

A PRELIMINARY STUDY OF THE CHANGES ASSOCIATED WITH LIPID BREAKDOWN IN OIL SARDINE (*SARDINELLA LONGICEPS*) STORED AT REFRIGERATED TEMPERATURES

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INTRODUCTION

THE spoilage of a fatty fish like oil sardine is the cumulative effect of many phenomena controlled or influenced by various factors. When the fish is stored in ice, it has been known that during the first week of storage, the enzymes which continue to be active influence the various changes occurring in the tissue. After this period when the dominant bacterial strains become established, these exert marked influence on the spoilage pattern. The spoilage changes are further complicated by the changes in the oxidation of fat leading to rancidity and associated changes.

The fat of oil sardine shows qualitative and quantitative seasonal variation, its component fatty acids being mostly unsaturated. In this respect the observations are parallel to those exhibited by *Clupea harengus* (Lovern, 1938). The fat content and the degree of unsaturation of fat besides the amount of lipoxidase enzyme in the tissue are the major factors influencing the rate of development of organoleptic rancidity in cold stored fish (Banks, 1938). The development of this oxidative rancidity adversely affects the odour and flavour and probably the texture of fish during processing (Banks, 1937; Tarr, 1955; Lovern *et al.*, 1959). Since lipids of fatty fish contribute much to the undesirable flavour, even a slight oxidation of these may contribute to serious flavour changes. It has also been observed that higher the temperature of storage of fish, the more rapid becomes the process of decomposition of fat (Banks, *loc. cit.*). In the case of fish frozen after short-term storage in ice, the free fatty acids formed during initial hydrolysis destroy the stabilizing effect of lipids on actomyosin (Dyer and Fraser, 1959). The present investigation has been designed to study the effect of storage at two temperatures on the course of breakdown of lipids in oil sardine side by side with such changes as are brought out by the proteolytic action in the tissue during storage.

EXPERIMENTAL

Fresh oil sardine (*Sardinella longiceps*) of uniform size obtained in June-July were used for the study. The fish were washed in clean water,

sealed in thin polythene bags, divided into two lots, one lot stored in clean crushed ice in an insulated box and the other kept in refrigerator maintained at 10° C. The polythene bag prevented the desiccation of fish during storage at the same time permitting access of oxygen. For chemical examination of various constituents, the fish was scaled (skin not removed) head and viscera removed and the meat separated and minced in a waring blender. 10 gm. quantities of minced meat were weighed out accurately and the soluble constituents extracted into organic and aqueous phases of chloroform-methanol-water mixture according to modification (Rhodes and Lea, 1961) of the extraction procedure of Folch *et al.* (1957) facilitating the simultaneous recovery of nonlipid substances present in the tissue into the aqueous phase. The two phases were analysed for total phosphorus (TP) (Chen *et al.*, 1956), total nitrogen (TN) (MicroKjeldahl procedure), and total solids. For determination of total solids, the aliquot volume evaporated, dried in vacuum oven to constant weight and the residue weighed. Free fatty acid (FFA) was determined in the residue from the organic phase by taking up with hot 75% alcohol and titrating with N/100 NaOH using phenolphthalein as indicator. Inorganic phosphorus was also estimated in the aqueous phase besides total phosphorus. The minced meat is analysed for peroxide value (Tarr, 1947) and volatile acid number (V.A.N.) (A.O.A.C., 1960). The thiobarb turic acid number (TBA number) is determined in the lipid extract after removal of the solvent by the distillation method (Tarladgis *et al.*, 1960).

