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Determination of Biogenic Amines in Squid and White Prawn by High-Performance Liquid Chromatography with Postcolumn Derivatization

QING-XI ZHAO, JIE XU, CHANG-HU XUE,* WEN-JING SHENG, RUI-CHANG GAO, YONG XUE, AND ZHAO-JIE LI

Department of Food Science and Technology, Ocean University of China, Qingdao, Shandong Province, 266003, People's Republic of China

A simple method was developed for the determination of biogenic amines in aquatic food products using a reverse-phase high-performance liquid chromatography with postcolumn automatic *o*-phthalaldehyde derivatization and fluorescence detection. The linearity, repeatability, and recovery of the method for seven amines (tyramine, putrescine, cadaverine, histamine, agmatine, spermidine, and spermine) were evaluated. This optimized method was applied to detect biogenic amines in squid and white prawn during refrigerated storage. Sensory analysis, pH value, and total volatile base nitrogen value were combined to evaluate the freshness quality of these two raw materials. Agmatine and cadaverine in squid, cadaverine, and putrescine in white prawn were the most obviously changed amines during the storage at two different temperatures, and these biogenic amines could be the effective quality indicators for the freshness of the raw aquatic materials.

KEYWORDS: Biogenic amine; HPLC; postcolumn derivatization; squid; white prawn

INTRODUCTION

Biogenic amines (BAs) are low molecular weight organic compounds that can be found in various foods and beverages, such as wine, beer, fish, and meat, normally as a consequence of microbial activity (enzymatic decarboxylation of free amino acids) (*1*). Several countries have established regulations for BAs intake from various kinds of foods because amines, especially tyramine (Tyr) and histamine (His), can be toxic when their levels are high. Polyamines, such as putrescine (Put), cadaverine (Cad), spermidine (Spd), and spermine (Spe), although not having a direct toxic effect, can enhance the toxic effects of Tyr and His by competing for the detoxifying enzymes in humans (*2*). Nowadays, the regulatory limits are focused on the content of His for 50 mg/kg (Food and Drug Administration, United States), while other BAs have no accordant limits. Some BAs can appear during food fermentation processes or food storage under certain conditions if amino acid decarboxylase positive microorganisms are present. These compounds are the chemical indicators of spoilage of fish, as well as those of maturation of wines and cheeses, and can be formed in relatively high concentrations due to prolonged storage time (**Figure 1**). The high content of proteins in meat results in an increased probability of fast decomposition processes (*3*). So BAs, irrespective of their important health significance, can serve as alternative food quality markers, especially their changes in nonfermented foods such as meat and fish during storage.

* To whom correspondence should be addressed. Tel: 086-532-82032468. Fax: 086-532-82032468. E-mail: xuech@ouc.edu.cn.

A number of methods to analyze BAs in food so far have been employed. These methods include thin-layer chromatography (*4*), gas chromatography (*5*), ion chromatography (*6*), capillary electrophoresis (CE) (*7*), liquid chromatography (LC) (*8*, *9*), and some other methods (*10*, *11*). These methods have been compared by taking the factors of sensitivity, linearity, rapidity, repeatability, efficiency, and operational requirements into consideration (*12*, *13*). High-performance liquid chromatography (HPLC) is the most extensively used technique in determining BAs in different kinds of food due to its selectivity, sensitivity, great versatility, and simple sample treatment (*14*). Chemical derivatization is usually applied for their analysis by HPLC to increase the selectivity and sensitivity of detection because the majority of BAs does not possess chromophoric or fluorophoric traits. A pre- or postcolumn derivatization step to facilitate detection is used for the sample preparation. In general, *o*-phthalaldehyde (OPA) and dansyl chloride are the two most commonly used derivatization reagents; the advantages and disadvantages of using these derivatization reagents have been compared (*15*, *16*). With the development of automatic derivatization equipment, the time-consuming sample preparation of precolumn derivatization procedures will be replaced by the rapid, simple postcolumn derivatization method. In recent years, several methods have been employed for the determination of BAs in aquatic food products (*10*, *11*, *13*). However, these methods were time-consuming, and the results were not satisfactory. Therefore, a versatile methodology needs to be developed for the determination of BAs that can be used as a quality measure in the food industry.

