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N-Nitrosamine Concentrations in Fish Distributed in a Domestic Market

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Abstract

In order to provide data on N-nitrosamine (NA) and sanitation in fish available in domestic markets, this study analyzed the levels of NA and its precursors in 9 samples of sea breams and yellow croakers, 12 samples of red-flesh fish, 38 samples of white fish, 5 samples of Alaska pollacks and cod, and 8 species of imported fish. Sea breams and yellow croakers had nitrite concentrations ranging from non-detectable (ND) to 7.4 mg/kg, red fish ND to 5.3 mg/kg, white fish ND to 18.7 mg/kg, Alaska pollacks 0.3 to 2.2 mg/kg, and imported fish from 0.4 to 12.8 mg/kg. Nitrates in sea breams and yellow croakers ranged from 1.2 to 41.19 mg/kg, red fish 0.6 to 26.1 mg/kg, white fish 4.3 to 75.9 mg/kg, Alaska pollacks 0.4 to 3.1 mg/kg, and imported fish ND to 16.0 mg/kg. DMA concentrations were 69.8 to 219.9 mg/100 g in sea breams and yellow croakers, 4.1 to 336.3 mg/100 g in red fish, 1.3 to 331.9 mg/100 g in white fish, 15.7 to 312.3 mg/100 g in Alaska pollacks, and 1.0 to 71.8 mg/100 g in imported fish. TMA concentrations in sea breams and yellow croakers, red fish, white fish, Alaska pollacks and imported fish were 43.8～496.2, 12.3～127.0, 2.0～525.9, 15.4～122.4, and 4～70.6 mg/100 g, respectively. For NA in fish distributed in local markets, only N-nitrosodimethylamine (NDMA) was detected, and its concentrations ranged from 4.7 to 73.7 μg/kg in sea breams and yellow croakers, 2.2 to 56.5 μg/kg in red fish, ND to 143 μg/kg in white fish, 3.8 to 33.3 μg/kg in Alaska pollacks, and 2.1 to 102.2 μg/kg in imported fish.

Key words: N-nitrosamine, N-nitrosodimethylamine (NDMA), nitrite, nitrate, dimethylamine, trimethylamine, imported fish

INTRODUCTION

Rapid changes in the world economy have resulted in increased international trade. Consequently Korea imports a large variety of marine products from around the world, making it imperative that imports of fish and shellfish be monitored to insure that unhealthy seafood is not imported from producers that do not conform to acceptable standards of sanitation and quality. With Korea being a member of OECD in the WTO system, the free trade policy has mandated that Korea increase the import of fish, shellfish, and their processed products. Most fish, such as Alaska pollacks, cod, and flounder arrive in a frozen state (1). Korea's annual per capita consumption of fish and shellfish is rather very high, amounting to about 40 kg per capita, and fish accounts for about 45% of gross protein consumption (2). As Koreans have recently become more health-conscious, the demand for fish has also increased. However, most of the fish are imported in a simply processed state from developing countries such as China and the Philippines. Generally speaking, such fish are poorer in quality and may be contaminated because of the poor hygiene and a lack of rigorous quality control (3). In addition, a cancer-causing substance, N-nitrosamine and its precursors are produced during storage of fish. Because of this situation, many countries have become more stringent, with quarantines and testing of marine products including all varieties of fish and their processed products that are distributed in their own countries using HACCP analysis to assure a safe and healthy food supply. Korea has also begun to recognize the necessity of this system.

N-nitrosamine, an extremely toxic substance, is likely to be ingested through the consumption of marine products. Therefore, the importance of research on countermeasures to prevent contamination can not be overemphasized for preserving the health of the current and future generations. The reason why researchers are more interested in N-nitrosamine than in all the other carcinogens is summarized as follows;

First, it is more carcinogenic than any other carcinogen, and an extremely small quantity can cause cancers in humans (4). Second, its precursors are extensively distributed in fish, fruits, and vegetables that are consumed

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daily (5-8). Third, most carcinogens can cause cancer in a particular organ or system, but N-nitrosamine acts as a cancer-causing agent in all parts of the body, including: liver, lungs, pharynx and larynx, gullet, bladder, kidneys, stomach, and ovaries (4,9). The opportunity for N-nitrosamine to manifest itself in humans can be attributed to both internal and external factors. External factors include lifestyle conditions such as food, cigarettes, cosmetics, air, medication, and hazardous job conditions as found in the processing of rubber, leather, metal processing; as well as in industries that result in exposure to hazardous materials such as mining and fish processing byproducts, agricultural chemicals, chemicals, laundry detergents. Internal factors include the consumption of nitrites or nitric acid, nitrous oxygen gas and amines generated by saliva after the consumption of nitrates (10). It has been discovered that this substance is generated via the interaction of amines (primary, secondary, tertiary amines, and quaternary ammonium compounds) and nitrites (8,11-13). Given the poisonous nature of N-nitrosamine and its extensive distribution in food, the blockage of these chemicals in fish and their processed products and the establishment of preventative measures are of great importance. Therefore, this study has been designed to provide basic data for evaluating public health and safety measures for domestically distributed fish by surveying the of distribution of N-nitrosamine and its precursors in commercially available fish.

