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The Content of *N*-Nitrosamine in Import Fishes

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수입어류 중 *N*-Nitrosamine 함량

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ABSTRACT

국내에서 유통되고 있는 수입어류 8종에 대한 발암성 물질인 *N*-nitrosamine과 전구물질들의 함량을 분석하여 위생학적 기초자료를 제공하고자 하였다.

수입어류의 질산염 및 아질산염 함량은 각각 0.4~12.8 mg/kg 및 N.D~16.0 mg/kg 이었다. Dimethylamine 함량은 1.0~71.8mg/100g 이었으며, Trimethylamine 함량은 15.4~70.6 mg/100g이었다.

수입어류 8종에 대한 발암성 물질인 *N*-nitrosamine은 *N*-nitrosodimethylamine (NDMA) 만 검출되었으며, 이들의 NDMA 함량은 2.1~102.2㎍/kg으로 러시아산 가자미에서 가장 높게 검출되었다. 또한 아르헨티나산 냉동새우는 35.3 ㎍/kg, 러시아산 냉동대구에서도 30.6 ㎍/kg으로 높은 함량을 보였다.

Key words : nitrosamine, nitrosodimethylamine(NDMA).

Ⅰ. INTRODUCTION

According as import or export is liberalized by the fast change of world economy composition recently, marine products of global village each place been importing in large quantities. With this, oversupply of inexpensive and insanitary fishes import in underdeveloped nation is expected. According to import opening plan by OECD adulterating, income of fish and it's products are controlled under WTO system in Korea. Import fishes are import in most frozen condition, and alaska pollack, codfish and flatfish of these fishes are occupying in large quantities[1]. In the meantime, the amount of fish intake in Korea is very high as 40kg level per person by year, and

demand about fish intake is increased gradually. The fish intake is supplied about 45% of total protein intake amount per person[2]. But, most of import fish from China, Russia, Philippines and so on could cause hygienic problem because the quality and safety is low than the domestic products[3].

Also, the fishes were regarded as a source of carcinogenic *N*-nitrosamine, since most of fish contain a lot of its precursors such as secondary amine and tertiary amine[4]. *N*-Nitrosamines were formed by reaction nitrite or nitrate with dimethylamine and trimerthylamine. These precursors are distributed extensively to fishes, vegetables and fruits that we are ingesting[5,6]. We must establish control and prevention system for this carcinogenic compounds in fish and its products. Recently, the many countries are enforcing HACCP system about all fish and its products circulated for domestic consumption as well as import fish.

Therefore, to offer hygienic data of *N*-nitrosamine in import fish, the contents of *N*-nitrosamine and its precursors such as nitrite, nitrate, dimethylamine and trimethylamine in 8 samples of import fish collected from domestic market were investigated.

Ⅱ. MATERIALS AND METHODS

1. Materials

Import fishes(Cod, Flounder, Atka mackerel, Mackerel, Whip-arm octopus, Shrimp, Trepang, and Roe of pollack) were collected from a commercial market, and these materials minced using a mixer after removing all viscera and bones. The minced fish tissues were used the materials for analysis of nitrite, nitrate, DMA, TMA and *N*-nitrosamine.

2. Determination of nitrite and nitrate

Nitrite and nitrate in fish extracted with distilled water by the method of Oh et al.[7]. The extract filtered through 0.22㎛ membrane filter(Corning Co., USA) and then determined by ion chromatography(IC). The analytical conditions by IC are shown in 〈Table 1〉.

3. Determination of dimethylamine(DMA) and trimethylamine (TMA)

The determination of dimethylamine and trimethylamine was performed according to the method by GC of Oh et al.[8]. Five grams of the minced muscle of fish was

〈Table 1〉 **Conditions of ion chromatograph for nitrite and nitrate in fish**

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

put into a 100ml beaker and then 50ml isopropyl alcohol was added to the beaker, homogenized for 10min, stood for 30 min at room temperature, and the homogenized mixture was filtered through filter paper. The filtered mixture was decanted into a 100ml volumetric flask and made up to the volume with isopropyl alcohol. This solution was used as a sample for the determination of DMA and TMA by GC. The analytical conditions by GC are shown in 〈Table 2〉.