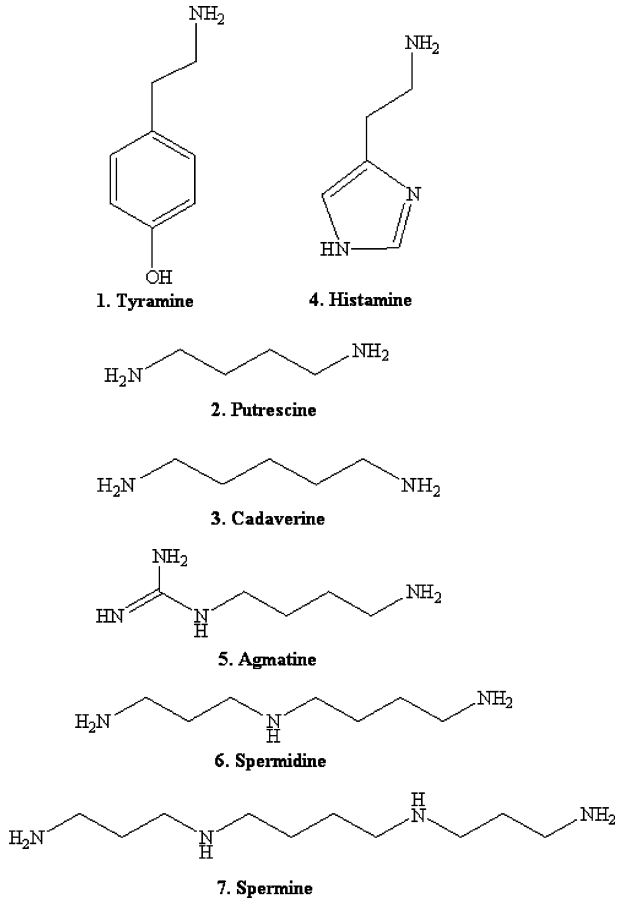

Figure 1. Structures of some BAs related to aquatic products.

Squid and white prawn are economically important aquatic food products in China. The objective of this work is to develop an analytical method in determining the quality of these aquatic products with the simple pretreatment of the sample for a reverse-phase HPLC analysis. In this study, an optimized HPLC method with automatic postcolumn OPA derivatization was applied to determine the changes in BAs contents in squid and white prawn during 8 days of storage at 0 and 4 °C. The obtained results were to test the possibility of finding useful indicators for the freshness of squid and white prawn samples in the quality control procedure of aquatic products processing.

MATERIALS AND METHODS

Chemicals. Acetonitrile and methanol were HPLC grade (Burdick& Jackson, Muskegon, United States). Ultrapure water was obtained from a Milli Q-System (Millipore, Bedford, MA). BA standards, Tyr, His free base, Cad, and Spe were from Nacalai tesque of Tokyo, Japan; agmatine (Agm), Spd, and Put were from Sigma Chemical (St. Louis, MO). 1,6-Diaminohexane (Fluka, Germany) was selected as the internal standard (IS). The stock solution of each amine was prepared in 0.1 M HCl with a concentration of 1000 mg/L, and solutions for further studies were prepared by diluting these standard solutions with 0.1 M HCl. The above solutions were filtered through a 0.45 μm filter (Millipore), stored at 4 °C, and protected from light.

Equipment. Chromatographic experiments were performed using Agilent 1100 Series (Palo Alto, CA) liquid chromatograph with an Agilent G1321A fluorescence detector (FLD), and a Pickering PCX5200 (Mountain View, CA) was used as the postcolumn reaction apparatus. The samples were injected with an Agilent G1313A automatic injector. The chromatographic separations were carried out using a Capcell Pak MG-C18 (150 mm × 4.6 mm × 5 μm) supplied by Shiseido (Tokyo, Japan). Chromatographic data were collected and recorded on the Agilent Chemstation software Rev. A. 10.02.