MATERIALS AND METHODS

Materials

Sixty four samples of domestic fish and 8 species of imported fish commonly sold in domestic markets were collected, minced using a mixer after removing all viscera and bones, and analyzed for nitrites, nitrates, dimethylamine (DMA), trimethylamine (TMA) and N-nitrosamine.

Determination of nitrite and nitrate

Nitrites and nitrates in fish were extracted with distilled water using the method of Oh et al. (14). The extract was filtered through 0.22 μm membrane filter (Corning Co., USA) and nitrite and nitrate were determined by ion chromatography (IC) under the conditions shown in Table 1.

Determination of dimethylamine (DMA) and trimethylamine (TMA) Levels

The concentrations of dimethylamine and trimethylamine were determined by gas chromatography (GC) as described by Oh et al. (15). A sample minced fish (5 g) was placed into a 100 mL beaker to which 50 mL of isopropyl alcohol was added, homogenized for 10 minutes, and allowed to stand for 30 minutes at room temperature. The homogenate was then filtered through filter paper, decanted into a 100 mL volumetric flask and filled to volume with isopropyl alcohol. This solution was used as a sample for determining the levels of DMA and TMA by GC under the conditions shown in Table 2.

Table 1. Conditions used for ion chromatography for nitrite and nitrate in fish

Ion chromatograph	DIONEX-100
Column	IonPac AS4A-SC 4 mm
Detector	Conductivity detector
Eluent	1.5 mM Na_2CO_3 / 2.5 mM $NaHCO_3$
Suppressor regenerant	25 mM H_2SO_4
Flow rate	1.7 mL/min, N_2
Sample injection	25 μL

Determination of N-nitrosamine

N-nitrosamine was extracted by steam-distillation by the method of Hotchkiss et al. (16) and then determined by gas chromatography (GC, Model 5890A, Hewlett Packard)-thermal energy analyzer (TEA, Model 543, Thermo Electron Corp. USA) The conditions for GC-TEA were as follows: a 10 foot 2 mm i.d glass column packed with 10% Carbowax 20 M/80 ~ 100 Chromosorb WHP; flow rate of helium gas, 20 mL/min; oven temperature programmed, 140 ~ 170 at 5°C/minute; injection port temperature, 180°C; pyrolizer temperature, 550°C; interface temperature, 200°C; cold trap temperature, -160°C; analyzer pressure, 1.9 torr. GE-TEA chromatogram for standard nitrosmine of 7 samples and imported fish is shown in Fig. 1. N-nitrosodipropylamine (NDPA) was used as an internal standard when N-nitrosamine was extracted.

RESULTS AND DISCUSSION

N-Nitrosamine was extracted from 64 samples of domestic fish and 8 species of imported fish that are commonly available in domestic markets. After separation and analysis by GC-TEA, N-nitrosodimethylamine

Table 2. Conditions for GC analysis of DMA and TMA

GC type	PYE UNICAM series 304 chromatograph
Column	Φ3 mm×2 m glass column
Packing material	Chromosorb 103 (60 ~ 80 mesh)
Column temp.	130°C
Injection temp.	180°C
Detector and temp.	FID, 250°C
Flow rate	Nitrogen: 40 mL/min, hydrogen: 40 mL/min and air: 200 mL/min

