BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part I.—The applicability of an improved iodine absorption and titratable acidity measurement methods to investigations on mechanism of fish soilage

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East Pakistan produces large quantities of fish of different species, the importance of which in contributing proteins of high biological values to our dietaries, has been previously pointed out by Basu, De and co-workers. [1,2] With a view to determine their suitability for providing protein-rich diet a careful examination of the various biochemical and nutritional factors has been undertaken and the present paper describes the work carried out on the assessment of the quality and spoilage of several species of East Pakistan fish regarding which no information is so far available in the literature.

The data obtained also throw light on the actual mechanism of fish spoilage which is specific for individual fish and has not been fully elucidated in the hitherto available literature, as for example, Jacobs[3] has remarked, "in the absence of comprehensive studies on the subject our knowledge in this field is very limited". It is hoped that the information in this field will be utilised for the development of fish processing industries in this region on a more scientific basis.

In the course of the present study new iodometric and titratable acidity measurement methods have been developed for evaluation of fish spoilage and by these new methods eleven species of fresh water fish have been studied in storage under ordinary laboratory conditions.

Experimental

Collection of fish:—Eleven species of fish as listed in Table I were collected from the market early in the morning when only 2 to 3 hours had elapsed after their capture from the river or tank.

Puti and Chapila fish belong to the small fish group and their average length was nearly 4 inches in the sample collected. These fish die very soon after they are caught. Rohu, Katla, Mrigale, Kali Baus and Āir collected were under 8 inches in size, while the Hilsa fish used was of full adult size as one of a smaller size could not be procured at that time.

Koi and Singi fish belong to air-breathing group which may thrive in muddy shallow water, in paddy fields and also in tanks and ponds. These fish may live for a longer time even when kept in small quantity of water. They were alive at the beginning of the experiment though died soon after.

Small size Baila still showed movements of fins and tail when collected. This fish also survives longer but not to the same extent as Koi and Singi.

Specimen samples of the same batch of fish were sliced immediately after collection and analysed for the determination of the values as detailed below and the rest was stored in an open condition in the laboratory at a temperature of 80-84°F. From this stored batch also samples were collected after 5 hours and 24 hours storage and were analysed in the same way as before.

It may be mentioned here that after 24 hours storage at the above room temperature the flesh of the above fish became soft and putrid, showing the definite onset of spoilage.

Old Iodometric Titration method:—A number of methods have been developed for the study of the fish spoilage based on the examination of the decomposition products

of protein, fat and glycogen either by autolysis or by bacterial invasion. Each method has been devised for the study of a particular product or group of products. Truttwin's[4] iodometric titration method was developed to study different oxidisable compounds having -SH group, unsaturated double bonds etc. which in ordinary condition remain in the tissues and accumulate further by degradation of tissue protein etc. during storage.

According to this method 1 gm. sample of fish, thoroughly mixed with aqueous suspension in an iodometric flask, was titrated with 0.16% and 0.08% iodine solution, in presence of starch, until a permanent blue colour persisted for fifteen minutes. A limiting value of 14 cc. of 0.08% iodine was considered admissible on the basis of the previous findings. As a result of applying this method on seven species of fish Orlandi[5] advocated the limiting value of 7 cc. of 0.01 N iodine per g. of fresh fish.

The method is time-consuming, as it is necessary to wait at least for fifteen minutes for noting the persistance of blue colour with starch. Moreover, the duplicate and triplicate determinations from the same sample did not yield very concordant results. In order to avoid these difficulties and to make the method more precise and quicker in application, the original procedure has been considerably modified in this laboratory as described below.

Improved Iodometric Titration Method.—According to the present method excess of iodine is added to the aqueous suspension of fish and unabsorbed iodine is back titrated by thiosulphate. 1 gm. of fish tissue was ground in 20cc. water with a little quartz sand and when finely ground was immediately transferred to an iodometric flask with washings by three aliquots of 20 cc. of water each time. The contents were then shaken in a gyroshaker for 15 minutes, filtered through glass wool and the volume made upto 100 cc. By trial experiments with different batches shaken for different periods from 5 to 30 minutes, it was noted that the maximum liberation of the oxidisable compounds takes place in 10 minutes shaking. But for margin of safety, shaking for a period of 15 minutes was considered adequate in the present determinations. To each 50 cc. aliquot in an iodometric flask was added 10 to 20 cc. of 0.01N iodine solution and after 10 minutes this was titrated back with 0.01N thiosulphate solution. The titration values for replicate samples of the same fish tissues varied only within a small limit of 0.05 cc.

