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Research Article
Quality Indicator Hypoxanthine Compared with Other Volatile Amine Indicators of Sea Foods Stored in Refrigerator

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Abstract

Background and Objective: Study evaluates the suitable chemical method for the determination of the freshness of refrigerated seafood. Hypoxanthine content (Hx) of the refrigerated seafood was analysed and it was compared with the level of volatile amines production due to bacterial spoilage. **Materials and Methods:** Fresh seafood such as *Stolephorus commersonii*, *Scomberomorus koreanus* and *Portunus sanguinolentus* were collected from the fishing harbour of Tuticorin. The fishes were analyzed initially for TMA-N, TVB-N, hypoxanthine and pH content and it was stored at -4°C for 30 days. Sampling was done for every 10 days interval and it was checked for all the above quality indicators. **Results:** The results showed that volatile amines (TVB-N) and (TMA-N) were not found in stored seafood till the end of 10 days storage. Changes in pH and hypoxanthine content were noticed at the initial storage period itself. Refrigeration of sea foods are common at homes and at this condition even the superior quality stored fish has hypoxanthine formation and it gradually increase during the storage period. Hx assay reflects ATP break down and ATP-related compounds and the degradation of ATP to Hx has been attributed to muscle endogenous autolytic enzymes. The enzymatic reaction occurs in the initial stage of storage at low temperature but the contents of trimethylamine-nitrogen and total volatile base-nitrogen increase progressively during the later period of spoilage. Thus, hypoxanthine is regarded as the major catabolite of adenosine triphosphate while it was formed in refrigerated situation and it is a valuable freshness marker in stored fish. **Conclusion:** Thus, the study reveals that Hx measurements have some advantage over TMA-N and TVB-N analyses and Hx could be used as a superior spoilage indicator in the refrigerated sea foods. This study proves that the Hx could be used as a spoilage indicator and the sea foods stored at -4°C, should be consumed within 10 days of storage period to avoid Hx accumulation.

Key words: Hypoxanthine, trimethylamine-nitrogen, total volatile base-nitrogen, refrigerated sea foods, storage period, quality assessment

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Frozen storage is an important method for preservation of food articles and it is commonly used in meat, fish and other animal protein-based industries, because under frozen storage the quality of seafood is retained for a long period and also this method has several advantages such as insignificant alterations in the product dimensions and minimum deterioration in products color, flavor and texture[1]. However, the loss of quality by seafood is unavoidable when stored in frozen state[2]. The changes in fish muscle fibers, proteins, lipids and textural properties during frozen storage have been studied for several decades because of their economic importance[3]. Aberoumand and Jooyandeh[4] found that a proper storage temperature depending on the freezing properties of different species should be maintained because of its impact on the quality of fish. Changes in quality and freshness of the fish flesh depend on the length of storage period and temperature. People prefer the various frozen methods usually to maintain the quality of seafood. On a commercial scale, freezing of fish is done at -50 to -60°C. Since the entire water content in the fish is frozen in this method, the seafood can be stored for a long period and the stored fish almost remains as good as fresh fish. In deep or quick freezing methods the fish is frozen at -20°C and it retains its physical properties and nutritive values. Freeze drying is a modified deep freezing method which completely eliminates all chances of denaturation. Apart from this, there are other methods, employed by the small scale industries and households, such as chilling and frozen storage methods using refrigerators (-4°C).

Seafood spoilage occurs rapidly after harvesting. The spoilage process begins within 12 h of their catch in high ambient temperatures of the tropics[5]. The rate of fish spoilage depends on several factors such as the species of the seafood; their fat content, size and shape; the season of catch and the nature of the fishing grounds[6,7]. Most of the fish species degrade as a result of digestive enzymes and lipases, microbial spoilage from surface bacteria and oxidation[8]. During fish spoilage, there is a breakdown of various components and also formation of new compounds. These new compounds are responsible for the changes in odor, flavor and texture of the fish meat. Bacterial numbers and levels of decomposition products have been used as indicators of post-mortem quality of fish. These changes have been assessed using nucleotides, ATP and related compounds, biogenic amines and volatile amines (TVB-N and TMA-N) as indicators[9].

Among the above-mentioned indicators, TMA-N and TVB-N are considered as the real quality indicators. However, the measurement of nucleotides such as hypoxanthine (Hx) used in fish quality assessments provides several distinct advantages over other chemical tests such as trimethylamine (TMA), dimethylamine (DMA) and total volatile bases (TVB) and others for these tests basically measure only the bacterial spoilage of sea foods. But the accumulation of Hx in fish tissue determines the initial phases of autolytic enzymatic deterioration as well as later contributions caused through bacterial spoilage at storage. Hx concentration is not affected by heat processing, cold storage and irradiation and is very useful in the case of certain seafoods containing little or no trimethylamine oxide (TMAO), which renders the TMA analysis useless[10].

After the death of fish, oxygen supply to the tissues ceases due to disruption of the circulatory system. Soon after rigor mortis, the mitochondrial system ceases to function. Adenosine triphosphate (ATP) is gradually depleted through the action of various ATPase enzymes. After the residual supplies of creatine phosphate are depleted, anaerobic glycolysis continues to regenerate some ATP with the accumulation of the end product, lactate[11]. ATP is degraded at the post-mortem stage by endogenous enzymes in the fish flesh: ATP→ADP→AMP→IMP→HxR→Hx. Hypoxanthine could be broken down by xanthine oxidase. The breakdown of ATP of fish muscle during the death struggle or subsequent to it liberates inosine 5'-monophosphate (IMP). The IMP is transformed into hypoxanthine during storage-in a progressive deprivation by dephosphorylation and the action of ribonuclease enzymes. The concentration of free hypoxanthine increases in the stored muscle of most species. IMP contributes to the pleasant flavor of fresh fish. Its degradation to hypoxanthine is a factor in the progressive loss of desirable flavor and in the development of the bitter 'off' flavor of sea foods.