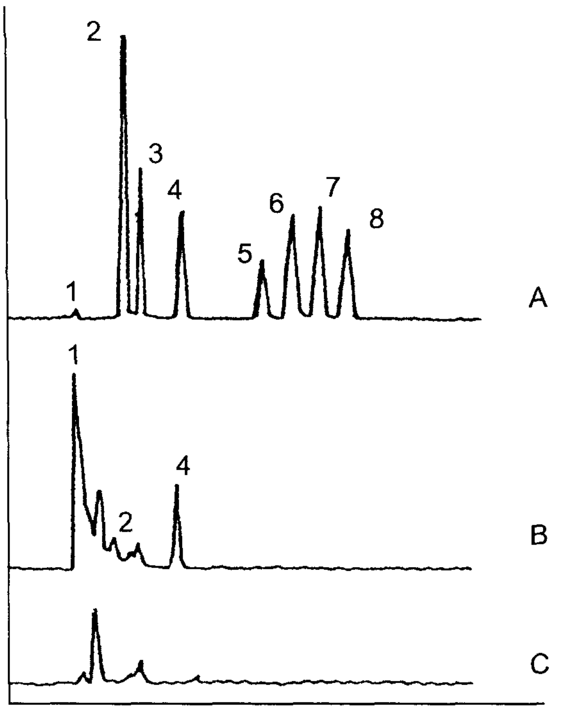

Fig. 1. GC-TEA chromatograms for standard nitrosamines (A), fish sample (B), and fish sample irradiated by UV light for 3.5 hr (C). 1, Solvent; 2, N-nitrosodimethylamine (NDMA); 3, N-nitrosodiethylamine (NDEA); 4, N-nitrosodipropylamine (NDPA); 5, N-nitrosodibutylamine (NDBA); 6, N-nitrosopiperidine (NPIP); 7, N-nitrosopyrrolidine (NPYR); 8, N-nitrosomorpholine (NMOR).

was the only N-nitrosamine detected; a typical chromatogram is shown in Fig. 1.

The levels of nitrates, nitrites, DMA, TMA and NDMA in sea breams and yellow croakers

The concentrations of nitrites, nitrates, DMA, TMA and NDMA in 9 samples of 6 species of sea breams and yellow croakers are shown in Table 3. Nitrite levels were high in dried geniun porgies (ND ~ 7.4 mg/kg) and dried yellow croakers (4.8 mg/kg). However, dried cherry porgies, damsel fish, large yellow croakers, yellow croakers had non-detectable (ND) concentrations of nitrites. Nitrate levels were the highest in damsel fish (41.1 mg/kg), followed by dried blanquillos (23.9 mg/kg), and dried cherry porgies (3.8 mg/kg). Kim and Oh (17) noted that the nitrite and nitrate levels in dried blanquillos were 5.3 mg/kg and 7.7 mg/kg respectively. Sung et al. (18) reported that the nitrite and nitrate levels in dried yellow croakers were 5.3 mg/kg and 7.7 mg/kg each, but less than 1.0 mg/kg in dried blanquillos. Kim et al. (19) found that the nitrate-N concentrations in damsel fish were 4.0 mg/kg.

High levels of DMA were detected in yellow croakers (69.6 ~ 219.9 mg/100 g). Out of the sea breams, dried blanquillos had the most highest DMA at 188.4 mg/100 g, followed by large yellow croakers (169.2 mg/100 g), dried cherry porgies (150.2 mg/100 g), damsel fish (128.4 mg/100 g), and genuin porgies (107.9 mg/100 g).

The levels of TMA were the highest in dried yellow croakers (43.8 ~ 469.2 mg/100 g), followed by yellow croakers (104.7 mg/100 g), blanquillos (91.6 mg/100 g), damsel fish (52.6 mg/100 g), and genuin porgies (49 mg/100 g). Sung et al. (18) reported levels of DMA and TMA-N of 10.4 mg/kg and 40.1 mg/kg in dried blanquillos respectively, and 10.4 mg/kg and 40.1 mg/kg in dried yellow croakers, respectively. The DMA levels were 1.79 mg/kg in yellow croakers (20) and 136 mg/kg and 201 mg/kg in wart perches and yellow croakers.

The NDMA levels ranged from 4.7 to 73.7 μg/kg, and were the highest in large yellow croakers. Among the sea breams, genuin porgies had 62.4 μg/kg of NDMA,

Table 3. Concentrations of nitrite, nitrate, DMA, TMA and NDMA in sea bream and yellow croaker (dry weight basis)

Sample		Nitrite (mg/kg)	Nitrate (mg/kg)	DMA (mg/100 g)	TMA (mg/100 g)	NDMA (μg/kg)
Blanquillo	fresh	4.2[1)]	13.0	69.8	91.6	4.7
Branchiostegus japonicus	dried	3.6	23.9	188.4	90.1	58.6
Genuin porgy	fresh	5.5	13.0	107.9	49.0	62.4
Chrysophrys major	dried	7.4	3.8	97.2	43.8	56.2
Cherry porgy *Sacura margaritacea*	dried	ND[2)]	15.2	150.2	63.6	55.0
Damsel fish *Chromis natatus*	fresh	ND	41.1	128.4	52.7	10.2
Yellow croaker	fresh	ND	7.4	219.9	104.7	40.9
Pseudosciaena polyactis	dried	4.8	1.2	76.0	496.2	27.5
Large yellow croaker *Pseudosciaena crocea*	fresh	ND	1.5	169.2	88.3	73.7