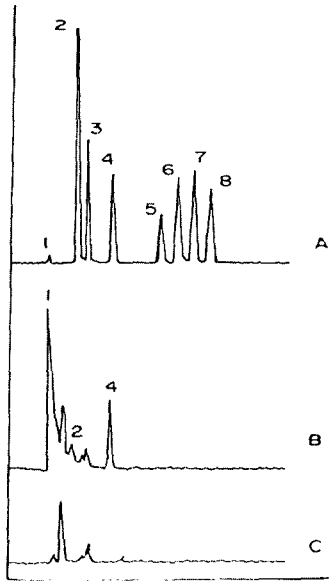
〈Table 2〉 **Conditions for GC analysis of DMA and TMA**

GC Type	PYE UNICAM series 304 chromatograph
Column	Ø3mm × 2m glass column
Packing material	Chromosorb 103 (60~80mesh)
Column temp.	130℃
Injection temp.	180℃
Detector and temp.	FID, 250℃
Flow rate	Nitrogen: 40ml/min, Hydrogen: 40ml/min and Air: 200ml/min

4. Determination of *N*-nitrosamine

N-Nitrosamine were extracted according to steam-distilled by Hotchkiss et al.[9] and then determinated by gas chromatography(GC, Model 5890A, Hewlett Packard)-thermal energy analyzer(TEA, Model 543, Thermo Electron Corp.) The conditions for GC-TEA were as follows: 10ft×2mm i.d glass column packed with 10% Carbowax 20M/80~100 chromosorb WHP; flow rate of helium gas, 20ml/min; oven temperature programmed, 140~170℃ at 5℃/min; injection port temperature, 180℃; pyrolizer temperature, 550℃; interface temperature, 200℃; cold trap temperature, -160℃; analyzer pressure, 1.9 torr.

GE-TEA chromatogram for standard nitrosmine of 7 samples and import fish are



〈Fig. 1〉 GC-TEA chromatograms for standard nitrosamines(A), fish sample(B), and fish sample irradiated by UV light for 3.5hr(C).

1. Slovent; 2. NDMA; 3. NDEA; 4, NDPA; 5, NDBA; 6, NPIP; 7, NPYR; NMOR

shown in 〈Fig. 1〉. Internal standard is used *N*-nitrosodipropylamine(NDPA) when extract *N*-nitrosamine.

Ⅲ. RESULTS AND DISCUSSION

1. Content of nitrite and nitrate in import fishes

The contents of nitrite and nitrate in import fish are shown in 〈Table 3〉. The nitrite content in fish were N.D~16.0mg/kg, and dried sea cucumber from Philippines showed the highest content by 16.0mg/kg. Octopus from China showed 3.0mg/kg, roe of pollack from Japan 1.6mg/kg, the fishes import another countries showed low contents of 1.0mg/kg. The nitrate contents in import fish were 0.4~12.8mg/kg and showed the highest content in dried sea cucumber in Philippines by 12.8mg/kg. According to report of Sung et al.[10], nitrite and nitrate contents of dried marine food products were N.D~9.6mg/kg, and N.D~16.8mg/kg, respectively. And these showed similar contents to import fish content, but be not same kind of fish. Nitrite and nitrate are main precursors for *N*-nitrosamine that can be formed by reacting with secondary and tertiary amines, and also induce methemoglobinemia and anemic hypoxia. The use

of these compounds should be a serious problem in human health during food processing where nitrite have been used as food additives for maintaining color and texture, and growth inhibition of *Clostridium botulinum* in meat products[11~13]. Nitrite and nitrate are detected in almost food, and are detected in vegetables and meat products specially[14]. Also, nitrite content increases by during storage or cooking of vegetables, cereals, meats and its products[13].