Hypoxanthine is one of the products of nucleotide degradation mediated by bacterial activity and is the cause of the bitter, off-flavors of sea foods[12]. Studies have shown that the progress in the degradation of nucleotides varies greatly from one fish to another but is often in direct proportion to the period of preservation[13]. Any kind of quality control parameter should accumulate or disappear rapidly during spoilage. In addition, it should be absent or present in variable amounts in fresh fish. Hypoxanthine is regarded as the major catabolite of adenosine triphosphate (ATP) and it is a useful indicator of freshness because of its gradual accumulation in seafood. Hx value rapidly increases in ice storage and the content of the muscle is used for the determination of the post-mortem age in seafood. Different preservation methods such as drying, smoking, freezing, chilling, brining,

fermentation and canning have been reported to extend the shelf-life of seafood. However, low-temperature storage and chemical techniques are the most common methods in the industry today for controlling water activity, enzymatic, oxidative and microbial spoilage[14]. Nowadays people store fish for long in refrigerating frozen condition and they are unaware of the effect of nucleotide degradation products on stored fish. Volatile amines in stored seafood samples are the preferred quality indicators, but very few studies are on Hx as an authentic quality indicator. The aim of the present study is to observe the content of hypoxanthine in seafood stored in refrigerators and compare it with the production of volatile amine at (-4°C) and to determine which method is suitable for monitoring the quality of frozen stored seafood.

MATERIALS AND METHODS

Sample collection: Fresh seafoods such as *Stolephorus commersonii, Scomberomorus koreanus* and *Portunus sanguinolentus* (crab) (Fig. 1a-c) were collected from the fishing harbour of Tuticorin during December, 2016 and transported to the laboratory in an ice bag. They were immediately washed, degutted, cleaned and stored in the freezer at -4°C (Fig. 2a-c). Hypoxanthine content of the seafoods was analyzed initially and in frozen samples at 10 days interval for 1 month.

Processing of samples: Initially the stored samples were taken out from the refrigerator (Whirlpool of India Ltd, Gurugram, India) after 24 h for the purpose of analysis. Five individual samples from each seafood were randomly taken from the frozen blocks for the analysis. Before the analysis, the samples were defrosted, filleted and cut into pieces of 5 mm.

TVB-N and TMA-N: Levels of TVB-N and TMA-N were determined according to the procedure of Siang and Kim[15] by using Conway micro diffusion unit (Bel-Art™ F409410000, India). The extracts were prepared by mixing 2 g of the sample with 4% TCA in a 50 mL beaker and were homogenized properly. It was left for 30 min at room temperature with occasional grinding and was filtered and the filtrate was labeled and stored. Three thoroughly cleaned Conway units were taken and the edge of the outer ring of each unit was sealed using a sealing agent (Vaseline). Using a micropipette (Labline, India), 1 mL of boric acid solution was added to the inner ring of each unit. Into the outer ring of each unit, 1 mL of the sample extract was added. One milliliter of saturated K_2CO_3 solution was carefully pipetted into the outer ring of each unit and closed with a clip. The solutions in the units were then mixed gently, to prevent any solution mixing from one ring to the other. The units were placed in an incubator at 37°C for 60 min. Then the covers of the units were removed and the inner ring solution (a green color) was titrated with 0.02 N HCl

Fig. 1(a-c): Seafood samples, (a) *Stolephorus commersonii*, (b) *Scomberomorus koreanus* and (c) *Portunus sanguinolentus*

Fig. 2(a-c): Seafood samples in stored condition, (a) Stored *Stolephorus commersonii*, (b) Stored *Scomberomorus koreanus* and (c) Stored *Portunus sanguinolentus*

using a burette until the green color solution turned to pink. An average titrate volume of HCl was found from the results of three titrations for each sample. For each sample, the TVB-N values were calculated. A blank test was also carried out using 1 mL of 1% TCA, instead of sample extract. Trimethylamine in the sample was also determined by the Conway technique, which is similar to TVB-N determination except that prior to the addition of potassium carbonate, 1 mL of 10% neutralized formalin was pipetted into the extract with ammonia and this allows only the TMA-N to diffuse over the unit.

pH: Samples were prepared according to Woyewoda *et al.*[16] using a tissue homogenizer. The pH level was monitored using a digital pH-meter (HANNA pH 123 microprocessor pH meter) in triplicate.

Hypoxanthine determination: Hypoxanthine content of the sea foods was determined according to the method of Luong *et al.*[17]. All the chemicals used were of analytical grade and procured from Sigma-Aldrich, USA, Reagents used for the Hx estimation:

- Perchloric acid (6%)
- Xanthine oxidase was prepared by 10 mg mL^{-1} xanthine oxidase in a ratio of 1:5 U with 0.05 M phosphate buffer. Buffer was added very gradually to avoid loss of enzyme activity. Dilution was done immediately before use; diluted enzymes may be stored at a frozen temperature up to 6 months
- Phosphate buffer 0.05 M was prepared by dissolving 17.01 g potassium dihydrogen orthophosphate in about 250 mL distilled water. The pH was adjusted to 7.6 with 1 M NaOH and diluted to 500 mL with distilled water. A further five-fold dilution with distilled water gave a final concentration of 0.05 M
- Potassium hydroxide/phosphate buffer (pH 7.6) was prepared by dissolving 27.22 g potassium dihydrogen orthophosphate in about 250 mL water and 171 mL of 1 M sodium hydroxide was added. The pH was adjusted with orthophosphoric acid or sodium hydroxide. Then 557 mL of 1 M KOH was added and made up to 1 L with water
- Hypoxanthine standards were prepared in a 100 mL volumetric flask by dissolving 5.0 mg (0.005 g) of

hypoxanthine in 100 mL distilled water. Overnight stirring or agitation in an ultrasonic bath may be necessary to complete dissolution

Procedure: Fifty gram of seafood samples were blended with 200 mL of perchloric acid to make protein extract which is used for hypoxanthine content determination. Ten milliliter of the perchloric acid extract was neutralized with 10 mL of KOH-buffer solution. The neutralized extracts are not stable for long periods of time under refrigeration, so prompt analysis is essential. However, neutralized extracts may be frozen at -30°C. Determination of hypoxanthine content in samples was done by preparing the following set of test tubes for each sample extracts: Tube A: 1 mL neutralized extract+2 mL buffer +2 mL water. Tube B: 1 mL neutralized extract+2 mL buffer+ 1.5 mL water+0.5 mL enzyme. If very high or low hypoxanthine concentrations are expected, the volume of extract and the volume of water are altered, so that the total volume remains at 5 mL. The samples were incubated in a water bath at 37°C for 30 min and the absorbance of the samples was measured at 290 nm.