[1)]Mean of triplicate experiments.
[2)]ND, not detected.

dried blanquillos 58.6 μg/kg, dried cherry porgies 55.0 μg/kg, dried genuin porgies 56.19 μg/kg, and yellow croakers 40.94 μg/kg. Sung et al. (18) reported that NDMA levels were 3.5 μg/kgin dried blanquillos and 45.9 μg/kg in dried-salt yellow croakers. Kim and Oh (20) found NDMA concentrations of 20 μg/kg in yellow croakers.

NDMA concentrations at potentially hazardous concentrations were detected in sea breams and yellow croakers. The levels of these substances change according to drying methods, storage conditions, and distribution routes: therefore, appropriate methods of storage, processing, and transport if fish must be established in order to minimize the formation of N-nitrosamines during processing and distribution.

Nitrites, nitrates, DMA, TMA and NDMA in red fish

Nitrite, nitrate, DMA, TMA and NDMA concentrations in 12 samples of 8 species of red-colored meat fish commonly available in the domestic market are shown in Table 4. The nitrate concentrations ranged from ND to 7.6 mg/kg and had a tendency to be higher in raw samples than in dried ones. Dried Pacific sauries had the highest levels out of all of the samples. However, no nitrites were detected in mackerel, anchovies, frozen Pacific sauries, snipefish, yellow tails and sharks. The concentrations of nitrates which ranged from ND to 26.1 mg/kg were higher in sharks (26.1 mg/kg) and anchovies (23.2 mg/kg) than in any other fish. Nitrate concentrations in mackerel, horse mackerel, and Pacific sauries had a tendency to be higher in dried rather than frozen products. Yim et al. (21) reported that the levels of nitrates were 1.7 mg/kg in mackerel and 2.15 mg/kg in horse mackerel; and that the concentrations of nitrites and nitrates were 2.1 mg/kg and 3.7 mg/kg in dried sharks respectively, and less than 1.0 mg/kg in dried anchovies.

DMA concentrations ranged from 4.0 to 336.3 mg/100 g and were highest in sharks followed by atka mackerel (164.9 mg/100 g). The TMA levels ranged from 6.6 ~ 127.0 mg/100 g and were highest in sharks, followed by Pacific sauries (31.8 mg/100 g), yellow tails (28.9 mg/100 g), anchovies (12.8 and 24.5 mg/100 g), mackerel (10.7 and 24.9 mg/100 g), and horse mackerel (12.3 and 19.9 mg/100 g). Sung et al. (18) reported that the levels of DMA in dried anchovies were 5.0 mg/kg, and those of TMA were 18.5 mg%. Wootton et al. (22) stated that the levels of DMA and TMA were 323.0 mg/kg and 239.0 mg/kg each in dried anchovies (Hong Kong), 0.49 mg/kg and 3.03 mg/kg in mackerel each, 0.09 mg/kg and 0.5 mg/kg in gizzard-shads, and 0.4 mg/kg and 0.96 mg/kg in sardines (23). Park et al. (24) also commented that their levels were just 0.84 mg/100 g and 0.44 mg/100 g in Pacific sauries, but that the contents of DMA were comparatively higher in shark's fins (895.0 mg/kg), Pacific sauries (106.0 mg/kg), dried anchovies (133.0 mg/kg), and dried cat-sharks (391.0 mg/kg) (25). These results demonstrate that nitrites and

Table 4. Concentrations of nitrite, nitrate, DMA, TMA and NDMA in red fish (dry weight basis)

Sample		Nitrite (mg/kg)	Nitrate (mg/kg)	DMA (mg/100 g)	TMA (mg/100 g)	NDMA (μg/kg)
Horse mackerel	fresh	0.2[1)]	0.6	10.4	19.9	3.9
Trachurus japonicus	dried	3.5	1.9	11.6	12.3	35.0
Mackerel	fresh	ND[2)]	3.9	5.5	10.7	2.4
Scomber japonicus	dried	5.3	7.4	4.1	6.6	2.2
Anchovy	fresh	ND	23.2	27.2	12.8	56.5
Engraulis japonica	dried	3.0	10.2	21.9	16.5	13.1
Atka mackerel *Pleurogrammus azonus*	frozen	0.3	5.8	164.9	69.6	7.7
Pacific saury	frozen	ND	3.8	47.1	31.8	21.6
Cololabis saira	dried	7.6	5.8	29.4	70.9	36.7
Snipefish *Hemirhamphus sajori*	fresh	ND	17.8	42.4	29.7	3.7
Yellow tail *Seriola quinqueradiata*	fresh	ND	6.5	49.7	28.9	9.4
Shark *Squalus fernandius*	fresh	ND	26.1	336.3	127.0	19.9

