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Sensory and histamine assessment of the freshness of Sardine (*Sardine sindensis*) during different storage conditions

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Abstract

Background: Storage of fish under refrigerated conditions from the time it is caught until when it is consumed has been found to be very important in reducing outbreaks of histamine poisoning.

Methods: Low temperatures control bacterial histamine formation during fish processing. The shelf life of sardine (*sardine sindensis*) during storage at ambient temperature (33°C), ice box temperature (0°C) and freezing temperature (-7°C) were studied in terms of sensory and histamine production. The sensory acceptability limit was up to one day at ambient temperature and 11 days at ice storage condition. However, freezing storage had a good preserving effect on sensory acceptability at the end of experiment. The formation of histamine was determined at day 0, 1, 2, 3, 4, 7, 8, 9, 11, 14, 16 and 18 of experiment using high performance liquid chromatography (HPLC) fluorometeric method.

Results: Histamine development had not exceeded the permissible level (200 mg/kg) recommended by the FAO (2012) during storage condition at -7°C throughout the experiment. At 0°C, histamine concentration was lower than safe level for up to 16 days (135 mg/kg). At ambient temperature, the sardine was spoiled on 3rd day and histamine concentration was found 500.48 mg/kg which was above the FAO recommended level for histamine.

Conclusion: Freezing storage condition has a good preserving effect on sensory acceptability and histamine production and seems the best means of storage.

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Introduction

The consumption of fish is often found associated with different pathological processes including histamine poisoning among the users all over the world. Histamine fish poisoning is a significant concern regarding public health, safety and trade. Histamine belongs to a group of compounds known as biogenic amines. Biogenic amines are biologically active compounds normally produced by decarboxylation of free amino acids and are present in a variety of food like fish, fish products, meat, wine, cheese and fermented food. The presence of biogenic amines in these food items is an indicator of food spoilage [1].

The intestinal tract of humans contains the enzymes diamine oxidase (DAO) and histamine-N-methyl transferase (HMT), which degrades histamine to harmless products. However, for large doses of histamine, the capacity of DAO and HMT to detoxify histamine is limited which ultimately allows it to mix in bloodstream [2]. Histamine exerts its effects through the activation of four different types of histamine receptors (H1, H2, H3 and H4) on and/or in the cellular membrane. These histamine receptors are expressed on different cell types and work through different signaling pathways, resulting in multiple biological responses. Ingestion of histamine-rich food that release histamine or block DAO and HMT may provoke diarrhea, headache [3], congestion of the nose, asthmatoid wheezing [4], hypotension, arrhythmia, urticaria [5], pruritus, flushing, and other conditions in these patients. Approximately 1.0% of the world population has histamine intolerance, and 80% of those patients are middle-aged [6].

Histamine is not present in the fish when caught. Three conditions play important role in the formation and accumulation of histamine in fish. The fish must contain abundant free histidine in the muscle. Microorganisms (including *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05) that can produce histidine decarboxylating enzymes must also be present. A variety of microorganisms that are normal constituents of the surface of the fish have histidine decarboxylase activity. Time and temperature conditions usually allow production and accumulation of histamine and other biogenic amines in the fish [7].

Histamine poisoning is increasing internationally in countries including the USA, Great Britain, Japan [8] and Taiwan [9]. Traditional foods such as fish sauce from Korea and Malaysia [10,11] fermented fish paste from Taiwan [12] and a cooked fish paste (Rihaakuru) from the Maldives have been reported to contain high levels of histamine [13]. Rihaakuru is generally stable in terms of bacterial deterioration [14], bacterial growth in the fish used to manufacture this product, has been speculated to be responsible for the high histamine levels in the product [13] as refrigeration is not widely available in the Maldives. In the absence of effective temperature control alternative methods are needed to control histamine levels in Rihaakuru, which continues to be a popular and widely consumed food.

Sensory evaluation of the fish is not sufficient to detect the absence or presence of histamine; therefore chemical testing is required. Unlike many bacterial pathogens, histamine is not destroyed when fish are frozen or cooked, adhering to temperature requirements along all stages of the food supply chain essential [15].

