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Survey of Quality Control in Whole Yellowfin Tuna (*Thunnus albacares*) Using Relation of Biogenic Amines with Psychrophilic Bacteria Load During Frozen Storage

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Abstract: Samples of *Thunnus albacores* are stored stored after caught on board in frozen storage till the time of discharge. In the first day of discharge, the samples are provided for microbiological and chemical analysis. The psychrophilic bacteria load of this sample became dominant at frozen storage and the numbers found lower than International Commission on Microbiological Specifications for Foods (ICMSF) limit of 10^7 cfu/g (more than 10^5 cfu/g in this study). In this research, three biogenic amines were investigated (histamine, putrescine, cadaverine) for whole Yellowfin tuna (*Thunnus albacares*). According to biogenic amine analyses, we couldn't find any histamine in all *Thunnus albacares* samples which stored in frozen method. Also in some samples putrescine and cadaverine are not detected. The maximum putrescine and cadaverine levels reached to 8.27 µg/g and 4.89 µg/g in one of the samples, respectively. In this study, the diamine putrescine had the best correlation with psychrophilic bacteria load (r^2=0.91). in the present work and according to International Standard Limit the mean concentration of putrescine, cadaverine and total mean psychrophilic bacteria load were detected in samples of this project lower than acceptable range, therefore the fishes that are stored in the catch vessels with frozen storage method, are suitable for consumption.

Key words: Yellowfin tuna · Biogenic amine · Psychrophilic bacteria · Frozen storage · Catch vessels

INTRODUCTION

Marine species deteriorate rapidly post mortem. The degradation process is carried out at first by muscle enzymes and later by microbial enzymes [1, 2]. Unlike other muscle food, fish are usually harvested in remote locations, making the time between the catch and the landing of the fish material much longer than the time between landing and selling [3]. Amines, such as putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), histamine (HI), tyramine (TY) and tryptamine (TR), being low molecular weight compounds, are likely to arise via several biochemical pathways [4]. Histamine, one of the biogenic amines, has been known as the causative toxin of scombroid fish poisoning [5]. The symptoms include rash, urticaria, edema, localized inflammation, nausea, vomiting, diarrhea, abdominal cramps, headache, palpitation, flushing and severe respiratory distress [6]. Some studies reported that histamine seems to be formed by post-catching microbial contamination on board, at the processing plant, in the distribution system, in restaurants, or homes [7]. In order to safeguard the health of the public, the US Food and Drug Administration (FDA) revised the compliance guide for decomposition and histamine poisoning [8]. It was announced that fish may be considered as decomposed when histamine level reaches 50 ppm. For the European Community (EC), an acceptable level of 100 ppm has been established for histamine in tuna and other fish belonging to the Scombridae and Scomberesocidae families [9]. Moreover, fish could be implicated in histamine poisoning outbreaks when histamine reaches 500 ppm [10]. During chilled storage of fish, significant deterioration of sensory quality and loss of nutritional value have been detected as a result of changes in chemical constituents, that lead to a strong effect on the commercial value [1,2,11]. This degradation process is carried out in the initial stage by muscle enzymes and later

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by microbial enzymes. The rate of alteration depends on factors such as the nature of the fish species, size, lipid content, state at the moment of capture, importance and nature of the microbial load and storage temperature. Tuna and tuna-like species are important fish species due to their high global economic value and their prevalence in international trade for canning and sashimi. Yellowfin tuna (*Thunnus albacares*) are large pelagic fish that prevail in the tropics and subtropics; with landings accounting for about 22% of the world's tuna catch [12]. They are commercially important in many countries and there is high demand from international markets. In storage trials with naturally contaminated seafood, toxic concentrations of histamine have been observed frequently at storage temperatures above 7-10°C [6, 13, 14]. However, toxic concentrations of histamine have also been observed in naturally contaminated seafood stored at 0-4°C [15-17]. Yellowfin tuna (*Thunnus albacares*) are large pelagic fish, commercially important in the international market. Research on fresh tuna meat has focused on the risk of poisoning due to biogenic amines formation by several mesophilic as well as psychrotolerant bacteria including *Morganella Psychrotolerans* and *Photobacterium* spp. [18- 20]. In some of catch vessels (Chabahar port, Iran), store the caught fishes in frozen method on-board catch vessels, therefore in the day of discharge, the current study was undertaken to assess the effect of frozen on quality control of yellowfin tuna by microbiological and chemical analyses.

MATERIALS AND METHODS

Preparation of the Fish Samples: The species used in this study were Yellowfin tuna (*Thunnus albacares*) caught in the Chabahar port, near Oman sea and stored in frozen method on-board catch vessels and immediately brought to the laboratory. The sampled fishes (15 samples) were caught during the period of April. Samples were analyzed in duplicate for the total psychrophilic bacterial load and biogenic amines analysis.