〈Table 3〉 **The contents of nitrite and nitrate in import fish**[1] (dry weight basis)

Sample	Import country	Nitrite (mg/kg)	Nitrate (mg/kg)
Cod, frozen	Russia	0.7	0.4
Flounder, frozen	Russia	0.6	5.2
Atka mackerel, frozen	Russia	0.6	5.2
Mackerel, frozen	Norway	ND[2]	3.8
Whip-arm octopus, frozen	China	3.0	2.1
Shrimp, frozen	Argentina	0.2	2.4
Trepang, dried	Philippines	16.0	12.8
Roe of pollack, frozen	Japan	1.6	0.6

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

2. Content of dimethylamine and trimethylamine in import fishes

The contents of DMA and TMA in import fish are shown in 〈Table 4〉. The DMA contents in fish were 1.0~71.8mg/100g and frozen shrimp from Argentina showed the highest content of 71.8mg/100g. Also roe of pollack from Japan and frozen cod from Russia showed high content of 54.8mg/100g and 47.7mg/100g, respectively. The TMA contents in import fish were 15.4~70.6mg/100g and was highest of 70.6mg/100g from Argentina frozen shrimp, and frozen flatfish from Russia showed high content of 52.9mg/100g. The DMA contents of import fish showed higher than domestic fish and DMA and TMA contents in dried fishery products of domestic were reported trace~31.2mg/kg and 57.2mg%, respectively[10,15]. DMA and TMA are distributed widely to various food including fish and is noted as precursors of carcinogenic *N*-nitrosamine. These compounds are increasing during storage for a long time in freezing condition as well as fish processing[16~18].

3. *N*-Nitrosodimethylamine content in import fishes

GE-TEA chromatogram for 7 kinds of standard nitrosamines and import fish are

<Table 4> The contents of DMA and TMA in import fish[1] (dry weight basis)

Sample	Import country	DMA (mg/100g)	TMA (mg/100g)
Cod, frozen	Russia	47.7	47.8
Flounder, forzen	Russia	13.0	52.9
Atka mackerel, frozen	Russia	36.6	30.7
Mackerel, frozen	Norway	28.6	24.9
Whip-arm octopus, frozen	China	25.2	24.0
Shrimp, frozen	Argentina	71.8	70.6
Trepang, dried	Philippines	1.0	37.4
Roe of pollack, frozen	Japan	54.8	15.4

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

shown in Fig. 1. Internal standard for the analysis of *N*-nitrosamine is used *N*-nitrosodipropylamine(NDPA). The only NDMA was detected in 8 kinds of import fish and the content is shown in <Table 5>. The NDMA contents of all tested samples were the range of 2.1~102.2μg/kg and detected the highest by 102.2μg/kg in flatfish from Russia. The contents of NDMA were 35.3μg/kg in freezing shrimp from Argentina, 30.6μg/kg in freezing codfish from Russia, 24.2μg/kg in roe of pollack from Japan, and 22.6μg/kg in freezing small octopus from China. Walker[13] reported that NDMA is detected 40~9,000μg/kg in salted fish ingesting in Oriental countries. Song and Hu[19] reported that the NDMA contents of Chinese fishery products were N.D.~131.5 μg/kg and was specially high as 5.4~131.5μg/kg in dried shrimp. Also, the NDMA contents of salted fish from Japan were known as 2.5~3.9μg/kg[6]. the NDMA contents of dried domestic fishery products,[20,21] and salted and fermented fish were reported as N.D.~7.7μg/kg and N.D.~86μg/kg, respectively[11].

Since *N*-nitrosamine precursors such as amine, nitrite and nitrate are contained a great deal in fish, NDMA is occasionally detected in fishes and these products. Also, the many countries are adding salt according to diet habit or taste preference when process or stores fish. The fish intake amounts in Korea were very high by year 40kg level per person, and demand for fish were increased gradually about 45% of total protein intake amount per person. Thus we must establish control system on hygiene about all fish and its products circulated for domestic consumption as well as import fish.

〈Table 5〉 **The contents of *N*-nitrosodimethylamine in import fish[1]**

(dry weight basis)

Sample	Import country	NDMA (μg/kg)
Cod, frozen	Russia	30.6
Flounder, frozen	Russia	102.2
Atka mackerel, frozen	Russia	13.1
Mackerel, frozen	Norway	2.1
Whip-arm octopus, frozen	China	22.6
Shrimp, frozen	Argentina	35.3
Trepang, dried	Philippines	3.7
Roe of pollack, frozen	Japan	24.2

[1] Mean of triplicate experiments.

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