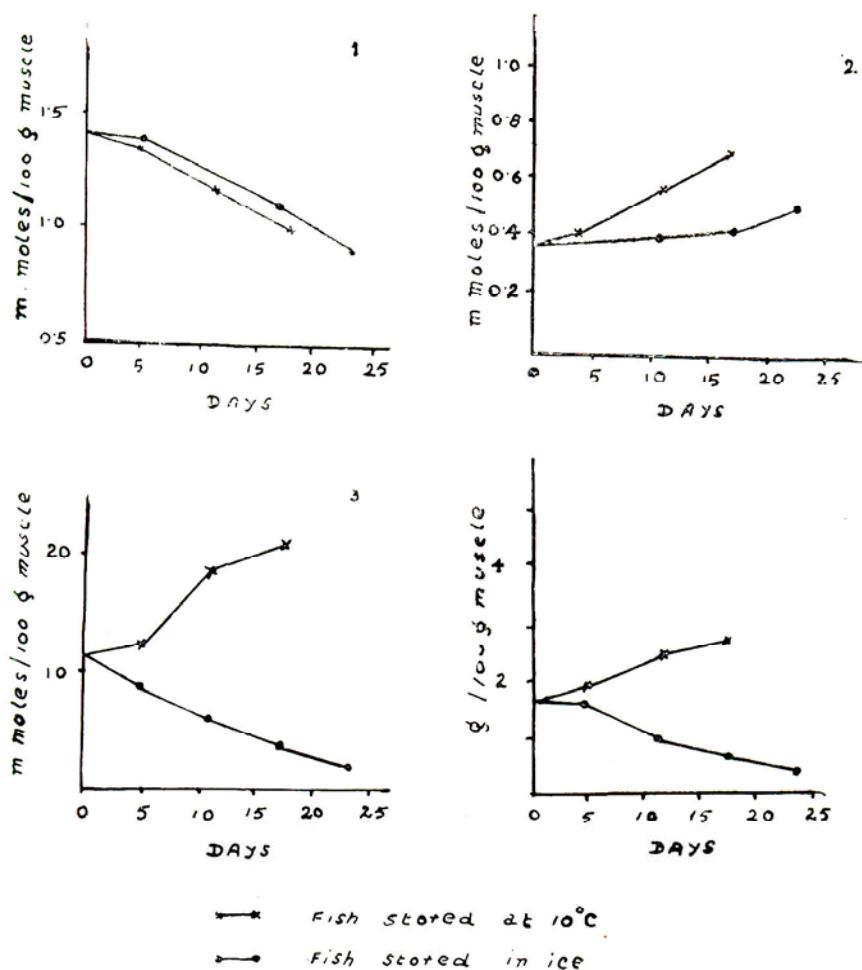
RESULTS AND DISCUSSION

The total lipid content of fresh fish muscle was 2.505% (wet weight basis). The initial analysis of the tissue is given in Table I.

TABLE I
Analysis of fresh fish tissue

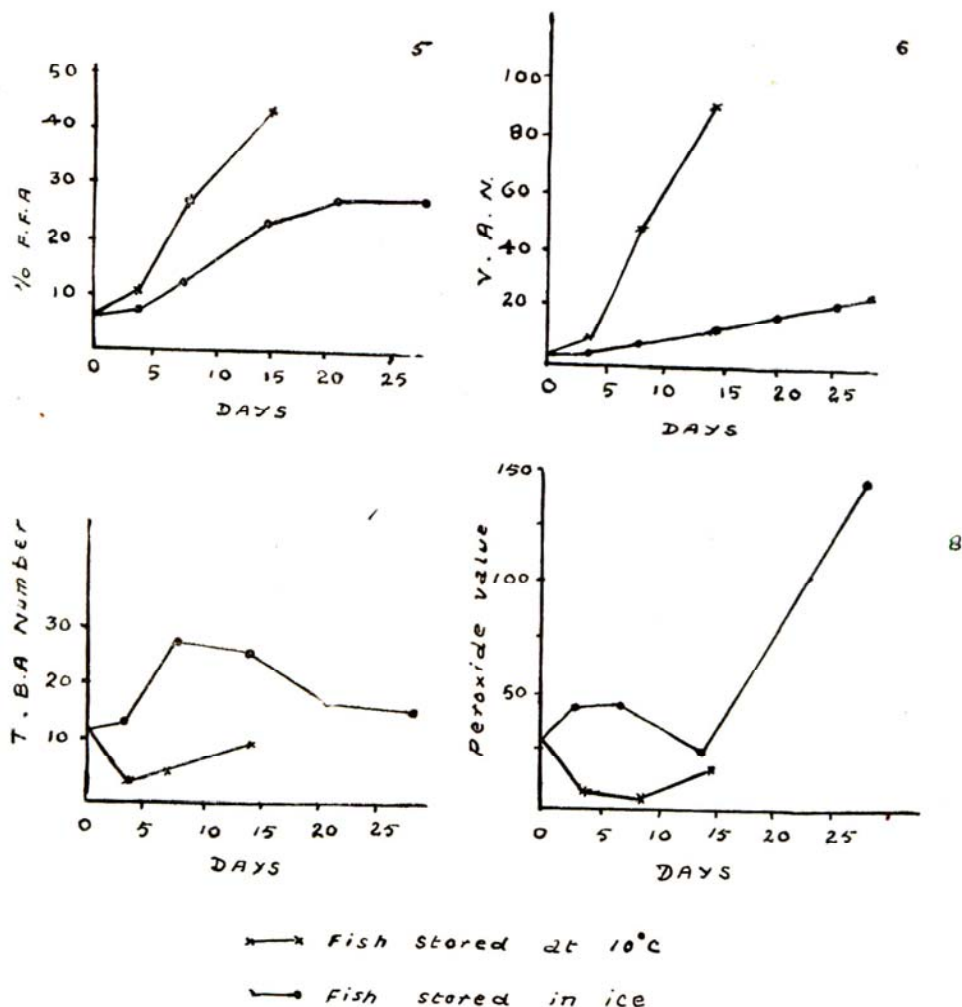
Organic extract		Aqueous extract	
Total P m.moles/100 gm.	.. 1.406	Total P m.moles/100 gm.	.. 6.380
Total N	.. 4.786	Inorganic P	.. 0.126
Dry solids g. %	.. 2.818	Total N	.. 11.77
		Dry solids g. %	.. 1.793

