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Analysis of free and bound formaldehyde in squid and squid products by gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 24 June 2012
Received in revised form
17 November 2012
Accepted 4 December 2012
Available online 1 June 2013

Keywords:
Aquatic products
Free formaldehyde
GC–MS
HPLC
Squid

ABSTRACT

Formaldehyde can be added illegally as a food preservative in addition to the endogenous formaldehyde that naturally occurs in aquatic products. In this study, formaldehyde was derivatized from 2,4-dinitrophenylhydrazine and analyzed using gas chromatography–mass spectrometry to investigate free and reversibly bound formaldehyde in 10 squid and squid products. The results were compared to those obtained by high-performance liquid chromatography (HPLC). The limit of detection was 2.0 mg/kg. The total concentrations of free and reversibly bound formaldehyde were, on average, higher than the free formaldehyde concentration by 26.6 mg/kg. Free formaldehyde made up, on average, 39% of total free and reversibly bound formaldehyde. The sum of the concentrations of free and reversibly bound formaldehyde was, on average, higher than the free formaldehyde concentration by 19.3 mg/kg in the HPLC method. Free formaldehyde made up an average of 39% of total free and reversibly bound formaldehyde in the HPLC method. The use of gas chromatography–mass spectrometry to detect formaldehyde in aquatic products allowed confirmation through retention time and molecular mass information. The monitoring of free formaldehyde in aquatic products and proper control of the manufacturing process could help to reduce the formaldehyde level in shredded squid products. Finally, exposure to formaldehyde from consumption of shredded squid was estimated: it was less than 0.2 mg/kg, which is the oral reference dose suggested by the US Environmental Protection Agency.

1. Introduction

Formaldehyde is classified as a carcinogen by the International Agency for Research on Cancer [1]. The oral reference dose (RfD) suggested by the US Environmental Protection Agency (EPA) is 0.2 mg/kg [2]. Formaldehyde occurs endogenously in many foods. According to a study by the World Health Organization, formaldehyde content ranges from 3.3 mg/kg to 60 mg/kg in fruits and vegetables, 8–20 mg/kg in meats, 1–3.3 mg/kg in milk and 1–98 mg/kg in fish [3]. In 2001, the Rapid Alert System for Food and Feed from the European Commission made an alert notification after finding that shiitake mushrooms from China contained 300 mg/kg of formaldehyde [4] and suggested the possibility that the formaldehyde had been added deliberately. After the incident, the French food safety agency Agence Française de Sécurité Sanitaire des Aliments required that the level of formaldehyde cannot exceed 63 mg/kg for fresh mushrooms [5]. In response to this alert, the China Quality Inspection Administration set a tentative maximum limit standard of 63 mg/kg in fresh

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1021-9498/$ – see front matter
http://dx.doi.org/10.1016/j.jfda.2013.05.010

mushrooms and 300 mg/kg in dry mushrooms [6]. However, the Center for Food Safety of Hong Kong revealed that 100–320 mg/kg of formaldehyde occurs naturally in dry mushrooms [7] and 6–54.5 mg/kg of formaldehyde occurs endogenously in fresh mushrooms. Mason et al from the Central Science Laboratory of the UK Government commissioned a survey of formaldehyde content in shiitake mushrooms using liquid chromatography–mass spectrometry (LC–MS) [8]. The concentrations of formaldehyde found were 100–320 mg/kg. Mason et al stated that under the harsh conditions of steam distillation with acid, the formaldehyde released was the sum of free and reversibly bound formaldehyde. Therefore, the measured formaldehyde concentrations were relatively high. They suggested that the method used for formaldehyde analysis should only determine free formaldehyde.

In addition to naturally occurring formaldehyde in foods, formaldehyde is added into some food products as a preservative [9]. European Commission Directive 95/2/EC allows formaldehyde in provolone cheese at a residual concentration of 25 mg/kg [10]. In 2006, the European Food Safety Authority concluded that formaldehyde is not carcinogenic by the oral route [11]. European Commission Directive 2009/10/EC allows a maximum level of formaldehyde residues of 50 mg/kg in gelling additives [12]. With the exception of its use as food additives, the therapeutic use of formalin in the aquaculture industry is approved in both the US and Canada [13]. However, Australia, Europe and Japan have not approved formaldehyde as an aquatic chemotherapeutant because of its association with oncogenesis. Although formaldehyde occurs endogenously in fish, Bianchi et al pointed out that in 1985, the Italian Ministry of Health proposed standard maximum limits of 60 mg/kg for Gadidae and 10 mg/kg for crustaceans [14].

