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Toxicity of Biogenic Amines and the Chemical Indices for Spoilage in Peeled White Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Introduction: Shrimps are extremely perishable seafoods, which could threaten human health by causing foodborne diseases and intoxications. Therefore, seafood is generally stored at the temperature of -18 ˚C until consumption. The present study aimed to determine the safety of white shrimp in terms of biogenic amine toxicity and investigate some of the rapid chemical indices in this regard.

Methods: After preparation, the samples were stored at the temperature of -18°C for six months and analyzed monthly for the levels of trimethylamine nitrogen (TMA-N), biogenic amines (histamine, putrescine, and tyramine), and total volatile base nitrogen (TVB-N).

Results: The values of TMA-N and biogenic amines had a rising trend during storage, while TVB-N had fluctuated values. Furthermore, putrescine and TMA-N had strong correlation-coefficients with time (r=0.933 and r=0.91, respectively). The biogenic amines remained below the limit value during the six-month storage.

Conclusion: Histamine, putrescine, and tyramine did not reach the toxic dose in the study period, posing no significant risk to human health during the appropriate storage of shrimp. Therefore, it is recommended that the values of TMA-N and putrescine be considered as potential quality indicators for frozen white shrimp.

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Introduction

Various types of shrimps are categorized as a major class of healthy food in the human diet. White shrimp (*Litopenaeus vannamei*) is a highly demanded shrimp species in Iran and is expanding worldwide (especially in Southern Asia and China) owing to its high nutritional value. The major nutrients of white shrimp include peptides, polyunsaturated fatty acids, and amino acids.

Although seafood is often stored at cold temperatures, it has the potential for rapid spoilage. The growth of microorganisms is the major cause of food spoilage (1). *Pseudomonas*, *Flavobacterium*, *Micrococcus*, *Aeromonas*, *Moraxella*, and *Shewanella* are the most commonly reported pathogens in perished freshwater fish (2-4).

By releasing autolytic enzymes and excessive values of non-protein nitrogen compounds, microbial contaminations lead to the rapid postmortem deterioration and spoilage of shrimp, thereby developing a soft texture and odor in the products (5). Therefore, evaluating the shelf-life and quality of frozen white shrimp plays a key role in maintaining human health. Moreover, the identification of a rapid chemical indicator of spoilage in shrimp could help determine the safety of these products.

Biogenic amines (BAs) are certain basic nitrogenous compounds. Due to microbial metabolism (amino acid decarboxylation), BAs could be found in fish, meat, and cheese (6). Histamine is produced after the decarboxylation of histidine by some bacterial species, such as *Raoultella* (*Klebsiella*) *planticola*, *Morganella morganii*, and *Enterobacter aerogenes* which are naturally occurring bacteria and the main causes of histamine fish poisoning (7).

In general, low concentrations of BAs in food and drink do not threaten human health. Histamine

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methyltransferase, monoamine oxidase (8), and diamine oxidase form a detoxifying system in the intestinal tract, which prevents histamine poisoning in case of the ingestion of low amounts (9). However, vasoactive and psychoactive effects (e.g., headaches, breathing problems, hypertension/hypotension, and nausea) have been reported as the clinical signs observed at the higher concentrations of histamine.

Histamine poisoning and even death have been reported after the consumption of some foods, such as cheese and particularly seafood (10, 11). However, there have been no reports on histamine poisoning due to the consumption of shrimps in Iran. As the symptoms of food poisoning are similar to various other conditions, they may remain unnoticed despite occurrence (12).

The present study aimed to determine whether the typical storage of white shrimp for six months at the temperature of -18˚C could preserve its safety in terms of BA toxicity, while also measuring the chemical indices for the assessment of the quality and spoilage of this food product.

Materials and Methods

Sample Collection and Preparation

White shrimp samples were obtained from Choebdeh in the vicinity of Abadan, located in Khuzestan province, Iran. The shrimps were caught from an aquaculture farm and immediately transferred to the food hygiene laboratory under cooling conditions in a polyethylene ice box. Subsequently, they were scalped and stored in sealed polyvinyl chloride bags at the temperature of -18˚C for six months. In total, 21 samples were evaluated before freezing on the first day and every month during the six-month storage. The experiment was carried out on three packs of the samples each time.

