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BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part III.—Investigation on the Mechanism of Fish Spoilage by Study of the Influence of Visceral Contents and Boiling Treatment on the Autodehydrogenase Activity of Fish Tissues

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It has been found that both viscera and the fillet of a fish act in a symbiotic manner, with the onset of spoilage by generation of dehydrogenases. The liver in normal condition possesses high dehydrogenase activity, indicating its rapid autolysis; and the spoilage in the fleshy portion is partly due to the influence of visceral autolysis and partly due to bacterial invasion from air.

Introduction

In the previous paper it has been shown by Qudrat-i-Khuda, De and Khan[1] that during spoilage of whole fish there is generation of the activity of total dehydrogenases in the fillet, the rate of which, however, depends on the nature of fish as well as on the total titratable acidity at the initial stage before spoilage had started. The present investigation was undertaken to see how far the presence of viscera in the intact fish influenced such generation of dehydrogenases during spoilage in the fillet and also to see whether storage after boiling may help the delay of the onset of spoilage by retarding the generation of dehydrogenases or their activities.

It has been noted that the powerful digestive enzymes in the gut even at ice temperature perforate its wall and these along with bacteria from the gut carry on the decomposition of belly wall as well as of the viscera.[2] The viscera itself has a high rate of autolysis and this combined with the above enzymes and bacteria from gut attack the belly and render it semi-fluid. Jacob[3] in discussing the above aspects has remarked that in case of unevisceratedfish autolysis may be considerable and general softening of flesh adjacent to the visceral cavity occurs. Although by gutting of fish, visceral autolysis may be completely retarded, it is still a debatable issue as to whether this process will also help in the retardation or delay in the onset of spoilage in the fleshy portion. Jacob[3] in the same review has opined that this process of gutting may contribute to a more rapid bacterial decomposition of the fillet at a later stage and this is probably because of the exposure of a large surface of the interior of the belly to the air. Dyer and Dyer[4] in their work on white fish have noted that the fillet of gutted fish might have higher content of spoilage products than similar ungutted fish. This, they attribute, is due to damage caused to intestine by such gutting process for which the spoilage products formed within the intestine diffuse into the belly cavity. Experiments with mackerol, on the contrary, did not show any difference between gutted and ungutted fish which had been iced for 6 days.[5]

From all the above reports, although it seems very possible that softening of flesh is partly due to the effect of visceral contents, yet the major decomposition of the fillet occurs due to the influence of bacteria from air or their enzymes as has been observed by a number of workers.[6,7]

The present investigation has been undertaken to have a clear picture of the above aspect

specially with regard to the individual or/and mutual influence of fillet as well as of the viscera on the spoilage mechanism in the intact fish.

Experimental

Fresh fish was collected from the market early in the morning, about 3 hours after catch. Immediately they were divided into different batches—each batch having at least three fish of equal size and all these batches were then subjected to the following series of experiments.

Experiment 1.—One batch of fish was sliced immediately after purchase and the auto-hydrogenase activity of liver as well as of the fillet was determined.

Experiment 2.—A batch of intact fish was stored under ordinary condition (80-84°F.) for 24 hours and then the liver and the fillet were analysed for dehydrogenase activity. By such storage condition the fillet became semi-fluid and emitted putrid odour after 24 hours and hence storage for further period was not possible.

Experiment 3.—At the beginning of experiment, the fish was eviscerated and both the liver and the fillet were stored separately in the same manner as before, after which the enzyme activity was recorded at different storage periods. Since under such storage condition the fish could be stored for 72 hours, the activity up to that stage of storage could be recorded easily.

Experiment 4.—One batch of fresh intact fish was boiled and stored under laboratory condition as before and thereafter the enzyme activities of both liver and flesh were determined.

Experiment 5.—The intact fish after boiling was eviscerated and both boiled liver and fillet were stored separately and thereafter their dehydrogenase activities were recorded at each stage of storage.