The fish species most commonly involved in histamine fish poisoning are scombroid dark meat fish such as tuna, mackrele, skipjack, bonito, marlin and non- Scombroid fish such as sardine, mahi-mahi (dolphin), yellowtail, herring and bluefish [16]. Six different species of sardine are found in the marine Catch of Karachi fish harbor i.e. *Sardine albella, Sardine gibbosa, Sardine longiceps, Sardine melanura,* and *Sardine scindensis.*

Storage of fish under refrigerated conditions from the time it is caught until when it is consumed has been found to be very important in reducing outbreaks of histamine poisoning. Low temperatures control bacterial histamine formation during fish processing. In the retail market, sardine fish are usually covered with ice or simply placed on ice to prevent spoilage. However, storage under these conditions for long periods of time can result in microbial growth and conversion of histidine to histamine. A better understanding of the lower temperature limit for bacterial growth and histamine production is therefore important to control fish quality and safety [2]. For this reason, this current study was planned to investigate the effect of different storage conditions on the concentration of histamine in sardine (*Sardine scindensis*) tissue.

Methods

Raw material, sampling and processing

Two large fillets of sardine were purchased from fishing port of Karachi, Pakistan on September 2012. Samples of the two fish were selected for replicate determination and sub-samples from each fish were selected for duplicate determination. The Sardine fillets were cut into single samples that were smaller, more manageable portions, and stored in individual sterile plastic jars. Care was taken to minimize bacterial contamination from benches and equipment used in the preparation. To this end, knives and chopping boards were sterilized in an autoclave prior to use. Prior to, and during sample preparation, a solution of 85% methanol was used to clean equipment and surfaces. Gloves were worn at all times.

Fresh sardines were divided into three groups, one was stored at ambient temperature (30± 3°C, and at 4°C at night) and the second one was stored in ice box containing ice flake with temperature 0°C. Third one was stored in freezer with temperature at -7°C. Samples of all groups were taken for analysis at day 0, 1, 2, 3, 4, 7, 8, 9, 11, 14, 16 and 18 of experiment. The pH value was determined for homogeneous mixtures of fish and distilled water (1:10, w:v), using a digital pH meter (315i, Germany). Before histamine analyses, specimens of samples were taken for sensory evaluation (whole fish appearance of skin, blood on gill cover, texture, texture of belly, odor, eyes appearance, shape and gills color).

Sensory evaluation

For storage trials, fish was inspected at each time of sampling and subsequent freshness grades were assigned by six experienced panelists including two veterinarians familiar with quality control and quality assurance of fish and fish products. The freshness state of sardine was assessed by the quality index method (QIM) shown in Table 1. This sensory scale is based on the freshness quality grading system for herring developed by Nielsen and Hyldig [17]. Each assessor was given simple descriptors, scoring demerit points from 0 to a maximum of 3, where 0 represented best quality and any higher score indicated poorer quality. Panelists were asked to state whether the fish were acceptable or not. This was used to determine the shelf life of sardine.

Histamine analysis method

Histamine concentration was analyzed using high performance liquid chromatography (HPLC) fluorometeric method determined by AOAC [18]. Histamine concentration (mg/kg) was analyzed at day 0, 1, 2, 3, 4, 7, 8, 9, 11, 14, 16 and 18 of experiment.

Standards and chemicals

Histamine dihydrochloride and o-phthalaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reagents were of chromatographic grade. Water was purified in Milli-Q (Millipore Corp., Milford, MA, USA).

Sample extraction

10 g fish meat was homogenized in 50 ml of 75% methanol for 2 minutes. The homogenate was transferred to 100 ml volumetric flask. The flask containing homogenate was heated in water bath to 60°C for 15 minutes. Homogenate was cooled to room temperature and filtered through Whatmann No.1 filter paper (Maidenstone, UK). The extract was collected in 100 ml volumetric flask and filled up to 100 ml with 75% methanol. The extract was passed through ion exchange resin column. The elute was collected in 50 ml volumetric flask containing 5 ml of 1M HCl and then filled the flask up to 50 ml with deionized water.

Sample derivatization

5 ml elute was taken in a beaker. 10 ml of 0.1M HCl and 3 ml of 1.0M NaOH was added and mixed. 1.0 ml of 0.1% o-Pthaldialdihyde, derivatization agent, was added and mixed. After four minutes, 3 ml of 3.57N H_3PO_4 was added and mixed immediately.

Sample analysis

Derivatized sample was added to the HPLC sample vial by the help of disposable syringe and analysed on Shimadzu HPLC system equipped with RF-10AXL spectrofluorometric detector (Histamine in seafood: Fluorometric method) [18]. Histamine was identified by comparison of the retention time of peaks in the sample in relation to standards and confirmed by the addition of histamine to the sample. The concentration of histamine was determined by direct interpolation in standard curves with $R^2 \geq 0.9926$. The limit of determination was 0.56 mg/kg.

