BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part II.—Assessment of Dehydrogenase Activity in Fish Tissue and Investigation on the Mechanism of Fish Spoilage by this New Method

M. QUDRAT-I-KHUDA, H. N. DE AND N.M. KHAN

East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

(Received December 14, 1959)

A new method for the study of spoilage of fish has been developed. The dehydrogenase activity of the tissue has been investigated by noting the time of discharge of methylene blue according to Thunberg technique after allowing the tissue enzyme to act on the tissue substrates. On the basis of this new technique, 1 g. flesh of Koi, Singi, Bele and Hilsa showed slight activity whereas younger samples of Rohu, Katla, Kali Baus, Mrigale and Air and smaller species, Chapila and Puti showed almost negligible activity of dehydrogenase at the initial stage just after purchase from the market. On storage for 24 hours, a high rate of dehydrogenase activity was noted in each of the above fish samples—comparatively Singi, Koi, and Boyal showed less generation of activity than the others. The significance of these data and their relationship with the titratable acidity values as were presented in Part I of this paper have been discussed.

Introduction

In the previous paper by M. Q. Khuda, H.N. De and J. C. Debnath,[1] it was postulated that difference in the accumulation of acids in various species of fish at the initial stage and during spoilage, after storage, may be due to imbalance between activities of glycolytic enzyme system for pyruvic acid and lactic acid production and that of Kreb's cycle enzymatic chain of reaction for their complete utilisation.

In order to get clear information on this important aspect, a series of investigations have now been undertaken.

Although some work [2–5] has been done in the past on various constituents for determining fish spoilage, yet no attempt has yet been recorded on the above line so as to assess the mechanism of spoilage in a precise manner. The study of the above aspects of glycolysis and Kreb's cycle oxidation processes, which universally occur in every organism, involves the investigation of a number of dehydrogenases and oxidases for each of which a specially prepared substrate is required for laboratory experimentation. Nevertheless, it was thought worthwhile to study the laboratory enzymatic reaction under conditions almost identical with those present in the tissue cell structure by allowing the tissue enzyme extract to act on the natural substrates of the cell constituents. In the present case the activity of dehydrogenase has been studied in the above way. The basis for this technique was noted in our previous study on succinic acid dehydrogenase in spoiled fish tissue extract, in which it was observed that the blank experiment without any addition of the substrate, i.e., sodium succinate, elaborated almost equal activity as that conducted with the substrate. Details of the technique are given below.

Experimental

Thunberg Methylene Blue Technique [6, 7, 8].—According to this technique the oxidation of any substrate due to dehydrogenase system is measured by noting the time of decolorisation of methylene blue (M.B.) due to its reduction to leuco M.B. by hydrogen transfer from the metabolite under vacuum. In the present case a series of trial experiments were first conducted to ascertain the minimum amount of tissue, buffer mixture and methylene blue to be used for proper elaboration of enzyme activity. The following procedure was adopted.

In a series of Thunberg tubes, 0.9 ml. of a mixture of 8 ml. methylene blue solution and 6 ml. phosphate buffer (Sorensen pH 7.2) was introduced. Then 1 g. fish tissue, finely ground with a little quartz sand in 10 ml. buffer solution, was added to the mixture. Under ordinary condition the tissue extract is generally used for noting the enzyme activity, but in the present case the ground tissue along with the extract was also introduced into the tube so as to allow the enzymes to act on the substrates already present in the tissue. Thus the identical conditions as are present in the tissues for enzyme action have been maintained to a certain degree for the performance of "autodehydrogenation" process. The tubes were then evacuated for 1 minute by vacuum pump and incubated in a constant temperature bath at 37°C. The time of discharge of the methylene blue colour was then noted in each individual tube. Since the time of decolorisation is inversely proportional to the enzyme concentration, i.e., the activity, the reciprocal value of this time of discharge in minutes may be arbitrarily accepted as the relative autodehydrogenase activity per g. wet fish. But, since the moisture contents of eleven species of fish studied in the present investigation showed a wide variation from 64.66 to 82.57%,

the expression of the activity per g. wet basis as above and comparison and interpretation of these values will always lead to erratic conclusion. In order to make better judgement of the activities, the time of discharge per g. wet fish (T_1) has been corrected for expression on per g. dry basis (T_2) according to the following formula taking into consideration the moisture percentage of individual fish.

$$T_2 = T_1 \times \left(\frac{1 - \% \text{ moisture}}{100} \right)$$

From these values of T_2, i.e., time of discharge, for 1 g. dry basis, the activity was then expressed as $1/T_2$.

