	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR	NMEA	Total	Ref
Cereals and potato	22#	ND-1.71	ND-0.72	ND-0.76	ND-0.38	ND-0.15	ND-0.18	ND		‡
Cereals	8	ND-0.41	-	-	-	-	-	-		30
Kind of flours	5#	ND-0.92	ND-0.66	ND-0.19	ND	ND	ND	ND		‡
Pickled vegetable	4#	ND-0.42	ND-0.26	ND-0.25	ND-0.2	ND	ND-0.66	ND		‡
Kimchi	11#	0.13-2.64	ND-2.05	ND-0.22	ND	ND-0.26	ND	ND		‡
	7	0.8-6.9	-	-	-	-	-	-		23
Fresh vegetables	6	< 3.3	-	-	-	-	-	-		30
	53#	ND-6.01	ND-1.53	ND-1.61	ND	ND-0.67	ND-0.4	ND		‡
Frozen vegetable	1	-	3.9	-	-	1.7	-	-		30
Mushroom	7#	ND-4.9	ND-0.62	ND-6.11	ND	ND	ND	ND		‡
Rice cake	11#	ND-0.8	ND-0.23	ND-0.46	ND	ND	ND	ND		‡
Fruit	29#	ND-6.21	ND-0.60	ND-1.03	ND	ND	ND-0.3	ND		‡
Tofu	3#	ND	ND-0.2	ND	ND	ND	ND	ND		‡
Vegetable jelly	3#	ND	ND-0.11	ND	ND	ND	ND-0.15	ND		‡
Bread, croquette, doughnut	20#	ND-0.98	ND-0.91	ND-0.64	ND	ND-0.22	ND-0.29	ND		‡
Noodle	9#	ND-0.72	ND-2.81	ND-0.80	ND	ND-0.11	ND-0.12	ND		‡
Snack	17#	ND-2.95	ND-2.22	ND-1.63	ND-0.66	ND-0.39	ND-0.62	ND-0.24		‡
Beverage	28#	ND-3.57	ND-0.56	ND	ND-0.47	ND-0.22	ND-0.26	ND		‡
Fruit juice	8	-	-	-	-	-	-	-	< 2.75-45.70	31

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.

Table 5. N-nitrosamine contents of in seafoods (μg/kg)

Product	The number of samples	NDMA	NDEA	NPYR	NPIP	NDBA	NMOR	NMEA	Total	Ref
Tuna	1#	0.12	ND	ND	ND	ND	ND	ND		‡
Frog flounder, porgy	2#	0.3-0.72	ND-0.14	ND	ND	ND	ND-0.53	ND		‡
Harvest fish	1#	0.99-2.06	ND	ND	ND	ND	ND	ND		‡
Freshwater eel	1#	3.93-4.18	ND	ND	ND	ND	ND	ND		‡
Shell fish	5#	0.21-1.35	ND	ND-0.14	ND	ND	ND-0.19	ND		‡
Mullet, spoon wormand short arm octopus	3#	ND	ND	ND	ND	ND	ND	ND		‡
Salted fish	20	12.64-322.92	7.65-50.27	-	-	-	-	-	20.29-373.19	32
	10	0.2-2.13	-	-	-	-	-	-	0.2-2.13	28
Salted shell fish	2	0.2-0.59	-	-	-	-	-	-	0.2-0.59	28
salted pollack roe	3	0.2-4.08	-	-	-	-	-	-	0.2-4.08	28
Salted shrimp	5	ND-0.9	-	-	-	-	-	-		28
Salted anchovy	6	ND-1.2	-	-	-	-	-	-		23
	1	-	-	< 0.02	< 0.01	-	-	-		24
Shellfish aekjeot	3	< 0.2	-	-	-	-	-	-	< 0.2	28
Anchovy aekjeot	6	-	-	< 0.02	< 0.01	-	-	-		24
Shellfish aekjeot	2	0.2-0.57	-	-	-	-	-	-	0.2-0.57	28
Crab stick	18	0.2-9.36	-	-	0.2-1.01	-	-	-	0.2-9.36	28
Fish cake	28	0.2-6.44	0.2-0.74	-	0.2-0.80	-	-	-	0.2-6.44	28
Fish sausage	16	0.2-2.71	-	-	-	-	-	-	< 0.2-2.71	28
Fish	33	0.04-3.5	0.06-4.2	-	-	0.11-5.3	-	-		30

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.