Experiment 6.—In this batch the viscera was removed and the eviscerated fish was then boiled. Unboiled viscera and boiled fillet were then stored separately and their activities determined after storage.

Technique of Evisceration.—Evisceration of the whole intact fish was done by making a small horizontal opening by sharp knife at the junction of the head and the belly. This process of evisceration is followed in this part of the country to minimize the chances of exposure of the fleshy portion surrounding the belly to bacterial invasion. In other countries evisceration is done by completely opening the belly, thus exposing the entire belly wall to bacterial attack for which the real autolytic effect of viscera on the spoilage of fillet cannot be fully judged. The process of evisceration adopted in the present investigation is expected to yield better information on the above aspect.

The dehydrogenase activity of 1 g. tissue sample was determined according to the technique detailed in our previous paper.[1] It may be noted here that for analysis of the fillet, the portion below the back bone and above the belly was collected from each sample. All the analyses were done in replicate and the difference in such replicate values was not much significant under the condition in which the above experiments were conducted.

Three types of fishes like Rohu Naola (below 10 inches) and small size fishes like Puti and Chapila were selected for the work. Since in all the three cases the effect of the above treatments was almost the same, specimen data for Rohu Naola only are given here.

Results

The results of the experiments presented in Table 1 show that in Experiment 1, the flesh at the initial stage just after purchase did not show any appreciable autodehydrogenase activity, whereas the liver, immediately after removal showed a certain amount of activity.

After 24 hours storage of intact fish (Experiment 2) the autodehydrogenase activity of liver tissue separated from the fillet after storage increased by nearly 3 times from the original value and that of the fillet approached to a considerable amount from almost negligible value.

The results of Experiment 3, in which eviscerated fillet and the separated liver were stored individually, indicate that the fillets of eviscerated fish after 24 hours storage also elaborated sufficient dehydrogenase activity but the rate in such case was much lower than that of the fillets of uneviscerated fish of Experiment 2. The extent of apparent inhibition of activity in this case was such that rate of activity after 48 hours storage did not even approach the limit as was elaborated in case of uneviscerated fillet after 24 hours storage. In contrast to the fillets, the separated liver of eviscerated fish on storage, however, showed rather increase in the values as compared to that of uneviscerated fish so much so that after 48 hours storage the activity almost approached to unity.

Study of the effect of pre-storage boiling treatment of intact fish on the generation of dehydro-

genase activity has shown (Experiment 4) that the inherent high activity of the enzyme in liver of fresh fish as was noted in Experiment 1 is completely destroyed during the process of boiling before any storage was started. When uneviscerated boiled fish was stored for 24 hours the activities of fillet as well as of liver, separated from each other after the above storage period, showed lesser values than those of unboiled and uneviscerated fish stored for similar period as in Experiment 2. The activities of both fillet and the liver increased with progress of storage period but it may be noted that the ultimately increased values even after 72 hours storage in this case did not reach the same limit as was noted in case of 24 hours storage of unboiled and uneviscerated fish of Experiment 2. When the fish after boiling was eviscerated (Experiment 5) and both liver and the fillet were stored separately, the dehydrogenase activity of both these tissues showed much lower values as compared to those of boiled and uneviscerated fish as recorded in Experiment 4. When only the fillet was boiled after evisceration and both the boiled fillet and the unboiled liver were stored separately, the fillet in this case also showed lower dehydrogenase activity as compared to that of uneviscerated intact boiled fish. Liver, not being boiled in this case, behaved in a similar way as that of separated liver of eviscerated unboiled fish (Experiment 3) stored under identical conditions.

TABLE 1.—THE EFFECT OF EVISCERATION AND BOILING TREATMENT ON THE DEHYDROGENASE ACTIVITY IN FISH TISSUES (ROHU NAOLA) DURING STORAGE.