Further, the initial values of titratable acidity and the fall in these values after 24 hours storage, as were reported in the previous paper, have also been included in the Table 1 (columns E and F) so as to correlate the dehydrogenase activity with these values.

As in the previous investigation, the fish was purchased from the market in the early morning and one sample of each species was sliced immediately in the laboratory and subjected to the above investigation. Another sample was stored for 24 hours in open (temperature, 84° to 90°F.) and the activities of these stored samples were noted next day.

Results

Table 1 shows that the fresh tissues of Puti, Chapila, Katla Naola, Rohu Naola, Kali Baus Naola, Mrigale Naola and Air did not discharge methylene blue colour even after incubation for a period of over 10 hours. This indicates that these fishes at the initial stage cannot elaborate a significant amount of dehydrogenase activity. Singi, Koi, Bele and Hilsa seem to possess some amount of dehydrogenase activity at the initial stage.

After 24 hours storage at ordinary temperature (84°—90°F.) in the laboratory, the methylene blue colour was discharged within a few minutes in all cases. Calculated on the basis of expected activity per g. dry basis, the time of discharge of methylene blue was 8.64, 10.44 and 10.15 minutes for Boyal, Koi and Singi, respectively, and from 2.44 to 4.07 minutes for the rest of the fishes.

TABLE I.—THE TIME OF DISCHARGE OF METHYLENE BLUE COLOUR (M.B.) OF FRESH AND STORED FISH AND THE RELATIVE DEHYDROGENASE ACTIVITY.

Local name of fish	Zoological name of fish	Moisture %	Fresh Fish	After 24 hr. - storage			Titratable acidity values (0.01 N) in ml. for fresh fish per g. dry basis	Fall in titratable acidity (0.01 N) in ml. after 24 hrs. per g. dry basis	D × F
			Discharge of M.B. (in hrs.) per g. wet fish	Discharge of M.B. (in min.) per g. wet fish	Discharge of M.B. (in min.) per g. dry basis	Enzyme activity per g. dry basis			
			A	B	C	D	E	F	
Singi	Palemon peneus	81.2	5 to 6 hrs.	54	10.15	0.098	27.37	6.29	0.61
Koi	Anabestestudineus.	76.26	,,	44	10.44	0.096	21.66	7.88	0.75
Baila	Glossogobius giuris	78.38	,,	40	8.64	0.115	27.19	10.64	1.23
Hilsa	Hilsa ilisa	64.66	,,	11	3.88	0.257	23.42	18.61	4.79
Puti	Barbus Puntius	79.14	18 to 24 hrs.	13	2.71	0.369	37.99	30.72	11.33
Rohu	Labeo rohita	82.57	,,	14	2.44	0.409	35.05	28.47	10.02
Air	Mystus Aor	80.2	,,	15	2.97	0.336	34.29	24.59	8.28
Chapila	Danoacquipinnatus	78.8	,,	15	3.18	0.314	40.00	34.15	10.73
Kali Baus	Labeo nandina	74.54	,,	16	4.07	0.245	31.34	18.62	4.57
Mrigale	Cirrhina Mrigala	81.11	,,	17	3.21	0.311	48.35	33.64	10.48
Katla	Catla catla	78.86	,,	19	4.01	0.248	33.55	23.36	5.82

