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Analysis of Dimethylamine and Trimethylamine in Fishes by Gas Chromatography

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Abstract

To develope a rapid analytical method of dimethylamine(DMA) and trimethylamine(TMA) in fish, the contents of DMA and TMA in squid(*Illex illecebrosus* and *Sepiell maindroni*), cod(*Gadus marcrocephalus*) and plaice(*Paralichthys olivaceus*) by gas chromatographic(GC) and colorimetric method were determined. Recoveries for DMA in fish were 86.8~102.5% by GC and 74.2~94.5% by colorimetric method, while those for TMA were 93.0~101.1% by GC and 62.9~117.5% by colorimetric method. The contents of DMA and TMA in fish by GC were 29.7~325.3mg/kg and 145.6~356.0mg/kg, respectively, and those by colorimetric method were 20.0~251.2mg/kg and 139.1~304.3mg/kg, respectively. The analysis of DMA and TMA in fishes by GC after the solvent extraction was simpler and faster and showed better recovery than colorimetric method.

Key words: *N*-nitrosamine, dimethylamine, trimethylamine, gas chromatographic and colorimetric method

INTRODUCTION

With development of culture and enrichment of diet, a number of compounds inducing cancer have been found in various foods. *N*-Nitrosamines and their precursors widely distributed in food and environment(1-3) have a strong carcinogenicity.

Dimethylamine(DMA) which is the precursor of *N*-nitrosamine biosynthesis did not receive much attention until it was found that its content was increased with progress of spoilage in fishes(4,5) and their products(6, 7), salt-fermented fishes(8-11), soy sauce and soybean pastes(12), and Kimchi(13) and so forth. The data for DMA in these foods have been published up to date.

Trimethylamine oxide(TMAO) is abundant in marine fishes, and functions as an important osmoregulatory compound in fishes and shellfishes(14,15). The contents of TMAO in fishes vary with season, habitat and species (14,15). Togunaka(15) reported that the contents of TMAO were greater in dark muscle than in ordinary muscle of red-fleshed fish, and greater in ordinary muscle than in dark muscle of white-fleshed fish. As TMAO is decreased, TMA and DMA are increased because TMAO is reduced to TMA by enzymes secreted by bacteria and then further transformed to DMA(16-18).

The methods for determination of secondary and tertiary amines were divided broadly into colorimetric and gas chromatographic(GC) methods. A number of reports on quantitative and qualitative methods for secondary and tertiary amine determinations in food have been published, but the analytical methods have been mainly based on the colorimetric method which requires improvement because of its limited sensitivity, the use of harmful reagents and complicated analytical procedures. Moreover, the colorimetric method was not able to separate and determine DMA and TMA independently but able to determine only total amines including volatile and non volatile amines(19).

On the other hand, the determination of volatile amine by GC has been obstructed by the loss of sample response, a ghosting phenomena, and badly tailed peaks because of the adsorptive effects between aliphatic amines and chromatographic support or adsorbent. Also, the method used after the formation of the derivatives exhibited good attenuations on GC analysis of DMA and TMA and was able to separate between other amines, but simpler pretreatment procedures for sample are required(20) These difficulties could not have been remarkably improved until a strong basic material such as KOH or ammonia was added to the carrier gas(19,20). Despite these difficulties, GC method has been generally applied for the determination of DMA and TMA.

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The present paper describes an improved method applicable to the flesh of fish by comparing GC with colorimetric method.

MATERIALS AND METHODS

Preparation of materials

Squid(*Illex illecebrosus* and *Sepiell maindroni*) and cod(*Gadus marcrocephalus*) were purchased from a local market, and plaice(*Paralichthys olivaceus*) from a fish farm in Cheju city in Korea. The fishes were removed of all viscera and bones, and then minced thoroughly. The minced muscles were used as materials for analysis of DMA and TMA.

Methods

Determination of DMA and TMA by colorimetric method

The extraction and determination of DMA and TMA for colorimetric method was performed according to Cu-dithiocarbamate improved by Kawabata et al.(21) and according to modified Dyer's method by Hashimoto and Okaichi(22), respectively.

Determination of DMA and TMA by GC

The determination of DMA and TMA by GC was performed according to the revised method of Baba et al.(19). Five grams of the minced muscle of fish was put into 100ml beaker and 50ml isopropyl alcohol was added to the beaker, homogenized for 10min, stood for 30min at room temperature, and the homogenized mixture was filtered through filter paper(Toyo, No. 5A). The filtered mixture was decanted into 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. In addition, a recovery test was performed by adding 5 and 10ppm of the standard solutions of DMA and TMA respectively. The analytical conditions by GC are shown in Table 1.

