BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part VI.—Bacterial Decomposition in Scaly and Non-scaly Fish and Assessment of their Spoilage Mechanism

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The total bacterial count in gills, intestine, fillet and skin of some scaly and non-scaly fish and also in the scales of the scaly ones was investigated in the fresh and decomposed conditions after storage for 24 hours at the room temperature of 80-85°F. The apparent rate of multiplication of bacteria due to storage of the tissues was also studied. From the results it would appear that skins of the scaly fish showed greater increase of the bacterial population due to storage as compared to those of non-scaly ones. This has ultimately caused higher bacterial invasion in the fillet of the scaly fish. As compared to other tissues, the fillet of both scaly and non-scaly fish and the skin of the scaly fish are considered to be better medii for the growth and multiplication of the bacteria. The significance of these rusults in the assessment of the mechanism of fish spoilage has been discussed.

Introduction

A large amount of work carried out in different laboratories with respect to the bacterial decomposition of fish has been reported in literature[1,2] and among the various probable routes of bacterial invasion so far investigated, the importance of skin has been stressed by Stewart,[3] Beathy and Gibbons,[4] Luncke and Frereke,[5] Reay and Shewan[1] and others. Qudrat-i-Khuda, De and Khan[6] in their previous investigation on the study of the mechanism of fish spoilage have made similar suggestion regarding the involvement of skin in allowing the passage to bacteria for partial decomposition of fillet.

In certain species of fish (non-scaly) the skin is directly exposed to the air and water, and in others it is imbedded with the scales of different shapes and sizes which keep them concealed from air and water. In spite of the accumulation of sufficient literature on the various aspects of bacterial invasion, no information has yet been reported regarding the characteristic behaviour of the skin of the two classes of fish (scaly and non-scaly) in allowing the passage of bacteria for causing spoilage in the fillet. In East Pakistan a large number of fish such as *Wallago atu* (Boal), *Mystus vittatus* (Tengra), etc. belong to the non-scaly group, and its was thought desirable to study the bacterial invasion in their fillet and in other parts of their bodies by comparison with similar invasion in scaly fish like *Labeo rohita*, (Rohu), *Cirrhina mrigale*, (Mrigale), *Catla catla* (Katla), etc. This aspect of investigation was desirable in order to collect information on the rate of spoilage of fillet or other parts of different species of fish of scaly and non-scaly groups, as this would ultimately facilitate the proper handling, transport and storage of fish in this tropical region.

Experimental

Collection of the Fish Sample.—Eight species of fish as shown in the table were examined in the course of the present work. Some of these belong to the non-scaly group as Air, Boal, Singi, Tengra, Pabda and some to the scaly group like Rohu, Koi, Punti and Leta.

Carps and other big species of fish were selected at their young age between 8 to 10 inches in size. The smaller species like Punti, Tengra etc. were collected at their maximum age of maturity.

The collection of fish samples was made after a lapse of 4 hours from the time of catch. Small range of bacterial population which might have diffused into the fillet during that period could not cause any perceptible decomposition as was evident from visual freshness by organoleptic and sensory tests.

Media.—The determination of the total bacterial count was carried out by the solid agar plate culture method. The medium of the following composition was found suitable for the present work.

A concentrated beef extract was prepared by boiling chopped beef in the proportion of 1 lb. beef in 1 litre of water for 4 hours. The volume was kept constant during this boiling process. The solid medium was then prepared by dissolving 2 g. NaCl, 1 g. glucose, 2 g. peptone and 1.5 g. agar in each 100 ml. lot of the above beef extract. The medium, after adjustment to pH 7.0, was ready for use.

Preparation of the Inoculum.—The inoculum of the following tissues was prepared for total bacterial count.

(a) SCALES: The scales of the scaly fish were carefully removed from the skin occupying the region covering the dorsoventral muscles between the pectoral and ventral fins.

(b) SKIN: The combined epidermis and epithelial layers of skin of the above region of both scaly and non-scaly fish was carefully separated from the fleshy portion.

(c) FILLET: The fleshy portion beneath the above skin was collected for the same purpose from both sides between the skin and peritoneal lining.

(d) INTESTINE: The intestine separated from the stomach, spleen and liver was opened and after removal of the food residue weighed in a sterile weighing bottle.

(e) GILLS: Samples of gills from both the pouches were collected for the present series of work.

About 0.5 to 2 g. samples were immediately weighed in a sterile bottle for total bacterial count. All the samples were first disintegrated in 10 ml. sterile 1% salt solution and then diluted to a definite volume with the sterilised water—the final volume made depended on the nature of the tissue used. A number of plates with the agar media was inoculated with 0.2 ml. of the above diluted inoculum. For each tissue sample, more than six plates were inoculated and for each tissue at least six sample inoculum from different fish of the same size and from same batch were prepared.