The increase of absorbance, Abs, for each sample was calculated as follows:

$$Abs = B+A8-A7-A$$

Where:

A = Absorbance from sample tube A
A8 = Absorbance from tube 8 used in standard curve preparation
A7 = Absorbance from tube 7 used in standard curve preparation
B = Absorbance from sample tube B

$$Hx = H \times \frac{[V1+ (0.01 \times M \times W)]}{4} \times W \times \frac{V2+V3}{V3} \times \frac{1}{G}$$

Where:

H = Hx from standard curve
M = Moisture content of fish expressed in percentage
V1 = Volume (mL) of perchloric acid used in 1:4 extraction
V2 = Volume (mL) of KOH/phosphate buffer used for neutralization
V3 = Volume (mL) of extract neutralized by KOH/phosphate buffer
V4 = Volume (mL) of sample extract added to test tube
W = Weight (g) of sample used in 1:4 extraction
G = Gram molecular weight of Hx, (136.1)

Statistical analysis: Values were expressed as mean±standard deviations. All analysis were carried out in triplicates.

RESULTS AND DISCUSSION

Post-mortem deteriorative changes in seafood samples were formerly assessed by evaluating their sensory quality, microbial population and chemical changes. Spoilage is the result of a whole series of complicated deteriorative changes brought about in dead fish tissue by their own enzymes, by bacteria and by chemical action[18]. The early reaction of spoilage is autolytic and bacterial enzymes become progressively more active during the later stages[19].

The concentration of volatile amines and the pH values of the samples are shown in Table 1. TMA-N, TVB-N and pH increases during frozen storage, causing loss of freshness and spoilage acceleration as a result of enzymatic and microbiological activity. The initial quality of TMA-N and TVB-N depends on many factors, including fish handling before freezing and it is a spoilage index indicating the beginning of the spoilage process. Thus the limits for some economically important species are already known. In most frozen stored fish the allowable maximum quantity of TMA-N

Table 1: TMA-N, TVB-N contents and pH in refrigerated sea foods

	Days of storage			
Name of the sea foods	Initial	10	20	30
TMA-N content (mgN/100 g)				
Stolephorus commersonnii	nil	nil	1.80±0.10	9.65±0.17
Portunus sanguinolentus	nil	nil	0.09±0.11	5.70±0.3
Scomberomorus koreanus	nil	0.05±0.01	1.37±0.60	7.20±0.03
TVB-N content (mgN/100 g)				
Stolephorus commersonnii	nil	nil	6.54±0.13	22.05±0.3
Portunus sanguinolentus	nil	nil	16.50±0.40	27.28±1.53
Scomberomorus koreanus	nil	2.42±0.05	12.11±2.10	19.63±1.22
pH				
Stolephorus commersonnii	6.51±1.55	6.53±1.11	6.58±0.05	6.97±2.8
Portunus sanguinolentus	6.53±2.3	6.56±8.2	6.59±1.90	6.70±2.22
Scomberomorus koreanus	6.85±0.5	6.87±0.9	6.93±2.10	7.05±0.6

*Values were expressed as Mean±standard deviations

Table 2: Hypoxanthine content of refrigerated (-4°C) sea foods

	Hypoxanthine content (mg/100 g)			
		Days of storage		
Name of the sea foods	Initial	10	20	30
Stolephorus commersonnii	1.45±0.09	3.38±0.01	3.94±2.1	7.05±0.05
Portunus sanguinolentus	0.98±1.12	5.11±0.17	6.19±0.11	7.81±0.09
Scomberomorus koreanus	2.33±0.04	3.05±0.1	5.72±1.23	6.47±0.15

* Values were expressed as mean±standard deviations

is 10-15 mgN/100 g and the TVB-N acceptability limits are 30-35 mgN/100 g wet weight[20]. TMA-N concentration in fresh fish is low and it increases progressively in later stages of spoilage and therefore, not a suitable indicator of quality of fish stored in ice for less than 10 days[21]. But most of the researchers use the volatile amines production in stored fishes as a primary quality indicator. TVB-N values of fish species increased during the storage period but it was observed only after 10 days. In the present study, initially the TMA-N and TVB-N content of stored frozen seafood was below the detectable level and after 10 days storage it gradually increased to 0.05 mg N/00 g (TMA-N) in *Scomberomorus koreanus.* A TVB-N content of 2.42 mg N/100 g was found in the frozen samples of *Scomberomorus koreanus* after 10 days. Amplified levels of both TMA-N and TVB-N formation were observed in stored seafood after 20 days. The present study agrees with the results of Simat *et al.*[22], who stated that volatile amines production occurred in fishes stored for long during the later stages of their storage. Kamal *et al.*[23] reported that TVB-N increased from 5.60-27.20 mg/100 g in frozen Hilsa fish at -20°C throughout 75 days and it was detected after 15 days. Some other researchers found that in pike fish TVB-N value was 4.19 at the end of 17 days but increased to 14.90 at the end of 7 months storage at -18°C[24]. This lower increase in volatile amines might have resulted from lower storage temperature. Amegovu *et al.*[25] reported that freezing inhibits bacterial activity and so is expected to inhibit TMA accumulation too. Orak and Kayisoglu[26] observed that little change occurred at -5°C and none at -12 and -20°C in frozen hake (*Merluccius merluccius*). El-Deen and El-Shamery[27] found that TMA value of banana shrimp (*Penaeus merguiensis*) did not change during the 6 months storage.