1)Mean of triplicate experiments.
2)ND, not detected.

nitrates in red fish tend to be higher in dried rather than frozen fish. This phenomenon can be explained by the influence of the drying environment on the nitrogen oxide compounds as well as by effects of food additives before drying. DMA and TMA concentrations were higher in raw and frozen samples than in dried ones. TAMO, a substance which alters osmotic pressure, reduced the TMA levels at a rapid rate either by modulating bacterial action or by changing pH by enzymatic or non-enzymatic action in the fish after death (26). The levels of NDMA in red fish were higher in frozen or dried products than in fresh fish, which is a reflection of the formations of precursors during freezing or frozen storage (9,27,28) and by heating (29,30). The presence of the precursors is essential to the development of NDMA during processing or storing fish.

The levels of nitrites, nitrates, DMA, TMA and NDMA in white fish

Concentrations of nitrite, nitrate, DMA, TMA and NDMA in 38 samples from 28 species of white fish distributed in local markets are shown in Table 5. Nitrite concentrations ranged from ND to 18.7 mg/kg and were highest in dried conger eels. Nitrites were higher in dried

Table 5. Concentrations of nitrite, nitrate, DMA, TMA and NDMA in white fish (dry weight basis)

Sample		Nitrite (mg/kg)	Nitrate (mg/kg)	DMA (mg/100 g)	TMA (mg/100 g)	NDMA (μg/kg)
Flounder *Limanda aspera*	fresh	ND[1]	16.5[2]	213.1	108.0	9.0
File fish *Stephanolepis cirrhifer*	fresh	4.2	13.0	52.9	52.0	51.6
	dried	3.6	23.9	16.3	36.6	18.0
	fillet	7.4	21.7	6.2	3.3	16.8
Sole *Areliscus joyneri*	dried	9.0	7.6	92.2	47.2	73.4
Harvest fish *Pampus argenteus*	fresh	ND	6.5	120.2	99.4	37.5
	dried	6.3	8.7	30.1	149.5	12.6
Chicken grunt *Parapristipoma trilineatum*	fresh	ND	28.1	139.9	62.7	3.1
Slender *Ilisha elongata*	frozen	4.2	13.0	150.2	179.2	143.0
Conger eel *Conger myriaster*	fresh	5.5	19.7	83.0	137.9	28.6
	dried	18.7	27.8	160.8	74.1	21.9
Japanese eel *Anguilla japonica*	fresh	1.0	75.9	8.0	6.0	2.0
Loach *Misgurnus anguillicaudatus*	fresh	4.9	15.2	1.3	2.0	2.9
Long shanny *Stichaeus grigorijewi*	fresh	11.2	12.3	200.6	93.5	35.0
Croaker *Nibea imbricatus*	fresh	ND	14.6	181.7	79.8	42.8
Japan seabass *Lateolabrax japonicus*	fresh	6.9	47.7	126.8	56.2	38.2
Common mullet *Mugil cephalus*	fresh	6.4	53.1	46.1	28.3	65.2
Ray *Dasyatis zugei*	fresh	4.9	15.6	37.2	293.3	23.2
Target dory *Zeus japonicus*	fresh	ND	46.0	135.0	46.5	27.8
Dusky spinefoot *Siganus fuscescens*	fresh	3.3	6.2	141.3	68.2	37.4
Striped puffer *Fugu xanthopterus*	fresh	8.2	19.0	86.2	46.2	ND

Table 5. Continued (dry weight basis)