Chromatographic Conditions. Two eluent reservoirs containing the following eluents (*17*): (A) 10 mM sodium octanesulfonate in 0.1 M sodium acetate (Dima, United States), adjusted to pH 5.3 with acetic acid, and (B) a mixture of solvent B−acetonitrile (2/1, v/v). Solvent B was a solution of 10 mM sodium octanesulfonate in 0.2 M sodium acetate adjusted to pH 4.5 with acetic acid. Eluents A and B were filtered through a 0.45 μm filter and then degassed before use.

The postcolumn derivatization reaction reagents were prepared as follows: 31.0 g of boric acid and 26.0 g of potassium hydroxide were dissolved in the mixture of 1 L of ultrapure water and 3.0 mL of polyoxyethylene (23) lauryl ether (30% Brij-35, Sigma). Then, 0.2 g of OPA (Fluka, Austria) dissolved in 5.0 mL of methanol was added to the mixture. At last, 3.0 mL of 2-mercaptoethanol (Sigma) as a reducing agent was added into the above solution. The derivatization reagents was prepared fresh daily and kept in darkness, filtered, and degassed before use.

The gradient elution program was controlled by a system controller at the flow rate of 1.0 mL/min, and the flow rate of the derivatization reagent was 0.3 mL/min. The optimized gradient program was implemented as follows: time = 0 min, A−B (80:20); time = 40 min, A−B (50:50); time = 42 min, A−B (40:60); time = 46 min, A−B (50:50); time = 50 min, A−B (40:60); time = 55 min, A−B (80:20). The eluted OPA derivatives were detected by monitoring the FLD at 330 and 465 nm for the excitation and emission wavelengths, respectively (*18*). The analytical chromatographic column was set at 40 °C, and the column reaction equipment was kept at 45 °C throughout the experiment. Prepared standard or sample solution (20 μL) was injected by an automatic injector.

Samples. Two aquatic samples were obtained from the fish market in Qingdao, Shandong Province, China. Samples of squid (*Illes argentinus*) had been kept under −20 °C before the cargo reached Qingdao. The muscle from each squid was cut into small pieces (2 cm × 2 cm), divided into two portions randomly, put in two beakers, and stored in a refrigerator at 0 and 4 °C. Samples of white prawn (*L. vannamei*) were alive. White prawn samples with crust were divided into two groups and stored in a refrigerator at two different temperatures, the same as for the squid samples. A small amount of sample was taken out at random from each portion and subjected to experiments every 2 days during 8 days of storage.

Analytical Methods. The degree of decomposition of the samples was evaluated by sensory test by six laboratory panelists and classified in the following three stages: acceptable stage (original color, no smell, and firm texture—sensory rating 1), stage of initial decomposition (slight color change, faintly putrid smell, and fairly soft texture—sensory rating 2), and stage of advanced decomposition (apparent color change, putrid smell, and very soft texture—sensory rating 3) (*19*).

The sensory rating could be the freshness index cursorily, and the values of pH and total volatile base nitrogen (TVBN) could be combined to evaluate the freshness quality of aquatic products. The muscle pH value was measured on the homogenate of the sample. For the determination of TVBN, the microdiffusion method of Conway was applied, and the TVBN values were expressed as mg/100 g muscle of squid or white prawn (*20*).

For the determination of BAs, 3.0 g of muscle of squid or white prawn was homogenized with 15 mL of cold perchloric acid (4 °C) in a high-speed homogenizer at 10000 r/min for 30 s and centrifuged at 4 °C (10000 r/min × 5 min). The procedure was repeated, and the removed supernatants from the two extractions were combined (*19*). The extraction solution was filtered through a 0.45 μm filter and kept in darkness at 4 °C until the HPLC analysis.

Statistical Analysis. The IS method was used for accurate determination of BAs. The concentration of each BA was calculated directly by interpolation of the ratio (each amine peak area/IS peak area) in the corresponding linear calibration curve (amine peak area/IS peak area against amine concentration) between 0.01 and 25.0 mg/L. All statistical analyses were performed by means of the Statistical Software Package for Windows SPSS, version 11 (SPSS, Chicago, IL). For testing the reliability of the method, analysis of variance of linear regression and the *t* test for mean comparison were applied. Stastical comparisons of BAs contents, TVBN values, and pH values during storage at different temperatures were analyzed.