In the present study, an increase in pH was observed during the storage period of the seafood samples. These results are in accordance with Erkan and Ozden[28], who stated that the increase was due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes. Obemeata *et al.*[19] observed that the increase in pH was higher in stored sample of Tilapia at -4°C, indicating that the biochemical and microbial changes are occurring faster in the stored fish at -4°C. Pawar[29] reported a slightly increased pH in *Catla catla* from 6.50-6.79 when stored at chilled temperature of -2 to -4°C. The change in pH of fish muscle is usually a good index for quality assessment. The increase in pH is caused by the enzymatic degradation of fish muscle. The highest pH value was obtained in *Scomberomorus koreanus*. Lakshmanan *et al.*[30] reported that there was a decline in pH after 36 weeks in whole, gutted and fillet frozen rock cod, *Epinephelus* species at -20°C. In catfish the muscle pH values after frozen storage at various time intervals were not significantly different from fresh muscle pH[31]. In the present study, a slight increase was observed in seafood throughout the 30 days of storage.

Shortly after the harvest, chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules[32]. Ozyurt *et al.*[33] stated that textural quality was reduced by the action of autolytic enzymes during the early stages of deterioration but it did not produce the characteristic spoilage off-odors and off-flavors. This indicates that autolytic degradation can limit shelf-life and product quality even with relatively low levels of spoilage organisms[34]. Most of the impact is on the textural quality in addition to the production of hypoxanthine and formaldehyde. The digestive enzymes cause extensive autolysis which results in meat softening and rupture of the belly wall. It also results in the draining out of the blood water which contains both protein and oil[35].

The freshness index was determined on the basis of the results of the content in muscle tissue of ATP as well as the products of its breakdown viz. ADP, AMP, IMP, inosine and hypoxanthine[36]. This index is a good indicator of meat freshness although it is specific to particular species[37]. During the initial stage of storage hypoxanthine (Hx) begins to accumulate; volatile amines are absent initially but they can be increased during later stage of the storage period. The hypoxanthine content of the collected seafood samples initially and during the storage is presented in Table 2. In the present study initially *Stolephorus commersonnii* fish sample showed the Hx level of 1.45 mg/100 g and it increased to

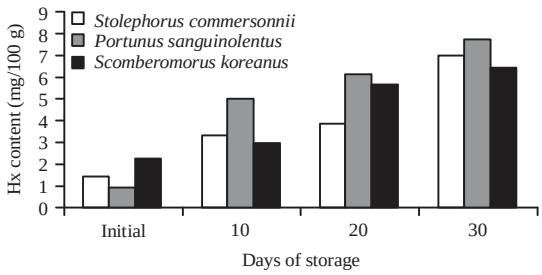

Fig. 3: Hypoxanthine content during the storage

7.05 mg/100 g during 30 days of storage at -4°C. In the case of *Scomberomorus koreanus* sample Hx content was 2.33 and it increased to 6.47 mg/100 g and in *Portunus sanguinolentus*, Hx was 0.98 in fresh crab and it increased to 7.581 mg/100 g during 30 days of storage (Fig. 3). An increase in ATP levels triggers the onset of rigor mortis and the accompanying nucleotide degradation.

Initially, the Hx concentration was noticed during the storage period but the TMA-N and TVB-N were absent in all the samples. Hx concentration increases with progressive spoilage of the sea foods. Ozogul and Balikci[38] reported that the acceptable limit of Hx in sea food was 4 mg/100 g whereas the unacceptable limit was 6-9 mg/100 g for consumption. However actual levels of Hx have been found to be dependent on genus, sampling procedures, seasonal differences and environmental factors[39].

Barnett *et al.*[40] reported IMP concentration in very fresh coho salmon less than 24 h of post-harvest to be greater than 7.0 mg/100 g. However, there was a decrease of IMP content in the coho salmon during storage, followed by a corresponding increase in hypoxanthine. After 9 days of storage, the changes in nucleotides appeared to affect the organoleptic attributes of the iced salmon in such a way as to cause loss of normal cooked odor and flavor characteristics due to increases of Hx. Sofyan[41] reported that increases in Hx value were noticed in Kembung fish (*Rastrelliger neglectus*) stored at 2-5°C for 12 days. Ozogul *et al.*[42] reported that the concentration of Hx increased gradually from the initial storage time for herring kept under modified atmospheric packaging (MAP) in iced storage conditions. In the present study, Hx increases almost linearly with storage time, indicating higher freshness index for frozen sea foods.

In the present study, Hx content was at the acceptable limit in all the three sea foods up to 20 in days storage in the refrigerator but after 20 days storage, all were found to be at the unacceptable limit. Greene and Bernatt-Byrne[43] studied Hx values in Pacific cod and pollock during storage. Accumulation of Hx in Pacific cod (*Gadus macrocephalus*) fillets was slower than that in Atlantic cod (*Gadus morhua*), but similar to that in North Sea cod (*Gadus callarias).* The variation in initial nucleotide contents is associated with differences among species, seasons, catching gear, stress during fish death, water temperature and the time lapse between catch, slaughter and storage[44]. Massa *et al.*[45] reported that at the beginning the Hx concentration increased during storage until the values leveled off at 4.7-5.0 M g^{-1}. The results of the present study are in concordance with those of the above-mentioned study.

Hx concentrations in hybrid striped bass irradiated with different radiation doses of 2.0, 3.0 and 20 kGy were similar to those of non-irradiated samples[46]. Brown *et al.*[47] stored rockfish and silver salmon under modified atmosphere and the level of Hypoxanthine was very erratic in modified atmosphere-stored samples and hence determined Hx cannot be eliminated at various types of storage. Since the Hx content in fish depends on species and quality of storage, it can be used as a freshness indicator of stored fishes[48]. Irradiation and MAP are good storage methods for seafoods, but Hx was not reduced using the above-mentioned methods and based on the present study it was concluded that Hx was not eliminated in refrigerated (-4°C) sea foods also.