Sample		Nitrite (mg/kg)	Nitrate (mg/kg)	DMA (mg/100 g)	TMA (mg/100 g)	NDMA (μg/kg)
Angler *Loppiomus setigerus*	fresh	ND	11.4	38.5	139.2	65.0
Bar-tailed flathead *Platycephalus indicus*	fresh	7.4	13.0	149.3	66.8	73.3
Pike gudgeon *Pseudogobio esocinus*	frozen	6.7	12.6	149.4	86.5	36.5
	dried	ND	19.0	13.3	48.4	15.3
Hair tall *Trichiurus lepturus*	fresh	1.4	4.3	148.6	72.0	15.6
	salt-dried	1.5	9.3	205.8	80.8	26.0
Pacific ocean perch	frozen	ND	22.8	331.9	525.7	14.7
Armorclad rockfish *Sebastes hubbsi*	fresh	7.6	17.4	197.9	100.7	91.2
Rock fish *Sebastes inermis*	fresh	ND	7.6	157.5	83.8	85.4
Lizard fish *Saurida elongata*	fresh	9.9	26.1	141.9	35.2	47.9
Japan. spanish mackerel *Scomberomorus niphonius*	fresh	ND	11.4	123.9	60.8	39.9
	frozen	11.8	17.4	108.0	49.5	59.1
	dried	0.5	13.0	70.5	33.6	45.9
Goog salmon *Sphyraena pinguis*	fresh	8.2	15.2	126.7	74.7	23.2
Sand smelt *Sillago sihama*	frozen	3.0	6.7	23.9	24.5	45.9
Icefish	dried strip	ND	74.5	ND	12.4	11.3
Roe of flying fish	seasoned	4.9	7.6	9.8	4.8	52.2

[1]ND, not detected.
[2]Mean of triplicate experiments.

file fish fillet than in dried file fish. They were 7.6 mg/kg in dried sole, 7.6 mg/kg in armorclad rockfish, 9.9 mg/kg in lizard fish, 11.2 mg/kg in long shannies, 11.8 mg/kg in frozen Japanese Spanish mackerel, and 8.2 mg/kg in striped puffers. However, no nitrites were detected in flounder, harvest fish, chicken gruntrs, target dories, croakers, anglers, dried pike gudgeons, Pacific ocean perch, and rockfish. Nitrate concentrations ranged from 4.3 to 75.9 mg/kg and were highest in Japanese eels, followed by Japanese sea bass (47.7 mg/kg) and target dories (46 mg/kg).

DMA concentrations ranged from 1.3 to 331.9 mg/100 g and were highest in target dories, followed by raw flounder (213.1 mg/100 g), dried hair tails (205.8 mg/100 g), and long shannies (200.6 mg/100 g). Over 100 mg/100 g of nitrates were detected in armorclad rockfish, rockfish, lizard fish, dusky spinefeet, target dories, slenders, croakers, Japanese seabass, bar-tailed flatheads, pike gudgeons, hair tails and dried conger eels; however, the levels were lower in loaches and Japanese eels. TMA concentrations which ranged from 2.0 to 525.9 mg/100 g tended to be higher in Pacific ocean perches and rays. Over 100 mg/100 g of TMA was detected in flounder, dried harvest fish, armorclad rockfish, slenders, anglers and conger eels. Kim and Oh (20) reported that the levels of DMA were 0.64 mg/kg in anglers, 1.67 mg/kg in hair tails, 1.61 mg/kg in flounder, 0.32 mg/kg in loaches, 1.23 mg/kg in harvest fish, 0.57 mg/kg in conger eels, 1.19 mg/kg in spiny rasp skates, and 1.92 mg/kg in atka mackerel. Lin (25) stated that the levels were 552.0 mg/kg in scallops, 320.0 mg/kg in sword fish, and 258.0 mg/kg in dried Japanese seabass. Maga (7) reported that the concentrations were 180.0 mg/kg in Japanese sea bass.

NDMA ranged from ND to 143 μg/kg and was highest in slenders, followed by armorclad rockfish (91.2 μg/kg), rock fish (85.4 μg/kg), dried soles (73.4 μg/kg), bar-tailed flatheads (73.3 μg/kg), common mullets (65.17 μg/kg), anglers (65.0 μg/kg), and file fish (51.63 μg/kg). However, NDMA was not detected in striped puffers. Lyengar et al. (31) reported that the levels of NDMA in white fish were 18.0 µg/kg in cods, 11.0 µg/kg in scallops, and 8.0 µg/kg in flounder. Kim and Oh (20) also reported NDMA concentrations of 7.0 µg/kg in local croakers, 12.0 µg/kg in hair tails, 4.0 µg/kg in loaches, 1.0 µg/kg in harvest fish, and 9.0 µg/kg in conger eels.

These results indicate that white fish tend to be higher in nitrites, nitrates, DMA and TMA than red fish in local markets. The DMA and TMA concentrations were higher than nitrites and nitrates, and higher in raw rather than dry samples. Even fresh fish contained significant amounts of DMA and TMA. Therefore, they are presumed to function as the main precursors for generating NA, that will eventually be consumed by humans, during the transportation, processing, storage, cooking or consumption processes of commercial fish. In addition, the levels of NDMA in white fish varied widely according to the fish type, and tended to be higher in frozen samples such as frozen slenders, pike gudgeons and Japanese Spanish mackerel than raw or dried white fish. It is possible that the levels were high before freezing and storage because the formation of NA is frequently caused by contaminated habitats. Another possibility is that the phenomenon was influenced by the increase in its precursors, DMA and TMA, during the freezing and storage processes.

