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Seasonal variation of background formaldehyde content in large squid from scientific research research vessel

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Abstract The background formaldehyde (FA) in large squid is determined through high performance liquid chromatography (HPLC). Its seasonal variation was further investigated. In autumn and summer, background FA content in Todarodes pacificus was higher, with the mean value up to 36.8 mg/kg in September. This study provide scientific data for the monitoring management of FA during the circulation and sale of squid.

1. Introduction

Formaldehyde (FA) is commonly using for keeping fresh, insecticide and sterilization. Beside the artificially added FA, background FA widely exists in various kinds of food such as meat, fruits, vegetables and aquatic products [1-5]. Background FA shows bound states and free states. Therefore, the determined content of FA depends on the methods applied, where different amount of bound state FA released from the matrix. However, some researchers believe that the detection of free FA can be enough due to its physiological toxicity [6-8]. Yeh. compared 10 kinds of squid and its processed products, and found that free FA was about 20 mg/kg, accounting for 39% of the total FA [9]. Rehbein found that the free FA in cod was 22.8 mg/kg, which accounts for 19.9%; Haddock was 7.6mg/kg, accounting for 19.7% [10]. The disadvantage of determining free FA is that its recovery rate is relatively low, because of the transform from free state to bound state. However, the recovery value is stable and could be corrected by the recovery factor.

The detection principle of this method is that, background FA is extracted by water under room temperature, then free FA is derivatised with 2,4-dinitrophenylhydrazine (DNPH) to form a chromophore for HPLC detection. In addition, background FA in far out fishing of Todarodes pacificus and Loligo beka is investigated. It will provide a theoretical basis for controlling the FA content of squid in storage, which is also very important for solving the problem of excessive FA in aquatic products, especially squid.

2. Experimental section

2.1Chemicals and Equipment

Squid: Far out fishing of Pacific frill and musket squid was bought directly from the returning ship, and stored at -20 °C back to the lab. The fishing time and location were conformed, and no artificial addition FA.

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI. Published under licence by IOP Publishing Ltd 1 Agilent Technologies 1100 HPLC (the USA) is consisted of a pump, a UV detector, a column chamber, which is controlled through an Agilent ChemStation software; Water was provided through Millipore water purification system (Millipore, the USA). Acetonitrile of chromatographic grade (Baker, the USA) was used. DNPH and other reagents were of analytical grade; FA standard solution: 10 mg/mL was obtained from Aladdin Reagent (Shanghai) Co., Ltd..

2.2*Experimentation*

2.2.1 Preparation of Standard Solution and Derivative Solution.

FA standard solution (200 μ g/mL): 2 mL of FA standard solution was diluted to 100 mL with water and stored at 4 °C, which is used for at least six months.

The derivative solution: 500 mg of DNPH was dissolved in 1 L acetonitrile as derivative reagent; and then 5.28 g sodium acetate was mixed with 2 mL glacial acetic acid and further diluted to 1 L with water as buffer solution; Finally, 10 mL of each solution was mixed as derivative solution.

2.2.2 Sample Derivatization and Extraction.

For the determination of FA, homogenized sample (10 ± 0.01) g and 20.0 mL water in 50 mL centrifuge tube were mixed and vortexed. And then, sample was treat with ultrasonic for 5min and centrifugation at 10000r/min for 10min. 2ml of the extract was filtered through 0.22 µm aqueous phase film, and 1ml filtrate was completely mixed with 2.5ml acetonitrile and 0.4ml derivative reagent. The reaction was carried out after stirring for 30s. Then 0.4ml phosphoric acid buffer (pH6.8) and 0.7ml sodium hydroxide solution (1N) were added, and reacted for 2min. The mixture was filtered through a 0.45 µm HV filter before detection with HPLC. For each sample, five replicates were analyzed.

2.2.3 Data Processing.

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

3. Results and discussion

3.1Seasonal variation of background FA content in fresh squid

Every month, at least 16 Todarodes pacificus were taken from scientific research vessel. Since the fishing time was closed from May to July every year in China coast, samples of these three months were obtained from ocean-going ships, and it usually took about 35 days from capture to detection. Therefore, the background FA in May, June and July was after 35 days of refrigeration. Seasonal change of background FA content in Todarodes pacificus was shown in Table 1, background FA content was of the range 1.51- 36.8 mg/kg in the whole year, with a mean of 17.0 mg/kg. The FA content from the April to October (13.8-36.8 mg/kg) was significantly higher than that in other months. FA content in the September (36.8 mg/kg) was the highest. On spring and winter, the average content of FA (1.51- 5.84 mg/kg) was lower. In the November, FA content (1.51 mg/kg) was the lowest, where 3 out 16 fresh squid samples were detected negative. In the December, the FA content of 13 samples was near the LOD, only 2 squid samples were higher than the LOQ. In conclusion, in the months of hot weather, background FA content in Todarodes pacificus was higher, while in the cooler months, background FA content was lower (Figure 1).

 Table 1. Seasonal change table of background FA content in Todalodes pacificus.			
Month	Rang of FA conten (mg/kg)	Mean \pm standard deviation (mg/kg)	
 1	2.98-6.01	3.65±0.904	
2	2.88-6.21	4.33±1.06	
3	3.19-9.50	$5.84{\pm}1.61$	
4	5.09-33.1	13.8±7.07	
5	3.76-47.2	19.5±12.1	

Table 1. Seasonal change table of background FA content in Todarodes pacificus.

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6	10.9-41.3	23.9±7.87	
7	14.9-47.6	33.8±10.9	
8	12.2-47.4	32.7±10.9	
9	18.4-46.7	36.8±9.74	
10	12.6-47.0	25.2±10.7	
11	Non detection-2.80	1.51 ± 0.60	
12	1.50-13.8	2.53 ± 2.98	



4. Conclusions

This paper studies the seasonal change of background FA in squid by HPLC, and squids were bought from the outgoing ship in order to obtain samples without artificial addition of FA. Results showed that in autumn and summer, background FA content in Todarodes pacificus was higher, with the mean up to 36.8 mg/kg, while in spring and winter, it was lower. This study would provide scientific data for the monitoring of FA content in the process of circulation and sale of squid.

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