The increase in Hx production with time is related to increase in autolytic enzymes[49]. FDA[50] indicated that Hx levels in fish relate to the length of storage and also to the amount of autolytic enzymes in the flesh. During the comparative storage study on specimens of cold-smoked salmon with reduced autolytic enzymes (with short storage) and those with normal autolytic enzymes (with long storage), it was found that 68% of the Hx in normal samples with long storage samples was due to enzymatic activity. The present study suggests that a month in the refrigerator is a prolonged storage period which increases the production of Hx and this increased Hx production, due to the nucleotide degradation of the sea foods, is responsible for the enzymatic worsening. Amos[51] reported that the first step in the degradation of adenosine triphosphate to Hx is governed by autolytic processes and the next step partly by bacterial enzymes. Duun and Rustad[52] reported that hypoxanthine can be formed by the autolytic decomposition of nucleotides and that it can be formed in fresh sterile fish muscles and the physical storage condition was one of the dependable factors for this. These inferences agree with the results of the present study.

Abbas *et al.*[53] reported that in most of the cases the enzymes involved in the breakdown of adenosine triphosphate (ATP) to inosine monophosphate (IMP) are believed to be autolytic whereas the conversion of IMP to inosine (Ino) and then to hypoxanthine (Rx) is believed to be mainly due to spoilage bacteria although Hx has been shown to accumulate slowly in sterile fish tissue. The factors which

have been shown to affect the nucleotide breakdown pattern include species, the temperature of storage and physical disruption of tissues. In addition, since nucleotide breakdown reflects the combined action of autolytic enzymes and bacterial action, the types of spoilage bacteria would no doubt affect the nucleotide patterns[54]. The present study correlates with the above findings.

Generally refrigeration storage freezes only 75% of the water present in the samples. This does not include the water which is chemically bound to specific sites such as carbonyl and amino groups of proteins and hydrogen bonding[55]. Ocano-Higuera *et al.*[56] reported on the variation of the proportion of water which is converted to ice in the muscle tissue of fish against temperature. The final quality depends on the condition of the fish at the time of freezing as well as on other factors including freezing/cold storage temperature, freezing rate and distribution. Therefore, based on the above reports the present study suggests that the bound water containing protein and lipid under enzymatic degradation (proteolysis and lipolysis) leads to the production of Hx in partially frozen sea foods.

The most crucial factor which affects the quality of refrigerated sea foods in the present study is slow freezing. Slow freezing, in comparison to fast freezing, results in the formation of large ice crystals, which damages the walls of the cells and causes denaturation of the protein. On the other hand, denaturation also depends on the concentration of enzymes and other compounds present in them[57]. The enzymatic reactions can still continue in refrigerated fish from the beginning of the storage at a temperature of -4°C[58] which involve a number of other metabolic activities such as glycolysis, nucleotide degradation and proteolysis[59]. These endogenous enzymatic activities cause intrinsic chemical and physical changes. Although enzymatic activities are slow, they can still support microbial growth and metabolism[60].

Shortly after capture, the dead fish undergoes chemical and biological changes due to enzymatic breakdown of major molecules[61]. Getu *et al.*[62] stated that autolytic enzymes reduce textural quality during early stages of deterioration but did not produce the off-odors and off-flavors characteristic of spoilage. So researchers are focusing more attention on the volatile amines and bacterial spoilage produced at the later stages rather than on the initial end product (Hx). This indicates that autolytic degradation can limit shelf-life and product quality even with relatively low levels of spoilage organisms. Tys and Pieters[63] reported that the autolytic changes occur in chilled/frozen stages. Most of the impact is on textural quality along with the production of hypoxanthine and formaldehyde. The digestive enzymes cause extensive autolysis which results in meat softening, rupture of the belly wall and draining out of the blood water which contains both protein and oil[64].

Under improper or inadequate storage conditions proteolysis is responsible for degradation of proteins and is followed by a process of solubilization[65]. At the same time, peptides and free amino acids can also be produced as a result of autolysis of muscle proteins, which lead towards the spoilage of fish meat as an outcome of microbial growth at later stage and production of hypoxanthine at the initial stage of storage[66]. Aoki and Ueno[67] reported that postmortem degradation of mackerel muscle tissue was due to the activity of Cathepsin L. enzyme liberated from lysosomes during aging for 7 days at -4°C. The enzymatic hydrolysis of fats by lipases is termed lipolysis (fat deterioration). During this process, lipases split the glycerides forming Hx initially and later it is converted to free fatty acid which is responsible for the common off flavor, frequently referred to as rancidity and reducing the oil quality[68]. The present study clearly indicates that the presence of hypoxanthine could be used as a quality indicator instead of the analysis of free fatty acids, other volatile amines and the microbial spoilage, for Hx is produced at the initial stages of storage and our results correlate with the previous studies made by other researchers.

Arannilewa *et al.*[69] investigated the effect of frozen storage on the chemical, microbiological and sensory profile of tilapia fish (*Sarotherodun galiaenus*). They reported decrease in the values of protein and fat and increase in nucleotide degradation products. Miladi *et al.*[70] reported that *Salmonella typhimurium* and *Anasakis simplex* (nematode) can be killed by freezing of fish but the nucleotide breakdown was not reduced in refrigerated storage.

Al-Jasser and Al-Jasass[71] stated that freezing and cold storage are efficient methods of fish preservation but they do not improve product quality. It is necessary to preserve the fish at 0°C after the catch as its spoilage is very rapid[72]. Humaid and Jamal[73], put forward two possibilities for storing fresh fish at low temperatures: cooling at -1 to +4°C, which inhibits the growth of microorganisms; and freezing at -18 to -30°C, which completely stops the growth of bacteria and nucleotide degradation. Seafood under prolonged refrigeration is highly prone to nucleotide breakdown and Hx formation.

CONCLUSION

The present study was carried out to determine Hx as the best spoilage indicator when compared with the other commonly used indicators (TMA-N and TVB-N).This study clearly shows that Hx is formed at the initial stage of storage

period though the rate of formation varies from species to species. So, it is concluded that the nucleotide degradation product is increased in refrigerated product and can only be used as a temporary method of preservation. Therefore, for long time storage of seafood the household refrigerator is not recommended.