Statistical analysis

For data analysis, ANOVA and standard deviation were used. The means were compared by the Tukey test at 5% probability using SPSS version 10.0 for Windows (SPSS Inc., Chicago, IL).

Quality Parameter	Description	Score
Whole fish appearance of skin	Very bright	0
	Bright	1
	Mat	2
Blood on gill cover	None	0
	Some	1
Texture	Hard	0
	Firm	1
	Soft	2
Texture of belly	Firm	0
	Soft	1
	Burst	2
Odour	Fresh sea odor	0
	Neutral	1
	Slight off odor	2
	Strong off odour	3
Eyes appearance	Bright	0
	Somewhat lustreless	1
Shape	Convex	0
	Flat	1
	Sunken	2
Gills colour	Characteristic red	0
	Somewhat pale, mat brown	1

Table 1. Quality Index Method (QIM) scheme for sensory evaluation of Sardine modified by Nielsen and Hyldig (2004)

Results

Concomitant pH determinations (non-published data) yielded a value of 6.1, indicating that fish was in the rigor-mortis phase at time of purchase. The pH values in sardine increased (7.84; 6.50, respectively) at the end of the storage period under ice flakes and freezing conditions. The results of freshness grading of stored sardine under different storage conditions are shown in Table 2. The initial quality characteristics of sardine were very bright appearance, hard texture, bright and convex eyes and fresh odour.

Sardine at the time of purchase had a QIM score of 0. The very fresh state is limited to day 0 of storage, but fish were still of excellent freshness up to the one day at ambient temperature and demerit points increased with storage time. The limit of sensory acceptability of sardine at ice storage condition was up to 11 days. However, freezing storage showed a good preserving effect on sensory acceptability throughout the experiment.

The histamine levels of the sardine fish samples stored at ambient temperature, in ice flakes and in freezer are given in Table 3. On the day 0, all the samples have same concentration of histamine i.e. 1.665 mg/kg. The data at ambient temperature indicated that histamine was produced in significantly ($p < 0.05$) large quantities as the fish degrades during the life of the experiment as compared to other storage conditions.

At ambient temperature (30± 3°C, and at 4°C at night), very low histamine production was found initially at day 0 and increased to the order of 60.08 mg/kg at day 2 of trial. At day 4, the level of histamine in the fish was found as much as 1224 mg/kg. The samples stored in ice flakes had significantly ($p < 0.05$) less value than ambient temperature and higher ($p < 0.05$) value than freezing condition throughout storage period.

On day 18, samples stored at ice flakes had about 200% more concentration of histamine (230.27 mg/kg) as compared with freezing samples (21.35 mg/kg). Data showed that samples stored at freezing condition had significantly ($p < 0.05$) less values of histamine than those of samples stored at ambient temperature and ice flakes storage conditions. On day 18, maximum histamine concentration (21.35 mg/kg) was noticed at freezing conditions.

Days of Storage	QIM Score		
	Ambient	Ice flakes	Freezing
0	0	0	0
1	9	0	0
2	11	1	0
3	17	3	0
4	18	3	0
5	-	4	0
6	-	4	1
7	-	5	1
8	-	5	1
9	-	6	1
10	-	8	1
11	-	8	2
12	-	9	2
13	-	9	2
14	-	10	2
15	-	10	2
16	-	12	3
17	-	12	3
18	-	13	3

Table 2. Freshness assessment of stored sardine under different storage conditions by Quality Index Method (QIM) scheme

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Days	Storage conditions		
	Ambient	Ice flakes	Freezing
0	1.66±0.010	1.66±0.010	1.66±0.010
1	13.52±0.197[a]	5.05±0.035[b]	1.73±0.021[c]
2	60.083±0.078[a]	15.25±0.091[b]	3.74± 0.134[c]
3	500.48±4.3[a]	19.99±0.509[b]	3.29± 0.2545[c]
4	1224.3±3.6[a]	34.27± 0.65[b]	3.49±0.487[c]
7	-	38.17±1.69[a]	5.31±0.276[b]
8	-	51.1±0.27[a]	5.79±0.325[b]
9	-	71.96±3.16[a]	6.42±0.389[b]
11	-	75.77±1.12[a]	6.46± 0.353[b]
14	-	77.52±1.4[a]	14.49±0.474[b]
16	-	135.11±3.32[a]	14.30±0.092[b]
18	-	230.27±10.4[a]	21.35±0.559[b]

Table 3. Average concentration of histamine (mg/kg) in *Sardine sindensis* during different storage conditions

Discussion

The increase in the pH during storage period may be related to the accumulation of alkaline compounds, such as ammonia mainly derived from microbial action during fish muscle spoilage. Similar pH values were found for other fish species during storage in ice by Erkan and Ozden [19] and Ozogurt & co-workers [20].

