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EFFECT OF ICE STORAGE ON FREE AMINO ACIDS OF VARIOUS EDIBLE FISHES

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Free amino acids (FAA) of six marine fish muscle tissues were separated on polyamide sheet by "Dansylation" in fresh state, after 1 week (8-day) and 2 weeks (15-day) ice storage. Sensory evaluation of the fish tissue was also carried out at appropriate intervals in order to find relationship between the FAA content and the sensory quality during the test period.

The patterns of FAA were found to be characteristic for different species of the fish examined. Predominant FAA present in almost all the fish species examined were : taurine, proline, glycine, alanine, valine, isoleucine, leucine, threonine and serine. During ice storage, the FAA in white pomfret (*Chondroplites sp.*), catfish (*Arius spp*), flat fish (*Pseudorhombus spp.*) decreased during the 1st week and then increased in the 2nd week. However, in mackerel (*Scomber spp.*), snapper (*Lutijanus spp.*) and Indian shad (*Hilsa spp.*). FAA decreased gradually upto 2 weeks. With the exception of mackerel, the FAA isoleucine, leucine and valine either decreased or disappeared during the 1st week of storage in all other fish examined showing a relationship with sensory properties. No remarkable accumulation of individual FAA was found during ice storage. The ammonia content was also high in all species. No distinct change in taurine content was observed during ice storage.

Key words: Free amino acids, Ice storage, Edible fishes.

INTRODUCTION

Freshness is one of the principal factors influencing the flavour of fishery products. It is well known that some fish species taste better sometime after death than immediately after death, while some others taste progressively flatter with the lapse of time. This is attributed to the diversity in species specific enzymatic actions, which bring about rise and fall in the levels of FAA, nucleotides, etc. [1,2].

Enzymatic dephosphorylation of inosine 5-monophosphate (IMP) via inosine to hypoxanthine occurs within the period of edibility during ice storage and these changes are involved in the loss of desirable fresh flavours and development of better off flavour in staling fish [3].

Microbial activity is also one of causes of quality deterioration of fish and shellfish during ice storage. Several reports are available on microbiological and/or chemical changes in fish and shellfish stored in ice for various lengths of time [4-6].

Deterioration in the quality of fish muscle during frozen and iced storage has been attributed to changes in lipid and protein fractions, which are very important in fish processing industry.

Undesirable changes in flavour during the storage of fish in ice are believed to be due to the formation of low molecular weight compounds from lipid oxidation and protein degradation. Levels of some amino acids may also be affected by the leaching action of ice drip [7] and by the activity of tissue and bacterial enzymes [8]. Changes in the levels of certain amino acids such as glycine could significantly affect the taste and result in bitterness in shrimp [9,10]. It has also been shown that certain amino acids are important precursors of flavour components released when foods are heated.

Several studies have been reported on the FAA present in different foods [11-15].

A definite role of amino acids in flavour has also been proposed in various fishery products. This study was undertaken to determine qualitative changes in the FAA of some edible fish during ice storage and to determine whether these changes were associated with sensory characteristics of fish.

MATERIALS AND METHODS

The fishes used in this study (Table I) were caught by the traditional fishing methods. Fishing was confined to 10-15 km off the Karachi-Makran coast by boats making trips of 5-10 days. A period of 5 day elapsed between the catching and landing of fish.

All fishes were covered with ice immediately after purchase and transported to the Laboratories, where they were gutted, deheaded and packed in clean polythene bags, placed in crushed ice and stored in crushed ice and stored in the refrigerator maintained at 5 ± 1°. Melted ice was drained off every day and ice was continuously replenished. After definite time intervals one bag of each fish was removed, washed in running tap water and three fish from each group were deboned, skinned, pooled together and analysed for FAA and sensory quality. All fishes used were of common size for the particular species ranging from 30-35 cm in length.

Sensory analysis. Sensory evaluation was carried out by the Laboratory staff (7-member panel). Samples were placed in boiling water for 5 min. and their appearance visually judged. They were then tasted for flavour and texture. The scoring difference test as described by Larmond [16] was used. A 9-point scale was worked out with score 9 for extremely good and 1 for very poor as judged by appearance, texture, and flavour. The score of each parameter was calculated in terms of average score points awarded by the taste panel to each sample.

Free amino acids analysis. The dansyl (dans.) amino acids were separated and identified by Thin Layer Chromatography (TLC) on polyamide layers by the technique described by Neuhoff [17]. Briefly the following method was used: 1 g tissue muscle of fish was homogenized with 2 ml of 0.05 M $NaHCO_3$ [ph 10.2) and centrifuged at 1500 rpm for 30 min. For dansylation, the supernatant was incubated for 30 min. at 37° with dansyl chloride and then dried under vacuum.