SIGNIFICANCE STATEMENT

This study discovers the content of hypoxanthine in seafood stored in refrigerators and compare it with the production of volatile amine at (-4°C) and to determine which method is suitable for monitoring the quality of frozen stored seafood and that can be beneficial for seafood consumers and exporters. This study will help the researcher to uncover the critical areas of best quality indicators on the post harvest processing of seafood's under storage that many researchers were not able to explore. Thus a new theory on quality indicators of stored food may be arrived at.

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REFERENCES

1. Sanjee, S.A. and M.E. Karim, 2016. Microbiological quality assessment of frozen fish and fish processing materials from Bangladesh. Int. J. Food Sci. 10.1155/2016/8605689.
2. Aberoumand, A., 2013. Impact of freezing on nutritional composition of some less known selected fresh fishes in Iran. Int. Food Res. J., 20: 347-350.
3. Gandotra, R., M. Koul, S. Gupta and S. Sharma, 2012. Change in proximate composition and microbial count by low Temperature preservation in fish muscle of *Labeo rohita* (Ham-Buch). IOSR J. Pharm. Biol. Sci., 2: 13-17.
4. Aberoumand, A. and H. Jooyandeh, 2010. Storage quality and chemical and structural changes of fresh and frozen-thawed fish. World J. Fish Mar. Sci., 2: 251-253.
5. Daramola, J.A., E.A. Fasakin and E.O. Adeparusi, 2007. Changes in physicochemical and sensory characteristics of smoke-dried fish species stored at ambient temperature. Afr. J. Food Agric. Nutr. Dev., 7: 1-16.
6. Ayas, D. and Y. Ozogul, 2011. The effects of sex and seasonality on the metal levels of different muscle tissues of mature Atlantic blue crabs (*Callinectes sapidus*) in Mersin Bay, North-Eastern Mediterranean. Int. J. Food Sci. Technol., 46: 2030-2034.
7. Yilmaz, A.B., M.K. Sangun, D. Yaglıoglua and C. Turan, 2010. Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from Iskenderun Bay, Turkey. Food Chem., 123: 410-415.
8. Ghaly, A.E., D. Dave, S. Budge and M.S. Brooks, 2010. Fish spoilage mechanisms and preservation techniques: Review. Am. J. Applied Sci., 7: 859-877.
9. Gui, M., J. Song, Z. Zhang, P. Hui and P. Li, 2014. Biogenic amines formation, nucleotide degradation and TVB-N accumulation of vacuum-packed minced sturgeon (*Acipenser schrencki*) stored at 4°C and their relation to microbiological attributes. J. Sci. Food Agric., 94: 2057-2063.
10. Jinadasa, B.K.K.K., 2014. Determination of quality of marine fishes based on Total Volatile Base Nitrogen test (TVB-N). Nat. Sci., 12: 106-111.
11. Zhong-Yi, L., L. Zhong-Hai, Z. Miao-Ling and D. Xiao-Ping, 2010. Effect of fermentation with mixed starter cultures on biogenic amines in bighead carp surimi. Int. J. Food Sci. Technol., 45: 930-936.
12. Rodriguez, M.B.R., C.D.S. Carneiro, M.B.D.S. Feijo, C.A.C. Junior and S.B. Mano, 2014. Bioactive amines: Aspects of quality and safety in food. Food Nutr. Sci., 5: 138-146.
13. Hu, Y., Z. Huang, J. Li and H. Yang, 2012. Concentrations of biogenic amines in fish, squid and octopus and their changes during storage. Food Chem., 135: 2604-2611.
14. Park, J.S., C.H. Lee, E.Y. Kwon, H.J. Lee, J.Y. Kim and S.H. Kim, 2010. Monitoring the contents of biogenic amines in fish and fish products consumed in Korea. Food Control, 21: 1219-1226.
15. Siang, N.C. and L.L. Kim, 1992. Determination of Trimethylamine Oxide, Trimethylamine and Total Volatile Basic Nitrogen by Conways Micro-Diffusion Method. In: Laboratory Manual on Analytical Methods and Procedures for Fish and Fisheries Products, Miwa, K. and L.S. Ji (Ed.). Southeast Asia Fisheries Development Center, Thailand, pp: B3.1-B3.6.
16. Woyewoda, A.D., S.J. Shaw, P.J. Ke and B.G. Burns, 1986. Recommended laboratory methods for assessment of fish quality. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1448, Department of Fisheries and Oceans, Canada.
17. Luong, J.H.T., K.B. Male, C. Masson and A.L. Nguyen, 1992. Hypoxanthine ratio determination in fish extract using capillary electrophoresis and immobilized enzymes. J. Food Sci., 57: 77-81.
18. Koral, S., S. Kose and B. Tufan, 2009. Investigating the quality changes of raw and hot smoked garfish (*Belone belone euxini*, Gunther, 1866) at ambient and refrigerated temperatures. Turk. J. Fish Aquat. Sci., 9: 53-58.
19. Obemeata, O., P. Nnenna and N. Christopher, 2011. Microbiological assessment of stored *Tilapia guineensis*. Afr. J. Food Sci., 5: 242-247.

