BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part X.—Reducing Sugar Content of some Fresh Water Fish as Influenced by Boiling Treatment and Storage under Room Temperature and in Ice

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The reducing sugar content of the muscles of some fresh water fish of East Pakistan as influenced by boiling treatment, ice storage and by spoilage due to storage at ordinary room temperature, has been investigated. The average value in the fresh fish is 42.9 mg. percent and this on parboiling of the whole fish drops to 26.6 mg. percent. Storage of the fresh fish at ordinary room temperature of 80-84°F which leads to decomposition, reduces the value by 47.1 % from the original value. Storage of the boiled fish, on the contrary, reduces the value by 25.6 %. Storage in ice for 2 days did not show any change of the values of fresh and boiled fish samples. The significance of the difference in the reduction of of the reducing sugar due to storage of fresh fish and boiled fish at ordinary room temperature and constancy due to storage in ice has been discussed in the light of the enzymes from two sources—one from endogenous source of the fish tissues and another from the exogenous source of spoilage microorganisms.

Introduction

In the previous communication from this Laboratory[1] it has been hypothesised that glycolytic decomposition is one of the factors to cause spoilage in fish, the extent of which depends on the species and their ecological behaviour. In offering explanation for less decrease in the titratable acidity values in the air-breathing species like Shingi (*Ophicephalus striatus*), Koi (*Anabas testudineus*) etc. as compared to those of the non-air-breathing ones like carps, it was further postulated that in the former species, because of continuous struggling behaviour of their muscles, a large quantity of lactic acid may remain accumulated there even after utilisation in the subsequent respiratory cycle (Kreb's Cycle). This hypothesis about the involvement of Embden—Meyerhof pathway in the process of glycolysis in the fish muscle has been confirmed by the findings of Brown[2] with labelled glucose. In the sequence of this glycolysis it is expected that some reducing sugar in the free state might also be formed in the tissues of the various species of fish available in this region.

The necessity for a thorough and quantitative study of these reducing sugars in the fish tissues is strongly felt because of their possible effect on enhancement of the Maillard Browning Reaction[3] by combination with the limiting amino acids like lysine etc. due to heat treatment as adopted in canning of fish and in the preparation of fish flour. Such aldehyde-amino acid combination makes lysin[4] and tryptophane, methionine etc.[5] unavailable to the human body, as a result of which the nutritive values of the protein of the processed fish are greatly reduced.[6]

In addition to the above deteriorating effect on protein nutrition, the reducing sugar in the fish tissue may also play an important part in enhancing the spoilage mechanism by allowing the growth of microorganism when these are stored and transported under ordinary and refrigerated conditions.

With these possibilities in view, it was felt desirable to undertake thorough study of the reducing sugar values of some typical species of fresh water fish of this region and the effect of boiling treatmnet, and of storage of the fresh and boiled fish at ordinary and refrigerated temperature on the above values.

Literatures about the sugar content of fish of the different regions of the world have not yet sufficiently accumulated to yield any conclusive evidence of qualitative and quantitative changes brought about by various processing operations adopted in the preservation techniques. While Tar[7] reported complete absence of glucose in liquid-air-frozen muscle of sculpin (*Cottidae*) and tomcod (*Microgadus proximus*), Jones[8,9] and Burt,[10] on the other hand, reported the presence of glucose in the muscle of trawled coldling and of the rested codling after death to the extent of 44-200μ mole i.e. from 8 to 39 mg., 100 g. and 210-220μ mole i.e., 37-39 mg./100 g. respectively. Ribose on the other hand, has been found to be absent in every species of fish at death but gradually accumulates in varying degrees during storage in ice. [8,9,11 and 12] The different sugar phosphates have also been isolated from the above fish muscles. [10,13,14]

The results of the present investigations will, however, supplement to the above information by supplying some data on the distribution of sugar in the fresh water fish of this region about which no information are yet reported in the literatures.