A 0.2 µl portion was applied on a polyamide sheet (5x5 cm) for two-dimensional chromatography. Formic acid/water 1.5 : 10 (v/v) was used for the first dimension and benzene/acetic acid 9:1 (v/v) was used for the second as suggested by Wood & Wang [18]. Photographs were taken under UV light.

RESULTS AND DISCUSSION

Sensory evaluation. Data obtained from the sensory evaluation performed by the seven member panel are embodied in Fig. 1. Each point on the graph represents an average score for flavour, texture and appearance. The total sensory evaluation data represent the mean score of all sensory characteristics. According to the taste panel, the O-day sample of all experimental fish were acceptable and lacked any undesirable odour. During ice storage all experimental fish lost their characteristic flavour, texture and appearance except for *surmai* (mackerel), which is

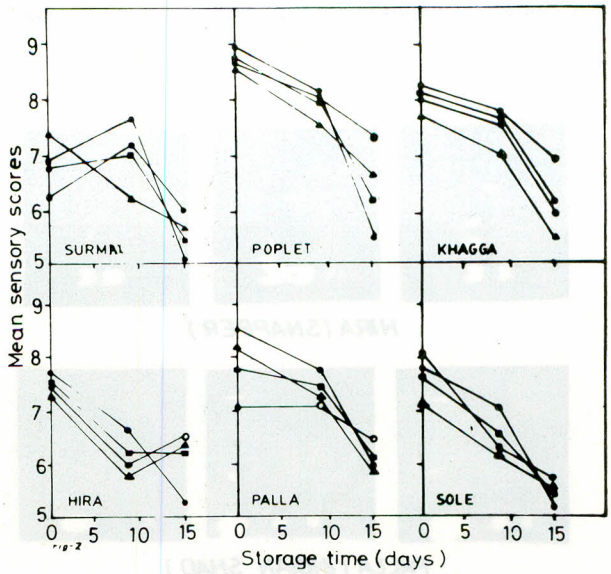

Fig. 1. Mean sensory scores assigned to different marine edible fishes stored in ice upto 15 days (2 weeks) data calculated from average scores submitted by a panel of 7 members. Total sensory scores (■) represent the combined average of flavour (.) texture (o) and acceptability (▲), 9=excellent, 8=very good, 7=good, 6=slightly good, 5=Neither good nor poor, 4=slightly poor, 3=poor, 2=very poor, 1=extremely poor.

evident by the decrease in sensory score awarded by the panel. In the case of *surmai*, the 8-day ice stored sample was awarded a higher score than the 0-day sample for flavour and texture (6.9, 6.3 at 0-day and 7.7, 7.2 at 8-day, respectively). The fifteen-day ice stored *hira* (snapper) sample was rated better and was awarded a higher socre for texture and appearance (6.2, 5.8 at 8-day 6.6, 6.4 at 15-day respectively). All other samples lost their desired sensory characteristics and were rated inferior after 2 weeks of ice storage.

Free amino acid pattern. Dansylated FAA patterns of fresh fish (0-day), one week (8-days) and 2 weeks (15-days) ice stored samples are presented in Fig. 2 and Table 1. Changes in individual fish species are discussed below.

1. *Surmai* (mackerel): Free amino acids taurine, aspartic acid, threonine, serine, proline, glutamic acid, glycine, alanine/NH_3, valine, isoleucine, leucine, tyrosine, lysine, phenylalanine and histidine, were separated in fresh state. During ice storage all these amino acids in addition to some unknown spots were present in the first week (8-day) samples. In the 2nd week (15-day) of storage, most of the amino acids disappeared with the exception of taurine, aspartic acid, alanine/NH_3 and arginine.

2. Poplet (white pomfret). At fresh state (0-day) FAA taurine, threonine, serine, proline, glycine, alanine/NH_3, valine, cystine/cysteine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, α-amino histidine and α-lysine were separated on a polyamide sheet. After one week (8-day) of ice storage tyrosine, phenylalanine disappeared, otherwise the pattern was the same with apparently less concentrations of individual amino acids. At the 2nd week (15-day) of storage the pattern was the same but showing more concentration of the acids together with some other unknown spots. Phenylalanine which disappeared during 8-day ice storage, appeared again after a 2-week storage.