As formaldehyde is sometimes used illegally as a food preservative in aquatic products, many countries have investigated the form and content of formaldehyde in seafood products for the sake of food safety regulations [14–19].

Nielsen and Jørgensen found that trimethylamine oxide aldolase could break down trimethylamine N-oxide (TMAO) into formaldehyde and dimethylamine even at freezing temperatures [20]. Formaldehyde can react with a number of amino acid residues in proteins, resulting in protein denaturation and cross-linking. Formaldehyde-deteriorated aquatic products are characterized as tough, hard, fibrous and dry.

In 1985, Tome et al studied the different types of bonding between formaldehyde and bovine serum albumin with Carbon-13 nuclear magnetic resonance [21] and found three types of bonding: reversible, acid-labile and acid-resistant bonds. Tome et al obtained the reversibly bound formaldehyde by dialysis. The reversible bond between formaldehyde and protein is in the form of reversible N-(hydroxymethyl) adducts. This N-hydroxymethylation is formed between the formaldehyde and the ε-amino group of lysine, α-amino group of N-terminal amino acids, the guanidyl group of arginine and the aromatic ring nitrogen of histidine and tryptophan. After bovine serum albumin dialysis treatment, Tome et al obtained the acid-labile fraction of formaldehyde by steam distillation in the presence of phosphoric acid. The acid-labile bond between formaldehyde and protein is made up of methylene bridges linking lysine to arginine, asparagine or glutamine. To obtain the acid-resistant formaldehyde, Tome et al added 6 M hydrochloric acid at 110 °C for 24 hours to the previously treated bovine serum albumin from which had been eliminated the free formaldehyde and acid-labile formaldehyde fractions. The acid-resistant bond between formaldehyde and protein is made up of methyl-lysine, formyl-lysine and a lysine–tyrosine methylene bridge.

Metz et al studied the bonding of formaldehyde with protein by LC–MS/MS [22]. The study found that arginine, tyrosine and lysine residues are very reactive with formaldehyde. The following types of bonding were formed between formaldehyde and protein: (1) methylol groups; (2) Schiff bases; (3) methylene bridges; and (4) imidazolidinone adducts. The formations of methylol and Schiff bases were reversible and hence could not be easily detected by LC–MS/MS. Furthermore, the Schiff base formed between formaldehyde and lysine residues could create stable cross-links with several amino acid residues, including arginine, asparagine, glutamine, histidine, tryptophan and tyrosine.

Rehbein studied the methods for the determination of free and bound formaldehyde in 22 fishery products [23]. He distinguished formaldehyde bonding types in fishery products as “free” formaldehyde, “bound” formaldehyde and “total” formaldehyde. Rehbein pointed out that due to the high reactivity of formaldehyde, the main difficulty in formaldehyde analysis is the release of formaldehyde from the sample matrix. Formaldehyde is able to react with proteins, nucleic acids and free amino acids, amines, creatine and nucleotides in fish tissues. Rehbein recommended the following sample preparation procedure for the determination of free and bound formaldehyde: (1) for measuring free formaldehyde, samples may be extracted using 6% perchloric acid at room temperature; (2) bound, acid-labile formaldehyde can be released by steam distillation using 1% sulfuric acid, giving a pH of about 1.

In a study by Rehbein et al [24], the free formaldehyde and free plus bound formaldehyde content of minced fish from cod, haddock and saithe were determined. In skinned fillet from cod, the free formaldehyde content was 22.8 mg/kg and the free plus bound formaldehyde content was 114.5 mg/kg. In skinned fillet from haddock, the free formaldehyde content was 7.6 mg/kg and the free plus bound formaldehyde content was 38.5 mg/kg. In skinned fillet from saithe, the free formaldehyde content was 6.5 mg/kg and the free plus bound formaldehyde content was 41.9 mg/kg. In each of the three minced fish samples, free plus bound formaldehyde content was considerably higher than free formaldehyde content.