Table 6. N-nitrosamine contents in milk and milk products (μg/kg)

Product	The number of samples	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR	Ref
Cheese	3#	ND-0.72	ND	ND	ND	ND	ND	‡
	21	< 0.04-3	< 0.06-4	-	-	< 0.09-2.6	-	30
Milk and milk products	4#	ND	ND	ND	ND	ND	ND	‡
Yogurt	3#	ND	ND	ND	ND	ND	ND	‡
Cake	5#	ND-0.56	ND	ND-0.23	ND	ND	ND-0.14	‡
Ice cream	4#	ND-0.28	ND	ND	ND	ND	ND	‡
Powdered non-fat milk	1	< 0.05	-	-	< 0.08	< 0.08	-	33
Powdered modified milk	1	< 0.05	-	-	< 0.08	< 0.08	-	33
Dried milk powder	5	< 0.3	< 0.3	-	-	< 0.3	< 0.3	20

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.

NDMA showed the highest detection rate in meat and meat products (Table 7 and 8). Dumplings which contain meat but also dozens of other ingredients, including seasoning, showed the highest amount of nitrosamines. In spite of the original concerns, NDMA ranged from 0.31 to1.54 μg/kg in processed meats, such as sausages, hams, and bacons, which were lower than the contents found in vegetables or fruits. However, NPIP and NMOR were detected in pro-

Table 7. N-nitrosamine contents in meat and meat products (μg/kg)

Product	The number of samples	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR	NMEA	Ref
Pork liver	1#	0.51-0.64	ND	ND	ND	ND	0.21-0.29	ND	‡
Pork intestine and pork products	5#	ND	ND	ND	ND	ND	ND	ND	‡
Pork flesh	3#	ND	ND	ND	ND	ND	ND	ND	‡
Pork belly	1#	0.13-0.21	ND	ND	ND	ND	ND	ND	‡
Dumpling	3#	0.75-2.26	ND	ND	ND	ND	ND	ND	‡
Beef intestine	4#	ND-0.54	ND	ND	ND	ND	ND-0.31	ND	‡
Beef and beef products	9#	ND-0.48	ND	ND-0.31	ND-0.24	ND	ND	ND	‡
Chicken	7#	ND-0.52	ND	ND-0.30	ND	ND	ND	ND	‡
Cow foot soup	1#	0.49-0.62	ND	ND	ND	ND	0.16-0.29	ND	‡
Pork meat, in salt	2#	0.08-0.28	0.06-0.07	-	-	-	-	-	30
Fat liver (duck)	1#	0.47	0.81	-	0.20	2.6	0.79	-	30
Duck	1#	ND-0.13	ND	ND	ND	ND	ND	ND	‡
Smoked pork brisket	19	0.04-4.0	0.06-9.5	0.11-2.7	0.16-11	0.09-37	0.19-2.1	-	30

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.

Table 8. Contents of nitrosamine in processed meats (μg/kg)

Product	The number of samples	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR	NMEA	Total	Ref
Cooked sausages	5•	0.33-0.5	0.3	-	-	0.3-1.0	< 0.3	-		20
Fried and cooked sausage	8	0.05-1.2	0.03-5.1	0.11-1.0	-	0.09-0.22	-	-		30
Sausages	4#	0.31-1.54	ND	ND	ND	ND-0.91	ND	ND		‡
	5•	0.04-4.5	0.02-7.9	0.11-1.0	-	0.09-0.90	-	-		30
	7•	0.3-0.6	0.3-0.6	-	-	0.3-1.1	0.3-0.4	-		20
	16	0.2-3.28	0.2-1.78	0.2-0.60	-	0.2-1.02	-	-	0.2-3.28	28
German sausage, pate, non-smoked	10	0.04-0.84	0.02-7.5	0.11-1.3	-	0.09-0.41	0.19-1.7	-		30
Sausage w/garlic	2	0.04-1.3	1.3-6.4	-	-	-	-	-		30
Sausage, salami type	4	0.04-2.1	1.5-12	0.29-2.0	-	0.09-0.58	-	-		30
Various smoked sausages	15	0.04-9.3	0.91-10.3	0.11-2.0	0.16-3.7	0.09-2.2	0.19-7.2	-		30
Smoked or dried Ham	11	0.04-4.8	0.06-12	< 0.11-1.1	-	0.09-1.3	0.19-2.0	-		30
Ham	2#	0.18-0.23	ND	ND	ND	ND	ND-0.39	ND		‡
	28	< 0.2-3.45	0.2-0.77	-	-	-	-	-	0.2-3.45	28
	4	0.04-0.72	0.06-0.07	< 0.11-0.23	0.16-0.23	0.09-0.97	-	-		30
Bacons	1#	0.67-0.78	0.17-0.19	ND	ND	ND	ND	ND		‡
	7•	1.21-3.92	0.2-0.77	-	-	0.2-0.50	-	-	0.2-3.92	28