Microbiological Analysis: A sample was taken from the flesh of the anterior-dorsal region of each whole fish. The skin was aseptically removed and 10 g of fish muscle were dissected aseptically from frozen storage fish specimens, mixed with 90 ml of 0.1% peptone water (Oxoid Ltd. London, UK) and homogenized in a stomacher (Seward Medical, London, UK) as previously described [21]. In all cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Psychrophilic bacteria were investigated by surface inoculation in plate count agar (PCA, Oxoid), after incubation at 20°C for 48h, microbiological data was collected and transformed into logarithms of the number of colony forming units' cfu g^{-1}.

Biogenic Amine Analysis

Preparation of Standard Solutions: Stock standard solution was prepared by dissolving standard accurately 0.1 g of Putrescine, Cadaverine and Histamine (Sigma) in 10 ml of 5% trichloroacetic acid (TCA) solution. The working standard solutions were prepared by diluting of 1 ml of each stock standard solution in 10 ml of distilled water.

Samples Preparation: Extraction: The amines were extracted following the method of Mietz and Karmas [22]. 50 g of fresh fish lateral muscle of anterior-dorsal half of each whole fish were homogenized in 75 ml of 5% trichloroacetic acid solution (TCA) for 2 min. and then, centrifuged at 4000 rpm for 10 min. The supernatant solution was recovered and filtered through Whatman No. 41 and into 250 ml flask, the residual material in the test tube, return to the homogenizer and this procedure was repeated for triplicate. in the next stage, 10 ml of the extracted solution with 4g NaCl, 1 ml NaOH (50%) and 5ml chloroform- butanol (1+1) mixed in the test tube and, shacked for 2 min. then, centrifuged at 3000 rpm for 5 min. and the supernatant layer (organic layer) was recovered, this stage was duplicate and 15 ml N- heptane and 1 ml of 0.2 N HCl added and was shacked, then the lower layer (water like) recovered. this procedure also was duplicate repeated. finally, 1 ml distilled water added and the solution dried under the N_2 or evaporation bathroom.

Drivatization: The amines were drivatizated following the method of Dawood [23]. To the extracted dry matter 1 ml of a 2 M NaOH solution and 5 µl of benzoyl chloride (derivatizator) were added. The mixture was shaken vigorously in a vortex mixer and allowed to stand for 20 min.; 2 ml of a saturated NaCl solution were added to stop the benzoylation. finally, 2 ml of Diethyl ether is added, then, centrifuged at 2500 rpm for 5 min.; the supernatant layer was recovered and dried under the N_2 or evaporation bathroom.

Injection in the HPLC: This stage, was achieved following the method of Dawood [23]. The extracted dry mater according to the procedures reported in

drivatization section, dissolved in 200 µl of HPLC-grade methanol, filtered through Millipore (0.45 µm) and 20 µl of the filtrate were injected in the HPLC using a Hamilton syringe. Amines were detected under the UV light at 254 nm and the separation were performed under inverse phase with isocratic conditions using a mobile phase composed of methanol/water (70/30) and the flow rate 1.1 ml/min.

RESULTS AND DISCUSSION

15 samples were analyzed. The mean amine contents (Histamine, Putrescine, cadaverine) and the total psychrophilic bacterial (log cfu/g) in all samples are given in Table1, Figure 1 and 2.

Biogenic amines occur either as a physiological constituent of live fish or as a result of bacterial growth and spoilage [24]. Formation of biogenic amines depends on aquaculture conditions, food (which affects microbes), fish species, body composition and storage and processing conditions [25-27]; and the presence of decarboxylase-active microorganisms and the availability of free amino acids [28]. The importance of determining the concentrations of biogenic amines in fish and fish products is related to their impact on human health and food quality. The most significant biogenic amines occurring in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, spermidine and agmatine [10]. In this study, the fish samples which stored after caught on board in frozen storage method till the time of discharge. In the first day of discharge we provided the samples immediately brought to the laboratory and analyzed microbiologically and chemically. At the first, in the laboratory analyzed microbiologically and then chemically for each samples. In this research, The maximum total mean psychrophilic bacteria load reached to 5.52 log cfu/g (more than 10^5 cfu/g). According to biogenic amine analyses, we couldn't find any histamine in all *Thunnus albacares* samples which stored in frozen method. Also in some samples putrescine and cadaverine were not detected. The maximum putrescine and cadaverine levels reached to 8.27 µg/g and 4.89 µg/g in one of samples, respectively. In this study, the diamine putrescine had the best correlation with psychrophilic bacteria load (r^2=0.91). Lysine can be converted by bacterial action into cadaverine and putrescine is the precursor of ornithine [5]. Fish muscle is able to support the bacterial formation of a wide variety of amines that come from the decarboxylation of amino acids.