Experimental

Fresh fish of the various species as shown in Table 1 were collected early in the morning from the market. These were then grouped into different batches and treated as below.

Batch A: Fresh fish without any treatment and storage.

Batch B: Fresh fish stored in the room temperature of 80-84°F. for 24 hours.

Batch C: Fresh fish heated in steam for ten minutes by gradual increase from the room temperature.

Batch D: Fresh Fish boiled as above and then stored for 24 hours at the room temperature of 80-84°F. like Batch B.

Batch E: Fresh fish stored at ice temperature for 2 days.

Batch F: Boiled fresh fish stored at ice temperature for 2 days.

Five grams of the muscle from each pooled sample of the above batches were then deproteinised by sodium tungstate and sulphuric acid and the total reducing sugar of the protein-free filtrate was then determined according to the standard Folin and Wu method.[15] The values determined represent mostly all the reducing sugars which might be present in the tissues along with small quantity of other reducing compounds and the results from all the batches examined are expressed in terms of glucose as shown in Table 1. The percent decrease of the reducing sugar values due to boiling and storage under ordinary and refrigeration temperatures are shown in Table 2.

Results and Discussion

The results presented in Table 1 show that the fresh fish (Batch A) of different species contain the total reducing sugar to the extent of 27 to 66 mg. percent of wet fish, with an average value of 42.9 mg. percent. On spoilage due to storage for 24 hours at the room temperature (Batch B) the above values decrease to the level of 17 to 31 mg. with an average value of 21.8 mg. percent. Boiling treatment decrease the values to the level of 14 to 40 mg. percent i.e., 26.6 mg. percent on an average. Storage of this boiled fish causes further reduction of the values to the level of 18.9 mg. on an average. Storage of the similar samples

Table 1.— Showing the Reducing Sugar Values of Some Fresh Water Fish under Condition of Boiling and Storage. The Results Indicate the Total Reducing Sugar in Terms of mg. of Glucose/100 g. Fish Tissues.

Sl. No.	Zoological name of the fish	Local name	Fresh fish	Fresh fish stored in ice for 2 days	Fresh fish stored at room temperature of 80-84°F. for 24 hours	Boiled fish	Boiled fish stored in ice for 2 days	Boiled fish stored at room temperature of 80-84°F, for 24 hours
1.	Ophicephalus straitus	Singhi	55	57	19	31	28	15
2.	Anabas testudineus	Koi	34	32	17	20	21	15
3.	Ophicephalus punctuatus	Leta	43	40	20	28	26	22
4.	Anabasis nama	Mala	55	56	31	33	33	24
5.	Gadusia chapra	Chapila	38	37	22	18	20	14
6.	Nandus nandus	Meni	44	46	22	32	31	22
7.	Mugil cascasia	Kechki	28	28	17	22	20	16
8.	Mystus vittatus	Tengra	27	26	17	14	14	11
9.	Labco rohita	Ruhee (Naola size 10°)	43	45	25	28	31	21
10.	Glossogobius giuris	Balia	62	64	28	40	37	29
		Average:	43.9	43.1	22.8	26.6	24.8	18.9

TABLE 2.—SHOWING THE PERCENTAGE DECREASE OF THE REDUCING SUGAR VALUES DUE TO BOILING TREATMENT AND DUE TO STORAGE OF FRESH AND BOILED FISH AT ROOM TEMPERATURE OF 80-84°F.

S. No. of fish as per Table 1	Decrease due to boiling treatment		Decrease due to storage of fresh fish at room temperature for 24 hours		Decrease due to storage of boiled fish at room temperature for 24 hours.	
	Total	As percent of fresh fish values	Total	As percent of fresh fish values	Total	As percent of boiled fish values
1.	24	43	36	65	16	50
2.	14	41	17	50	5	25
3.	15	35	23	53	6	21
4.	22	40	24	44	9	27
5.	20	52	16	42	4	22
6.	12	27	22	50	10	31
7.	6	21	11	40	6	27
8.	13	48	10	37	3	21
9.	15	35	18	42	7	25
10.	22	33	34	55	11	27
Average:	16.3	37.7	21.1	47.9	7.7	27.6

of fresh fish and its boiled product for two days in the ice temperature did not show any appreciable change from the initial values before storage. In all the batches studied, the minimum reducing sugar values were noted in those of Batch D i.e., in the batch which was stored for 24 hours at the room temperature after boiling.