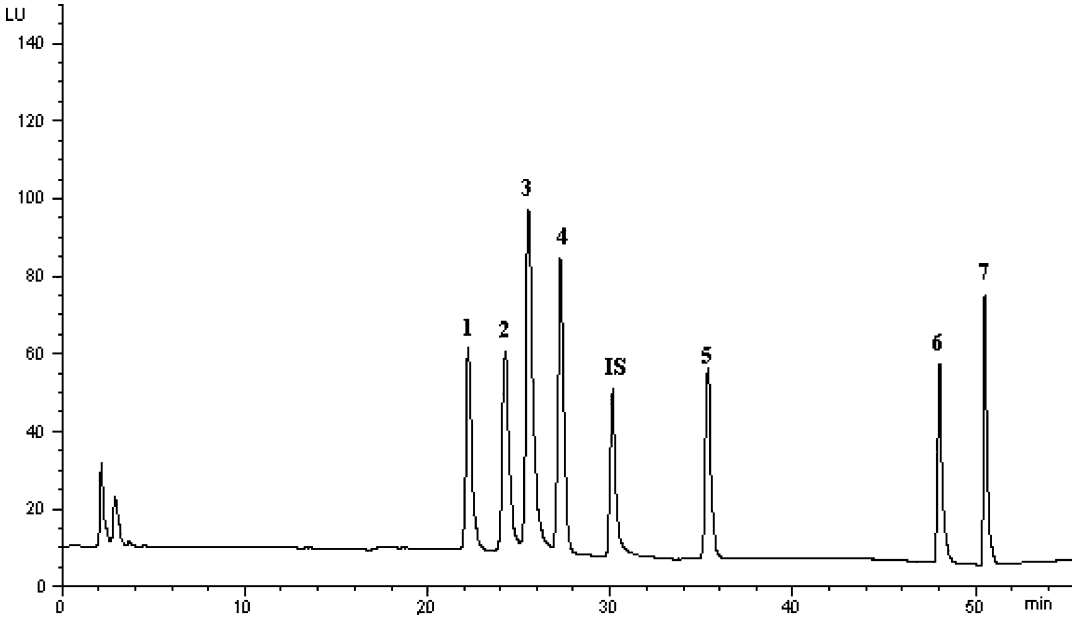

Figure 2. Chromatogram of BAs standard solution (5 mg/L) by HPLC-FLD. Abbreviations: 1, Tyr; 2, Cad; 3, Put; 4, His; 5, Agm; 6, Spd; 7, Spe; and IS, 1,6-diaminohexane.

RESULTS AND DISCUSSION

This method, based on the HPLC procedure, requires that the equipment consist of a reverse-phase stationary column, a postcolumn reaction system for OPA derivatization, and a good separation of amines from possible interferences of the complex matrix. In preliminary work, different wavelengths were tried and the largest peak areas were obtained at an excitation wavelength of 330 nm and an emission wavelength of 465 nm, also at the same term with Pickering laboratories manual (*18*). The optimization of HPLC procedure showed that the retention times of BAs standards depended on pH values of mobile phases. Particularly, the pH of eluent A was critical for the steadiness of the elution of the compounds throughout the analyses, and the proportion of acetonitrile in eluent B also obviously affected the separation and retention times of Cad and His. The elution program developed provided chromatograms of high-resolution peaks, allowing a complete pattern of seven amines in a single run of less than 1 h. The chromatogram of a standard solution (5 mg/L) is shown in **Figure 2**. As can be observed, chromatograms were simple without interferences, and the identification was certain. This HPLC condition may be applied to analyze other samples containing more kinds of BAs (*17*). Moreover, to obtain more precise quantitative analysis, the IS (1,6-diaminohexane) was added. As shown in the chromatogram, the IS was appropriate for the amine analysis and quantification.

Linearity. FLD response in the corresponding calibration curves (10 points from 0.01 to 25.00 mg/L) was linear. Linearity was verified by analysis of the variance of the regression and by the calculation of the relative standard deviation (RSD) of the response factor (each amine peak area/IS peak area against amine concentration). For Tyr, Put, His, and Agm, the correlation coefficient (R^2) was 0.9999 ($P < 0.001$), and the R^2 value of Cad, Spe, and Spd was 0.9998 ($P < 0.001$). Furthermore, the RSD of the response factors was less than 2.0% in all cases, ranging from 0.3 to 1.9% (**Table 1**).