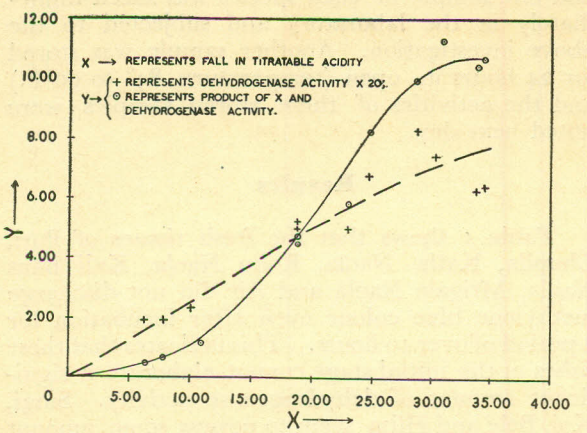

Fig. 1.—The relationship between dehydrogenase activity and fall in titratable acidity.

Figure 1 shows graph for (i) dehydrogenase activity (crosses) against the fall in titratable acidity, and (ii) the product D×F (in Table 1) against this fall in titratable acidity.

Discussion

From the experimental results and the graph it is evident that methylene blue can be used as a tool for study of spoilage in fish tissues. Reay and Shewan[2] in their review on fish spoilage have remarked: "It is possible that a suitable dye or dyes may be indicated. No success has, however, been achieved in this direction."

The results submitted herewith on the use of methylene blue is a partial success to the fulfilment of the above possibility.

While interpreting the above results one should keep in view that the glycolytic breakdown of glycogen to pyruvic acid or lactic acid involves the participation of one dehydrogenase system for which one pair of hydrogen ions is removed from the metabolite and the utilisation of the pyruvic acid to CO_2 by Kreb's cycle involves the participation of two dehydrogenases and three oxidases (functioning by electron transfer) for which five pairs of hydrogen ions are released. Thus when both the processes operate simultaneously the rate of dehydrogenase activity will be very high and consequently there will be less accumulation of acid. This is the position with respect to spoilage of some non-air-breathing species of fish like Chapila, Puti etc. during storage and is represented by the upper part of the broken curve of the graph. The tendency for slope of the curve at higher values would account for a limit of the utilisation of acid at which the enzyme activity would behave in this way. If only the Kreb's cycle system operates, there will be comparatively lesser activity than above and also very small quantity of acid will accumulate. But if the glycolytic system only operates, the tissues will then show very low amount of enzyme activity and so comparatively greater amount of acid will accumulate in the tissues. This second process is represented by the lower part of the broken curve and seems to be manifested by the air-breathing species like Koi, Singi etc. on their storage. In this respect the other fish may behave between the above two extremes.

An interesting correlation between the utilisation of acid, i.e., fall in titratable acidity for an individual fish after 24 hours storage, and the corresponding values of dehydrogenase activities is further evaluated by the data in the last column of Table I, which denotes the product of D, the dehydrogenase activity, and F, the fall in titratable acidity. From these data and also from the graphical representation in Fig. 1, it is noted that the values of the product initially show a rise and thereafter almost a constancy with the increase in the values of F. This curve has a saturation value like that of enzyme activity substrate relationship hypothesised by Michaelis and Menten.[9] Its interpretation however requires further experiments, which are in hand.

References

1. M. Q. Khuda, H. N. De and J. C. Debnath, Pakistan J. Sci. Ind. Research, **2**, 217 (1959).
2. G. A. Reay and J. M. Shewan, Advances in Food Research, **2**, 344 (1949).
3. Massachusetts Institute of Technology—Symposium paper No. 18, F. A. O. Fish Processing Technologists Meeting, June 1956, Rotterdam, the Netherlands.
4. F. Farber and P. Y. Lerke, ibid., Symposium paper No. 19.
5. W. Partmann, ibid., Symposium paper No. 21
6. T. Thunberg and G. Ahgren, Skand. Arch. Physiol 47, Supplement.
7. T. Thunberg, (1920), Skand. Arch. Physiol, **40**, 1.
8. P. B. Hawk, B. L. Oser and W. H. Summerson, *Practical Physiological Chemistry* (1954), 13th edition, p. 326.
9. L. Michaelis and M. L. Menten, Biochem Z., **49**, 333 (1913).