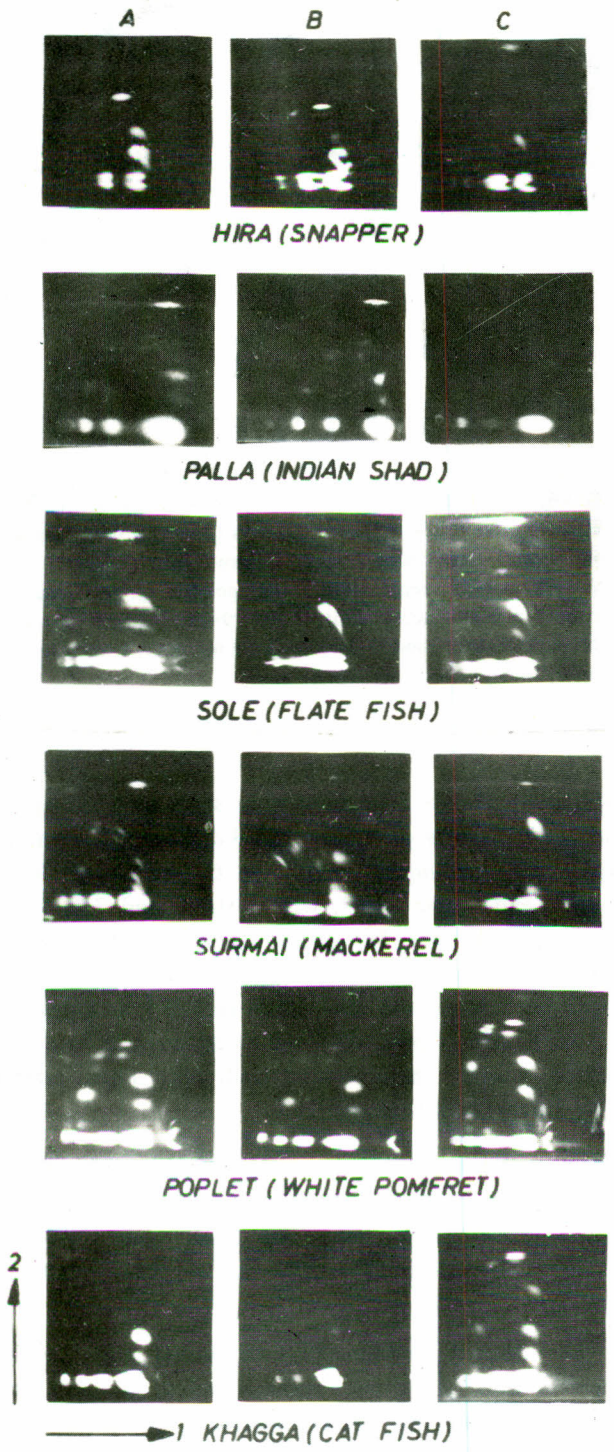

Fig. 2. Microchromatography of dansyl free amino acids in the muscles of marine fish A-fresh state B-After one-week ice storage C-After two-week ice storage.

3. *Khagga* (catfish). Free amino acids taurine, serine, proline, glycine, alanine/NH_3, valine, cystine/cysteine, isoleucine, leucine, arginine, α-amino histidine and α-lysine were separated in fresh state. After one week's ice storage all the FAA which were present in fresh state disappeared except for taurine, glycine and alanine/NH_3. At the 2nd week (15-day) of ice storage FAA taurine, serine, proline, glycine, alanine/NH_3 and cystine/cysteine were noticeable. It is worth noting that glutamine, which was absent in fresh state as well as after one week's storage was present in 2-week (15-day) ice storage.

4. *Hira* (snapper). Free amino acids taurine, threonine, serine, proline, glutamic acid, glycine, alanine/NH_3, valine, isoleucine and leucine, were separated in fresh state. The pattern during the 1st week (8-day) of ice storage shows the disappearance of only valine. During the 2nd week of ice storage, proline, glutamic acid, isoleucine and leucine also disappeared. With the exception of glycine and taurine the other amino acids were present in very small concentrations.

5. *Palla* (Indian shad). In fresh state (0-day) free amino acids, taurine, threonine, proline, glutamic acid, glycine, alanine/NH_3, valine, isoleucine, leucine, phenylalanine and lysine were detected on a polyamide sheet. After one-week (8-day) of ice storage also all the FAA which were present in the fresh state could be detected, although in lesser concentrations. After 2 week's storage all the FAA disappeared with the exception of alanine/NH_3. This is the only case in which taurine disappeared after 2 weeks (15 days) of ice storage.

6. Sole (flat fish). In fresh state (0-day) FAA taurine, serine, threonine, proline, glutamic acid, glycine, alanine/NH_3, valine, cystine/cysteine, phenylalanine, lysine, arginine, α-amino histidine and α-lysine were separated. After one weeks ice storage all the free amino acids which were present in fresh state disappeared with the exception of taurine and alanine/NH_3. The concentration of alanine/NH_3 increased significantly. After 15 days of ice storage taurine, threonine, serine, proline, glutamic acid glycine and

alanine/NH_3 were separated.

