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STUDIES ON THE AUTOLYTIC SPOILAGE OF TROPICAL SHRIMP (*PENEAUS MERGUIENSIS*)

Nikhat Shaukat, Masarrat Riaz and R.B. Qadri

PCSIR Laboratories, Karachi 39, Pakistan

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It has been found that the process of spoilage of tropical shrimp is accompanied with oxidation and saturation of different groups and double bonds. Dehydrogenase activity which is negligible in fresh shrimp increases, whereas the concentration of acetylcholine hydrochloride falls progressively during spoilage. No direct role of trypsin in the spoilage could be confirmed. The possible mechanism of the autolytic spoilage in the shrimp (*Peneaus merguiensis*) in the light of the above findings is discussed.

INTRODUCTION

The work described in this paper forms a part of the programme designed to study the factors responsible for the spoilage of tropical shrimps. On storage the shrimps undergo different phenomena of oxidation, hydrolysis and decomposition. Their spoilage is accompanied by the changes in protein, fat and glycogen content. A number of chemical compounds and their products have been used as indicators of spoilage. Polyamine and histamine content of shrimp have recently been used as an indicator of spoilage by Meitz and Karmas [1]. Farooqui *et al.* [2] have correlated the variation of some nitrogenous compounds to the changes in organoleptic response and spoilage when the shrimps were stored in ice for certain periods. In fish tissues the determination of pH has been accepted as a parameter of spoilage by several workers [3,4].

Some workers have used iodometric titration methods to study different oxidisable components having – SH groups and unsaturated double bonds [5].

Determination of certain acids, like lactic acid and volatile fatty acids, has also been used as a parameter of fish spoilage by certain workers [6,7].

Trypsin is reported to play an important part in autolytic spoilage of fish [8]. Attempts have also been made to study the activity of acetylcholinesterase in some species of fish (Qudrat-e-Khuda *et al* [9].

In order to study the pattern of spoilage in shrimps from tropical water and to differentiate it from similar phenomena in fish, a number of paremeters, such as iodine absorption, available acidity, dehydrogenase activity and the role of different enzymes involved in the process of spoilage, have been selected for investigation. In the present paper, therefore, attention is confined to a study of the phenomena of oxidation, of dehydrogenase, trypsin and acetylcholinesterase during the spoilage of shrimp. Changes in pH and total titratable acidity have also been measured.

MATERIALS AND METHODS

Freshly caught shrimp (4 – 6 hr old) were collected from Ibraheem Hyderi, packed in ice and transported to the Laboratory. The shrimp used in this study were *Peneaus merguiensis,* locally known as "Jaira", measuring 10 – 22 cm and weighing 20 – 25 g.

Samples were packed in perforated polythene bags and stored in wide mouth glass jars at ambient temperature (24 – 30°) and in the refrigerator maintained at 4 ± 1°. For analysis, samples were removed after 5 and 12 hr from ambient temperature and after 1, 4, 8 and 12 days from the refrigerator.

IODINE ABSORPTION METHOD

For the determination of iodine absorption, 1 g. of shrimp tissue was ground in 20 ml distilled water with a little quartz sand with the help of mortar and pestle. It was then immediately transferred to an iodometric flask and the mortar and pestle were washed three to four times with aliquots of 20 ml water. The contents were shaken for 15 min for the liberation of oxidisable compounds and then filtered through glass wool. Finally, the solution was made up to 100 ml and divided into two portions.

To each portion, in iodometric flask, 20 ml of 0.01 N iodine solution was added and left for 10 min. Unabsorbed iodine was titrated back with 0.1 N thiosulfate solution.

TITRATABLE ACIDITY AND pH

Shrimp tissue was homogenized with deionized distilled water (1:1, W/W) and the pH was measured using Cambridge pH meter.

50 ml aliquot of aqueous suspension of 1 g. tissue, prepared as described above, was titrated against 0.01 N Na_2CO_3 using phenophthalein as an indicator.