The levels of nitrites, nitrates, DMA, TMA and NDMA in Alaska pollacks and cod

The analysis of the nitrite, nitrate, DMA, TMA, and NDMA levels in Alaska pollacks and cod is shown in Table 6. The nitrite levels in pollacks and cod ranged from 0.3 to 2.2 mg/kg and were generally lower than in the other fish. The nitrate levels that ranged 0.4 3.1 mg/kg were the highest in frozen Alaska pollacks. Sung et al. (18) reported nitrite and nitrate levels of 1.0 ~ 5.7 mg/kg and 1.0 ~ 16.3 mg/kg, respectively. The levels of DMA ranged from 15.7 to 312.3 mg/100 g and were the highest in frozen pollacks, followed by dried immature Alaska pollacks (142.8 mg/100 g) and dried pollacks (130.0 mg/100 g). The TMA levels ranging from 4 to 122.4 mg/100 g, and were the highest in frozen pollacks. According to some researchers, the DMA levels were 738.0 mg/kg in cod (7), 740.0 mg/kg in frozen cod (8), and 1,105.0 mg/kg in dried cod (25). Sung et al. (18) reported DMA and TMA levels in frozen pollacks of 22.9 ~ 24.3 mg/kg and 15.4 ~ 17.7 mg/100 g, respectively.

NDMA was detected at concentrations ranging from 3.8 to 33.3 µg/kg in most of the frozen and dried pollacks, and was 20.8 µg/kg in frozen pollacks. Kim and Oh (20) reported 22.3 µg/kg of NDMA in pollacks, and Sung et al. (18) found 8.2 ~ 55.5 µg/kg in dried pollacks. In addition, it was reported that drying methods also influenced the levels, which were 25.1 µg/kg when dried in the sun, 43.5 µg/kg when dried by a hot fan, and 12.2 µg/kg when dried by a cold wind. The levels were higher in raw pollacks (2.8 µg/kg). Contamination of nitrogen oxide in the heated air during the hot wind drying and increases in the precursors through enzymatic action during sun-drying were identified as the main causes (32), it is highly likely that the levels may increase during frozen storage, processing or drying of pollacks. Therefore, careful attention must be paid to each phase of the process.

The results of N-nitrosamine and its precursors in 72 samples of fish distributed in local markets showed that the nitrite and nitrate levels varied in raw, frozen, or dried samples. However, it became evident that nitrite levels increased during the drying and cooking of the fish. One possible cause may be the Nox compounds generated in the air or during combustion (32). In addition, fish and shellfish naturally contain high concentrations of amines. TAMO, a substance that significantly influencing the control of osmotic pressure, reduces TMA levels at a rapid rate through the by modulationg bacterial activity or the changing of pH and thereby reaction rates by enzymatic or non-enzymatic action in fish after death (26). In addition, fish containing substances such as Fe^{2+}, cysteine, taurine, hemoglobin and myoglobin are known to promote the formation of TMA, DMA, and formaldehyde from TMAO (28,32). Since these substances are expected to increase during

Table 6. Concentrations of nitrite, nitrate, DMA, TMA and NDMA in Alaska pollack and cod (dry weight basis)

Sample		Nitrite (mg/kg)	Nitrate (mg/kg)	DMA (mg/100 g)	TMA (mg/100 g)	NDMA (µg/kg)
Alaska pollack *Theragra chalcogramma*	frozen	1.9[1)]	3.1	312.3	122.4	20.8
	dried	0.3	1.8	130.0	62.7	14.9
	frozen dried	0.5	1.3	70.6	20.3	33.3
	immature, dried	2.2	2.1	142.8	45.5	12.5
	dried strip	0.3	0.5	15.7	67.6	3.8
Cod *Gadus macrocephalus*	dried strip	4.1	1.9	11.0	80.5	1.4

[1)]Mean of triplicate experiments.

the processing and storage of fish, the formation of NA is likely to increase to similar levels. On the other hand, only NDMA was detected in this study and the levels varied significantly throughout the samples. The levels of NDMA in salted and dried fish (40~9,000 μg/ kg), which are popular foods among Asian people, were reported to be higher than in other foods (18). The levels of NDMA in processed fish from China were reported to range from ND to 131.5 μg/kg (25) whereas the dried marine products from Korea ranged from ND to 86 μg/kg (18).