For the study of the increase of the bacterial invasion due to spoilage under storage condition, some samples of fish of the same size and body weight collected from the same batch were stored at ordinary room temperature (80-85°F.), and after 24-hour storage the total bacterial counts from similar tissues were made in the same manner as before. The 'apparent' rate of multiplication of the bacteria per unit organism due to storage was then calculated by dividing the final count with the initial one. Ordinarily the multiplication and destruction of bacteria proceed very rapidly. The total bacterial count determined at any period does not, therefore, indicate the actual number of bacteria multiplied up to that period. For this reason the term 'apparent' has been used in the present study of the rate of multiplication per unit organisms. Determination of the above value will help to judge the characteristic behaviour of different tissues and organs for acting as the medii for the growth and multiplication of bacteria and finally to explore which of the tissues are more apt to bacterial invasion in preference to the others. This information is, however, not available in earlier investigations, reported in the literature.[1,2]

Results and Discussion

From the results presented in Table 1 it is observed that the scales of fish of group B harboured original bacterial population ranging from 1.93×10^5 to 1.15×10^6/g. of wet basis. On storage, the total count increased to the level of 5.6×10^6 to 4.2×10^7 and their apparent multiplication rate showed the value ranging from 16 to 93. On surveying the data of the skin it would appear that the bacterial count of the fresh skin of scaly fish is almost of the same order as that of non-scaly ones within the range of 1 to 5.2×10^5; though the skin of the scaly ones was not directly exposed to air or water like that of non-scaly one. Ultimately, however, bacterial invasion in the skin of scaly fish also occurs from the water and the air via the medium of the scale. On storage, the total count of the skin of scaly fish showed greater increase to the extent of 3.9×10^7 to 1.7×10^8 as compared to that of the non-scaly one, having a lower range of increase to the level of 1 to 8.6×10^6. The apparently high multiplication rate to the extent of 121 to 410 in the skins of scaly fish, after their storage, may be due to their anatomical structures and biological constituents which render them more favourable media for the growth and multiplication of bacteria and also allow the passage of more bacteria from the scales.

Regarding the invasion in gill it was observed that in fresh condition, the bacterial count in the gills of both the scaly and non-scaly group of fish was almost of the same order ranging from 1.8×10^5 to 2.2×10^6. This, on storage increased to the level of 1.3 to 6.7×10^7 at the apparent multiplication rate of 41 to 80. The intestinal count before and after storage, and the apparent multiplication rate showed similar range of values as noted in the gills.

A very striking feature was recorded in the case of the bacterial population of the fillet of the above two groups of fish. Unlike the other tissues discussed above, the fillet of the scaly fish in their fresh condition (after 4 hours from the period of catch) and in decomposed stage after storage showed comparatively larger bacterial population

TABLE 1.—THE TOTAL BACTERIAL COUNT OF FRESH AND DECOMPOSED FISH TISSUES. THE INITIAL VALUE REFERS TO THE COUNT IN THOUSANDS PER G. BASIS OF FRESH CONDITION BEFORE STORAGE AND THE FINAL REFERS TO THE DECOMPOSED TISSUES AFTER STORAGE FOR 24 HOURS AT 80-85°F. APPARENT RATE OF MULTIPLICATION (R.M.) REFERS TO THE MULTIPLICATION PER UNIT ORGANSIM AND IS OBTAINED BY DIVIDING THE FINAL COUNT WITH THE INITIAL ONE.

Zoological name of the fish	Local name	Scale			Skin			Gill			Intestine			Flesh		
		Initial	Final	R.M.	Initial	Final	R.M.	Initial	Final	R.M.	Initial	Final	R.M.	Initial	Final	R.M.
GROUP - A NON-SCALY FISH																
Wallago atu (Bl and Sch)	Boal	—	—	—	328	2,280	7	360	15,716	43	472	12,356	26	7	2,380	340
Mystus aor	Air	—	—	—	315	8,600	26	380	18,750	50	230	18,000	78	9	2,840	316
Heteropneustes fossilis (Block)	Singi	—	—	—	170	8,000	47	333	18,540	55	435	18,350	42	3.5	2,080	594
Callichrous bimaculatus (Block)	Pabda	—	—	—	450	5,580	12	320	25,670	80	380	28,380	80	8.5	4,270	500
Mystus vittatus ..	Tengra	—	—	—	260	1,080	4.2	180	13,800	77	370	12,500	34	3	1,860	620
(GROUP - B) SCALY FISH																
Labeo rohita ..	Rohu	193	18,000	93	330	39,950	121	1320	54,200	41	1 338	27,460	25	43	10,762	250
Barbus (Puntius) *Sophare*	Punti	750	15,000	20	100	19,500	195	695	33,250	47	1,040	44,060	43	65	24,440	376
Ophicephalus Puncuatus	Leta	1,155	42,070	30	520	170,000	326	2242	67,215	30	223	22,708	101	76	18,270	240
Anabaus testudinius ..	Koi	337	5,380	16	290	120,000	410	389	28,000	74	480	32,000	66	85	59,000	694

than those of the non-scaly fish. This high population is perhaps due to the higher level of the count in their skin from which probably some bacteria have penetrated into the fillet by direct contact. So, apparently, the fillet of the scaly fish will undergo bacterial decomposition more quickly than the fillet of the non-scaly ones.

It is further noted that the apparent rate of bacterial multiplication in the fillet of both scaly and non-scaly fish is very high to the extent of 240 to 694 as compared with those of other tissues excepting the skin of scaly fish. This indicates that probably the fillet is a better and more favourable medium for the growth and multiplication of bacteria than the other tissues.

Another noteworthy feature of the observation was the lower total bacterial population of the fillet of the stored fish of both scaly and non-scaly group as compared to those of their intestines. This would indicate that probably the intestinal bacteria cannot penetrate through the belly wall and cause decomposition in the fillet. These observations would rather substantiate the previous concept of Qudrat-i-Khuda, De and Khan[6] that the enzymes of the bacteria of the intestine rather than the bacteria themselves find passage through the belly wall and cause partial decomposition of the fillet in addition to the direct decomposition effected by the bacteria penetrating through the skin.

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