Bechmann proposed that when reporting formaldehyde content in fish products, it is of utmost importance to describe exactly which analytical method was employed [25]. Bechmann also suggested that it is the free formaldehyde which is of toxicological interest that should be measured.

Previous sample preparation methods for the determination of formaldehyde in food required steam distillation of acidified food samples to release formaldehyde from the sample matrix. Then formaldehyde was derivatized with acetylacetone [15,18,19,26] or 4-amino-3-hydrazino-5-mercapto-1,2,4-triazol [16] and detected by UV spectrometry or high-performance liquid chromatography (HPLC). Under

these harsh conditions, the formaldehyde measured included both free and reversibly bound formaldehyde. This present study derivatized formaldehyde directly from 2,4-dinitrophenylhydrazine (DNPH), followed by gas chromatography–mass spectrometry (GC–MS) or HPLC detection. A comparison of free formaldehyde with free and reversibly bound formaldehyde was made for both detection methods. Formaldehyde exposure from consumption of squid products in the present study was also estimated, and ways by which formaldehyde generation can be reduced in the manufacturing process are discussed.

2. Methods

2.1. Reagents and chemicals

Formaldehyde (H_2CO, 36–38%) was bought from Union Chemical Works Ltd. (Hsinchu, Taiwan). Acetaldehyde, propionaldehyde and DNPH ($C_6h_6N_4O_4$) were purchased from Alfa Aesar (A Johnson Matthey Company, Ward Hill, MA, USA). Hydrochloric acid (HCl) was from Fluka (Sigma-Aldrich, St Louis, MO, USA), dichloromethane (CH_2Cl_2) was from TEDIA (Fairfield, OH, USA), and phosphoric acid (H_3PO_4, 85.6%) was from Sigma-Aldrich.

A stock solution containing 50 mg/L of formaldehyde, acetaldehyde and propionaldehyde was prepared. The stock solution was then diluted with deionized water to obtain standard solutions of 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20 and 50 mg/L.

2.2. Instrumentation

The HP-5MS capillary column (Agilent Technologies, Santa Clara, CA, USA) was employed for chromatographic separation. An HP-7890A gas chromatograph equipped with a MSD-5975C mass spectrometer (Agilent Technologies) was used for GC–MS analysis. The shaker was a SA31 from Yamato Scientific Co. (Tokyo, Japan). A CR21G III high-speed refrigerated centrifuge (Hitachi, Tokyo, Japan) was used. A DC400H ultrasonic bath was provided by Delta (Taipei, Taiwan). The HPLC system employed was a Shimadzu LC-20AT with a SPD-20A UV-VIS detector (Shimadzu, Kyoto, Japan). The column used for HPLC analysis was an Agilent XDB-C18 (4.6 × 150 mm, 5 μm).

2.3. Samples and sample preparation

Squid samples were obtained from the Taiwan Food and Drug Administration for border inspection of imported food, not from the local market.

For free formaldehyde analysis, 5 g of homogenized sample was added to a volumetric flask that was then filled with deionized water to the 50 mL mark. The flask was then capped and sonicated for 40 minutes at 20–25 °C. The sample was placed into a centrifuge tube and centrifuged at 7000 rpm. Then, 20 mL of supernatant and 2 mL of DNPH were mixed into an unused centrifuge tube and placed in the dark for 6 hours at 20–25 °C for the reaction to be completed. Next, 2 mL of CH_2Cl_2 was added to the sample solution and vortexed for 10 minutes; it was centrifuged at 7000 rpm for 5 minutes and the bottom CH_2Cl_2 layer was taken and filtered before GC–MS analysis.

For free plus bound formaldehyde analysis, 5 g of homogenized sample was added to 40 mL of deionized water and 1 mL of 20% phosphoric acid. The sample underwent steam distillation and 100 mL of distillate was collected. After steam distillation, 20 mL of distillate was taken and then the procedure for DNPH derivatization as described above for free formaldehyde analysis was followed. As DNPH is light-sensitive, all solutions and samples were protected from light.