Coefficient of variation(CV) calculated follow as :

$$CV(\%)=\frac{S}{\overline{X}}\times 100$$

where S is standard deviation of these data and $\overline{X}$ is the mean of these data

Table 1. Condition for GC analysis of DMA and TMA

GC Type	PYE UNICAM series 304 chromatograph(UK)
Column	Φ3mm×2m glass column
Packing material	Chromosorb 103(60~80mesh)
Column temp.	130°C
Injection temp.	180°C
Detector temp.	250°C(FID)
Flow rate	Nitrogen : 40ml/min
	Hydrogen : 40ml/min
	Air : 200ml/min

RESULTS AND DISCUSSION

Recovery of DMA and TMA

The results of recovery tests for DMA and TMA in fish by GC and colorimetric method were shown in Table 2. Because the fleshy substances in fish were different in species, the recoveries for each fish were determined. GC chromatograms for DMA and TMA were shown in Fig. 1.

The recoveries of DMA and TMA by GC method showed 90% or more in all fish except codfish(86.8%) and the differences among fish species were very small.

Table 2. Recoveries of DMA and TMA in fishes by colorimetric and GC methods[1)]

Sample	Standard solution (μg/ml)	Recovery (%)			
		Colorimetry		GC	
		DMA	TMA	DMA	TMA
Squid(Pacific)	5	—[2)]	—	94.5	100.6
	10	117.5	78.5	97.3	100.2
Squid(Cheju)	5	—	—	101.3	95.3
	10	96.3	91.0	102.5	96.2
Cod	5	—	—	92.2	101.1
	10	82.6	94.2	86.8	96.0
Plaice	5	—	—	99.0	98.5
	10	62.9	81.0	94.5	93.0

[1)]Mean of triplicate experiments
[2)]Not analyzed

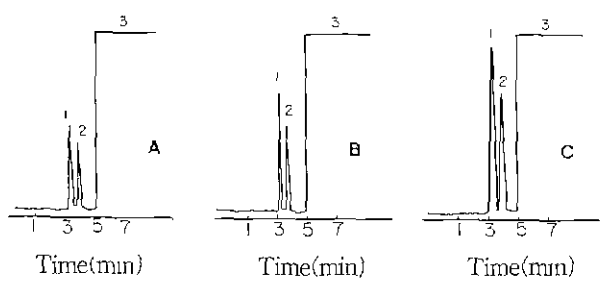

Fig. 1. Chromatograms of dimethylamine and trimethylamine by GC.
A. Standard solution(10μg/ml), B. Sample, C. Sample + Standard solution.
1. Dimethylamine, 2. Trimethylamine, 3. Solvent.

While the recoveries of DMA by colorimetric method showed 62.9~117.5% and the differences by fish species were bigger. On the other hand, the recoveries for TMA by GC method showed 92% or more in all fish tested, while those by colorimetric method showed 78.9~94.25%. Moreover, DMA and TMA were measurable concurrently by GC method.

Based on these results, GC method was able to extract and determine DMA and TMA simultaneously from fish sample with only isopropyl alcohol. It showed good recoveries, and was much more effective than colorimetric method. By contrast, the colorimetric method required time consuming, was not able to determine DMA and TMA simultaneously, and showed poor recoveries. However, in order to resolve the problem for stabilization of GC baseline, the development of substitute solvents for isopropyl alcohol or the substitute supports for Chromosorb 103 was required for GC analysis.

The contents of DMA and TMA in fishes

The contents of DMA and TMA in fishes by GC and colorimetric method are shown in Table 3.

The differences between the contents of DMA measured by the two methods were not much different, but the content of DMA in Pacific squid was 1.3 times higher in GC(325.3mg/kg) than in colorimetric method(251.2mg/kg). The differences in the contents of DMA among fish species were enormous, and the contents of DMA by GC and colorimetric method were 2.7 times and 2.3 times higher in Pacific squid than in Cheju squid, respectively. These differences in both squids were attributed to the difference of storage periods by pelagic fishery. Moreover, the contents of DMA in codfish and plaice by both analytical methods were very low as compared to both squids.

