A Rapid Determination of Free Formaldehyde Content in Marine Products

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Abstract: Objective for rapid detecting free formaldehyde (FA) in marine products, a method with high sensitivity and little interference is described. Background value of FA in marine products would supplement the scientific evaluation index system. Free FA is derivatised with 2,4-dinitrophenylhydrazine (DNPH) to form a chromophore for high-performance liquid chromatography (HPLC) detection. The formation of the DNPH FA derivative is shortened to 30 min. It shows good linear correlation between the peak areas and FA concentrations with a dynamic linear range of 0.3-25.0 mg/L, the limit of detection (LOD) is 0.2 mg/Land the limit of quantitation (LOQ) is 0.5 mg/L. The recovery range of free FA in spiked squid was 70%-78% with relative standard deviation (RSD) of 5%-10% (n=5). FA content is detected in 14 species of seafood comparing to the past analysis method, results show no significant difference, FA content of tuna, tuna, cod, Surf Calm and cuttlefish is more than 40 mg/kg. The average of FA content in 28 species of packaging squid products is 14.7 mg/kg, ranging from 2.10-61.8 mg/kg. This method is simpler and easier to operate; it reduces the concentration of derivatives, shortens the reaction time, and is applicable to the determination of formaldehyde content in all kinds of seafood.

1 INTRODUCTION

FA is a highly active gas with low molecular weight at room temperature. It is sold in the form of formalin (containing 6-13 percent of FA), used as preservative, insecticide and acaricide in aquatic products. As a toxic substance (Liteplo et al., 2003; Zhang et al., 2018), FA is easy to react with nucleophilic material, causing DNA damage (IARC, 2004). Thrasher & Kilburn believes that FA could lead to fetal toxicity and aberration (Thrasher and Kilburn, 2001). FA ranked second on the priority control list of toxic chemicals in China (Tang et al., 2009). In 2004, FA was categorized in Group I as 'carcinogenic to humans' by the International Agency for Research on Cancer (IARC) (Noda et al., 2011) , The United States Environmental Protection Agency recommended daily intake of FA as no more than 0.2mg/kg of the body weight while WHO set it as 0.15mg/kg of the body weight. The American Cancer Society considers that FA in the air, food and water is a carcinogen. However, authorities of European Food Safety believe that oral FA is not carcinogenicand the oral reference dose is 0.2mg /kg (EFSA, 2006). In 1985, Italian health departments set limit of FA in cod and shellfish aquatic products respectively 60 mg/kg and 10 mg/kg (MINSAN-telegram, 1985). Chinese Ministry of Agriculture set it to "no detectable" in aquatic products in 2001, and 10 mg/kg in 2002, at present, two standards have been abolished, and no uniform standard of FA is put forward.

Detection methods of FA are spectrophotometry (Yasri et al., 2015; Chen et al., 2018), HPLC (Lv et al., 2010; Zhang et al., 2018) and gas chromatography (GC) (Ma et al., 2015; Shao et al., 2015). In this study, HPLC method was used because of its convenient operation, high accuracy and high sensitivity. The concentration, time and temperature of the derivative reagent were optimized, and the chromatographic conditions were optimized. At the same time, FA content in seafood was determined by the improved method.

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2 EXPERIMENTAL SECTION

2.1 Chemicals and Equipment

Marine products: fresh squid was bought directly from the returning ship, other seafoods were bought from aquatic product market in Shanghai, and seafoods were stored at -20 º C back to the lab. Squid products were purchased from supermarkets.
Agilent Technologies 1100 HPLC (the USA) consisted of a pump, a VU detect, a column chamber, and an Agilent ChemStation for LC system; Millipore water purification system (Millipore, the USA).

Acetonitrile was chromatographic pure (Baker, the USA), DNPH and the rest of the reagents were analytically pure; FA standard: 10 mg/mL, 2 mL (Aladdin, Shanghai).

In addition, FA in aqueous solution could form a stable hydrate with the formula H2C(OH)2: the hydrate exists in equilibrium with various oligomers. FA further forms an insoluble white trimer and further polymerises to solid paraformaldehyde in aqueous solutions. Sometimes even unopened bottles of formalin had insoluble white precipitate. Therefor we chose clear FA solution as the standard solution.