The sample preparation procedure for HPLC was basically the same as that for GC–MS. After the derivatization products were extracted with dichloromethane, the solvent was evaporated to dryness and re-dissolved with 1 mL of acetonitrile. The sample was then ready for HPLC analysis.

2.4. GC–MS and HPLC analyses

For GC–MS analysis, helium at a flow rate of 1 mL/min was used as the carrier gas. To avoid overloading the column, a split ratio of 10:1 was employed for the present study. The injection volume was 0.6 μL. The injection port temperature was maintained at 250 °C. The GC oven temperature was raised from 180 °C to 240 °C at 10 °C/min and held there for 10 minutes. Electron ionization at 70 eV was used for generating an ion source. The total ion chromatography of a standard solution of 50 μg/mL from the derivatization reaction products of formaldehyde-DNPH, acetaldehyde-DNPH and propionaldehyde-DNPH are shown in Fig. 1. Five main chromatographic peaks corresponding to the derivatization reaction products were found at 5.02 minutes, 5.88 minutes, 6.05 minutes, 6.55 minutes and 6.84 minutes. The peak at 5.02 minutes belonged to the derivatized product HCHO-DNPH from the formaldehyde standard solution. The peaks at 5.88 minutes and 6.05 minutes belonged to the syn- and anti-isomers of acetaldehyde-DNPH from the acetaldehyde standard solution. As the anti-isomer was more stable and the predominant form of acetaldehyde-DNPH, the corresponding peak of the anti-isomer had a higher peak height and larger peak area compared to that of the syn-isomer. The peaks at 6.55 minutes and 6.84 minutes were the syn- and anti-isomers of propionaldehyde-DNPH from the propionaldehyde standard solution. The anti-isomer of propionaldehyde-DNPH also possessed a higher peak height and larger peak area compared to that of the syn-isomer. The mass spectrum of formaldehyde-DNPH is shown in Fig. 2

The mass spectrometer was operated in the selected ion monitoring mode to enhance selectivity and avoid matrix interference. The ions selected are listed in Table 1.

The quantitation ion chosen for formaldehyde-DNPH was of *m/z* 210. The traditional UV detection method could not distinguish among the interference from acetaldehyde, propionaldehyde and other similar compounds with carbonyl functional groups. The present GC–MS detection method offered confirmatory results with enhanced selectivity and sensitivity.

For HPLC analysis, the mobile phase was acetonitrile–water (50:50, v/v) with a flow rate of 0.9 mL/min. The column temperature was 40 °C. The sample injection volume was set at 20 μL and the wavelength of the UV detector was set at 365 nm.

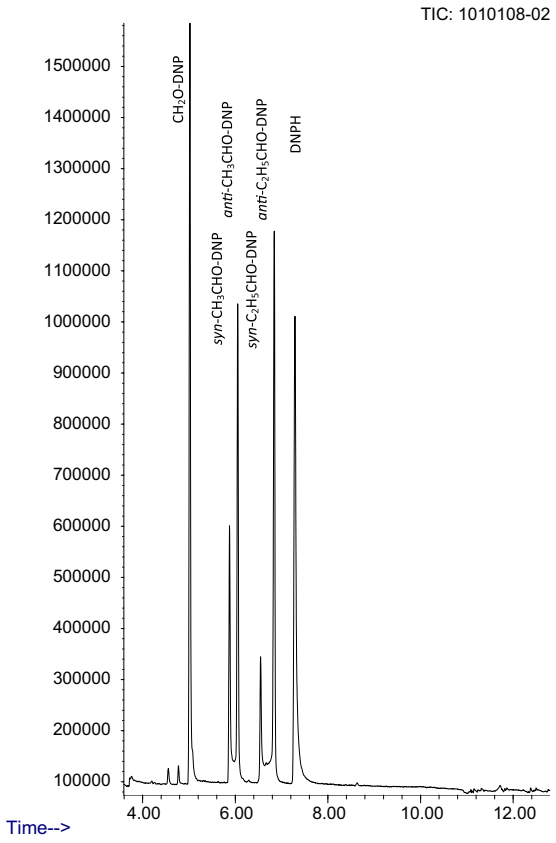

Fig. 1 – Total ion chromatogram of 50 μg/mL standard solution of formaldehyde-DNPH, acetaldehyde-DNPH and propionaldehyde-DNPH. DNPH = 2,4-dinitrophenylhydrazine.