By this method the time required was considerably reduced and the values of duplicate samples from the same tissue of each species of fish were also found to be concordant within smallest limit of error.

Determination of titratable acidity and pH.—The determination of pH of fish tissue has very often been accepted as a measure of spoilage of fish by many workers.[6–10] In the present investigation also the pH of fish tissue after 24 hours of storage has been determined. A small fleshy portion below the scale and epithelial cell was soaked in bidistilled water and the pH was measured with a micro-glass electrode in Beckmann pH-meter.

It has been observed that large amount of acids (volatile and non-volatile) accumulate in the tissues during spoilage but a part of this is buffered by the bases generated simultaneously with the acids. The determination of individual acids, like lactic acid and volatile fatty acids, has also been accepted as a measure of spoilage.[11–14] In the present investigation, however, the measure of total titratable acidity obtained, as detailed below, has been advantageously used in the determination of spoilage and the co-relation of these values with iodine absorption and pH changes. 50 cc. aliquot of aqueous suspension of 1 gm. tissue, prepared in a manner as described in the determination of iodine absorption values, was titrated against 0.01N Na_2CO_3 using phenolphthalein as an indicator.

TABLE 1.—IODINE ABSORPTION AND TITRATABLE ACIDITY VALUES OF FISH UNDER STORAGE CONDITION AT ROOM TEMPERATURE (80-84°F).

Local Name of the fish.	Zoological names.	Moisture per cent.	Iodine absorption at different storage periods. 0.01N.-I_2 absorbed in c.c.						Titratable acidity at different storage periods 0.01N.-Na_2Co_3 required in c.c.						pH values	
			0 hrs.		5 hrs.		24 hrs.		5 hrs.		5 hrs.		24 hrs.		Initial at 0 hrs.	Final at 24 hours.
			Per gm. wet fish.	Per gm. dry basis.	Per gm. wet fish.	Per gm. dry basis	Per gm. wet fish.	Per gm. dry. basis.	Per gm. wet fish.	Per gm. dry basis	Per gm. wet fish.	Per gm. dry basis	Per gm. wet fish.	Per gm. dry basis.		
1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18
Puti	.. *Barbus puntius.*	79.14	6.44	30.87	9.09	43.61	17.09	81.93	7.93	37.99	7.87	37.30	1.51	7.27	6.25	6.25
Chāpila	.. *Dano aequipinnatus.*	78.8	8.84	41.69	8.99	42.40	18.79	92.888	8.48	40.00	7.95	37.5	1.24	5.85	6.30	7.15
Rohu Naolā*	.. *Labeo rohita* ..	82.57	8.05	46.19	..	..	16.91	97.07	6.11	35.05	..	..	2.89	16.58	6.25	7.15
Kāli Baus Naolā*.	*Labeo nandina*..	74.54	8.09	31.78	..	..	13.62	53.51	8.08	21.34	..	..	3.24	12.72	6.35	7.25
Katlā Naolā*	.. *Catla catla* ..	78.88	5.54	26.24	..	..	13.15	62.28	7.08	33.55	..	..	2.15	10.19	6.25	7.0
Mrigele Naolā*	.. *Cirrhina mrigala.*	81.11	5.51	29.79	8.25	43.71	15.26	80.83	7.13	48.35	8.41	44.55	2.78	14.71	6.35	6.95
Hilsā	.. *Hilsa ilsa* ..	64.66	2.66	7.54	..	..	13.21	37.38	8.27	23.42	..	..	2.05	5.81	6.45	7.25
Bāiliā	.. *Glossogobius giuris*	78.38	6.01	27.74	8.07	37.35	17.0	78.63	5.88	27.19	4.82	22.29	3.57	16.55	6.35	6.85
Āir	.. *Mystus Aor* ..	80.2	4.34	21.92	..	..	11.94	60.30	6.79	34.29	..	..	1.92	9.70	6.20	7.05
Koi	.. *Anabastestudineus.*	76.26	7.45	31.41	..	..	24.71	104.07	5.14	21.66	..	..	3.27	13.78	6.35	6.80
Singi	.. *Pelaemonpeneus*	81.2	5.41	28.81	6.19	32.95	18.52	98.54	5.14	27.37	5.06	26.96	3.96	21.08	6.20	6.75

* Naola is the local name of these species of fish under 10 inch size.

