Food Chemistry 235 (2017) 220–226

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Towards improved quality benchmarking and shelf life evaluation of black tiger shrimp (*Penaeus monodon*)

Le Nhat Tam [a,b,*], Doan Nhu Khue [a], Nguyen Ba Thanh [a], Tran Thi Van Thi [b]

[a] Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Viet Nam
[b] Department of Chemistry, Hue University of Science, Hue University, Viet Nam

ARTICLE INFO

Article history:
Received 4 December 2016
Received in revised form 8 May 2017
Accepted 10 May 2017
Available online 11 May 2017

Keywords:
Black tiger shrimp
Histamine
Hypoxanthine
QIM
TVB-N

ABSTRACT

An improved quality benchmarking and shelf life evaluation of freshly harvested black tiger shrimp (*Penaeus monodon*) was pursued by combining sensory and chemical methods. This involved developing a quality index method (QIM) to further assess both freshness and shelf life of the studied shrimp samples. The quality index included the use of trimethylamine (TMA-N), total volatile basis nitrogen (TVB-N), histamine, and hypoxanthine, which were performed at scheduled times during the ten days of ice storage (0 °C). Shelf life of the studied shrimp was most likely to be 8 days, and there were positive linear correlations between quality indices (QI) and storage period. The quality of shrimp decreased over storage time. In fact, significant changes of chemical and sensory characteristics of the shrimp samples would become more obvious from day 5 onwards. Besides, quality classification of black tiger shrimp involved four main levels, namely: excellent, good, moderately acceptable, and just acceptable.
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1. Introduction

Both black tiger shrimp (*Penaeus monodon*) and pacific white shrimp (*Penaeus vannamei*) are economically important fishery products increasingly studied in recent times (Okpala, 2015a). Primarily, here in Vietnam, they are found at Southwestern and Southern Provinces, for example Ca Mau. After seafood dies, there is a reduction quality due to series of bacterial and enzymatic reactions, which result in undesired odors and off flavors associated with certain organic compounds (Okpala, Choo, & Dykes, 2014). Also, many microorganisms developed in death seafood are hazardous for the consumers (Dabadé et al., 2015). Therefore, the quality of seafood has to be assessed by valid sensory and chemical methods. Specifically, quality index method (QIM) is a sensory method believed to be originally developed by the Tasmanian Food Research Unit in Australia (Bremner, 1985). It has evolved over a decade to become a rapid and reliable method to measure the freshness of whole fishes stored in ice (Botta, 1995). Many chemical quality indices have been established for determining fish quality during storage time. These indicators help to reveal the degree at which autolysis and degradation of glycogen, protein, ATP, the products of ATP and lipid oxidation take place. In line with this, the freshness of seafood products can be established, based on total volatile base nitrogen (TVB-N) and trimethylamine (TMA-N) concentration (Howgate, 2010). Several research groups (Mietz & Karmas, 1977; Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997) have put together quality assessment applicable to fishery products, through the use of biological indicators BAI amines (Biogenic Amines Index) and quality index (QI) as following:

$$QI = (\text{histamine} + \text{putrescine} + \text{cadaverine}) \,/\, (1 + \text{spermine} + \text{spermidine})$$

$$BAI = (\text{histamine} + \text{putrescine} + \text{cadaverine} + \text{tyramine})$$

In this case, the QI is determined based on the increase in putrescine, cadaverine, and histamine and the decrease in spermine and spermidine during storage of fish. The BAI is based on the increase in histamine, putrescine, cadaverine, and tyramine. Nucleotide breakdown involves both autolytic enzymes and bacterial action (Huss, 1988). The degree/emergence of adenosine triphosphate (ATP) and its products can also be a reflection of freshness and quality of seafood (Veciana-Nogués et al., 1997)

Because we are not completely convinced about extant quality index methods reported in relevant literatures, the current study sought for an improved assessment of freshness and shelf-life quality benchmarking by determinations of TVB-N, TMA-N, histamine, and hypoxanthine concentration of black tiger shrimp

* Corresponding author at: Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Viet Nam.
E-mail addresses: lenhattam@iuh.edu.vn (N.T. Le), doannhukhue@iuh.edu.vn (N.K. Doan), thanhngba@iuh.edu.vn (T. Nguyen Ba), ttvthi@hueuni.edu.vn (T.V.T. Tran).

http://dx.doi.org/10.1016/j.foodchem.2017.05.055
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(*Penaeus monodon*) stored at 0 °C. From the results of current study, we suggest quality benchmark criteria specifically to evaluate black tiger shrimp quality.

2. Materials and methods

A schematic overview of the experimental study is shown in the supplementary material. This flow chart describes the entire experimental process from harvesting to storing and analyzing the shrimp samples.

2.1. Shrimp collection and storage

Thirty (30) kilograms of black tiger shrimp was freshly harvested from three different farms located in Ca Mau Province, Vietnam. The shrimp visually seen to be with defect or breakage were removed. Upon harvest, live shrimp samples were quickly washed using filtered clean flowing water and placed in 300 sterile Reynolds zipper (26.8 × 27.9 cm) polyethylene bags (Alcoa Products Inc., Richmond, VA 23261, USA), consistent with that reported in Okpala et al. (2014).These bags were then distributed uniformly in the styrene foam boxes between layers of ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the laboratory after 8 h. At the laboratory, polyethylene bags containing shrimp samples were kept in cold room (0 °C) until required.