Table 1 – Gas chromatography–mass spectrometry retention times and *m/z* parameters for selected ion monitoring (SIM) mode.

	Retention time (min)	SIM *m/z*	Quantification ion
Formaldehyde-DNPH	5.02	63, 79, 210	210
Acetaldehyde-DNPH	5.88 + 6.05	78, 79, 224	224
Propionaldehyde-DNPH	6.55 + 6.84	78, 79, 238	238

DNPH = 2,4-dinitrophenylhydrazine.

3. Results and discussion

3.1. Method performance and validation

3.1.1. Method linearity and limit of detection

The linear range, calibration curve, correlation coefficient and limit of detection for the present method are listed in Table 2. As the peak area integration for the standard solution from 0.2 mg/kg to 1 mg/kg did not have good linearity, these three concentrations were not adopted in the calibration curve. The limit of detection for the present method was 2 mg/kg, which was lower than the 10 mg/kg limit of detection with the previous acetylacetone UV detection method without separation by HPLC [15].

3.1.2. Method precision and accuracy

The recovery rate for free formaldehyde was obtained by spiking formaldehyde into 5 g of sample matrix containing known formaldehyde concentrations in triplicate for 3 days. The same sample preparation procedure for free formaldehyde was followed and detected by GC–MS. The recovery rate and method precision for free formaldehyde are listed in Table 3. The mean recovery rates for 9.2 mg/kg and 22.2 mg/kg of spiked formaldehyde in 3 days were 108.88% and 103.61%, respectively.

The method precision was determined by spiking two formaldehyde standard solutions of 9.2 mg/kg and 22.2 mg/kg in triplicate for 3 days. The results of the precision study revealed that the intra-day repeatabilities for the two formaldehyde concentrations were 1.61% and 1.16%, respectively. The between-day precision values for the two formaldehyde concentrations were 8.75% and 5.60%, respectively. The results indicated that the precision and accuracy of the present method are adequate for the determination of formaldehyde in squid and squid products.

The recovery rate was also studied for free and reversibly bound formaldehyde by spiking formaldehyde into a sample of known formaldehyde concentration. By spiking 22.2 mg/kg of formaldehyde, the recovery rate was 0% for 3 days. Therefore, 60 mg/kg and 120 mg/kg were used for the recovery study. The recovery rates were 51.3% and 30%, respectively, which were quite poor compared to the recovery rate of free formaldehyde. The between-day precision values for the two formaldehyde concentrations were 26.97% and 60.97%,

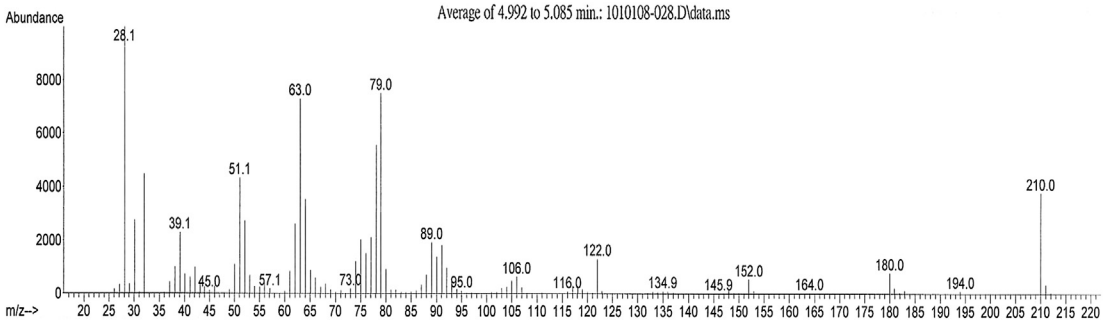

Fig. 2 – Mass spectrum of formaldehyde-DNPH. DNPH = 2,4-dinitrophenylhydrazine.