MOISTURE DETERMINATION

The moisture content was determined in an oven at $60 - 70^{o}$

DEHYDROGENASE ACTIVITY

Thunberg methylene blue technique was used for the determination of dehydrogenase activity. The oxidation of any substrate due to dehydrogenase activity is measured by noting the time of decolorisation of methylene blue (M.B.) due to its reduction to leuco methylene by hydrogen transfer from the metabolite under vacum [9 – 10].

Since the moisture content of the fresh sample was found to vary during the study, the time of decolorisation of M.B. was corrected acdording to the formula $T_2 = T_1 \frac{(\%\ Moisture)}{100}$.

Where T_2 = Time of decolorisation of M.B g. moisture free sample.

T_1 = Time of decolorisation of M.B./g. fresh shrimp. The activity was expressed as $1/T_2$

TRYPSIN IN SHRIMPS

A fresh shrimp homogenate containing 1 g. shrimp tissue/100 ml was prepared in the manner already described. Eight samples of 50 ml each (containing 0.5 g homogenate per sample) were prepared and kept in an iodine flask. In four of these samples, 0.1 ml of pure trypsin enzyme solution (10 mg/ml) was added and the contents were incubated at 37^{o} for enzymatic hydrolysis.

The control samples and two samples of hydrolysed homogenate were checked for iodine absorption and two of each for available acidity.

ACETYLCHOLINESTERASE ACTIVITY

To study the effect of spoilage, acetylcholinesterase activity (QAChE) was measured in dissected brain and muscles separately by Hestrin's [11] modified method of Fiegel *et al* [12]. The control was prepared by mixing 1.0 ml of 0.004 M acetylcholine hydrochloride, 1.0 ml of M/15 phosphate buffer (pH 7.2) and 2.0 ml of alkaline hydroxylamine (freshly prepared by mixing equal volumes of 2 M hydroxylamine solution and 3.5 M sodium hydroxide solution) with vigorous shaking. After 1 min, 1.0 ml of HCI (4N) was admixed. The O.D. of the purple coloured complex was measured at 540 nm. This is a measure of acetylcholine hydrochloride present in the control. In the case of test sample, 1.0 ml of enzyme source (muscle homogenate) was added instead of buffer. The rest of the procedure was the same as described for the control. Since acetylcholinesterase inhibits the formation of the purple end product by hudrolysing the substrate through a different route, the optical density of enzyme containing specimen was lower than that of the control. The difference in O.D. of control and the test sample gives a measure of acetylcholine hydrochloride hydrolysed by the enzyme. Acetylcholinesteras activity in the extract of 1 g flesh was also estimated by the electrometric titration method of Stedman, *et al* [13] using acetylcholine chloride (A ch) as the substrate.

RESULTS AND DISCUSSIONS

Iodine Absorption: Results represent an average of 3 – 4 expriments. Table 1 shows that iodine absorption decreases on storage at room temperature. Thus in the case of fresh samples, iodine absorbed was found as 20.6 and 76.5 per gram for wet and dry shrimp, respectively, in 24 hours these valurs were reduced to 8.0 and 29.7 respectively. Comperable values of iodine absorption were obtained in shrimps stored in the refrigerator for about 12 days (Table 1) This suggests that the autolytic spoilage of shrimps passes through the same route whether they are kept in refrigerator or at room temperature.

The values of total titratable acidity of shrimp decreased from an initial value of 4.0 ml wet shrimp (14.8 ml dry shrimp) to 2.1 ml wet shrimp (7.7 ml dry shrimp) after 24 hr storage at ambient temperature. The pH increased from 6.9 to 8.1 in 24 hr. Similar changes were observed in samples stored for 12 days in the refrigerator.