Precision. Method reproducibility was evaluated. Six determinations of a standard solution of all amines (10.0 mg/L) were performed using the same reagents and equipments on the same day. The RSDs obtained from all determinations indicated less than 2% (**Table 2**). Because no aquatic products contained all seven amines, two different samples were prepared by adding known amounts of all BAs (0.5, 5.0, and 10 mg/L, respectively) to test the precision at three levels of concentration. The RSDs obtained for all amines of intraday calibration were always less than 5.0% (data not shown). These results demonstrated that the method was reproducible for the detection of all amines.

Table 1. Linearity, Precision, and Sensitivity for Seven BAs Determination

				sensitivity (μg/L)	
BAs	linear regression equations	R^2 [a]	RSD[b] ($n = 6$, %)	LOD[c]	LOQ[d]
Tyr	$y = 0.2429x + 0.001$	0.9999	0.77	2.00	6.50
Put	$y = 0.2849x + 0.0166$	0.9999	0.38	0.45	1.50
Cad	$y = 0.4858x + 0.0912$	0.9998	0.69	0.20	0.65
His	$y = 0.3362x + 0.0152$	0.9999	1.96	0.75	2.50
Agm	$y = 0.2111x + 0.0073$	0.9999	1.54	0.50	1.65
Spd	$y = 0.1844x + 0.0012$	0.9998	1.55	0.65	2.10
Spe	$y = 0.1740x + 0.0111$	0.9998	1.72	0.80	2.80

[a] R^2, linear regression correlation coefficients. [b] RSD, RSD for six determinations. [c] LOD, limit of detection. [d] LOQ, limit of quantification.

Table 2. Precision and Mean Recovery of the Method for Determination of BAs in Squid and White Prawn

	squid ($n = 18$)			white prawn ($n = 18$)		
BAs	RSD[a] (%)	mean recovery (%)	CI_{95} global recovery[b] (%)	RSD (%)	mean recovery (%)	CI_{95} global recovery (%)
Tyr	5.47	95.3	92.1–98.5	5.28	87.1	86.9–91.4
Put	6.05	88.3	86.2–90.4	6.69	88.9	88.0–93.8
Cad	8.27	89.5	86.8–92.7	8.20	92.0	88.7–95.4
His	7.49	99.1	94.6–101.6	5.16	89.7	89.2–94.3
Agm	5.32	97.2	94.3–100.1	4.80	94.6	92.4–96.8
Spd	6.78	89.8	87.5–93.2	3.41	92.9	91.5–94.1
Spe	8.22	98.6	94.1–99.1	7.56	93.8	92.3–97.3

[a] RSD, RSD for six determinations. [b] CI_{95}, confidence interval (95%) of the mean recovery from all three addition levels.

Table 3. Changes in BAs of Squid during Storage at 0 °C (mg/kg)[a]

storage time (days)	Tyr	Put	Cad	His	Agm	Spd	Spe	TVBN	pH	sensory rating[d]
0	0.17 ± 0.11[b] A	0.61 ± 0.38	ND[c]	ND	0.16 ± 0.07 A	3.17 ± 0.80	1.09 ± 0.33	0.63	6.66	1
2	0.18 ± 0.15 A	1.12 ± 0.24	0.24 ±0.21 A	ND	0.31 ± 0.11 A	3.47 ± 0.71	0.96 ± 0.42	0.89	6.64	1
4	0.73 ± 0.21 AB	1.46 ± 0.32	0.63 ± 0.35 A	0.26 ±0.10	0.55 ± 0.45 A	3.21 ± 0.94	1.23 ± 0.68	1.88	6.71	1
6	0.81 ± 0.16 B	1.97 ± 1.07	2.20 ± 1.31 A	0.35 ± 0.27	13.5 ± 2.05 B	4.24 ± 1.61	1.04 ± 0.39	2.57	6.82	1
8	2.03 ± 1.01 B	2.11 ± 1.50	21.5 ± 4.01 B	0.48 ± 0.41	101.1 ± 7.41 B	2.09 ± 1.04	0.75 ± 0.40	3.76	6.91	2

[a] (A–B) Mean values of the same BA bearing different superscripts differ significantly ($p < 0.01$). [b] The values were expressed as means ± standard deviations ($n = 3$). [c] ND, not detected. [d] Key: 1, acceptable; 2, initial decomposition; and 3, advanced decomposition.