20. Ali, M.Y., M.I. Sharif, R.K. Adhikari and O. Faruque, 2010. Post mortem variation in total volatile base nitrogen and trimethylamine nitrogen between Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*). Univ. J. Zool. Rajshahi Univ., 28: 7-10.
21. Joshi, P.A. and V.S. Bhoir, 2011. Study of histamine forming bacteria in commercial fish samples of Kalyan city. Int. J. Curr. Sci. Res., 1: 39-42.
22. Simat, V., J. Marsic-Lucic, M. Tudor and I. Mladineo, 2009. Long-term storage influence on volatile amines (TVB-N and TMA-N) in sardines and herring utilized as food for tuna fattening. J. Applied Ichthyol., 25: 766-770.
23. Kamal, M., M.N. Islam, M.A. Mansur, M.A. Hossain and M.A.I. Bhuiyan, 1996. Biochemical and sensory evaluation of Hilsa fish (*Hilsa ilisha*) during frozen storage. Indian J. Mar. Sci., 25: 320-323.
24. Olgunoglu, I.A., A. Polat and I. Var, 2002. Chemical and sensory changes of pike-perch (*Sander lucioperca* Bogustkaya & Naseka, 1996) fillets during frozen storage (-18°C). Turk. J. Vet. Anim. Sci., 26: 879-884.
25. Amegovu, A.K., M.L. Sserunjogi, P. Ogwok and V. Makokha, 2012. Nucleotide degradation products, total volatile basic nitrogen, sensory and microbiological quality of nile perch (*Lates niloticus*) fillets under chilled storage. J. Microbiol. Biotechnol. Food Sci., 2: 653-666.
26. Orak, H.H. and S. Kayisoglu, 2008. Quality changes in whole, gutted and filleted three fish species (*Gadus euxinus*, *Mugil cephalus*, *Engraulis encrasicholus*) at frozen storage period (-26°C). Acta Scient. Pol. Technol. Aliment., 7: 15-28.
27. El-Deen, G. and M.R. El-Shamery, 2010. Studies on contamination and quality of fresh fish meats during storage. Egypt. Acad. J. Biol. Sci., 2: 65-74.
28. Erkan, N. and O. Ozden, 2008. Quality assessment of whole and gutted sardines (*Sardina pilchardus*) stored in ice. Int. J. Food Sci. Technol., 43: 1549-1559.
29. Pawar, P., 2011. Preparation of battered and breaded product from fresh water fish catla (*Catla catla*). M.F.Sc. Thesis, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth(Agricultural University), Dapoli, Maharashtra, India.
30. Lakshmanan, P.T., P.R.G. Varma, T.S.G. Iyer and K. Gopakumar, 1990. Quality changes in seafrozen whole and filleted rock cod (*Epinephelus* spp.) during storage. Fish. Res., 9: 1-12.
31. Abbas, K.A., A. Mohamed, B. Jamilah and M. Ebrahimian, 2008. A review on correlations between fish freshness and pH during cold storage. Am. J. Biochem. Biotehcnol., 4: 419-421.
32. Boran, M. and S. Kose, 2007. Storage properties of three types of fried whiting balls at refrigerated temperatures. Turk. J. Fish. Aquat. Sci., 7: 65-70.
33. Ozyurt, G., E. Kuley, S. Ozkutuk and F. Ozogul, 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. Food Chem., 114: 505-510.
34. FAO., 2012. The State of World Fisheries and Aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome, Italy, ISBN-13: 9789251072257, Pages: 209.
35. FAO., 2013. Fishery and aquaculture country profiles: Sri Lanka. Food and Agriculture Organization of the United Nations, Rome, Italy.
36. Hong, H., J.M. Regenstein and Y. Luo, 2017. The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: A review. Crit. Rev. Food Sci. Nutr., 57: 1787-1798.
37. Al Bulushi, I., S. Poole, H.C. Deeth and G.A. Dykes, 2009. Biogenic amines in fish: Roles in intoxication, spoilage and nitrosamine formation-a review. Crit. Rev. Food Sci. Nutr., 49: 369-377.
38. Ozogul, Y. and E. Balikci, 2013. Effect of various processing methods on quality of mackerel (*Scomber scombrus*). Food Bioprocess Technol., 6: 1091-1098.
39. Kumolu-Johnson, C.A., N.F. Aladetohun and P.E. Ndimele, 2010. The effects of smoking on the nutritional qualities and shelf-life of *Clarias gariepinus* (BURCHELL 1822). Afr. J. Biotechnol., 9: 73-76.
40. Barnett, H.J., R.W. Nelson, P.J. Hunter, S. Bauer and H. Groninger, 1991. Studies of the use of carbon dioxide dissolved in refrigerated brine for the preservation of whole fish. Fish. Bull., 69: 433-442.
41. Sofyan, I.R., 1978. Biochemical effects of gamma radiation on Kembung fish (*Rastrelliger neglectus*). Proceedings of the International Symposium on Food Preservation by Irradiation, November 21-25, 1977, Wageningen, Netherlands, pp: 407-410.
42. Ozogul, F., K.D.A. Taylor, P.C. Quantick and Y. Ozogul, 2000. A rapid HPLC-determination of ATP-related compounds and its application to herring stored under modified atmosphere. Int. J. Food Sci. Technol., 35: 549-554.
43. Greene, D.H. and E.I. Bernatt-Byrne, 1990. Adenosine triphosphate catabolites as flavor compounds and freshness indicators in Pacific cod (*Gadus macrocephalus*) and pollock (*Theragra chalcogramma*). J. Food Sci., 55: 257-258.
44. Ozogul, F., Y. Ozogul and E. Kuley, 2007. Nucleotide degradation in Sardine (*Sardina pilchardus*) stored in different storage condition at 4°C. J. Fish. Sci., 1: 13-19.
45. Massa, A.E., D.L. Palacios, M.E. Paredi and M. Crupkin, 2005. Postmortem changes in quality indices of ice-stored flounder (*Paralichthys patagonicus*). J. Food Biochem., 29: 570-590.
46. Karahadian, C., R.G. Brannan and J.L. Heath, 1997. Electron beam irradiation, oxygen and temperature effects on nucleotide degradation in stored aquaculture hybrid striped bass fillets. J. Food Qual., 20: 157-169.
47. Brown, W.D., M. Albright, D.A. Watts, B. Heyer, B. Spruce and R.J. Price, 1980. Modified atmosphere storage of rockfish (*Sebastes miniatus*) and silver salmon (*Oncorhynchus kisutch*). J. Food Sci., 45: 93-96.