Table 2 – Gas chromatography–mass spectrometry method linearity and limit of detection (LOD).

Retention time (min)	Linear range (mg/kg)	Calibration curve	Correlation coefficient	LOD (mg/kg)
5.02	2–50	y = 25937.558x – 20543.87	0.9996	2

respectively. Owing to the poor recovery, no attempt was made to check the within-day precision by spiking in triplicate.

3.2. *Comparison between direct derivatization and derivatization after steam distillation*

The results of the determination of free formaldehyde and free plus reversibly bound formaldehyde by GC–MS are shown in Table 4. The sum of the concentrations of free and reversibly bound formaldehyde was, on average, higher than the free formaldehyde concentration by 26.6 mg/kg in the GC–MS method. Free formaldehyde made up an average of 39% of the total free and reversibly bound formaldehyde in the GC–MS method. The ratio of free formaldehyde to free and reversibly bound formaldehyde ranged from 13% to 69%.

The results of the determination of free formaldehyde and free plus reversibly bound formaldehyde by HPLC are shown in Table 5. The sum of the concentrations of free and reversibly bound formaldehyde was, on average, higher than the free formaldehyde concentration by 19.3 mg/kg in the HPLC method. Free formaldehyde made up an average of 39% of the total free and reversibly bound formaldehyde in the HPLC method. The ratio of free formaldehyde to free plus reversibly bound formaldehyde ranged from 10% to 82%.

The results of the HPLC and GC–MS methods were comparable. The HPLC method could be nonspecific and subjected more to matrix interference. GC–MS could provide positive confirmation from the additional information provided by mass fragmentation.

There have been several studies on the effect of the distillation procedure in increasing the formaldehyde concentrations detected in different food samples. Kaminski et al [27] found that steam distillation of milk increased the measured level of formaldehyde compared to the non-distillation method. Lagace et al [28] revealed that heating greatly increased the amount of formaldehyde detected in maple syrup. Claeys et al [29] from the Federal Agency for the Safety of the Food Chain of Belgium detected free formaldehyde in mushrooms by direct derivatization without steam distillation and found only 0.08–0.65 mg/kg of formaldehyde, which differs significantly from the 100–320 mg/kg reported by Mason et al [8]. The method employed by Mason et al determined the sum of free formaldehyde and reversibly bound formaldehyde, so their reported value is much greater than the free formaldehyde value.

Similar to the problem posed by formaldehyde analysis in other food samples, the distillation process also produces a greater amount of formaldehyde in squid products, caused by TMAO decomposition in squid products. Lin and Hurng [30] showed that squid may contain up to 2558–8064 mg/kg of TMAO, which would break down into formaldehyde, dimethylamine and trimethylamine during heating. TMAO decomposes into formaldehyde and dimethylamine through two pathways, i.e., via enzymatic catalysis by trimethylamine oxide aldolase and via non-enzymatic break down during processing. The work done by Kolodziejska et al [31] revealed that heating would increase the dimethylamine and formaldehyde content in squid, but result in only negligible changes in cod. Zhu et al

Table 3 – Gas chromatography–mass spectrometry method recovery rate and precision (n = 3).

Spiked (mg/kg)	Detected (mg/kg)	Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)
9.2	10.02	108.88	1.61	8.75
22.2	23.00	103.61	1.16	5.60

RSD = relative standard deviation.

Table 4 – Free formaldehyde and free plus reversibly bound formaldehyde determined by gas chromatography–mass spectrometry.

Sample	Free FA (mg/kg)	Free + RB FA (mg/kg)	Method difference (mg/kg)	Free FA/(Free + RB FA) (%)
Shredded squid 1	22.3	60.2	37.9	37
Shredded squid 2	19.0	46.2	27.2	41
Shredded squid 3	24.7	76.8	52.1	32
Shredded squid 4	7.5	23.7	16.2	32
Shredded squid 5	48.5	70.7	22.2	69
Shredded squid 6	4.1	32.2	28.1	13
Shredded squid 7	14.2	56.8	42.6	25
Shredded squid 8	8.7	29.4	20.7	30
Shredded squid 9	12.1	24.0	11.9	50
Frozen squid 10	10.4	17.1	6.7	61
Mean			26.6	39

FA = formaldehyde; RB = reversibly bound.

