OBJECTIVE TESTS FOR FISH QUALITY

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Evolution of a universally applicable objective test for determination of the quality of fresh fish is a problem that has so far been evading solution in spite of sustained research by fish processing technologists and offers no prospects in sight of arriving at a satisfactory one. Attempts so far made towards this end have been centred round measurements of products of spoilage released during progressive staling of fish either at room temperature or in ice storage. The important spoilage products which have been made use for determining the fish quality are trimethylamine and dimethylamine produced by bacterial reduction of trimethylamineoxide generally found in varying quantities in fresh marine fish muscle, total volatile basic nitrogen formed by degradation of several compounds like amines, amino acids etc., bacterial loads including total plate count and specific pathogenic organisms, all of which multiply in leaps and bounds as spoilage progresses, volatile acids formed by deamination of lower amino acids etc. and volatile reducing substances. Several other tests making use of one or more of the above spoilage products have also been developed subsequently, such as the picric acid turbidity test (a meaaure of basic compounds), tetrazolium and resazurin reduction tests (a measure of reducing substances), colour development in sugar media (acid production by bacteria), methylene blue indicator test (discolouration of methylene blue due to reduction by bacteria), ninhydrin colour tests (based on ninhydrin reacting substances produced during spoilage) and several test papers making use of the changes in pH of the fish muscle due to spoilage reactions. Peroxide value and free fatty acids are determined in the case of fatty fishes as a measure of oxidative and hydrolytic deteriorations that have taken place in the fish muscle. Rapid chemical methods for the diagnosis of putrefaction in toleost marine fishes have been reported making use of hydrogen sulphide, ammonia and indole which are liberated during spoilage. These consist in testing the aqueous extracts of the muscle with lead acetate paper for the presence of hydrogen sulphide, with Nessler reagent for the presence of ammonia and treating a hydrochloric acidisoamyl alcohol extract of the muscle with Ehrlich Kowac's reagent for detecting the presence of indole.

H. Wittfogel who studied direct bacterial counts, pH and volatile basic nitrogen in the case of whole and filleted unfrozen and frozen cod, reports that direct bacterial count could not be used as a measure of freshness of frozen fish, but could be used only as an index of sanitary conditions under which the fillets were prepared, whereas pH and volatile basic nitrogen could be used as indices of the condition of the frozen fish and limits for acceptability could be fixed as 6.8 and 30 mg% respectively. S. Bethea and M. E. Ambrose of the U. S. Bureau of Commercial Fisheries have observed that bacterial counts, trimethylamine and volatile acid contents are not sufficiently sensitive to detect deterioration in frozen shrimp prior to spoilage; but measurement of pH and amino nitrogen contents are of value in indicating loss of freshness. However, the magnitude of change is small. J. M. Shewan and A. C. S. Ehrenberg of Torry Research Station, Aberdeen, have recorded in the case of iced North Sea Cod that volatile base content of fish muscle is a less precise index of eating quality than was expected, although it has considerable value. Scientists of the Fisheries Research Board of Canada like S. A. Beaty and N. E. Gibbons, C. H. Castell and R. E. Riggs and C. H. Castell etc. have found trimethylamine content as a useful index of freshness and they have applied this for grading of fish for quality.

According to Bethea and Ambrose, a freshness test for a fishery product must conform to certain criteria, viz; it must be capable of sensitively and accurately estimating the product or products of spoilage, which must be either absent or present in small but constant concentrations in the unspoiled sample and must increase or decrease regularly and rapidly when once spoilage starts. But what we find practically is that being a biological material, different varieties of fishes show widely varying biochemical compositions in the fresh condition depending upon the species, sex, maturity, environment from which they are caught and season. For example, the elasmobranch fishes are cartileginous in structure containing high proportions of urea which decompose easily and emenate strong ammoniacal odours (volatile bases) as soon as spoilage starts. Marine fishes contain comparatively higher proportions of trimethylamineoxide in the muscle which is reduced to trimethylamine during spoilage, whereas fishes of fresh water origin contain practically little of this compound and it is futile to search for its breakdown product to assess the state of spoilage of such fishes. Shell fishes like prawn, lobster, crab etc. contain relatively high proportion of free amino acids in the fresh condition which fall rapidly in ice storage, whereas teleost fishes behave in an entirely different way with respect to this compound. Stage of growth of fish and season also play a very important role in the overall biochemical composition of the different species of fishes.