It has been found that the total solids extracted amounted to 4.61% of the wet weight of the muscle. Of the total phosphorus soluble in aqueous phase 33.1% has been worked out to be due to inorganic phosphorus. The changes in P in organic phase and TP, TN and total solids in aqueous phase are represented in Figs. 1-4.



FIGS. 1-4. Fig. 1. Changes in TP in Lipid extract. Fig. 2. Changes in TP in Aqueous phase. Fig. 3. Changes in TN in Aqueous phase. Fig. 4. Changes in Total solids in Aqueous phase.

Figures 5-8 represent changes in FFA, V.A.N., TBA number and P.V. with storage period. The total solids soluble in aqueous phase showed rise in samples stored at 10°C and this is accompanied by rise in amounts



FIGS. 5-8. Fig. 5. Changes in FFA in lipid. Fig. 6. Changes in volatile acid number. Fig. 7. Changes in TBA number. Fig. 8. Changes in Peroxide value.

of Total N too. However the increase in TN cannot be accounted alone by the degradation of phospholipid N and therefore this indicates that rapid proteolysis might be occurring at this temperature releasing large amounts of other water-soluble nitrogenous compounds, mainly amino-acids. There is good correlation between the increase in total solids and increase in TN of aqueous extract (Fig. 9).

In the case of ice-stored fish, the proteolysis might be occurring at a slower rate. However, the observed changes in total solids and total

nitrogen of the aqueous phase did indicate that these were continuously leached out by the washing action of ice.

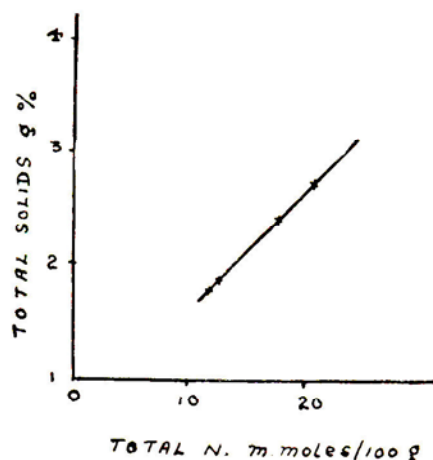


FIG. 9. Relationship between total solids and TN in Aqueous phase.

The changes in TP in organic phase show on one hand that phospholipid breakdown is of considerably little significance during storage in ice and at 10° C. The slight increase in TP in aqueous phase may be accounted for as being due to the partial decomposition of phospholipid releasing water-soluble inorganic phosphate. The breakdown of TP of lipid is however rapid at 10° C. than at 0° C. That the degradative changes of the fish tissue occur at a faster rate at 10° C. is further evidenced by the rapid accumulation of water-soluble solids, and production of volatile acids in the meat (Figs. 4 and 6). The volatile acids which are essentially the products of bacterial action showed a steep rise after the 4th day in fish kept at 10° C. Concomitant rise has been observed in the amounts of water-soluble nitrogen and total solids in aqueous phase beyond this period which should represent the maximum storage life of fish at this temperature.

The increase in FFA occurring as spoilage proceeds appears to be interesting. de Silva and Hughes (1962) observed that FFA production is not affected in fish by treatment with tetracycline antibiotics even when the bacterial counts have been lowered considerably. However the present study has shown that the activity of lipolytic bacteria influenced to a great deal the production of FFA at higher temperature (10° C.) as compared with enzyme system existing in fish. Alternatively, the lipase enzymes which were originally separated from the lipids of cell in the initial stages might

have been released by cellular disorganisation brought about by proteolysis thereby facilitating their action on the substrate (Lovern and Olley, 1962). In the case of fish stored in ice, the slow rise in FFA up to about 1 week indicates on the other hand that bacterial lipases are not involved under this condition, an observation identical with that made by Lea (1957).