Table 5 – Free formaldehyde and free plus reversibly bound formaldehyde determined by high-performance liquid chromatography.

Sample	Free FA (mg/kg)	Free + RB FA (mg/kg)	Method difference (mg/kg)	Free FA/(Free + RB FA) (%)
Shredded squid 1	17.4	45.5	28.1	38
Shredded squid 2	13.2	31.9	18.7	41
Shredded squid 3	20.1	49.0	28.9	41
Shredded squid 4	2.4	16.2	13.8	15
Shredded squid 5	45.6	55.4	9.8	82
Shredded squid 6	2.6	26.3	23.7	10
Shredded squid 7	13.5	51.5	38	26
Shredded squid 8	7.8	20.6	12.8	38
Shredded squid 9	9.9	20.8	10.9	48
Frozen squid 10	9.0	17.6	8.6	51
Mean			19.3	39

FA = formaldehyde; RB = reversibly bound.

[32] demonstrated that the non-enzymatic decomposition of TMAO during thermal processing of squid is the key pathway for formaldehyde generation. As there would still be TMAO remaining after the squid product manufacturing process, the formaldehyde level in shredded squid would increase after the distillation step in sample pretreatment.

Although the purpose of acid-aided steam distillation sample pretreatment is to eliminate matrix interference, its side effect is the greatly increased formaldehyde content. Other drawbacks include the large amount of solvent used, as well as poor repeatability and recovery of steam distillation. More important than these abovementioned drawbacks, the difference between free formaldehyde and free plus reversibly bound formaldehyde is significant and causes great consumer concern and confusion with regard to food safety regulations. When reporting formaldehyde content in food, it is important to distinguish between free formaldehyde and free plus reversibly bound formaldehyde.

3.3. *Comparison with results from previous studies*

A comparison of the formaldehyde content in squid and squid products with results from previous related studies are shown in Table 6 [14,16,19,33–36]. Most fresh squid contained less than 20 mg/kg of free and reversibly bound formaldehyde, which should be the endogenous content. It has been reported that the concentration of formaldehyde deliberately added to squid could be as high as 4250 mg/kg [16]. The free formaldehyde of 10.4 mg/kg found in squid in the present study is comparable to the free formaldehyde content of 2.91–3.27 mg/kg in cuttlefish as measured by Bianchi et al [14].

As the process of making shredded squid requires heating and drying, processed squid products like shredded squid usually contain more formaldehyde than raw squid [15,16,34,36]. According to Li et al's study [34], mean formaldehyde concentration in raw *Dosidicus gigas* was 17.3 mg/kg, but that in the shredded squid product made from *Dosidicus gigas* was 35.3 mg/kg. The mean formaldehyde concentration of raw Japanese ocean squid was 10.7 mg/kg, but that in the shredded squid product made from Japanese ocean squid was 34.4 mg/kg. Shentu et al [36] showed that different drying temperatures greatly influence the formaldehyde content in shredded squid. Drying by heating at 50 °C increases the formaldehyde content in squid from 3.25–4.78 mg/kg to 14.1–25.8 mg/kg, while a heating temperature of 80 °C markedly increases the formaldehyde content to 266.0–377.3 mg/kg. In the present study, the free formaldehyde concentration in fresh squid was 10.4 mg/kg while that in shredded squid was 48.5 mg/kg.

Table 6 – Comparison of formaldehyde content in squid and squid products among different studies.