Lin(23) reported that the contents of DMA in squid from Argentina, Japan, New Zealand and Taiwan were 1882, 2043, 1225 and 956mg/kg, respectively. Kawamura et al.(24, 25) reported that those in cod and plaice were 8.1 and 0.1mg/kg, and the contents of DMA in egg and milk products were 0.05~34.65mg/kg. Neurath et al.(26) reported that the contents of DMA in vegetables were 1.4~14mg/kg. Kawamura et al.(24) reported that the secondary amine was increased in fresh fish and broiled or salted shellfish and these contents were 96.8mg/kg in salted squid and 12.1mg/kg in salted salmon.

Table 3. The contents of DMA and TMA in fishes by colorimetric and GC methods[1)] (mg/kg)

Sample	Analytical method			
	Colorimetry		GC	
	DMA	TMA	DMA	TMA
Squid(Pacific)	251 2	304.3	325.3	356.0
Squid(Cheju)	110.0	143.5	119.4	168 1
Cod	34.7	139.1	36.4	145.6
Plaice	20.0	217.4	29.7	253 9

[1)]Mean of triplicate experiments

The differences between the contents of TMA measured by the two methods were not much different, but the content of TMA in Pacific squid was approximately 1.4 times higher in GC than in colorimetric method. Because TMAO was rapidly reduced to TMA by such various factors such as enzymes, pH and storage conditions(18) and the storage periods by pelagic fishery, it was possible that the different TMA contents in both squids were observed. Moreover, fresh fishes containe such low and high molecular weight compounds as Fe^{2+}, cysteine, taurine, hemoglobin and myoglobin and these compounds catalyze the production of TMA, DMA and formaldehyde from TMAO(18,27). While the contents of TMA in cod and plaice were 145.6 and 253.9mg/kg by GC method and 139.1 and 217.4mg/kg by colorimetric method, respectively. No significant differences between the contents of TMA by two analytical methods or among fish species were found.

Meanwhile, Tokunaka(18) reported that the contents of TMA were 2.66mg% in mackerel, 0.54mg% in pacific saury and 0.8mg% in alaska pollack. Takahashi(28) reported that the content of TMA in fresh squid meat was 5.1mg%, but its content was increased to 30.0mg% after sun-drying. Sung et al.(7) reported that the content of TMA of yellow corvenia was increased after salting and sun-drying.

Coefficient of variation for colorimetric and GC methods

To estimate the confidence for the results analyzed by GC and colorimetric methods, the calculated coefficients of variation(CV) for DMA and TMA are shown in Table 4. The calculated CV of DMA by GC and colorimetric method were 3.01 ~ 6.75% and 1.02~4.82%, respectively, while those of TMA were 3.11~6.98% and 1.52~3.87%, respectively. The calculated CV for DMA and TMA were somewhat smaller in GC than in colorimetric method in all fishes tested. From this, it was confirmed that the analyses of DMA and TMA in fishes showed better precision

Table 4. The coefficients of variation(%)[1] for different fishes

Sample	DMA		TMA	
	Colorimetry	GC	Colorimetry	GC
Squid(Pacific)	6.75	4.84	4.56	3.87
Squid(Cheju)	3.01	1.02	6.78	1.52
Cod	4.09	3.04	6.97	1.94
Plaice	4.80	3.27	3.11	2.15
Detection limit (ug/ml)	1.0	0.5	10.0	0.5

[1]Coefficient variation(%) = $CV(\%)=\frac{S}{\overline{X}}\times 100$
where S is standard deviation of these data and $\overline{X}$ is the mean of these data

by GC than by colorimetric method.

The limits of detection for DMA and TMA by GC method were 0.5mg/kg, and those by colorimetric method were 1.0mg/kg for DMA and 10.0mg/kg for TMA. From these results, it was concluded that the GC method was able to be applied to the microanalysis and was better than colorimetric method.

Comparison of GC and colorimetric method

Based on the analyzed results of DMA and TMA by GC and colorimetric methods in fishes, the colorimetric method has several disadvantages of its complicated and time-consuming extraction procedures, low recovery rate and poor reproducibility, and the use of harmful reagents and organic solvents for determination of DMA and TMA, while GC method has advantages for the good recovery rate, the simple extraction procedures, and the simultaneous determination of DMA and TMA.

The determination of DMA and TMA by the GC method may be widely applied to other food products as well as to fishes.

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