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples, •: sample contained nitrite.

cessed meats, albeit in low concentration. Compared with the results reported by another research group, nitrosamine contents in the present study tended to be lower.

In oil samples, about 1 μg/kg of NDMA was detected on average. NDMA was detected in all of the samples and NMOR was detected in soybean, olive, canola, rape, and sun follower oil; however, other nitrosamines were not detected (Table 9). Hedler and co-authors (25) reported 1 to 10 μg/kg of NDMA and NDEA in most edible oils. The present study showed similar results except for the fact that

Table 9. N-nitrosamine contents in oils (μg/kg)

Product	Number of samples	NDMA	NMEA	NDEA	NDBA	NPYR	NPIP	NMOR	Sum	Ref.
Olive oil	2#	0.98-1.5	ND	ND	ND	ND	ND	ND-0.49		‡
	16	1.9-3.5	-	1.7-3.6	-	-	-	-		25
	12	0.51 (avg*)	-	0.45 (avg)	ND	ND	ND	-	0.96	26
Vegetable oil	7#	ND-2.39	ND-0.16	ND	ND	ND	ND-0.43	ND		‡
	21	23 (highest), 1-10	-	21 (highest) 1.4 (avg)	-	-	-	-		25
	22	0.24-0.5	-	0.18-0.24	ND	ND	ND	-		26
Grapeseed oil	2#	ND-1.12	ND	ND	ND	ND	ND	ND-0.39		‡
	11	0.36 (avg)	-	0.19 (avg)	ND	ND	ND	-	0.55	26
Sunflower oil	2#	1.01-2.83	ND	ND	ND	ND	ND	ND-0.7		‡
	11	11 (highest)	-	9.3 (avg)	-	-	-	-		25
Sunflower oil (refined)	12	0.64 (avg)	-	0.41 (avg)	ND	ND	ND	-	1.05	26
Sunflower oil (not refined)	10	0.71 (avg)	-	0.47 (avg)	ND	ND	ND	-	1.18	26
Perilla oil	2#	1.81-2.26	ND	ND	ND	ND	ND	ND		‡
Sesame oil	3#	1.09-2.1	ND	ND	ND	ND	ND	ND		‡

* Avg: average. ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.

Table 10. N-nitrosamine in butter and margarine (μg/kg)

Product	The number of samples	NDMA	NMEA	NDEA	NDBA	NPYR	NPIP	NMOR	Sum	Ref
Margarine	14	0.2-0.3	-	-	-	-	-	1.7-3.8		30
	3	2.5-5.8	-	1.4-5.5	-	-	-	-		25
Butter	10	-	-	-	-	-	-	0.1		30

-: Not tested.

Table 11. N-nitrosamine in soybean paste, soy sauce, seasoning, and sauce (μg/kg)

Product	The number of samples	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR	NMEA	Ref
Market soy sauce	1#	ND	1.17-1.49	0.13-0.19	0.74-0.96	ND	ND	ND	‡
Market soybean paste	2#	ND-0.21	ND-0.12	ND-0.48	ND	ND	ND	ND	‡
Sauce[a)]	9#	ND-3.02	ND-0.71	ND-0.43	ND-0.18	ND	ND	ND	‡
Seasoning[b)]	5#	ND-13.48	ND-1.01	ND-1.88	ND-6.48	ND-6.53	ND-0.21	ND	‡
Market soybean sauce	1	1.1-2.7	-	-	-	-	-	-	27
Traditional soy sauce	1#	ND	ND	ND	ND	ND	ND	ND	‡
	7	-	-	-	< 0.02	< 0.01	-	-	24
	1	< 0.5-1.87	-	-	-	-	-	-	27
Market soybean paste	4	-	-	-	< 0.02	< 0.01	-	-	24
	1	1.6-2.4	-	-	-	-	-	-	27
Traditional soybean paste	7	-	-	-	< 0.02	< 0.01	-	-	24
	1	1.5-3.1	-	-	-	-	-	-	24

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.
[a)]Sauce samples included commercial sauce sold in market.
[b)]Seasoning included artificial flavored seasoning, black pepper, and salt.