The early freshness stages of fish stored in ice flakes are characterized by a pleasant seaweedy odor of the gills and a faint green odor in skin. The "fishy" odor character in skin and the gradual loss of seaweedy odor of gills are good indications that fish are of lower sensory grades (QIM score >12). The odor of sardine in ice flakes on day 10 started to be somewhat objectionable, although fish were still fit for consumption. Sardine in ice storage condition used in this trial had a shelf life of 11 days as determined by sensory evaluation. These results are in line with the findings of some studies [21, 22] which showed that sardine (*Sardina pilchardus*) could be stored for 6 to 10 days in ice storage conditions. Similarly, another study [20] showed that the sensory acceptability limit for goldband goatfish (*Upeneus moluccensis*) was 8 days and 11 days for red mullet (*Mullus barbatus*) stored in ice. In contrast of above studies, Triqui and Bouchriti [23] reported that sardine (*Sardina pilchardus*) stored in ice had a shelf life of 4 days as determined by sensory evaluation. Factors such as season and inadequate handling practices of fish onboard and after landing may accelerate the rate of spoilage.

The results of the present study showed that the samples stored at ambient temperature, histamine concentration progressively increased with storage time. A few hours after capture, initial histamine content in sardine was low (1.66 mg/kg). It started exceeding to the toxic level (13.52 mg/kg) in muscles rapidly after 24 hrs of storage at ambient temperature. In agreement with some studies on mackerel [24, 25], this excessive production is probably due to a high amount of histidine in red muscle of fish, proliferation of mesophilic bacteria capable of histidine decarboxylation, optimum temperature (26°C) and a suitable pH for the synthesis and activity of histidine decarboxylase (from 2.5 to 6.5) [26]. Histamine is produced in raw fish from the action of bacterial histidine decarboxylase following temperature/time abuse. Production of histamine is greater at high abusive temperatures (21.1˚C or higher) than at moderate abusive temperatures (7.2˚C), while its generation is particularly rapid at temperatures near 32.2˚C [27] as in case of the present study.

The results of the present study showed that maximum level of histamine at day 3 or 4 was noted to be higher than safe limit of 200 mg/kg recommended by Australian Food Standards Code [28] and FAO/WHO [29]. During storage at ambient temperature, sardine was found unfit for consumption beyond two days. These reports are consistent with other results, including 3 days in sardine [30], 4 days in *Trachurus mediterraneus* [31]. Delays a little longer were reported: 5 or 6 days in *Trachurus trachurus* [32, 33], 5 days [21] in sardine and 4-6 days in anchovy [34]. These relatively short periods of acceptability can be explained by the predominance of psychrotrophic bacteria and gram-negative psychrophilic in fish caught in cold and temperate waters [35]. These differences in shelf life can be attributed to fishing areas that influence the character of psychrophilic or mesophilic natural flora of fish [36].

According to literature, effectiveness of icing in control of histamine production has already been proven on mackerel and bonito [25, 35]. According to the most recent HACCP guidelines for the control of histamine production, a core temperature of 4.4˚C or less should be achieved and maintained throughout handling, processing, and distribution of potentially hazardous fish. The primary goal of these guidelines is

the growth inhibition of spoilage bacteria capable of producing histamine through proper handling and chilling of fish [27].

However, in frozen sardine, results showed a decrease in histamine content after 18 days of freezing. These results agreed with findings of Oucif & coworkers [35], who reported that by adopting freezing method, the samples remained in good condition for 2 months of storage and unacceptable beyond 3 months of storage. They explained that histamine content depends on histamine-producing bacteria as well as on degrading bacteria (bacteria with histaminase activity). In conclusion, the limit for sensory acceptability was up to one day at ambient temperature and 11 days at ice storage condition. Freezing method for storage has a good preserving effect on sensory acceptability and histamine production. It seems as the best means of storage even after two days following capture. Freezer provides constant low temperature thus inhibits the growth of bacteria and enzyme activities responsible for the increase of histamine concentration. Ice flakes are feasible for only 16 days of storage in respect of histamine production. It is suggested that all boats should keep the fish in ice immediately after landing aboard the vessel so that the temperature at sites of microbiological concern is reduced at levels capable of controlling the growth of histamine-producing bacteria. Using ice chilling and freezing storage conditions are usually the best methods to control the histamine production.

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