Study	Product	Formaldehyde content (mg/kg)
Bianchi et al, 2007 [14]	Cuttlefish[a] ($n = 2$)	2.91–3.27 (mean, 4.4)
Lee et al, 1989 [16]	Squid[b] ($n = 11$)	2.4–6.4
	Shredded squid[b] ($n = 39$)	43.4–169.6 (mean, 96.7)
Kim et al, 2011 [19]	Squid[b] ($n = 51$)	0.71–12.38 (mean, 4.4)
Teerasong et al, 2010 [33]	Rehydrated squid[b] ($n = 12$)	0.26–12.37
Li et al, 2007 [34]	Squid[b] ($n = 4$)	10.7–19.7 (mean, 17.4)
	Shredded squid[b] ($n = 4$)	0–35.3 (mean, 17.4)
Chen et al, 2009 [35]	Squid[b] ($n = 3$)	15.1
Shentu et al, 2006 [36]	Squid[b]	3.25–4.78
	Shredded squid[b]	27.84–154.37
This study	Squid[a] ($n = 1$)	10.4
	Squid[b] ($n = 1$)	17.1
	Shredded squid[a] ($n = 9$)	4.1–48.5 (mean, 17.9)
	Shredded squid[b] ($n = 9$)	23.7–76.8 (mean, 46.7)

a Free formaldehyde.
b free plus reversibly bound formaldehyde.

Table 7 – Formaldehyde exposure from consuming shredded squid containing 48.5 mg/kg of free formaldehyde.

Age (y)	Male			Female		
	19–30	31–64	65	19–30	31–64	65
Aquatic product consumption (g)	12.53	14.84	13.09	7.98	12.32	9.03
Body weight (kg)	64	64	60	52	54	52
Formaldehyde intake (mg/kg/d)	0.009	0.011	0.011	0.007	0.011	0.008

3.4. *Formaldehyde exposure from consumption of squid products*

To provide different scenarios of formaldehyde exposure from squid, the aquatic product consumption data from a Nutrition and Health Survey in Taiwan were adopted [37]. Body weight data were taken from the Dietary Reference Intake Tables of Taiwan [38]. Assuming that all of the aquatic product consumption is solely from squid and squid products and that the highest level of free formaldehyde concentration is 48.5 mg/kg, the calculated data for formaldehyde exposure from squid products are shown in Table 7. The calculated highest formaldehyde exposure is 0.011 mg/kg/d, which is less than the 0.2 mg/kg/d RfD suggested by the US EPA.

If all of the protein consumption is from shredded squid, the calculated exposure levels are shown in Table 8. The calculated highest formaldehyde exposure is 0.074 mg/kg/d,

Table 8 – Formaldehyde exposure assuming that all protein ingestion is from shredded squid containing 48.5 mg/kg of free formaldehyde.

Age (y)	Male			Female		
	19–30	31–64	65	19–30	31–64	65
Protein consumption (g)	94.5	98.1	72.1	76.7	71.4	55.4
Body weight (kg)	64	64	60	52	54	52
Formaldehyde intake (mg/kg/d)	0.072	0.074	0.058	0.072	0.064	0.052

which is also less than the 0.2 mg/kg/d RfD suggested by the US EPA. Therefore, there appears to be no risk associated with the consumption of shredded squid with 48.5 mg/kg of free formaldehyde.

4. Conclusion

In this present study, free formaldehyde in squid and squid products was successfully detected with GC–MS. Steam distillation with acid and derivatization releases both free formaldehyde and reversibly bound formaldehyde, resulting in a high reported formaldehyde concentration. Formaldehyde exposure from the consumption of squid and squid products in the present study was found to be less than the 0.2 mg/kg/d RfD suggested by the US EPA. Free formaldehyde content should be employed in food safety monitoring instead of the sum of the concentrations of free and reversibly bound formaldehyde. Confirmatory determination of harmful free formaldehyde with GC–MS could assist in food safety regulations and alleviate consumer concerns about formaldehyde levels reported in food.

Naturally occurring free formaldehyde in different foods should be investigated. With the knowledge that there is endogenous formaldehyde present in foods, proper regulations should be put in place against the use of formaldehyde in food preservation. The monitoring of deliberately added formaldehyde by using the proper analytical method that only detects free formaldehyde is very important. During the manufacturing of squid products, proper attention should be given to reduce the formaldehyde content in the production process.

Acknowledgments

This project received financial support from the Department of Health of Taiwan (100TFDA-FS-20). The views expressed herein are solely those of the authors.

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