Evaluation of the BAs

The assay for evaluating the BAs was performed using the method described by Dawood et al. and Noori et al. with slight modifications (13, 14). Each sample (10 g) was mixed with trichloroacetic acid (TCA 5% w/v, 75 ml; Merck, Germany) by a blender for two minutes and centrifuged at 2,000 grams for 10 minutes. The supernatants were filtered through a Whatman filter (No. 1) and filled up to 100 milliliters with 5% TCA.

At the next stage, two milliliters of the solution was combined with one millimeter of NaOH (2N; Merck, Germany) and 10 microliters of benzoyl chloride (Merck, Germany) in a 25-milliliter glass tube and placed in a water bath at the temperature of 30°C. Afterwards, two milliliters of saturated NaCl and three milliliters of diethyl ether were added to the tubes, and the mixtures were centrifuged at 3,000 grams for 10 minutes. Following that, the organic phase of the supernatants was transferred to another glass tube and dried in an oven with an air current at the temperature of 70˚C.

At the next phase, the residue was dissolved in 200 microliters of methanol (HPLC grade; Merck, Germany) and filtered through a micropore membrane filter (pore size: 0.45 µl; Millipore). Subsequently, 20 microliters of the filtrates was used for high-pressure liquid chromatography (HPLC) analysis. Furthermore, the BAs were measured using the HPLC device (Shimadzu, Japan), which was equipped with an ultraviolet detector. The isocratic mobile phase was a combination of methanol, water (70:30 by volume), and the flow rate of one milliliter per minute at room temperature. To detect the peaks, a 254-nanometer absorbance wavelength was used.

Determination of Trimethylamine Nitrogen

Navarro-Segura et al. evaluated the amounts of trimethylamine nitrogen (TMA-N) (15). In the present study, 10 grams of each sample was initially homogenized in 90 milliliters of 7.5% TCA and filtered through a Whatman filter paper. The filtered solutions (4 ml) were transferred into the glass tubes and mixed with 10 milliliters of anhydrous toluene, one milliliter of formaldehyde, and three milliliters of saturated K_2CO_3 solution. After shaking the tubes, five milliliters of the supernatant with toluene was removed, and 0.02% picric acid (5 ml) was added to the solution. Finally, the 410-nanometer wavelength was used to assess the absorbance against the blank control.

Determination of the Total Volatile Base Nitrogen

The level of the total volatile base nitrogen (TVB-N) was measured using the method proposed by Sun et al. (16). Initially, 10 grams of each sample was blended in 50 milliliters of distilled water. The mixture was transferred to a 500-milliliter flask, followed by adding 200 milliliters of

distilled water and two grams of MgO for 20 minutes. Meanwhile, 2% boric acid (25 ml), methylene blue, and methyl red (indicator) were added to an Erlenmeyer flask (250 ml), which was used for receiving distilled water. Consequently, the TVB-N distilled water and alkaline changed the color of the boric acid solution to green. Following that, a hydrochloric acid solution (0.1 N) was applied for titration and continued until a pink color appeared. The TVB-N amount (mg/100 g of the shrimp muscle) was measured using the formula below:

$$\%\boldsymbol{mg}\mathbf{TVB-N} = \frac{(\boldsymbol{V}\times\boldsymbol{C}\times\mathbf{14}\times\mathbf{100})}{\mathbf{10}}$$

Where *V* is the volume of the hydrochloric acid, and *C* signifies the concentration of hydrochloric acid.

Statistical Analysis

All the measurements were carried out in triplicate, and data analysis was performed in SPSS version 16 using the one-way analysis of variance, Tukey's test, regression analysis, and correlation-coefficients. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Results

Table 1 shows the formation of BAs in the white shrimp stored at the temperature of -18°C for six months. According to the findings, histamine remained undetectable until the second month, and its highest mean concentration was 6.34±0.38 µg/g, which was determined in the sixth month. The initial mean value of putrescine was estimated at 2.99±1.98 µg/g and reached 18.30±1.01 µg/g on the final day of storage, which was the highest value compared to the other BAs. On the other hand, tyramine was detectable from the second month, and its highest concentration was observed in the sixth month (mean: 9.23±0.78 µg/g).