Experiment No.	Nature of treatment	Duration of storage in hours	Nature of tissue studied	Time of decolorisation of M.B. per gm. tissue in minutes (T)	Activity $\frac{1}{T}$
Expt. 1	immediately after purchase	0	Flesh	More than 12 hrs.	Negligible
			Liver	25	0.04
Expt. 2	Stored as intact fish	24	Flesh	7	0.142
			Liver	8	0.125
Expt. 3	Stored after evisceration	24	Flesh	23	0.043
			Liver	6	0.167
		48	Flesh	18	0.055
			Liver	Instantaneous	1.00
		72	Flesh	10	0.10
			Liver	Totally decomposed	
Expt. 4	Stored as intact fish after boiling	0	Flesh	Decolorised after 24 hrs.	Negligible
			Liver	,,	,,
		24	Flesh	23	0.043
			Liver	10	0.10
		48	Flesh	16	0.062
			Liver	8	0.125
		72	Flesh	7	0.142
			Liver	5	0.20
Expt. 5	Fish boiled, eviscerated and stored	24	Flesh	37	0.027
			Liver	29	0.034
		48	Flesh	28	0.036
			Liver	22	0.045
Expt. 6	Eviscerated fish, boiled and stored	24	Flesh	37	0.27
			Liver	5	0.02
		48	Flesh	18	0.055
			Liver	Instantaneous	1.00

Discussion

The results of Experiment 1, clearly indicate that the fillets of the Rohu Naola although do not possess any appreciable amount of dehydrogenase activity at the initial stage just after purchase from the market, the liver tissue, on the contrary, yields evidence of the presence of high level of these enzymes. This is indicative of the survival stage of the liver tissue even under apparently dead condition of the intact fish and this is perhaps the cause of autolysis of the visceral contents. This process of autolysis involves the participation of a large number of dehydrogenases and other enzymes for the performance of glycolysis, Kreb's cycle oxidation and other processes for the breakdown of different constituents.

The results of Experiments 2 and 3, in which it was observed that fillets of unevisecrated fish on storage at the room temperature of 80°-84°F. elaborated comparatively more activity than that of eviscerated fish stored under similar conditions, suggest that the generation of dehydrogenases in fillet of intact fish is not only the result of decomposition by bacteria invading through the skin and other routes but may also partly be due to the effect of liver from which its autolytic enzymes along with the food bacteria of the gut or their enzymes reaching the liver after penetration

through the intestinal wall, might have diffused into the fillet and had accelerated the spoilage process there.

Whereas the results of Experiments 2 and 3 indicate the influence of visceral contents (liver) on the spoilage of fillets, those of Experiments 4, 5 and 6, on the contrary, submit a new picture regarding the influence of fillet on the decomposition of visceral contents. The observation as to the complete destruction of dehydrogenase activity of liver tissue after boiling treatment, the regeneration of the activity of both fillets and liver of boiled uneviscerated fish on storage and comparatively lesser generation in both the above tissues in case of boiled but eviscerated fish, suggest that the enzymes of the liver of fresh fish even after complete destruction or inhibition in their activities due to boiling treatment are regenerated when left in contact with the fillet in intact fish and this is probably due to the influence of fillet. While the liver remains within the belly under sterile condition after boiling treatment, the fillet on the contrary even after sterilisation by boiling remains exposed to the air and thus the bacteria from air get access into the fillets through skin and other routes and initiate decomposition therein. The enzymes of these bacteria of fillets perhaps diffuse into the liver and other visceral contents and initiate the spoilage in this tissue also.

Summarising the results of the above experiments it may be postulated that both the fillet as well as the viscera (liver) act in a symbiotic manner in the causation of spoilage of whole fish when stored under ordinary atmospheric condition in the laboratory.

This symbiosis with respect to the mechanism of fish spoilage will depend on the rate of autolysis of the liver and other visceral contents coupled with the presence of intestinal bacteria on the one hand, and on the extent of bacterial invasion in the fillets on the other.

Further work is in progress for investigating the influence of evisceration process adopted in other countries on the spoilage of fish so that the right process of evisceration to cause less spoilage in the fillet may be evaluated.

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