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Biogenic amine changes in barramundi (*Lates calcarifer*) slices stored at 0 °C and 4 °C

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ABSTRACT

The biogenic amines formation in barramundi (*Lates calcarifer*) slices kept for 15 days at 0 °C and 4 °C were investigated using nine biogenic amines, total plate counts and biogenic amines formers. Significant differences in biogenic amines concentrations of barramundi slices stored at 4 °C and at 0 °C after 3 days of storage were observed. All amines, except tryptamine, 2-phenylethylamine, tyramine and agmatine in the slices increased with time during storage at both temperatures. At the end of the storage period, histamine concentrations were 82 mg/kg and 275 mg/kg for samples kept at 0 °C and 4 °C, respectively. At day 15, the total plate count was approximately 8.6 log CFU/g for sample kept at 0 °C and 9.7 log CFU/g for samples kept at 4 °C. Histamine-forming bacteria (HFB) in all samples ranged from 5.4 to 6.1 log CFU/g at 0 °C and 4 °C, respectively. The observed shelf-life of barramundi slices were 6–9 days.

1. Introduction

Biogenic amines (BAs) in foods are formed by microbial decarboxylation of amino acids. Amines such as cadaverine and putrescine are very important in food, especially in fish and fish products, since they have been shown to potentiate the toxicity of histamine (Shalaby, 1996). Histamine, one of the biogenic amines, has been known as the contributory toxin of scombroid fish poisoning (Önal, 2007), however, histamine formation was reported to not be related to the activity of endogenous enzymes in fish, but due to histidine content in the fish muscle. Biogenic amines are produced at very low levels in fresh fish and their formation is related to bacterial spoilage (Özogul & Özogul, 2006). Spermine and spermidine are usually the major amines present in fresh muscle at concentration of less than 10 mg/kg flesh, but depending on the fish species, the free amino acids present in the tissue and the conditions of exposure to spoilage bacteria, other amines such as histamine in fish of mackerel and herring families (*Scombridae* and *Clupeidae*) can rise to 2000 mg/kg flesh (Clifford & Walker, 1992). They can be produced during the storage or processing of the products by thermal or bacterial enzymatic decarboxylation of free amino acids (Önal, 2007).

The most common fish associated with histamine fish poisoning or scombroid fish poisoning or scombrotoxicosis are scombroid fish including tuna, mackerel and saury, and non-scombroid fish including bluefish, mahi-mahi, sardine, anchovy, herring, and marlin (Flick, Oria, & Douglas, 2001). Different concentrations have been gazetted for the establishment of guidelines such as 'safe for consumption', or 'acceptance' for different fish species (Arnold & Brown, 1978). The Food and Drug Administration (FDA) of the United States of America had established a guidance level for histamine at 50 mg/kg for assuring the safe consumption of scombroid or scombroid-like fish. The FDA has also recommended the use of other data to judge fish freshness, such as the presence of other biogenic amines associated with fish decomposition (USFDA, 2002). A maximum histamine content of 200 mg/kg has been established in the European Community (EC) for acceptance of tuna and other fish belonging to the *Scombridae* and *Scomberesocidae* families (EC, 1991). The EC has suggested that in the future, a maximum of 300 mg/kg for total biogenic amines in fish and fish products may be an appropriate legal limit (EC, 1991).

The biogenic amine contents in several fish such as sardine, tuna and herring are quite abundant in the literature; however, none has been reported for Barramundi (*L. calcarifer*), a brackish water fish. It is a high value fish, which is cultured and also found wild in Malaysia. They are marketed live or in the fresh form on ice. Therefore, this study was carried out to investigate the formation of biogenic amines in barramundi slices kept at 0 °C and 4 °C. The findings from the study can contribute to the database on biogenic amines in farmed, tropical fish, which at present is negligible.

2. Materials and methods

2.1. Reagents

Standard amines, containing tryptamine hydrochloride (TRT), 2-phenylethylamine hydrochloride (PHE), putrescine dihydrochloride (PUT), cadaverine dihydrochloride (CAD), histamine

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dihydrochloride (HIS), spermidine trihydrochloride (SPD), spermine tetrahydrochloride (SPR), tyramine (TYR) and agmatine (AGM) were obtained from Sigma (St. Louis, MO, USA). All chemicals used were of analytical grade.

2.2. Sample preparation

Ten fresh barramundi (*L. calcarifer*) weighing approximately 1 kg and of 33 cm in length were purchased from a local wet market. The duration between catch and arrival of the fish at the laboratory was less than 15 h where they were always kept in ice. To ensure uniformity of the sample characteristics, all fish were bought from the same supplier and were treated in the same manner. Upon arrival, the whole fish were washed under running tap water, headed, gutted, filleted and rinsed. Then, they were cut to slices of approximately 1.3 cm thick. Slices were then randomly divided into homogenous groups of approximately 200 g each, then packed into two separate bags. One pack was analysed immediately and the data collected was labelled as the data for 0 day. The rest of the samples were then stored at two storage temperatures, 0 °C and 4 °C, for 15 days. The whole experiment was repeated twice within a space of a month.