The results suggest that the process of spoilage in shrimps is accompanied by oxidation and saturation of different groups of double bonds. Since the pH is the recipro cal of log $[H^+]$, and is increased (goes to alkaline scale) on storage, the phenomenon of decrease in iodine absorption

Table 1. Iodine absorption and titratable acidity values of shrimp when stored at room temperature (24 – 30°) and in refrigerator (4°). Initial moisture (73.1%)

Room temp. (24 – 30°)						Refrigerator (4°)					
Time (hr)	0.0.1N I_2 (ml) Absorbed		0.01 N Na_2CO_3 (ml) Required		pH	Time (days)	0.01N I_2 (ml) Absorbed		0.01N Na_2CO_3 Required		pH
	wet weight/g	dry weight/g	wet weight/g	dry weight/g			wet weight/g	dry weight/g	wet weight/g	dry weight/g	
0	20.6	76.5	4.0	14.8	6.9	1	20.6	76.5	4.0	14.8	7.0
1	19.8	73.6	4.0	14.8	7.0	4	15.8	58.8	3.3	12.2	7.2
5	18.4	64.6	3.4	12.6	7.4	8	9.4	34.9	3.0	11.1	7.6
24	8.0	29.7	2.1	7.8	8.1	12	7.0	26.0	2.0	7.4	8.0

Table 2. Time of discharge methylene blue color (M.B.) of fresh and spoiled shrimp and the relative

Dhydrogenase activity

Fresh shrimp	Spoiled shrimp		After 24 hr	Fresh	Spoiled
Discharge of M.B./ hr/g wet shrimp	Discharge of M.B./ g wet weight (min) T_1	Discharge of M.B./ g dry weight (min) T_2	Enzyme activity/ g dry weight $1/T_2$	Titratable acidity (0.01N) Na_2CO_3 in ml for fresh shrimp/g dry weight	Tritratable acidity (0.01N) Na_2CO_3 in ml after 24 hr/g dry weight
Color does not discharge after 24 hr.	280	75.6	0.0132	14.86	7.7

(since the iodine absorption is a measure of oxidisable groups present in the specimen at any time) seems more convincing. However, this observation in the spoilage of shrimps is contrary to what has been reported in the spoilage of fish by Qudrat-e-Khuda *et al.* [5]. They have observed an increase in ioine absorption during spoilage of a number of fresh water fish.

Dyhydrogenase Activity: Fresh shrimp tissues were found not to discharge the M.B. colour completely at room temperature even after incubation for 24 hr. This shows that fresh shrimp does not show an appreciable amount of dehydrogenase activity.

After 24 hr storage at room temperature (24 – 30°) however, the M.B. colour was discharged in 280 min. Caluclated on/g. dry weight basis the time of discharge of M B. in shrimp was 75 min.

A decrease in available acidity was also observed upon spoilage of shrimp. (Table 2).

Role of Trypsin: As shown in Table 3 the phenomena of iodine absorption and titratable acidity in trypsin-hydrolysed shrimps are reversed to what is found in spoilage. This suggests that trypsin which is reported to be important in the autolytic spoilage of fish, has no apparent role in shrimp spoilage.

Acetylcholinesterase Activity: Shrimp brain and ventral nerve cord have high acetylcholinesterase activity, which is slightly reduced in spoiled shrimp. Shrimp muscles show an unusually high concentration of acetyl choline. Some of this substrate is hydrolysed on exposure to atmosphere. As shown in Tables 4 and 5, the concentration of acetyl choline in spoiled shrimp is lesser as compared to fresh shrimp.

It is further reduced when the shrimp is allowed to spoil with the head intact. It is likely that during spoilage some of acetylcholinesterase enzyme penetrates through the muscles from brain and nerves. This could be one explanation of why the hydrolysis of acetylcholine hydrochloride is greater in shrimp allowed to spoil with head intact. It appears that in shrimp, utilization in normal course in fresh condition seems to be proportionally higher than the rate of synthesis of enzyme. This does not seem to be unreasonable in view of the fact that muscular activity due to struggling efforts is high in shrimp for which more enzyme is being constantly utilized.

