CHEMICAL CHANGES IN SKIN MUCIN AS AN INDEX OF EARLY STAGES OF SPOILAGE IN FISH

S. V. SURYANARAYANA RAO AND *S. J. LEE
Central Food Technological Research Institute, Mysore

Results of a preliminary investigation on the overall chemical nature of fish skin mucin in lung fish, *Clarias batrachus*, with special reference to water soluble low molecular weight compounds, are presented. Changes observed during room temperature spoilage have been studied with a view to present a new approach towards the assessment of freshness in fish inspection. pH of the mucin was distinctly alkaline (8.2) and remained unchanged during spoilage. Much of the nitrogen was found to be present in the glycoprotein fraction. Free amino acids and purine bases were present in apreciable quantities in the aqueous extracts which registered a significant increase after 10 hrs. Post-mortem increase in total solids was accompanied by a slight rise in protein nitrogen which may indicate tissue breakdown. Increase in TVN was also observed to occur earlier in the outside mucin as compared to the inside muscle. Presence of free sugars or sialic acid could not be confirmed nor was there any indication of cholesterol and lipoid material as stated in earlier literature.

INTRODUCTION

Although much attention is paid in fish inspection to the condition of the slime on the surface which turns turbid and shows discolouration in stale fish, very little information is actually available on the chemical nature of the fish skin mucin and its constituents. There have been some investigations, of late, on muco-proteins of fish skin fluid and the nature of the sialic acids present in particular (Enomoto, Nakagawa and Tomitasu, 1966). While such fundamental knowledge regarding the chemistry of fish skin mucin is of great interest, it is more appropriate to study the basic physico-chemical nature of the skin mucin

* M.Sc student; 1969-71 at International Food Technology Training Centre, CFTRI, Mysore.

and post-mortem changes, if any, in low molecular weight compounds, which may serve as indices of early spoilage of fish. It is well known from the work of Wood (1940) that fish slime, which might possess bactericidal properties in case of live fish, forms the main source of contamination after death. Results of the preliminary investigations at this Institute which indicate the presence of free amino acids in slime and the gradual increase in free amino acids, purine compounds and volatile bases on the onset of spoilage have been presented here.

Materials And Methods

Since marketed fish showed wide variation in the moisture content and pH of the mucin lung fish (*Clarius batrachus* Linn.), which could be obtained locally, maintained in live condition were employed for the study of room temperature spoilage under controlled conditions. The fish were kept in running water in the laboratory for 24 hours before the commencent of the investigation. Another air-breathing variety, murrel (*Channa marulius* Ham. as also one popular species of carp (*Barbus carnaticus*) were also examined in the initial experiments. Fish were killed by administering a powerful electric shock and stored in a closed dessicator over water since it was observed that fish skin mucin gets dried up quickly under tropical conditions in most species of fish. Light spraying with water promotes a fresh secretion in such cases. Condition of the slime was examined at intervals upto 10 hours and skin mucin extracts were prepared after spraying lightly whenever necessary, with distilled water. Each fish was shaken with 500 ml. distilled water or other media in a tall cylinder or closed glass container, using the same suspension repeatedly for all the fish. One dozen fish were extracted as above and transferred again to the dessicator.

Total solids were estimated in the skin mucin extracts by evaporation over water bath followed by drying *in vacuo*. Total solids in the skin mucin itself was estimated by rubbing off the skin with a previously weighed cotton swab and drying at 90°C to constant weight.

Total nitrogen (TN) was estimated in the mucin suspension by microkjeldahl distillation after digestion. Glycoprotein nitrogen (GN) was estimated by adding absolute alcohol (171 ml.) to 100 ml. of the mucin extract to obtain an ethanol concentration of 65% and estimating nitrogen in the filtrate obtained after removing the precipitated material (Dische, 1965). Protein nitrogen (PN) was estimated by adding an equal volume of 20% trichloracetic acid (TCA) solution to the mucin suspension. GN and PN were obtained by difference. It was observed that non-protein nitrogen (NPN) which is estimated in all the three cases form an insignificant portion (0.01%) of the total solids by this procedure. Total volatile nitrogen (TVN) was estimated in the mucin extract by distilling 10 ml. after adding sodium borate in a microkjeldahl distillation unit. TVN of the fish muscle was obtained by distilling 10 ml. of an alcoholic extract prepared from 5 g. of the muscle.