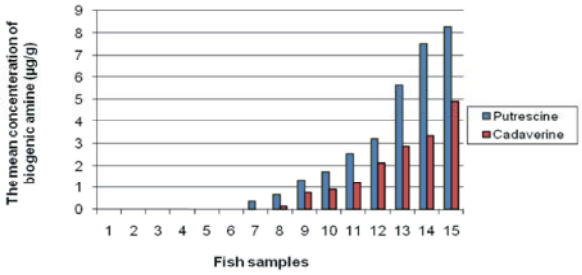

Fig. 1: Mean concentration of biogenic amines in each sample of *Thunnus albacares* during frozen storage

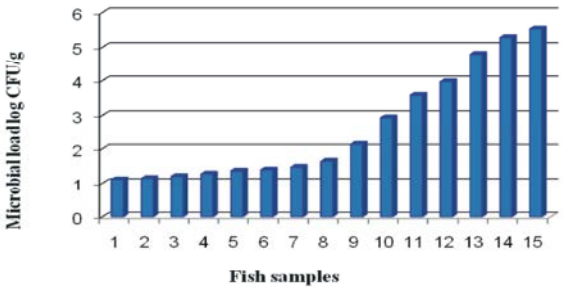

Fig. 2: Total mean psychrophilic bacterial load (log cfu/g) in each sample of *Thunnus albacares* during frozen storage

Table 1: Total mean of psychrophilic bacterial load (log cfu/g) and mean concentration of biogenic amines (µg/g)

Samples	Histamine	Putrescine	Cadaverine	Psychrophilic bacterial load
1	ND*	ND	ND	1.11±0.12
2	ND	ND	ND	1.14±0.17
3	ND	ND	ND	1.2±0.13
4	ND	ND	ND	1.27±0.22
5	ND	ND	ND	1.36±0.35
6	ND	ND	ND	1.39±0.11
7	ND	0.36±0.17	ND	1.47±0.43
8	ND	0.66±0.11	0.16± 0.11	1.64±0.17
9	ND	1.35±0.07	0.76± 0.17	2.13±0.19
10	ND	1.73±0.14	0.91±0.05	2.93±0.07
11	ND	2.54±0.31	1.19±0.15	3.58±0.18
12	ND	3.21±0.09	2.12±0.13	3.97±0.42
13	ND	5.65±0.45	2.86±0.19	4.79±0.16
14	ND	7.52±0.45	3.34±0.26	5.28±0.09
15	ND	8.27±0.41	4.89±0.11	5.52±0.11

The values are expressed as means ± SD (n =3). ND*: not detected

Biogenic amines are produced at very low levels in fresh fish and their formation is related to bacterial spoilage [29]. Inhibition of enzymatic activity of food or bacterial decarboxylase activity and prevention of bacterial growth are very vital to control amine production. The most important factor affecting the production of biogenic amines is storage temperature [30-32]. The bacteria which are in charge of producing histamine are mostly

mesophilice bacteria [6]. Also Arnold *et al.* [33] reported that the highest histamine level was found in fish stored at 30 °C, whereas no histamine was found when fish was stored at 1 °C. A similar key effect of temperature was observed by Baixas Nogueras *et al.* [34] in their study of Mediterranean hake (*Merluccius merluccius*). In this study, it was not suitable condition (aspect of temperature) for bacterial growth which produced histamine, that is why, histamine not detected in any fish samples during frozen storage. During the experiment, psychrophilic bacterial remained small (5.52 log cfu/g) and this may be the reason for the relatively low formation of putrescine in the samples. The psychrophilic bacteria load of this sample became dominant at frozen storage and the numbers found lower than International Commission on Microbiological Specifications for Foods [35] limit of 10^7 cfu/g (more than 10^5 cfu/g in this study). Several studies indicated that putrescine along with cadaverine were found in relative large quantities in toxic fish, facilitated the transportation of histamine and increased the fish toxicity [14, 36]. In order to safeguard the health of the public, the US Food and Drug Administration (FDA) revised the compliance guide for histamine poisoning [8]. It was announced that fish may be considered when histamine level reaches 50 ppm. For the European Community [37], an acceptable level of 100 ppm has been established for histamine in tuna and other fish belonging to the Scombridae and Scomberesocidae families [9]. Moreover, fish could be implicated in histamine poisoning outbreaks when histamine reaches 500 ppm [10]. A putrescine value of 14 μg/g was proposed by Chytiri *et al.* [38] as an upper limit for spoilage, also putrescine values higher than 10 mg/kg have been was proposed by Krizek *et al.* (39) for good quality values between 10 and 20 mg/kg for acceptable quality and values over 20 mg/kg for poor quality based on sensory scores. Krizek *et al.* [25] found higher levels of cadaverine (92.5 μg/g) and Rodriguez *et al.* [40] proposed cadaverine as indicator of alteration of muscle as a result of microbial activity. Yamanaka *et al.* [41] have suggested a level acceptable for cadaverine (10 mg/kg) as an indicator of freshness in fish meat. In conclusion, in the present study, according to the authors and International Standard Limit the mean concentration of putrescine, cadaverine and total mean psychrophilic bacteria load were detected in all samples of this project lower than acceptable range, therefore the fishes that are stored in the catch vessels with frozen storage method suitable for consumption after discharge and this storage method recommend in catch vessels as convenient method for maintaining the freshness and quality of catch fishes.

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