Table 12. N-nitrosamine contents in alcoholic beverages (μg/kg)

Product	The number of samples	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR	NMEA	Ref
Beer	3#	ND	ND	ND	ND	ND	ND	ND	‡
	12	0.28	-	-	-	-	-	-	30
	6	0.5-1.87	-	-	-	-	-	-	27
	16	-	0.2-1.14	-	0.2-0.84	-	0.2-1.14	-	28
Cognac, vodka	1#	0.02-0.2	-	-	-	-	-	-	30
Wine	3#	ND	ND	ND	ND	ND	ND	ND	‡
Rice wine	1#	ND	ND	ND	ND	ND	ND	ND	‡
Whisky	1#	0.22-0.23	ND	ND	0.3-0.39	ND	0.16-0.24	ND	‡
	2	0.68-2.5	-	-	-	-	-	-	30
Malt beverage	5	-	0.2-0.54	-	-	-	0.2-0.73	-	28
Other liquor[a)]	5#	ND	ND	ND	ND	ND	ND	ND	‡

ND: Not detected, -: Not tested, ‡: present study, #: number of composite samples.
[a)]Other liquor included soju, white wine, refined rice wine, plum wine, raspberry wine, and fermented herb liquor.

NDEA was not detected in the present study. Yurchenko *et al.* (26) reported less than 0.71 μg/kg in all nitrosamines, but they used the method we have used in the fatless sample, which may not be sufficiently efficient to extract all nitrosamines from oils. Also, the method may not be able to remove the interference of remaining lipids for detection sensitivity. NDMA was also reported in margarine and butter, though the amount was low (Table 10) (25,30).

The highest concentration of nitrosamines was observed in seasoning samples with 13.48 μg/kg of NDMA and 6.53 μg/kg of NPIP. Since previous studies on nitrosamines in seasoning are scarce, it needs to be investigated further concerning the formation mechanism in relation to the manufacturing process. In sauce samples, 3.02 μg/kg of NDMA was detected (Table 11).

In alcoholic beverages, no nitrosamine was detected in the present study in beer, wine, rice wine, soju, and other liquors, while trace amounts of NDMA, NPYR, and NMOR were detected in whisky. Kim *et al*. (27) reported the maximum of 1.87 μg/kg NDMA in beer (Table 12).

The analysis methods of nitrosamines have become more accurate and simple. From the 1960s, the nitrosamine analysis employed distillation (29). However, distillation could generate false results, since heat of the distillation procedure could facilitate the synthesis of nitrosamines; at the same time, low molecular weight nitrosamines could be lost during distillation due to the low vapor pressure. These days, various resins are used in the food analysis. In the present study, solid supported liquid extraction using Extrelut NT and Florisil SPE was employed (20). To analyze the whole spectrum of food items, the method had to be modified. For fatty foods, liquid-liquid extraction was used to extract polar organic nitrosamines from lipids. To speed up the extraction procedures, polar solvent, immiscible and lighter than oil, was searched. Acetone-water (3 : 1) mixture or acetone-acetonitrile mixture satisfied the terms. The extraction mixture was stored at −80°C freezer to help the separation of the phases. Any remaining fat in the extract may interfere and damage analytical instrument. C-18 SPE cartridge laid with aluminum oxide powder was employed to remove the remaining fats and emulsifier. The final procedure can be used for food matrix with a large amount of fats and emulsifiers, such as dressings and creamers.

To estimate a close-to-real exposure of any hazardous compound, it is always better to analyze as many food items as possible; therefore, a fast and easy method is necessary. The methods reported in the present study can cover a wide range of food items on the Korean dining table and are also easy and fast. Our study also demonstrated the application of the methods to various food items, including agricultural, animal, and fishery products.

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