Free amino acids present in the mucin extract were studied by two dimensional paper chromatography employing n-butanol-acetic-acid-water (4 : 1 : 5) and sec-

TABLE I

CHEMICAL CHANGES IN FISH SKIN MUCIN DURING ROOM TEMPERATURE SPOILAGE OF LUNG FISH (*CLARIAS BATRACHUS*)

Time (hrs)	pH	* Total Solids in mucin extract %	GPN expressed as % of total solids	PN expressed as % of total solids	GPN/PN
0	8.2	0.120	3.08	1.78	1.73
4	8.2	0.365	3.14	1.83	1.72
6	8.2	0.380	3.34	1.94	1.72
8	8.1	0.375	3.48	2.57	1.31
10	8.1	0.590	3.12	2.17	1.43

* 12 fish in size 15 - 31 cm. extracied with 500 ml. water; same fish used repeatedly for extraction at time intervals.

TABLE II

CHANGES IN FREE AMINO ACIDS IN SKIN MUCIN OF LUNG FISH (*CLARIAS BATRACHUS*) DURING ROOM TEMPERATURE SPOILAGE (VALUES EXPRESSED AS mg./100 ml. OF MUCIN EXTRACT)

Time (hrs.)	Glycine	Glutamic acid	α alanine	Iso-leucine/ Leucine	Lysine
0	1.092	—	—	1.28	—
4	4.370	2.84	2.54	2.57	2.30
6	4.920	3.55	1.09	5.14	—
8	6.560	4.26	2.90	6.42	—
10	8.750	5.68	5.07	9.00	2.30

butanol : 3% ammonia (3 : 1) as solvents. Those of the amino acids present in significant amounts were estimated quantitatively by elution of the spots with 70% alcohol using a Bosch and Lomb spectrophotometer. Purine bases were estimated by one dimensional paper chromatography employing n-butanol : acetic acid : water (4 : 1 : 1) and n-propanol : 3% ammonia (65 : 35) as solvents. The chromatograms were examined under U. V. light and quantitative examination was undertaken after elution from paper with 0.1 N hydrochloric acid. Molecular extinction coefficients (Block, Durrum and Zweig, 1955) were employed for calculation.

Mucin extracts were also tested for the presence of other substances like free sugars, sialic acid and sterols. Aniline phthalate was employed for the detection of free sugars (Patridge, 1949) and the procedure recommended by Zlatkis, Zak and Boyle (1953) was employed to reveal the presence of cholesterol. Paper chromatography utilising orcinol : trichloroacetic acid reagent was employed for the detection of free sialic acid as recommended by Zilliken and White House (1958). Solvent ether was employed for the extraction of lipoid material, if any, from the mucin extract.

Results And Discussion

Results of the present investigation presented in Tables I to IV reveal that the skin mucin of the cat fish studied, differs considerably from that of eel mucin in which albumin was said to be the main component (9%) besides lipoid material like cholesterol and lecithin (Brown, 1957). In the case of cod slime, the total solids were reported to be 15% of which 60% was made up of protein, the rest being presumably free sugars, amino acids, hexosamine and sialic acid besides mineral constituents (Shewan, 1971). As mentioned earlier, emphasis is placed in recent years on the presence of a sialic acid containing glycoprotein as also a sulfated mucopolysaccharide in Japanese eel mucin (Asakawa, 1970). Although the main objective of the present study was to follow the changes in skin mucin during room temperature spoilage, it will be of interest to consider the general features of the fish skin mucin in the fresh condition.

Results of the present investigation revealed a wide range of variation in the total solids of fresh fish skin musin. In freshly caught murrel (*Ophicephalus striatus* Day) it was only 8.34% while a market sample of carp (*Barbus carnaticus*) showed a high value of 29.33%. It may be observed from the controlled study on the thickening of surface mucin in lung fish (Table I) that there has been a five fold increase in total solids during spoilage at room temperature for 10 hrs. pH of the fish mucin was found to be distinctly alkaline (8.2) which was practically unchanged even upto 10hrs. after death. It is believed that in addition to the lubricating effect and protective action against bacterial and parasite infection in live fish, skin mucin may help the fish to move in muddy waters by precipitating clay colloids. Refractive index of the fresh skin mucin in murrel was found to be 1.340 by Abbe refractometer.