When different phenomena are compared in fresh and spoiled shrimp it appears that spoilage is associated with the exidation of double bonds along with an approach towards alkalinity. The fall in available acidity may be due to the accumulation of acid in the tissues on one side and utilisa-

Table 3. Iodine absorption value titratable acidity in fresh and trypsin hydrolysed shrimp

Iodine absorption value of shrimp 0.0IN Iodine absorbed in ml				Titratable acidity 0.0IN Na_2CO_3 in ml required for			
Fresh shrimp		Typsin hydrolysed shrimp		Fresh shrimp		Trypsin hydrolysed shrimp	
wet weight/g	dry weight/g	wet weight/g	dry weight/g	wet weight/g	dry weight/g	wet weight/g	dry weight/g
11.8	43.7	16.4	60.7	6	22.2	10.6	39.5

Table 4. Optical density at 540 nm and amount of acetylcholine hydrochloride at different stages.

Control		Fresh shrimp		Spoiled shrimp without head		Shrimp spoiled with head	
O.D. at 540 nm	mg of ach. HCI	O.D. at 540	mg of ach. HCI	O.D. at 540 nm	mg of ach. HCI	O.D. at 540 nm	mg of ach HCI
0:2875	0.72	0.562	1.417	0.316	0.7963	0.276	0.6955

Table 5. The acetylcholinesterase (qache) in fresh and spoiled shrimp moisture (75%)

Room temperature (24 – 30°)			Refrigerator (4°)		
Time (hr)	(QACHE) mg/g wet weight/g	(QACHE) mg/g dry weight/g	Time (days)	(QACHE) mg/g wet weight/g	(QACHE) mg/g dry weight/g
0	40.43	161.72	1	39.62	158.48
1	39.24	156.96	4	32.24	128.96
5	29.20	116.80	8	26.02	104.08
24	20.28	01.12	12	21.62	06.48

tion on the other according to the following possible mechanism.

1). Production of pyruvic and lactic acid by the glycolytic breakdown of muscle glycogen under anaerobic condition and of fatty acids by degradation of fats and proteins.

2). Aerobic utilisation of pyruvic acid with the formation of CO_2 through acetyl CO – A and through Kreb's cycle.

3). Utilisation of the acid by the buffering action of amines, ammonia and basic amino acids produced by proteolytic action.

Since the shrimp die soon after they are caught, in such a case glycogenesis stops whereas the utilisation of acids proceeds uninterrupted leading to more fall in titratable acidity.

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 simulataneously the rate of dehydrogenase acticity will be very high and consequently there will be less accumulation of acid. This is probably the situation with respect to spoilage of shrimp.

Trypsin does not seem to play such an important role in shrimp spoilage as it is reported to do in fish. Probably, the acetylcholinesterase activity is suddenly stopped in muscles at the time of death of shrimp. This accounts for high concentration of acetylcholine hydrochloride in fresh shrimp muscles. On storage some acetylcholinesterase enzyme penetrates from brain and ventral chord through the muscles and reduces the concentration of substrate in spoiled shrimps.

REFERENCES

1. J. Mietz, and E. Karmas, J. Assoc. Off. Anal Chem, **61**, 139 (1978).
2. B. Farooqui, R. B. Qadri, R. Fatima, R. Rafique and A. H. Khan, Pak. J. Sci. Ind. Res., **21**, 33 (1978).
3. F. Chanley and D. H. Goard, Can. J. Res **D20**, 20 (1942).
4. R. P. Elliot, Food Research, **12**, 87 (1947).
5. M. Q. Khuda, H. N. De, and J. C. Debnath, Pakistan J. Sci. Ind. Res., **2**, 217 (1950).
6. N. I. Macpherson, Ann. Ind. Food Invest. Bd. (Gt. Britain), 134 (1950).
7. J. G. Sharp, Biochem J., **29**, 850 (1935).
8. George Borgstrom, Fish as Food (Academic Press, New York and London, 1961), p. 275, Vol. 1.
9. M. Q. Khuda, H. N. De and N. M. Khan, Pak. J. Sci. Ind. Res., **5**, 156 (1962).
10. T. Thunberg, Skand. Arch. Physiol., **40**, 1 (1920).
11. S. Hestrin, J. Biol. Chem., **179**, 943 (1949).
12. F. Fiegal, V. Anger and O. Frehden, Mikrochemie., **15**, 12 (1934).
13. E. Stedman, L. Stedman and L. H. Easson, Biochem. J., **26**, 2056 (1932).