Dansylated FAA taurine, proline, glycine, alanine/NH_3, valine, isoleucine, and leucine were separated on a polyamide sheet in all the fish examined with the following exceptions. There was no threonine in *khagga,* no serine in *palla* and no lysine in *hira.*

It may be seen that all fish species examined have a characteristic FAA pattern (Fig. 2, Table 1). All precautions were taken to obtain fresh samples and were presented to a panel after the same treatment. The only difference was the species of the fish examined. The panelists awarded different scores to different samples. It can be concluded that FAA pattern has an influence on flavour of the samples.

It has been reported that the contents of FAA varies not only from species to species but also from one specimen to another [19]. This depends not only on environmental conditions [20], but also on the size of individual specimen, seasonal and salinity conditions [21-23]. Colly *et al.* [24] and Ahokas & Sory [25] reported that FAA play a significant role in osmoregulation in fish and in many species of shellfish, and that their amounts increase or decrease in accordance with change in external salinity. During osmoregulation fluctuations in the amounts of taurine, glutamic acid (including glutamine), glycine, alanine and proline have been reported [26, 27]. It has been generally accepted that a species of fish or shellfish shows different taste when salinity varies. This may be due to variations in the level of FAA. It has been reported that in shrimp the sweetness depends on the glycine content of the muscle [28]. Indeed, the flavour of cultured specimen of a fish has been reported to be quite different from that of corresponding wild specimen of the same species [29].

Effect of storage on FAA pattern. As already stated, there was no apparent difference between the FAA pattern of fresh state (0-day) and one week (8-days) of ice stored samples of *surmai* (mackerel). After one week of ice storage some extra spots were also visible. The stability of FAA

Table 1. Effect of ice storage on *free amino acids of various edible fishes.

	Ice storage time	Dans-taurine	Dans-aspartic acid	Dans-threonine	Dans-serine	Dans-glutamine	Dans-proline	Dans-glutamic acid	Dans-glycine	Dans-alanine/NH_2	Dans-valine	Dans-cystine/ cysteine	Dans-Isoleucine	Dans-leucine	Dans-tyrosine	Dans-Phenylalanine	Dans-lysine	Dans-histidine	Dans-(arginine α-amino histidine α-lysine)	No of un-identified spots	Total spots
Surmai	F	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	++	+	2	18
(Mackerel)	I	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	4	20
(*Scomber sp.*)	II	+	+	–	–	–	–	–	–	++	–	–	–	–	–	–	–	–	+	5	9
Poplet	F	+	–	+	+	–	++	–	++	+	++	+	++	++	+	+	+	++	+	5	20
(White Pomfret)	I	+	–	+	+	–	+	–	+	+	+	+	+	+	–	–	+	+	+	1	14
(*Chondroplites* sp.)	II	+	–	++	+	–	++	–	+	+	++	+	++	++	–	+	+	++	+	10	24
Khagga	F	+	–	–	+	–	+	–	++	+	+	+	+	+	–	–	+	–	+	5	16
(Cat Fish)	I	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	2	5
(*Arius sp.*)	II	+	–	–	+	+	+	–	+	+	–	+	–	–	–	–	–	–	–	8	15
Hira	F	+	–	+	+	–	++	+	+	+	+	–	+	+	–	–	–	–	–	6	16
(Snapper)	I	+	–	+	+	–	+	+	+	+	–	–	+	+	–	–	–	–	–	5	14
(*Lutijanus sp.*)	II	+	–	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	7	12
Palla	F	+	–	+	–	–	+	+	+	+	+	–	+	+	–	+	+	–	–	5	16
(Indian Shad)	I	+	–	+	–	–	+	+	+	+	+	–	+	+	–	–	+	–	–	7	17
(*Hilsa sp.*)	II	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	5	6
Sole	F	+	–	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–	+	3	15
(Flat Fish)	I	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	3	5
(*Pseudorhombus sp.*)	II	+	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	8	15

F = Fresh 1 = 1st week; II = 2nd week; *Value represent average of 2-determinations.

during ice storage coincided with increase in the scores of flavour and texture (7.2, 7.6 at 8 days and 6.9, 6.5 at 0 day) showing a relationship between FAA and sensory quality. On the contrary the concentration of FAA decreased remarkably in all other samples after 8 days of ice storage and the same was the case with the scores awarded for texture and flavour confirming the influence of FAA on sensory quality.