As compared to the earlier work quoted above, results of the present study failed to confirm the presence of lipoid material. Lipid content (ether soluble) was negligible. Test for cholesterol did not reveal the characteristic purple colour although a light brownish colour was noticed. Free sialic acid was not in evidence even after anion exchange resin treatment. Free sugars were also absent under the conditions of the experiment.

Much of the nitrogen is present in the glycoprotein fraction and the other proteins (N x 6.25) form about 15% (Table I) of the total solids. As it may be observed from the procedure adopted for the estimation of glycoproteins, precipitation at 65% ethanol concentration was employed as the criterion although glycoproteins separate out over a wide range of alcohol concentration upto 70%

TABLE III

CHANGES IN PURINE BASES IN SKIN MUCIN OF LUNG FISH (*CLARIAS BATRACHUS*) DURING ROOM TEMPERATURE SPOILAGE (VALUES EXPRESSED AS μ M./100 ml. OF MUCIN EXTRACT)

Hours	Unknown	Hypoxanthine	Xanthine
0	3.5	62.5	1456
4	156	0	1515
6	0	0	3000
8	0	0	4120

TABLE IV

CHANGES IN TOTAL VOLATILE NITROGEN (TVN) DURING THE ROOM TEMPERATURE SPOILAGE OF LUNG FISH *CLARIAS BATRACHUS*

Hours	TVN in fish muscle (mg /100 g. %)	TVN in mucin extrat (mg /100 ml.)
0	3.60	0.23
4	5.75	0.94
6	5.88	1.41
8	5.52	2.56
10	4.56	2.50

(V/V). PN was similarly estimated by difference after TCA precipitation since acidic glycoproteins are soluble under these conditions. It may be observed that PN registered an appreciable increase upto 8 hours during room temperature spoilage. This may be due to the breakdown of structural protein or a change in the nature of mucin secreted in spoiled fish. It may be remembered however that the changes observed in proteins pertain to water soluble constituents. When 2% alkali was employed for extraction, total solids in the extracts were 3.67% as against 0.17% by aqueous extraction.

Apart from the changes in the protein fraction, the main changes observed in

the fish skin mucin during room temperature spoilage relate to free amino acids and purine compounds. Although it was known that free amino acids occur in the outside mucin which might serve as ready substrates to the bacteria during early spoilage, a definite increase was observed in some of the free amino acids, namely glycine, glutamic acid, α-alanine and leucine/iso-leucine (Table II) Shewan (*loc. cit.*) has referred to the presence of α-alanine and leucine besides taurine and tyrosine in cod slime. It is of interest to note that lysine which is generally associated with early spoilage of fish does not show any change. Absence of cystine and proline is also significant since they occur prominently in skin and connective tissue proteins. Since there is no reduction in any of the free amino acids during the entire course of spoilage and an actual increase has been observed, further study is needed regarding the origin and role of free amino acids in fish spoilage by microorganisms. Moreover significant differences were observed in the free amino acid pattern between the mucin of lung fish and murrel. While lung fish mucin showed in addition to those estimated quantitatively, traces of methionine, serine, tyrosine and aspartic acid, the sample from murrel showed only lysine, glycine, serine and cystine.

Apart from free amino acids, another important finding is the presence of high amounts of purine bases in fish skin mucin (Table III). Although their presence in fish slime is probably related to the reported occurrence of guanine in fish scales (Zaitsev *et al.* 1969) high levels of purine bases like xanthine and hypoxanthine in the skin mucin and their striking changes observed during the present study calls for further investigation. It was observed that xanthine, the main component increases during the later stages of spoilage while hypoxanthine disappeared soon after death accompanied by a transient rise in an unidentified compound.

It may be observed from the comparative study of TVN levels in the fish skin mucin and in the inside fish muscle that the onset of spoilage occurs sooner in the outside mucin with a tenfold rise in TVN level in 10 hrs. (0.23 to 2.5mg./100 ml). Further work is in progress to characterise the nature of the volatile nitrogen in skin mucin and its relation to the early spoilage of uniced fish.

Based on these observations, it may be possible that a systematic study of the chemical changes in skin mucin will help in devising improved methods for the quality control of fish.

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