The reducing sugar values as described here mostly comprise of glucose and this may origin by the action of phosphatase on glucose-6-phosphate produced by glycolysis, or by amylase. The presence of the latter enzyme has been detected in the above fish muscle extracts and their results will be reported in due course. The possibility of the presence of other reducing sugars like ribose may not be overruled, and further investigations in this line are expected to yield new informations on the mechanism of formation of reducing sugars in fish tissues.

In offering the explanation for decrease of the reducing sugar values due to storage of both fresh and boiled fish at room temperature for 24 hours, it may be hypothesised that some enzyme systems are involved in the process of utilisation of the reducing sugar. These enzymes may be partly endogenous in character and partly bacterial in origin. This possibility is further confirmed from the results of Table 2 which show greater percent reduction of the values due to storage of fresh fish (47. 9%) as compared to those of boiled fresh fish (27. 6%). In case of storage of fresh fish enzymes from both the above sources are actively operative in the utilisation of sugar causing greater decrease by 47.9%, whereas in case of boiled fish showing less decrease of 27.6 percent, the bacterial enzymes are only operative, the endogenous enzymes in this case having been inactivated during boiling treatment. The above possibility of the involvement of bacteria in causing the decrease of the sugar during storage of fish has also been pointed out by Jones[9] in his work on cod.

About the nature of the endogenous and exogenous enzymes involved in the above process of utilisation of sugar during storage at ordinary temperature, the active participation of hexokinase may not be overruled because of the probable availability of large excess of ATP due to decrease in the activity of ATPase of fish tissues under storage in the above conditions as have been noted in these laboratories.

In the present investigation it has also been observed that pre-boiling of the fish causes a reduction of the sugar values by 37.5% (Table 2). This may be partly due to leaching out process and partly due to activation of the enzymes like hexokinase etc., which utilise the sugar at the initial stage of boiling when their optimum temperatures are reached before these are completely inactivated

at the final steam temperature. It may be mentioned here that in the present investigation the intact whole fish was boiled instead of opening up the belly for evisceration, and thus the leaching out of the sugar during steaming of the intact fish may not fully account for a considerable decrease of the reducing sugar value to the extent of 21.8 percent (Table 1), in the pre-steamed or parboiled sample.

The samples of fresh and steamed fresh fish on storage at ice temperature for 2 days did not show any change of the reducing sugar values when compared with their unstored values. These observations tend to show the cojoining effect of the inactivation of the endogenous tissues enzymes with the retardation of the invasion by the spoilage organisms. The above cojoining effect has been further intensified by the storage of the unevisceratedintact fish, whether boiled or fresh as a result of which the microflora get less chance for invasion of the fish tissues. But how far the storage for a prolonged period in ice or at a lower refrigeration temperatures may cause damage to the tissues to help invasion by the spoilage microflora is a problem which is now under investigation.

Maillard Browning reaction which poses a great problem even when the ice-stored rested or trawled fish are subjected to heat treatment for preparation of fish flour etc. as evidenced by the report of Jones, can be fairly controlled if the intact fish is pre-steamed as discussed in the present investigation. The high value of available lysin [16] to the extent of 8 to 9 g. per 16g. N in the three fish flour prepared in this laboratory as determined by carpenter's chemical method and high values of their available tryptophane methionine and Relative Nutritive value as determined by microbiological technique [17] *vis-a-vis* the lower values of the same in other sample examined in different laboratories [6], [18] give strong evidence of the superiority of the whole uneviscerated boiled fish, because of its lower sugar content, for subsequent processing operations when heat treatments are involved.

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