Peroxide value and TBA number recorded always lower values in the case of fish stored at 10° C. than in fish stored in crushed ice. Watson (1939) observed that this behaviour is due to the presence of increased numbers of aerobic bacteria on surfaces of fish at higher temperature of storage leading to the formation of an oxygen barrier against the availability of oxygen to subcutaneous fat layer. This explanation appears plausible because reduction of bacterial numbers by treatment with CTC has led to higher P.V. being recorded in the meat (de Silva and Hughes, 1962). Although FFA increased steadily with storage period, changes in P.V. were not found useful in judging the development of rancidity. It has also been observed by Gaddis *et al.* (1959) that detectable rancidity appears in different fats with different P.V. levels. As is expected, the oxidative breakdown of fatty acids considerably reduced the solubility of lipids extractable by fat solvent. The percentage of fat at the end of 17 days decreased to 7.65 (Dry weight basis) from the initial value of 8.45 (D.W.B.). According to Zipser *et al.* (1962) the lower TBA value observed in lipids obtained from fish stored at 10° C. may be due to incomplete extraction of TBA active material from oxidising fish tissue especially at later stages of storage.

SUMMARY

The course of lipid breakdown and proteolysis of muscle has been followed in oil sardine stored at two different temperatures, 10° C. and temperature of ice. The amounts of total solids and nitrogen extractable into the aqueous phase of chloroform-methanol-water mixture indicate that rapid proteolysis occurs at the higher temperature. Changes due to phospholipid breakdown are of relatively little importance at the two temperatures. Volatile acid number showed rapid rise after the 4th day in fish at 10° C. Free fatty acid production is found to be influenced by the increased proteolytic rate. TBA number and peroxide value were always higher in fish stored in ice. The various changes observed have been discussed.

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REFERENCES

- A.O.A.C. 1960 .. *Official Methods of Analysis*, 9th edn., Assoc. Official Agr. Chemists, Washington.
- Banks, A. 1937 .. Rancidity in fats. I. Effect of low temperature, sodium chloride and fish muscle on the oxidation of herring oil. *J. Soc. Chem. Ind.*, **56**, 13 T-15 T.
- 1938 .. The storage of fish with special reference to the onset of rancidity. I. The cold storage of herring." *Ibid.*, **57**, 124-28.
- Chen, P. S., Toribara, T. Y. and Warner, H. 1956 Microdetermination of phosphorus. *Anal. Chem.*, **28**, 1756-58.
- de Silva, N. N. and Hughes, R. B. 1962 Influence of tetracycline antibiotics on the spoilage of herring (*Clupea harengus*). *J. Sci. Fd. Agric.*, **13**, 161-68.
- Dyer, W. J. and Frazer, D. I. 1959 Proteins in fish muscle. 13. Lipid hydrolysis. *J. Fish. Res. Bd. Canada*, **16**, 43-52.
- Folch, J., Lees, M. and Stanley, G. H. S. 1957 A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509.
- Gaddis, A. M., Ellis, R. and Currie, G. T. 1959 Carbonyls in oxidising fat. I. Separation of steam volatile mono carbonyls into classes. *Food Research*, **24**, 283-97.
- Jensen, L. B. 1945 .. *Microbiology of Meats*, Gerrard Press, Champgn.
- Lea, C. H. 1957 .. Deteriorative reactions involving phospholipids and lipol proteins. *J. Sci. Fd. Agri.*, **8**, 1-13.
- Lovern, J. A. 1938 .. Fat metabolism in fishes. XII. Seasonal changes in the composition of herring. *Biochem. J.*, **32**, 676-80.
- , June Olley and Watson, H. 1959 Changes in the lipids of cod during storage in ice. *J. Sci. Fd. Agri.*, **10**, 327-37.
- and June Olley 1962 Inhibition and promotion of postmortem lipid hydrolysis, in the flesh of fish. *J. Food. Sci.*, **27**, 551-59.
- Rhodes, D. N. and Lea, C. H. 1961 Enzymic changes in lamb's liver during storage. *J. Sci.-Fod. Agri.*, **12**, 211-24.
- Tarladgis, B. G., Watts, M. B., Younathan, M. T. and Dugan, L. 1960 A distillation method for the qualitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.*, **37**, 44-48.
- Tarr, H. L. A. 1947 .. Control of rancidity in fish flesh. I. Chemical antioxidants. *J. Fish. Res. Bd. Canada*, **7**, 137-54.
- . 1955 .. Oxidative rancidity in frozen fish, *Chemistry in Canada*, **7**, 39-41.
- Watson, D. W. 1939 .. Fish spoilage. IV. The bacterial reduction of Trimethylamine oxide, *J. Fish. Res. Bd. of Canada*, **4**, 252-66.
- Zipser, M. W., Jacqueline Dupont, and Watts, B. M. 1962. "Extraction of lipids from oxidising mullet," *J. Food. Sci.*, **27**, 135-38.