Moisture determination.—Since the moisture contents of different species of fish vary, the results must relate to the dried substance in order to make them comparable. The moisture content for each species was determined separately.

Results

Iodine Absorption.—From the results presented in Table 1 it is noted that the absorption of 0.01N iodine per gm. of fresh fish just after purchase from the market shows a range from 2.66 cc. to 8.84 cc. per gm. wet fish i. e. 7.54 cc. to 46.19 cc. per gm. on dry basis. According to old method [5] the limiting value of 7 cc. of 0.01N I_2 per gm. wet tissue was considered as indicative of the freshness of fish. In the present case, however, the limiting value of nearly 8 cc. of 0.01N I_2 per gm. wet tissue may be considered as indicative of the freshness of fish. From these values it seems evident that the fresh fish tissues contain certain amount of oxidisable-SH group compounds and other such compounds in the free state.

After 5 hours storage, five fish as shown in the table, have been analysed with respect to the above and their iodine absorption values at that stage showed a little increase. After 24 hours storage the values for all the fish showed a high rise to the range of 11.94 cc. to 18.79 cc. per gm. of wet fish i. e. from 53.51 to 104.07 cc. per gm. dry basis.

However, the general rise in the iodine absorption values in all cases after storage for 24 hours suggests that -SH group compounds and other oxidisable compounds of like nature accumulate in a greater amount (probably by proteolysis) by spoilage during storage even in ordinary atmospheric conditions. The rate of such production or accumulation, however, depends on the nature of the fish and their age and size.

Titratable acidity.—The values of total titratable acidity of the eleven species of fish measured by titration with 0.01N Na_2Co_3 show a range of values from 5.14cc. to 8.48 cc. per gm. wet fish just after purchase from the market i.e. 21.66 cc. to 48.35 cc. when calculated per gm. dry basis. These values gradually decreased with the progress of storage period and ultimately reached to a low level ranging from 1.24 cc. to 3.96 cc. per gm. wet basis or 5.81 cc. to 21.08 cc. per gm. dry basis after 24 hours storage.

pH values.—At the initial stage the pH of the above species of fish ranged from 6.25 to 6.45 and these values increased to the limit of 6.75 to 7.25 after 24 hours storage.

Relationship between Iodine value, Titratable Acidity and pH.—From the data presented in Table 1 it was apparently noted that when the iodine absorption value increased with the progress of storage period the titratable acidity, on the contrary, maintained reciprocal relationship with the iodine values. To study this relationship in a more precise manner for individual fish, the data presented in Table 1 have been treated in a different way, in which the rise in iodine absorption value from initial level to that of ultimate storage period of 24 hours expressed as per gm. dry basis has been compared with the fall in the titratable acidity as well as with the increase in the pH value due to the same storage conditions. The ratio between the rise in iodine absorption values and the fall in titratable acidity values has also been calculated in each case to explore the nature of relationship maintained by these values and all these are presented in table 2. For better comparison of the behaviour of different fish with respect to their spoilage characteristics, the fish in table 2 have been listed according to the ascending order of the rise in iodine absorption values. From this table it is noted that for the first eight varieties of fish the rise in iodine absorption values maintains almost linear relationship with the fall in the titratable acidity values and the ratio between these two values remains within the low limit of 1.1 to 1.7. Thus eight varieties of non-air-breathing fish fall under the same group regarding their spoilage characteristics.

TABLE 2.—RELATIONSHIP BETWEEN THE INCREASE IN IODINE ABSORPTION, DECREASE IN TITRATABLE ACIDITY AND INCREASE IN PH VALUES AFTER 24 HOURS STORAGE OF FISH.