One possible explanation for the high levels of NDMA detected in fish and their processed products is the presence of NA's, which are precursors of the amines, nitrites and nitrates that were extracted from fish and shellfish. NDMA is generated when nitrates and nitrites in salt react with the amines in fish with the addition of salt during the consumption, processing and/or storage of fish.

The levels of nitrite, nitrate, DMA, TMA and NDMA in imported fish

Nitrite, nitrate, DMA, TMA and NDMA levels of 8 samples of imported fish are shown in Table 7. The concentrations of nitrites in imported fish that ranged ND to 16.0 mg/kg were the highest in dried trepang imported from the Philippines (16.0 mg/kg), followed by whip-arm octopus from China (3.0 mg/kg), the roe of octopus from Japan (1.6 mg/kg), and other imported fish (less than 1.0 mg/kg). The levels of nitrates ranged from 0.4~12.8 mg/kg and were highest in dried trepang imported from the Philippines, followed by flounder and atka mackerel from Russia (5.2 mg/kg of each). The dried domestic trepang was free of nitrites and nitrates, while the trepang from the Philippines had the highest levels out of all imported fish. The levels of nitrites and nitrates in dried local marine fish products ranged from ND to 9.6 mg/kg and ND 16.8 mg/kg, respectively (18), which is similar to the concentrations in imported fish, even if they are not the same species.

The concentrations of DMA ranged from 1.0 to 71.8 mg/100 g and was highest in frozen shrimp from Argentina, followed by Japanese roe of pollacks (54.8 mg/100 g), and Russian frozen cod (47.7 mg/100 g). The level of TMA ranged from 15.4 to 70.6 mg/100 g and was the highest in frozen shrimp from Argentina (70.6 mg/100 g), followed by Russian frozen flounder (52.0 mg/100 g), and Russian frozen cod (47.7 mg/100 g). The DMA levels in imported fish were higher than those in local fish: local flounder (1.61 mg/kg) (24), mackerel (0.49 mg/kg), and a trace in the local dried trepang. The levels of DMA and TMA in all of the local marine products were reported to range from traces to 31.2 mg/kg and 57.2 mg/kg respectively (18).

Analysis of NA in 8 species of imported fish revealed detected only NDMA. NDMA ranged from 2.1 to 102.2 μg/kg, and were highest in flounder from Russia (102.2 μg/kg), followed by frozen shrimp from Argentina (35.3 μg/kg), frozen cod from Russia (30.6 μg/kg), roe of pollacks from Japan (24.2 μg/kg), and frozen whip-arm octopus from China (22.6 μg/kg). Walker (33) reported that the levels of NDMA were higher in salted fish (40~9,000 μg/kg). Another study (25) revealed that the levels of NDMA in marine products from China ranged from ND to 131.5 μg/kg, and were highest in dried shrimp (5.4~131.5 μg/kg). NDMA levels were reported to be from 2.5 to 3.9 μg/kg in salted fish from Japan (6) and ranged from ND to 86 μg/kg in local dried marine produce (18). The precursors of amines, nitrites and nitrates were found in large concentrations in fish; therefore, NDMA was extracted from fish and their processed products. NDMA is formed when the nitrates and nitrites in salt react with the naturally-occurring amines in fish, especially when salt is added in accordance with some countries' eating habits or during the processing or storage of fish.

Table 7. Concentrations of nitrite, nitrate, DMA, TMA and NDMA in imported fish (dry weight basis)

Sample	Import country	Nitrite (mg/kg)	Nitrate (mg/kg)	DMA (mg/100 g)	TMA (mg/100 g)	NDMA (μg/kg)
Cod, frozen	Russia	0.7[1)]	0.4	47.7	47.8	30.6
Flounder, frozen	Russia	0.6	5.2	13.0	52.9	102.6
Atka mackerel, frozen	Russia	0.6	5.2	36.6	30.7	13.1
Mackerel, frozen	Norway	ND[2)]	3.8	28.6	24.9	2.1
Whip-arm octopus, frozen	China	3.0	2.1	25.2	24.0	22.6
Shrimp, frozen	Argentina	0.2	2.4	71.8	70.6	35.3
Trepang, dried	Philippines	16.0	12.8	1.0	37.4	3.7
Roe of pollack, frozen	Japan	1.6	0.6	54.8	15.4	24.2

[1)]Mean of triplicate experiments.
[2)]ND, not detected.

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