Development of rancidity affecting flavour is a problem during the storage of fatty fish, whereas such a problem is not frequently encountered in lean fish. The improvement of flavour during the storage of mackerel appears interesting. A possible explanation may be that the fish used in this study had low fat, since seasonal variation in lipid content has been reported in different fish varieties [30]. It is also possible that nucleotides play a role in flavour enhancement in this case in the initial stages of a storage and thereafter hypoxanthine accumulation takes place and the quality deteriorates.

In this study, an improvement in the texture of *hira* was noted after 15 days of ice storage. Changes in texture that take place after death during storage are due to the action of certain endogenous enzymes. However, the process of spoilage is so complex that there may be other factors interacting to produce this effect. Certainly more information is required to clarify the position.

Hira (snapper), however, did not show any loss of glycine similar to the loss of other FAA in the 2nd week (15 days). This was the case with the sensory score for texture assigned to the 8-day (6.0) and 15-day (6.3) stored samples also. However the flavour score dropped. It confirms the finding of Jiang and Lee [31] that the free glycine content in the muscle affects the stability of muscle protein during frozen storage.

During the first week of ice storage, a decrease in the levels of free lysine, leucine, isoleucine and valine and a loss in the scores for flavour and texture was noted in all the samples except for *surmai* (mackerel). A decrease in lysine, leucine, isoleucine and valine in these samples indicates an association of these acids with the flavour. It is possible that the degradative products of these acids may have contributed to changes in the flavour of the sample.

It has been reported [32,33] that certain amino acids are converted to substances imparting undesirable odours and affecting the flavour of fishery products. For instance, lysine is converted to cadaverine, transformed to piperidine and amino piperidine as well as pyridine, isoleucine to keto methyl-η-valeric acid and butyric acid.

No distinct change in the taurine content of the fish samples was observed except in the *palla* (Indian shad) two-week (15-day) ice stored sample, where the taurine level was appreciably low as compared to the fresh sample. Non-protein nitrogen (NPN) compounds have been shown to affect the flavour of fish [34]. The unchanging level of taurine during ice storage in these experiments is interesting, because it is a major constituent of NPN. Taurine is resistant to the attack of microorganisms and is not found in proteins [35]. In spite of the presence of taurine during storage, almost all the samples lost their characteristic flavour and were considered inferior. From these results it appears that taurine alone is not involved in flavour retention of fish during storage.

After 15-day ice storage, all FAA increased in poplet (Pomfret), *khagga* (catfish) and Sole (flat fish), when compared with the 8-day storage samples where a slight initial decrease was observed. However, in other samples (*surmai, hira* and *palla*) a continuous reduction was observed upto 2-week (15-day) storage.

Liston [36] has reported that FAA and non-protein nitrogenous compounds (NPNc) content in fish flesh are closely associated with autolysis as well as bacterial actions in the earlier stage of spoilage. Therefore, the reduction or disappearance of FAA may be due to bacterial activity. Chung [37] and Shewan [33] reported that bacteria utilized selected amino acids for their initial growth and their primary mode of utilization seems to be oxidation and deamination. Therefore, it is possible that the invading bacteria use FAA of the fish for their initial growth. In later stages sufficient amount of FAA will be released through autolysis. This in turn allows more rapid bacterial growth and subsequent changes occur in odour and taste.

The appearance and disappearance of FAA was not similar in all the samples examined. Sensory ratings show that each fish lost its characteristic flavour with the time of storage except for "surmai". It appears that all the FAA do not contribute to flavour, but only specific FAA are responsible for flavour.

The concentration of FAA may also contribute to the specific taste of fish. Hujita *et al.* [28] reported that the sweetness of shrimp is directly related to the concentration of glycine. In this study the relationship between the concentration of FAA and flavour was noted in *surmai*, poplet and *palla*. In the case of *surmai* the concentration of FAA did not decrease at 8-day ice storage, whereas in poplet and *palla* all the FAA were present in less concentrations as compared to fresh ones. Later these samples (*poplet, khagga* and *sole*) showed an increase in FAA at the end of storage (15 days). This increase in FAA did not contribute to flavour enhancement as evidenced by the

taste panel score. It is possible that the increase in FAA may take part in the enhancement of taste but the rapid appearance of rancidity would cancel the flavour. From this study it may be concluded that changes in flavour of fish during ice storage do not entirely depend on FAA. There are many other substances such as nucleotides and related compounds [38] which have been proved to be flavour enhancers.

This limited study reports qualitative changes in FAA during the storage of selected fish samples. In order to understand the role of FAA in the enhancement/stability of flavour, a study on quantitative changes in these amino acids is also necessary.

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