2.2. Reagents

Hypoxanthine (PubChem CID: 790), histamine (PubChem CID: 774), and TMA (PubChem CID: 1146) standards were purchased from Sigma –Aldrich Singapore. Methanol (PubChem CID 123132), ethanol (PubChem CID: 702) and water HPLC grade were obtained from Merck Vietnam Ltd. Co.

2.3. Developing another Quality Index Method (QIM) using sensory panel approach

The current study involved developing another Quality Index Method (QIM) using sensory panel approach. This involved three major steps namely: Identification of quality attributes, main sensory evaluation, and quantification of the developed (sensory) Quality Index (QI).

2.3.1. Step 1- Identification of quality attributes

Three experts independently performed sensory evaluation of shrimp wherein all observed changes (appearance, odor, carapace texture, carapace color, eye, shell color, the black spots appearing on the cuticle and the telson of shrimp etc.) were qualified and attributes apportioned and recorded. Each attribute was scored from 0 to 3 with the lower scores indicating best quality.

2.3.2. Step 2- Main sensory evaluation

Shrimp samples were evaluated daily for ten days under 0 null storage. There were six sensory trained experts examined the shrimp at different time periods in one month. The trained panel, at a practice phase, evaluated the changes of shrimp samples given the storage time periods. After completing this practice with good performance, the panellists evaluated the samples without knowing the storage period and the results were recorded. This process provided the privilege for slight adjustments/amendments to ensure to best of authors' ability, good data bias reduction, improved accuracy and reliability.

2.3.3. Step 3- Quantification of the developed (Sensory) Quality Index (QI)

This stage basically involved quantification of the data arising from this sensory QIM. The data covered the storage days of this study. The regression equation between storage days and quality score was also developed in this step. Regression equation was used to compare the projected shelf life with actual shelf life of shrimp of this study. The data were collected from ten different samples corresponding to each of the 10-day storage period.

2.4. Determination of TVB-N and TMA-N

TVB-N of shrimp was measured according to the Commission Regulation (EC) No 2074/2005 of 5 December 2005 (European Commission, 2005). An amount of (5.0 ± 0.01) g of minced shrimp samples without shells were blended with 90 mL of perchloric acid 6% in a 250 mL blender at high speed for 2 min. Extract solution was transferred into test-tube, followed by centrifuging at 3000*g* for 3 min, thereafter filtered using Qualitative Circles filter paper, and diluted to 100 ml. An aliquot of 50 ml was transferred into a distiller flask, added with 4 ml of 20% NaOH and distilled. The composition of TVB-N is absorbed into 100 ml of 0.1 M sulfuric acid solution. The residual sulfuric acid solution after absorption of TVB-N was titrated by 0.1 N sodium hydroxyl solution.

TMA-N concentration was measured by AOAC 971–14 method (Hungerford, 1998). Shrimp samples were blended to achieve a homogeneous mix. Then 10 ± 0.01 g of the mix was extracted with 30 ml of solvent 7.5% TCA (w/v). The process was repeated three times. Collected solution after extraction was centrifuged at 4000*g* for 10 min, filtered and diluted to 100 ml. Trimethylamine reacted with picrate salt to form complexes, which were measured at 410 nm.

2.5. Determination of histamine

Histamine has been used as an index of relative freshness of certain fishery products (Andrade, Mársico, de Oliveira Godoy, Franco, & Junior, 2014). Histamine reacts with o-phthalaldehyde (OPA) to form a stable fluorescent compound, which is the basis for determination (Gouygou, Sinquin, & Durand, 1987). Briefly, homogenous samples (10 g) without shells were mixed with 40 mL ethanol. The mixture was blended for 2 min in a blender (MX-SM1031S, Panasonic, Japan) and was then centrifuged at 3000*g* for 10 min by Hetich-EBA 20S (Sigma-Aldrich, Germany). The supernatant was transferred into a 150 mL volumetric flask. The process was repeated three times and the total extraction solution was diluted to 150 ml by ethanol. Next, 2 ml containing histamine was separated and purified by SPE C_{18} column (Agilent Technologies, USA), with 80% methanol as the elution solution, and then diluted to 10 ml. 250 µl of solution was injected into the HPLC system (Waters 600, Artisan Technology Group, USA), and reacted with OPA and 2-mercaptoethanol to form a fluorescent compound. Chromatographic separation was performed with C_{18} column (size 5 µm, length 250 mm × 4.6 mm ID) (Agilent Technologies, USA); the mobile phase consisted of 80% ethanol at a flow rate of 1 mL/min and a column temperature of 40 °C. Fluorescent compounds were detected using the fluorescence detector (Waters 474, USA) set at an excitation wavelength of 359 nm and an emission wavelength of 445 nm.

2.6. Determination of hypoxanthine

Hypoxanthine concentrations have been measured by HPLC method with DAD detector. In this method, hypoxanthine was extracted by 0.6 M acid perchloride (Veciana-Nogues, Izquierdo-Pulido, & Vidal-Carou, 1997), and was detected by the method

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