48. Andrade, S.D.C.S., E.T. Marsico, R.L. de Oliveira Godoy, R.M. Franco and C.A.C. Junior, 2014. Chemical quality indices for freshness evaluation of fish. J. Food Stud., 3: 71-87.
49. Morkore, T., M. Rodbotten, G. Vogt, S.O. Fjaera, I.O. Kristiansen and E. Manseth, 2010. Relevance of season and nucleotide catabolism on changes in fillet quality during chilled storage of raw Atlantic salmon (*Salmo salar* L.). Food Chem., 119: 1417-1425.
50. FDA., 2009. Food: Generally recognized as safe (GRAS). U.S. Food and Drug Administration, Silver Spring, MD., USA.
51. Amos, B., 2007. Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement. United Nations University, Uganda. http://www.unuftp.is/static/fellows/document/amos06prf.pdf
52. Duun, A.S. and T. Rustad, 2007. Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. Food Chem., 105: 1067-1075.
53. Abbas, K.A., A.M. Saleh, A. Mohamed and O. Lasekan, 2009. The relationship between water activity and fish spoilage during cold storage: A review. J. Food Agric. Environ., 7: 86-90.
54. Diei-Ouadi, Y. and Y.I. Mgawe, 2011. Post-harvest fish loss assessment in small-scale fisheries: A guide for the extension officer. FAO Fisheries and Aquaculture Technical Paper 559, Food and Agriculture Organization of the United Nations, Rome, Italy.
55. Ahmed, A.A., 2008. Post-harvest losses of fish in developing countries. Nutr. Health, 19: 273-287.
56. Ocano-Higuera, V.M., E. Marquez-Riosb, M. Canizales-Davilaa, F.J. Castillo-Yaneza and R. Pacheco-Aguilar, 2009. Postmortem changes in cazon fish muscle stored on ice. Food Chem., 116: 933-938.
57. Click, M.A. and R. Ridberg, 2010. Saving food: Food preservation as alternative food activism. Environ. Commun., 4: 301-317.
58. Panchakshari, V., P.V. Krishna, K.M. Rao and K. Prabhavathi, 2015. Microbiological and biochemical observations of *Lates calcarifer* in different types of processing with Hurdle technology. Int. J. Adv. Res., 3: 1232-1235.
59. Wang, Q., C. Xue, Z. Li, X. Fu, J. Xu and Y. Xue, 2007. Changes in the contents of ATP and its related breakdown compounds in various tissues of oyster during frozen storage. J. Ocean Univ. China (Engl. Edn.), 6: 407-412.
60. Castillo-Yanez, F.J., E.I. Jimenez-Ruiz, D.F. Canizales-Rodriguez, E. Marquez-Rios, N. Montoya-Camacho, S. Ruiz-Cruz and V.M. Ocano-Higuera, 2014. Postmortem biochemical changes and evaluation of the freshness in the muscle of tilapia (*Oreochromis niloticus*) during the storage in ice. J. Fish. Aquat. Sci., 9: 435-445.
61. Pacquit, A., J. Frisby, D. Diamond, K.T. Lau, A. Farrell, B. Quilty and D. Diamond, 2007. Development of a smart packaging for the monitoring of fish spoilage. Food Chem., 102: 466-470.
62. Getu, A., K. Misganaw and M. Bazezew, 2015. Post-harvesting and major related problems of fish production. Fish. Aquacult. J., Vol. 6. 10.4172/2150-3508.1000154.
63. Tys, D. and M. Pieters, 2009. Understanding a Medieval Fishing Settlement along the Southern North Sea: Walraversijde, C. 1200-1630. In: Beyond the Catch: Fisheries of the North Atlantic, the North Sea and the Baltic, 900-1850, Sicking, L. and D. Abreu-Ferreira (Eds.). Brill Publ., Leiden, The Netherlands, ISBN-13: 9789004169739, pp: 91-122.
64. Kabaherda, M.K., P. Omony and S.M.C. Hiisken, 2009. Post-harvest handling of low-value fish products and threats to nutritional quality: A review of practices in the Lake Victoria region. Regional Programme Fisheries and HIV/AIDS in Africa: Investing in Sustainable Solutions, The WorldFish Center, Project Report 1975.
65. Nemova, N.N., L.A. Lysenko and N.P. Kantserova, 2016. Degradation of skeletal muscle protein during growth and development of salmonid fish. Russian J. Dev. Biol., 47: 161-172.
66. Zhou, G.H., X.L. Xu and Y. Liu, 2010. Preservation technologies for fresh meat-A review. Meat Sci., 86: 119-128.
67. Aoki, T. and R. Ueno, 1997. Involvement of cathepsins B and L in the post-mortem autolysis of mackerel muscle. Food Res. Int., 30: 585-591.
68. Nieva-Echevarria, B., E. Goicoechea, M.J. Manzanos and M.D. Guillen, 2017. Fish *in vitro* digestion: Influence of fish salting on the extent of lipolysis, oxidation and other reactions. J. Agric. Food Chem., 65: 879-891.
69. Arannilewa, S.T., S.O. Salawu, A.A. Sorungbe and B.B. Ola-Salawu, 2005. Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (*Sarotherodun galiaenus*). Afr. J. Biotechnol., 4: 852-855.
70. Miladi, H., K. Chaieb, A. Bakhrouf, N. Elmnasser and E. Ammar, 2008. Freezing effects on survival of *Listeria monocytogenes* in artificially contaminated cold fresh-salmon. Annals Microbiol., 58: 471-476.
71. Al-Jasser, M.S and F.M. Al-Jasass, 2014. Study the chemical, physical changes and microbial growth as quality measurement of fish. Annu. Res. Rev. Biol., 4: 1406-1420.
72. Alasalvar, C., K. Miyashita, F. Shahidi and U. Wanasundara, 2011. Handbook of Seafood Quality, Safety and Health Applications. Blackwell Publishing Ltd., New Delhi, India, ISBN-13: 9781444347760, pp: 13-29.
73. Humaid, A.A. and M.T. Jamal, 2014. The effect of storage temperature (4°C, 15°C and 25°C) on the shelf life of whole marine fish (*Rastrelliger kanagurta*). IOSR J. Environ. Sci. Toxicol. Food Technol., 8: 46-71.