Name of the fish.	Increase in 0.01 I_2 absorption (in cc.) per gm. dry basis after 24 hours storage. (A)	Decrease in titratable acidity values by 0.01 Na_2Co_3 (in cc.)per gm. dry basis after24 hours storage. (B)	Ratio = A/B	Increase in pH values.
Kali Baus.	21.73	18.62	1.1	0.9
Hilsa ..	29.8	18.61	1.5	0.8
Katla ..	36.04	23.36	1.5	0.75
Air ..	38.38	24.59	1.39	0.85
Mrigele ..	50.54	33.64	1.49	0.60
Rohu ..	50.88	28.47	1.7	0.90
Puti ..	51.06	30.72	1.66	0.70
Chapila ..	51.19	34.15	1.49	0.85
Bailia ..	50.89	10.64	4.77	0.50
Koi ..	72.66	7.88	9.2	0.45
Singi ..	69.75	6.29	11.9	0.55

While studying air-breathing fish, Singi and Koi, it was observed inspite of high iodine absorption values to the extent of 69 cc. to 73 cc. the depression in titratable acidity values was limited only to 6 cc. to 8 cc. and the ratio between these values was also very high to the extent of 9 to 12. Bailia fish occupies an intermediate position between these two groups and against its increased iodine value of 50.89 cc. the fall in titratable acidity value was found to be very low to the extent of 10.64 cc. and the ratio between these values was 4.77.

The increase in pH values as presented in Table 1 also shows some relationship. The increase in pH values due to Koi, Singi and Bailia was nearly 0.45 to 0.55 whereas those for others ranged from 0.6 to 0.9.

Discussion

After a survey of the results it is noted that in case of non-air-breathing fish the fall in titratable acidity due to spoilage is very high as compared to that of air-breathing species. This striking feature may be explained on the background of our knowledge on enzymology and on the hypothesis that the accumulation of acid in the tissues depends on the production on the one side and utilisation on the other according to the following mechanism :—

(*a*) production of pyruvic and lactic acid by the glycolytic break down of muscle glycogen under anaerobic condition and of fatty acids by degradation of fats and proteins.

(*b*) aerobic utilisation of pyruvic acid with the formation of CO_2 through acetyl-CO-A and through Kreb's cycle.

(*c*) utilisation of the acid by the buffering action of amines, ammonia and basic amino acids produced by proteolytic action.

On the basis of the above picture the reason for lower fall of titratable acidity in case of the air-breathing fish like Koi and Singhi and also Baila may be due to the predominance of the process of formation of acid over utilisation. This seems to be plausible in view of the fact that these fish survive and always remain in a struggling condition even after they are removed from water and for this the glycolytic enzymes remain active producing a large amount of acid which remain accumulated in the tissues after utilisation. Quite an opposite phenomena happen in case of non-air-breathing fish which die soon after they are caught and in such a case glycogenesis stops whereas the utilisation of acids proceeds utinterrupted leading to more fall in titratable acidity.

How far all the previously stated reactions are caused by some enzymes in the different fish during their survival and spoilage stages are now under investigation. It is expected that this will help to understand the mechanism of spoilage of different species of fish and this will constitute the subject matter of a subsequent publication.

Conclusion

1. An improved iodometric titration and titratable acidity measurement method has been devised for the determination of the quality of fish, and by applying these methods the spoilage of eleven species of fish like Puti and Chapila and younger fish of Rohu, Katla, Mrigele, Kala Baus and Air and Singi, Koi, Bailia and adult-size Hilsa has been studied under storage condition at ordinary temperature of 80-84°F. for 24 hours in the laboratory.

2. Absorption value for 0.01N iodine by all the fish except Hilsa at the initial stage just after purchase showed a range of values from 21.92 cc. to 46.19 cc. per gm. dry basis. Hilsa showed a low value of 7.54 cc. in this respect perhaps because of its bigger size. After 24 hours storage the above value per gm. dry basis increased to 37.38 cc. in the case of Hilsa and from 53.51 cc. to 104.07 cc. in the case of others.

3. Titratable acidity values with 0.01 N Na_2CO_3 at the initial stage ranged from 21.66 cc. to 48.35 cc. per gm. dry basis and these values depressed to the limit of 5.81 cc. to 21.08 cc. after 24 hours storage.

4. The pH values increased from the initial level of 6.25 to 6.45 to the final level of 6.75 to 7.25 after 24 hours storage.

5. The rise in iodine absorption and in pH values, and the fall in titratable acidity values due to 24 hours storage showed some interesting relationship by which Koi, Singi and Bailia may be grouped under one class and the rest in another as regards their spoilage characteristics. The ratio between the above values also showed some interesting relationship.

6. The possible mechanism of spoilage in the above fish has been discussed in the light of the above findings.

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