2.2 Experimentation

2.2.1 Preparation of Standard Solution and Derivative Solution

FA standard solution (200 μg/mL): dissolved 2 mL of FA standard and constant volume to 100 mL with water, and the standard intermediate liquid could be used for six months saved at 4 ºC.

The derivative solution: took 500 mg weight of DNPH into 1 L acetonitrile, we got derivative liquid; then took 5.28 g weight of sodium acetate into 2 mL glacial acetic acid, and constant volume to 1 L with water, we got buffer solution; 10 mL of each solution was mixed to get the derivative solution.

2.2.2 Sample Derivatization and Extraction

For determining FA content, mixed (2+0.02) g homogenized sample and 20.0 mL derivative liquid in 50 mL polypropylene centrifuge tube, tighten the plug, and then blent through vortex device, then put in Water-bathing Constant Temperature Vibrator at 60 ºC, 150r/min for 30min. The mixture was filtered through a 0.45um HV filter before injection. For each sample five replicates were analyzed. Results were expressed as mg of FA /kg.

2.2.3 Chromatographic Condition

The HPLC column was a Hypersil ODS-C18, 4.6 mm ×250 mm, 5 μm. The sample vol ume was set at 20 μl, the absorb wavelength of detector was set at 365 nm, the column temperature was set at 40 ºC. The mobile phase was methanol-water (70:30, v/v) with a flow rate of 0.9 mL/min. The peak area was used for quantitative calculation of formaldehyde.

2.2.4 Calibration Curve

Respectively took 0.015, 0.025, 0.05, 0.25, 0.5, 1.0 mL FA standard solution (200 μg/mL) into 10 mL volumetric flask, added buffer solution to 5 mL, and derivative liquid to 10.0 mL, hence FA standard solution was respectively diluted into 0.3, 0.5, 1.0, 5.0, 10.0, 50.0 μg/mL as FA work solution. The FA work solution was derivatised and extracted according to described procedures. Three injections of each standard solution were made and the peak area was the corresponding FA content to obtain the calibration curve.

2.2.5 Data Processing

The data were statistically analyzed by Microsoft Excel and the anova was analyzed by SPSS.

3 RESULTS AND DISCUSSION

3.1 Liquid Chromatographic Analysis

The Figure 1 showed that calibration curve in the 0.3-25 μg/mL range was obtained and correlation coefficient was 1. Figure 2 showed chromatogram of 5mg/kg of FA in squid sample by HPLC. The peak in 3.597min was residual DNPH, the other peak in 5.413min was considered to be a derivative of HCHO-DNPH in squid. Table 1 showed that the average recoveries of this method were in the range of 70-78%, RSD was 5.3-10%.

Table 1: Recovery and precision data of FA (n=5).

Added[mg/kg]	Recovery[%]	RSD[%]
5	70	10
20	75	8.1
100	78	5.3

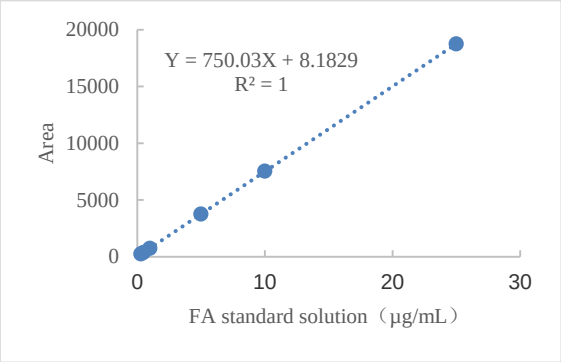

Figure 1: The Calibration Curve.

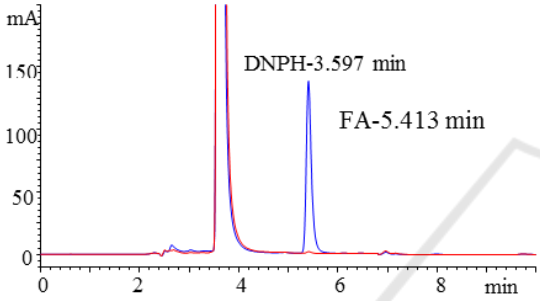

Figure 2: Chromatograms of the determination.