Table 4. Changes in BAs of Squid during Storage at 4 °C (mg/kg)[a]

storage time (days)	Tyr	Put	Cad	His	Agm	Spd	Spe	TVBN	pH	sensory rating[d]
0	0.17 ± 0.11[b] A	0.61 ± 0.38 A	ND[c] A	ND	0.16 ± 0.07 A	3.17 ± 0.80	1.09 ± 0.33	0.63	6.66	1
2	0.24 ± 0.12 A	1.36 ± 0.18 AB	0.42 ± 0.30 A	ND	0.51 ± 0.21 A	3.66 ± 1.44	0.62 ± 0.42	0.89	6.74	1
4	0.26 ± 0.19 A	1.68 ± 0.60 AB	1.59 ± 1.25 A	0.15 ± 0.15	1.22 ± 1.05 A	2.74 ± 1.34	1.14 ± 0.68	2.28	6.82	1
6	1.52 ± 0.36 A	6.97 ± 1.42 B	4.72 ± 1.48 A	0.29 ± 0.16	90.5 ± 2.35 B	4.78 ± 0.64	1.11 ± 0.39	3.92	6.94	2
8	12.2 ± 0.96 B	26.2 ± 3.20 C	77.6 ± 7.51 B	0.68 ± 0.46	188.9 ± 9.55 C	2.49 ± 1.41	0.83 ± 0.40	5.30	7.02	3

[a] (A–C) Mean values of the same BA bearing different superscripts differ significantly ($p < 0.01$). [b] The values were expressed as means ± standard deviation ($n = 3$). [c] ND, not detected. [d] Key: 1, acceptable; 2, initial decomposition; and 3, advanced decomposition.

Recovery. Recovery was tested by the standard addition procedure using three levels of concentration. The samples were prepared by adding known amounts 0.5, 5.0, and 10.0 mg/L of all BAs to test the precision and recovery at a low, medium, and high level of concentration. Six replicas were carried out for each additional level (**Table 2**). For squid samples, the average recovery was 88.3−99.1%, and the RSD ($n = 18$) was between 5.3 and 8.3%. For white prawn, the average recovery was 87.1−94.6%, and the RSD ($n = 18$) was between 5.1 and 8.2%. Depending on different amine, the recovery varied with the RSDs below 9.0%. The mean recovery could satisfy the need for quantification of BAs in our work. Because of the complex matrix of aquatic samples, the obtained recoveries were considered to be lower than the wine samples (*15*) but at a comparative level with meat samples, which were treated with precolumn derivatization (*3*, *9*).

Sensitivity. The detection limit was calculated from the amount of amines required to give a three-fold signal-to-noise ratio and was found to be between 0.2 and 2.0 μg/L for all of the BAs (**Table 1**). Because of the higher sensitivity of FLD than other methods (*4*), the quantification limit was established at 10-fold the signal-to-noise ratio and 0.01 mg/L was fixed as the first point in the calibration graphs. This may be useful to detect trace amount of BAs.

Determination of Aquatic Products. This method was applied to determine the BA content in two aquatic products during the storage at two different temperatures. After simple sample preparation, 20 μL of filtered solution was injected by the automatic injector. The peak areas of amines obtained from the integration software of Agilent 1100 Series HPLC system were emendated by adding the known amount of IS to obtain the response factors. Quantification was based on the calibration curve of each amine. Dilution was needed when necessary to match the amine calibration curve.