Table 1. Biogenic Amine Concentrations (µg/g) of White Shrimp Stored at -18°C for Six Months

Months of Storage	Biogenic Amines		
	Histamine (µg/g)	Putrescine (µg/g)	Tyramine (µg/g)
0	ND^{defg*}	2.99±1.98^{efg}	ND^{efg}
1	ND^{defg}	4.91±0.31^{efg}	ND^{efg}
2	ND^{defg}	7.65±0.72^{efg}	1.61±0.26^{fg}
3	2.29±0.36^{abcfg}	8.02±1.34^{fg}	2.20±0.42^{fg}
4	3.61±0.74^{abcfg}	13.94±1.44^{abc}	2.49±0.50^{abfg}
5	6.34±0.38^{abcde}	15.59±1.32^{abcd}	6.50±0.79^{abcdeg}
6	6.56±0.66^{abcde}	18.30±1.01^{abcd}	9.23±0.78^{abcdef}

*ND = not detected; *Means in same column with different letters are significantly different (P<0.05); Values are mean of three replications ± standard deviation*

Table 2 shows the changes in the TVB-N and TMA-N values. According to the obtained results, the initial TMA-N content of the shrimp samples was relatively low (mean: 1.3±0.20 mg/100 g), which had a rising trend (P<0.05) until the final month of storage (mean: 9.53±1.36 mg/100 g). Unlike TMA-N, the TVB-N values varied in the shrimp samples, indicating no specific trend (P<0.05).

Table 2. Trimethylamine Nitrogen (TMA-N) and Total Volatile Base Nitrogen (TVB-N) Values (mg/100 g) of White Shrimp during Storage at -18°C for Six Months

Month of Storage	TMA-N (mg/100 g)	TVB-N (mg/100 g)
0	1.3±0.20^{efg*}	17.73±1.86
1	2.66±0.34^{fg}	24.73±3.06
2	3.30±.20^{fg}	25.66±0.93
3	4.12±0.10^{fg}	25.40±0.69
4	5.63±0.36^{afg}	25.20±1.40
5	9.49±1.27^{abcde}	22.86±2.46
6	9.53±1.36^{abcde}	21.00±1.40

**Means in same column with different letters are significantly different (P<0.05); Values are mean of three replications ± standard deviation*

Discussion

Histamine, putrescine, and tyramine could be used as spoilage indicators. Notably, histamine and tyramine are the causative agents of food poisoning and exert direct adverse effects on health. However, putrescine acts as a potentiating factor for the toxicity of histamine and tyramine (17) due to its inhibitory effect on the detoxifying enzymatic system (18).

According to the results of the present study, histamine, putrescine, and tyramine had rising trends during the six-month storage. Moreover, the steepest increase in the contents of BAs was detected for putrescine. As putrescine is a natural constituent of living cells (19), its detection in the shrimp samples since the first day of the storage period was not surprising, while the other BAs in the samples had low levels at the beginning of the storage at the temperature of -18˚C. These findings are consistent with studies conducted by Restuccia et al. (20), Hu et al. (21), and Wang et al. (22). In addition, Restuccia et al. (20) investigated the production of BAs in mullet during a six-month storage at the temperature of 4˚C, reporting an increasing trend in the concentrations of histamine, putrescine, and tyramine during the storage compared to their low titers at the outset of the experiment. High-quality seafood should not exceed the putrescine and tyramine concentrations of 15 mg/kg (23). In the current research, white shrimp was stored at the temperature of -18˚C for six months, and putrescine and tyramine remained below the limit value.

According to our findings, histamine, putrescine, and tyramine had strong correlation-coefficients with time (r=0.929, r=0.933, and r=0.906, respectively). Among these components, putrescine showed the highest correlation-coefficient with time, as well as a consistently increasing trend (P<0.05). Furthermore, it was detectable from the beginning to the end of the storage period. Therefore, putrescine is suggested as the optimal BA quality index for white shrimp during storge. According to a study conducted by Ruiz Capillas et al., the concentration of cadaverine was correlated with the spoilage of hake (*Merluccius merluccius*) (24). In the present study, the histamine concentrations were undetectable in the samples until the third month and remained low until the end of the experiment, which is in line with the results obtained by Lee et al. (25). This could be due to the low histidine content in white shrimp tissues, microbial growth inhibition, and low activity of enzymatic decarboxylation at low temperatures (-18˚C). Regarding low-quality seafood samples, histamine levels generally exceed five milligrams per kilogram (23), while in the present study, the histamine content of the samples was below this limit. It has been suggested that foods containing 20 milligrams per kilogram of histamine should not be consumed due to their toxic effects on human health (23). Accordingly, white shrimp could be considered a healthy food for humans.