2.3. Biogenic amine quantification

The sample preparation, benzoylation and determination were according to the procedure of Hwang, Chang, Shiua, and Chai (1997) Tryptamine hydrochloride (61.4 mg), putrescine dihydrochloride (91.5 mg), cadaverine dihydrochloride (85.5 mg), 2-phenylethylamine hydrochloride (65.1 mg), spermidine trihydrochloride (87.7 mg), spermine tetrahydrochloride (86.0 mg), histamine dihydrochloride (82.8 mg) tyramine hydrochloride (63.4 mg), agmatine sulphate (87.7 mg) were dissolved separately in 50 ml 0.1 N HCl. The final concentration of the free base for each amine was 1000 mg/kg solution. A series of dilutions were prepared from the standard stock solution and used to obtain the standard curve. The benzoyl derivatives of all biogenic amines were then prepared according to the prescribed procedure (Hwang et al., 1997).

2.4. Sample preparation and amine extraction

Samples were homogenised in a Waring blender (Model 32BL79, USA) for 3 min. Ground samples (5 g) were transferred to 50 ml centrifuge tubes and homogenised with 20 ml 6% TCA solution for 3 min. The homogenates were then centrifuged at 8000*g* for 10 min in a refrigerated high speed centrifuge (KUBOTA7800) and the supernatant was filtered through Whatman No. 1 filter paper (Whatman, Maidstone, UK). The filtrates were then placed in volumetric flasks and 6% TCA was added to a final volume of 50 ml. After which, an aliquot of each extract was derivatised with benzoyl chloride using the same procedure as the benzoylation of the standard amine solution.

2.5. HPLC parameters

Amines were determined using a Perkin Elmer liquid chromatograph (Perkin Elmer Series 200, USA) system which was equipped with a UV–Vis detector, LC Chromato-integrator and Vacuum Degasser. A Lichrospher 100 RP-18 reserved-phase column (5 μm, 150 × 4.6 mm I.D., Merck) was used for the peak separation which was detected at 254 nm. The gradient elution program was set at 0.8 ml/min, starting with a methanol–water mixture (50:50, v/v) for 0.5 min and the program preceded linearly to methanol–water (85:15, v/v) at a flow rate of 0.8 ml/min over 6.5 min. This was followed by the same composition and flow rate for 5 min, then a decrease over 2 min to methanol–water (50:50, v/v) at a flow rate of 0.8 ml/min.

2.6. Total plate count (TPC)

Twenty-five grams of fish slices were aseptically weighed and homogenised in stomacher bags (BAGMIXER® 400, Model P) with 225 ml sterile peptone water for 1 min. The homogenised sample was serially diluted using 9 ml peptone water. Further serial dilutions were made and 0.1 ml of each dilution was pipetted onto the surface of the plate count agar (Merck), in triplicates, after which they were incubated for 2 days at 30 °C (AOAC, 2002).

2.7. Histamine-forming bacteria (HFB)

The histamine-forming bacteria was carried out according to the procedure of Nivein's (Niven, Jeffreg, & Corlett, 1981). Ten grams of fish muscle was homogenised with 90 ml of peptone water in a stomacher bag (BAGMIXER® 400, Model P) for 1 min. Serial dilutions of each sample were prepared. Aliquots of 0.1 ml were spread in triplicate over the Niven's medium plates, which were then incubated for 72 h at 37 °C. Purple colonies (surrounded by a purple halo on a yellowish background) were counted for each plate using a colony counter.

2.8. Quality index and biogenic amines index

The quality index and the biogenic amine index were calculated according to the procedures described by Mietz and Karmas (1977) and Veciana-Nogues, Marine-Font, and Vidal-Carou (1997) respectively. The formulae used were as follows:

(i) $\text{Quality index(QI)} = \frac{(\text{histamine}+\text{putrescine}+\text{cadaverine})}{(1+\text{spermidine}+\text{spermine})}$

(ii) $\text{Biogenic amine index(BAI)} = (\text{histamine} + \text{putrescine} + \text{cadaverine} + \text{tyramine})$

2.9. Statistical analysis

Data collected were analysed by one-way analysis of variance (ANOVA). The one-way ANOVA was used to analyse the effect of days by different temperature on the fish slices. The Tukey's test was used for mean comparison when a significant variation was found by the ANOVA test. The significance of results was at $P < 0.05$. The software used was Minitab release 14 (2005). Pearson correlation was conducted to determine if there existed any relationship amongst the HFB, TPC, and histamine contents of the two samples tested.

3. Results and discussion

3.1. Biogenic amines analysis

The concentrations of the nine biogenic amines present in the muscle of barramundi stored at 0 °C and 4 °C for 15 days are given in Table 1. On the whole, samples stored at 0 °C had lower concentrations of the biogenic amines as compared to those stored at 4 °C. No amines were detected on the first day of storage for both temperatures.

For 0 °C storage, only two amines, i.e. putrescine and tryptamine were detected on the third day and six amines were detected on the sixth day of storage. However, for the same sampling day, the presence of eight and nine amines was detected, respectively, for samples kept at 4 °C. The first appearance of hista-