Squid. The concentrations of BAs present in the muscle of squid stored at 0 and 4 °C are given in **Tables 3** and **4**. Because of different fresh levels of raw materials, the contents of BAs were a little higher than other researchers (*19*). It was obvious that squid quality deteriorated more rapidly during storage at 4 °C by generating an increased amount of the amines faster and earlier as compared to storage at 0 °C. Agm and Cad were the two most obviously changed amines in squid during storage. Increases in the amount of amines (Agm and Cad) during storage at 4 °C were more rapid than those at 0 °C. In case of the storage at 0 °C, Agm values rapidly increased from day 6 and reached 101.1 mg/kg (**Table 3**) at day 8. Cad concentrations, from 2.2 mg/kg (**Table 3**) after day 6, reached 21.5 mg/kg (**Table 3**) sample at day 8. Values of other amines were relatively low and did not change so much throughout the 8 day storage period. In contrast, in the case of the storage at 4 °C, Agm values started to increase rapidly from day 4, a day earlier than the storage at 0 °C, and reached 188.9 mg/kg (**Table 3**) sample at day 8. Cad levels, from 4.72 mg/kg (**Table 3**) before day 6, reached 77.6 mg/kg (**Table 3**) sample on day 8. In addition, when stored at 4 °C, Put and Tyr levels increased gradually, with a significantly higher increase than stored at 0 °C. However, His, Spd, and Spe fluctuated within negligible levels during the storage period in two conditions.

In **Tables 3** and **4**, the changes in pH and TVBN values of the squid muscle during storage at 0 and 4 °C were recorded, together with sensory ratings. Values of TVBN and pH in the squid muscle stored at 4 °C were clearly higher than those stored at 0 °C. At the beginning of the storage, the TVBN value of the squid muscle was low (0.63 mg/kg, **Table 3**). After storage at 0 °C for 6−7 days or at 4 °C for 5−6 days, the muscle gave off a faintly putrid smell, the pH value increased sharply, the TVBN values exceeded 3 mg/kg, which is the national limit of China for freshness quality of aquatic products, and this stage was designated as the sensory rating 2, the stage of initial decomposition. At the corresponding time, BAs started to increase sharply. This study also demonstrated that there was a good correlation between the production of BAs (Agm and Cad) and TVBN values as well as sensory rating. Similar results were obtained from other researchers (*19*). Moreover, according to the SPSS analysis, the changes in BAs, TVBN, and pH values of squid muscle during storage at different temperatures were really significantly different ($P < 0.05$).

Table 5. Changes in BAs of White Prawn during Storage at 0 °C (mg/kg)[a]

storage time (days)	Tyr	Put	Cad	His	Agm	Spd	Spe	TVBN	pH	sensory rating[d]
0	ND[c]	0.28 ± 0.15[b] A	0.15 ± 0.05 A	ND	0.66 ± 0.32 A	2.17 ± 0.48	11.1 ± 1.63 B	0.73	7.08	1
2	0.37 ± 0.25	3.81 ± 1.02 AB	1.80 ± 0.11 A	ND	1.56 ± 0.82 AB	3.02 ± 1.25	10.3 ± 2.33 AB	0.89	7.54	1
4	2.66 ± 1.01	2.79 ± 0.65 AB	10.2 ± 1.42 B	0.59 ± 0.30	4.25 ± 1.05 BC	1.49 ± 0.86	5.56 ± 0.58 AB	1.19	8.02	1
6	2.36 ± 1.16	6.16 ± 1.12 B	23.8 ± 2.36 C	0.35 ± 0.27	2.99 ± 1.22 ABC	1.91 ± 1.51	4.78 ± 1.99 A	2.97	8.11	1
8	3.03 ± 1.22	13.9 ± 1.50 C	51.7 ± 4.50 D	0.40 ± 0.12	5.03 ± 1.13 C	0.59 ± 0.44	4.87 ± 1.54 A	3.51	8.26	2

[a] (A–D) Mean values of the same BA bearing different superscripts differ significantly ($p < 0.01$). [b] The values were expressed as means ± standard deviation ($n = 3$). [c] ND, not detected. [d] Key: 1, acceptable; 2, initial decomposition; and 3, advanced decomposition.