The formation of histamine differs in fish species due to the varied histidine content (26). The histamine concentration in mullet bottarga has been reported to be within the range of 0.33±0.2-2.70±0.04 milligrams per kilogram (27) during the storage period to a high concentration in European anchovy (*Engraulis encrasicolus)* (28). However, histamine contents are negligible in some fish, such as carp roe (*Cyprinus carpio*) (23). The range of histamine concentration in the white shrimp stored at the temperature of 4˚C for 14 days has been reported to be 0.22-0.96 µg/g (29).

Overall, our findings indicated that the total BA contents were within the range of 1.61-18.30 µg/g in the white shrimp. In the study conducted by Lin et al., five different salted shrimp products were analyzed for the BA contents, and the obtained results indicated that the concentration of histamine, putrescine, and tyramine were -8.75 (non-detectable), -1.14 (non-detectable), and -16.14 mg/kg (non-detectable), respectively (30).

According to the results of the present study, the white shrimp had lower total BA contents compared to other seafood, and it seems that the formation of BAs during six months of storage at the temperature of -18˚C is not a major hazard to the health of the consumers. Several studies have evaluated TMA-N and TVB-N values in fish (31-34). The TMA-N is a useful indicator of the freshness of seafood (35). In the present study, the TMA-N had a high correlation-coefficient with time (r=0.91) and was detectable from the beginning of the storage period with a consistently increasing trend. Therefore, it could be considered a quality index for the freshness of white shrimp. In line with our findings, the TMA-N has been reported to be on a rising trend in northern shrimp (*Pandalus borealis*) (36). Moreover, TMA-N and TVB-N values of sardine (*Sardina pilchardus*) during six months of storage were estimated at 2.03-10.86 and 7.00-28.47 mg/100 g, respectively, which exceeded the permissible limits at the end of the storage period (37).

Shrimp with the TMA-N value of <10 mg/100 g are regarded as fresh samples (38). In the present study, the TMA-N value remained lower

than 10 mg/100 g, indicating proper preservation during storage at the temperature of 18˚C. This is consistent with the findings of a study (39) in which the TMA-N values in the white shrimp treated with modified atmosphere packaging remained below 10 mg/100 g during six months of storage. In the current research, TVB-N was applied as a biochemical index to determine freshness and quality, which is linked to the endogenous enzymes and growth of spoilage bacteria. According to the obtained results, the TVB-N was not an appropriate index for the evaluation of white shrimp quality during the six-month storage at the temperature of -18˚C since it had a low correlation-coefficient with time (r=0.097), and its values fluctuated during the storage period.

Researchers have suggested the standard TVB-N values of 20 mg/100 g for fresh shrimp samples (38); however, the corresponding values were below this limit in the present study. Similar results have been proposed for the white shrimp treated with modified atmosphere packaging stored at the temperature of -18˚C (39). On the other hand, the storage of white shrimp at the temperature of 4˚C has been shown to lead to the formation of higher TVB-N values, which exceeded the permissible limits after four days (39).

Conclusion

In this study, BA formation and some chemical indices of white shrimp (*Litopenaeus vannamei*) were evaluated during six months of storage at the temperature of -18˚C. According to the results, the contents of histamine, putrescine, and tyramine in the shrimp samples were within the range of 1.61±0.26-18.30±1.01, which remained below the toxic doses and food spoilage limits. Therefore, BAs were observed to be harmless for humans. Furthermore, putrescine and TMA-N could be used as the quality indicators of white shrimp at the temperature of -18˚C due to the strong correlation-coefficients with time (r=0.933 and r=0.91, respectively). It seems that the six-month storage of white shrimp at the temperature of -18˚C could preserve the quality of the food without causing any human health concerns.

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Conflicts of interest

None declared.

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