3.2 Discussion

3.2.1 The Concentration and States of FA in Marine Products

Trimethylamine oxide (TMAO) widely exists in food, TMAO could resolve into DMA, TMA and FA under enzymolysis condition of trimethylaminek-N-oxide (TMAOase). Besides the enzymatic pathway, FA is steadily accumulated during the thermal processing (Chen et al., 2017; Huang et al., 2017).

FA could react with protein, amino acid and creatinine, which makes free and bound forms of FA in organisms. “Total” formaldehyde is the sum of these two forms. Bound FA could be extracted through steam distillation under the sulfuric acid or phosphoric acid solution (1% -40%). Therefore, it is essential to specify whether free or bound formaldehyde is being determined when reporting FA content in tissue. Yeh et al. studied 10 different kinds of marine products, they found that total content of FA was 20mg/kg more than free FA, the proportion of free content of FA ranged 39 percent among total FA (Yeh et al., 2013). Rehbein et al. found that free FA was 22.8 mg/kg ranged 19.9% among total FA in cod, free FA was 7.6 mg/kg ranged 19.7% among total FA in Haddock, free FA was 6.5 mg/kg ranged 15.5% among total FA in Pollack (Rehbein and Schmidt, 1996). Literatures state that free FA is that which is of toxicological interest and that it should be measured (Bechmann, 1998). Low recovery is the disadvantage of detecting free FA, so the authors used a “recovery factor” (Treezl et al., 1997).

3.2.2 Detection Methods of Free FA

Detection methods of free FA include spectrophotometry, chromatography, fluorescence method, colorimetry and electrochemical method. Generally spectrophotometry and chromatography are used more, Table 2 Showed the comparation of different methods of detecting free FA using DNPH. The method in this paper was to react at room temperature with simple operation, the results showed high accuracy and sensitivity.

Table 2: Comparation of different methods.

References	Linearity range [mg/L]	LOD [mg/L]	LOQ [mg/L]	Derivative time	Derivative temperature	Recovery [%]
Zhang et al., 2018	0.5-50	0.3	0.5	60 min	60℃	63-74
Bechmann, 1998	-	0.00892	0.0268	Distillation	100 ℃	83-103
Treezl et al., 1997	0.05-2	0.005	0.05	15 min	100℃	97.5-106
Oliva-Teles et al., 2002	1-10l	0.319	0.957	30 min	Room temperature	>95
This paper	0.3-25	0.2	0.5	30min	Room temperature	70-78

3.2.3 FA Content in Seafood Detecting by Above Method

Table 3 showed comparison of free FA content in seafood by two methods. The method in this paper showed lower concentration of derivative and less detecting time, the reaction was under room temperature, which made it easier to operate. Anova ($P=0.923>0.05$) was analyzed by SPSS, it showed no difference between the two methods. The FA content of 14 kinds of sea products was detected, the results showed that FA content of various seafood was different. The FA content of tuna, cod, Surf Calm and cuttlefish was higher all above 40 mg/kg. Meanwhile this paper studied free FA in 28 types of packaged squid products, results showed that the average FA content was 14.7 mg/kg, ranging from 2.10-61.8 mg/kg.

Table 3: The comparison of free FA content in seafood by two methods.

FA content [mg/kg]	Suggested method	literature method
Penaeus vannamei Boone	10.8	11.4
Salmon	25.7	29.6
Tuna	51.5	56.3
Cod	55.7	53.8
Surf Calm	71.4	68.2
Cuttlefish	41.8	44.2
Octopus	33.7	37.2
Peru Squid	7.63	7.50
Todarodes Pacificus	27.2	26.5
Uroteuthis edulis	Not detected	Not detected
Loligo Chinensis	Not detected	Not detected
Loligo Duvaucelii	6.10	6.33

4 CONCLUSIONS

Based on the previous research, this paper improved the determination of free FA in aquatic products by HPLC. The free FA in Marine products was fully reacted with the derivative reagent at room temperature for 30 min, showing a good linear relationship, the reactant was stable for 24 h, and the LOQ was 0.5 mg/L. This method showed no significant difference comparing with the old method, while this method was simpler and easier to operate and was suitable for the determination of free FA content in all kinds of Marine products.

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