Table 6. Changes in BAs of White Prawn during Storage at 4 °C (mg/kg)[a]

storage time (days)	Tyr	Put	Cad	His	Agm	Spd	Spe	TVBN	pH	sensory rating[d]
0	ND[c]	0.28 ± 0.15[b] A	0.15 ± 0.05 A	ND	0.66 ± 0.32 A	2.17 ± 0.48	11.1 ± 1.63	0.73	7.08	1
2	0.48 ± 0.15 AB	3.16 ± 0.84 A	4.30 ± 0.35 A	ND	2.06 ± 0.76 AB	3.72 ± 1.37	9.65 ± 2.14	1.27	7.53	1
4	1.13 ± 0.74 AB	6.61 ± 1.56 A	37.7 ± 5.47 B	0.68 ± 0.22	5.33 ± 1.48 AB	1.76 ± 0.94	6.85 ± 1.65	1.80	8.09	1
6	3.36 ± 1.06 BC	102.8 ± 7.97 B	95.8 ± 7.61 C	0.23 ± 0.17	6.31 ± 2.27 B	7.45 ± 1.68	5.03 ± 1.25	3.64	8.23	2
8	6.03 ± 1.41 C	124.5 ± 8.44 C	233.7 ± 9.06 D	0.48 ± 0.21	6.14 ± 2.14 AB	4.29 ± 2.25	5.27 ± 2.41	4.21	8.32	2

[a] (A–D) Mean values of the same BA bearing different superscripts differ significantly ($p < 0.01$). [b] The values were expressed as means ± standard deviation ($n = 3$). [c] ND, not detected. [d] Key: 1, acceptable; 2, initial decomposition; and 3, advanced decomposition.

Chemical and microbial changes, together with sensory evaluation of squid muscle, are generally the main indices of squid quality (*21*). The efficiency of BAs indices for freshness evaluation was investigated in this work. Therefore, the obtained results suggest that characteristic Agm and Cad could be efficient quality indices for freshness of squid muscle.

White Prawn. During the storage of white prawn, nigrescence of the shell and softening of the muscle were noticed. It was observed that the lower the temperature, the slower the quality deterioration. At the stage of acceptable sensory rating (**Tables 5** and **6**), the TVBN value of white prawn muscle was 0.73 mg/kg, and it was in an excellent quality. The initial decomposition stage was reached when the TVBN value exceeded 3 mg/kg. The pH value went through a sudden increase at the start of storage, and then, the changes slowed down through day 8 at both storage temperatures. After storage at 0 °C for 7−8 days or at 4 °C for 5−6 days, panelists of sensory analysis denied the white prawn samples for the quality deterioration. These results were similar to other reports with similar raw materials (*22*).

Tables 5 and **6** also showed that large changes in the contents of Cad and Put were observed from less than 1 mg/kg (at day 0) to more than 50 and 13.9 mg/kg (**Table 6**) at day 8, when stored at 0 °C, respectively. Also, when stored at 4 °C, Cad concentrations started to increase rapidly from 4.30 mg/kg (**Table 6**) at day 2 and reached 233.7 mg/kg (**Table 6**) at day 8. However, in the case of Put, the values rapidly increased from 6.61 mg/kg (**Table 6**) at day 4, 2 days earlier than the samples stored at 0 °C, and reached 124.5 mg/kg (**Table 6**) at day 8. This period also generally matched with the results of sensory analysis and TVBN determination presented above. Spd and Spe were in relatively high concentrations at the original state of white prawn muscle but decreased to some degree as the storage time was extended. Like the squid sample, the concentrations of Tyr, His, and Agm were not stable to detect throughout the storage time. Combined with the changes of TVBN and pH values, the differences of BAs changes between 0 and 4 °C were significant ($P < 0.01$) by analysis of SPSS. Because of no regulatory limits for Cad and Put, it is necessary to find proper safety limits with these two components for the quality control.

In summary, Cad was the most potential index for the freshness of white prawn, and Put would be a supplementary index in the evaluation of the quality of white prawn. The *K* value was suggested as a freshness index of shrimp of high quality, and the TVBN value could be used as a decomposition index of shrimp at a late stage of storage (*22*). Therefore, BAs determined by a simple method carried out in this work could be effectively employed for the early control of quality deterioration as well as for the evaluation of acceptable stage of the prawn in aquatic food industry.

Characteristic production of Agm and Cad could be a useful indicator for the freshness of squid muscle, whereas that of Cad and Put could be used for the quality of white prawn. Determination of BAs using the method developed in this study can be applied to other aquatic food products as a part of the quality assurance measures.

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