We must also know something about the mechanism of spoilage in order to have a clearer view of this problem. To quote the words of H. Beard of U. S. Bureau of Commercial Fisheries, a fish starts to spoil the minute it is taken out of water. Fish is just about the most perishable of all foods and we cannot entirely stop the progress of its spoilage; but only slow it down. The principal factors causing spoilage in fish are bacteria and autolysis. (Of course, oxidation by atmospheric oxygen also plays an important part in the case of fatty fishes like our oil sardines.) The flesh of a fish is free from bacteria as it comes from water; but the surface slime and intestinal tract contain large numbers of spoilage bacteria. As long as the fish is alive, the number of these bacteria and their activities are kept under control; but when once death occurs, they multiply rapidly and quickly ruin the fish if their activities are not checked. Autolysis or self digestion is the process of breakdown of the proteins and other entities of the fish muscle into simpler substances by the enzymes or ferments naturally present in the fish muscle and assist in the useful biochemical transformations when the fish is alive.

As indicated before, the course of production of the various products of spoilage in the fish muscle primarily depends upon its biochemical composition; and their amounts at a particular stage of deterioration are determined by various factors like their quantities originally present in the fish muscle. temperature of holding, i.e., at room temperature or refrigerated temperatures, mode of holding, i.e., iced or without ice etc. In case the fish is held without ice, all products of spoilage, viz; trimethylamine, volatile bases, volatile reducing substances, volatile fatty acids etc. as well as bacterial loads show steady increases, the rates of which are decided by the temperature of holding. The higher the temperature of storage, the greater the rates of production of these materials. For example, at a storage temperature of 5°C, fish spoils twice as fast as it does at 0°C. The rate of spoilage at 11°C is four times that at 0°C. But when the fish is stored in ice as soon as it is caught, it presents an entirely different picture. The spoilage change are retarded by the low temperature and the rates of production of the spoilage products considerably reduced. Moreover, the melting ice exercises a remarkable washing effect on the fish, which consequently washes out these minor constituents which are highly soluble in water and even part of water soluble protein and non-protein constituents from the muscle. Hence in the initial stages of storage in ice, these spoilage indices exhibit a decrease. This washing effect affects the bacterial loads also. Part of the superficial bacterial contamination of the fish is washed away, while part of it as well as part of those present in the internal organs like intestinal tracts and gills get destroyed due to the lowered temperature, as only the psychrophilic or cold-loving types can survive at such temperatures. After a considerable period of holding in ice only, these parameters generally show a tendency to rise gradually.

A third type of contingency arises when the fish is held at room temperature for a considerable length of time and then iced. For example, in the case of country crafts and such of the mechanised crafts which do not carry ice on fishing trips, the fish is landed after a certain amount of spoilage depending upon the period for which it has been exposed to atmospheric temperatures. By this time lag, bacterial loads as well as the various indices of spoilage would have increased considerably; but the fish may still be in an edible state at which the stage of deterioration cannot be detected organoleptically. When such fish is further stored in ice, the above indices first decrease considerably for periods ranging from a couple of days to a week and then only show an increasing trend. In the case of bacterial load, the matter can be further complicated by external contamination during handling of the material from probable insanitary surroundings like improperly cleaned containers, unprotected water used for washing and unclean ice used for preservation.

Hence we see how complicated this problem of applying the objective indices of quality to fish actually is. Carrying out these tests on samples of fish of unknown history may not give any useful clue regarding their quality. In such cases sensory or organoleptic characteristics are more reliable, though highly subjective. Poor organoleptic quality accompanied by high bacterial loads and enhanced values for the chemical indices mean definite spoilage, whereas a high bacterial contamination alone (in the absence of the others) may mean only careless handling. Higher values of chemical indices in the absence of high bacterial loads, but accompanied by poor organoleptic rating can mean that some disinfectant has been used on the fish to suppress the bacterial loads. A high organoleptic rating and low bacterial load accompanied by comparatively higher chemical indices may mean only that either these compounds themselves or their precursors have been present in larger amounts in the fresh fish. It will be rather impracticable to associate certain absolute numerical values or limits of such indices with definite stages of spoilage or to fix up strict levels of them for acceptability, as they differ from fish to fish and according to conditions of storage. In such cases, visual and organoeptic rating will be the guiding factor and the others will act as supporting evidences to the organoleptic findings.

It therefore becomes clear that if these indices are followed right from a fairly fresh stage, whether the fish is stored in ice or at room temperature, they can give valuable clue towards corroborating the organoleptic findings. For example, in the case of marine fish under prolonged storage, a close follow up of the trimethylamine, voltile bases and total plate counts from an early stage can give definite indications of its state of freshness at a particular stage.

In conclusion we may say that under our existing level of knowledge on this subject, visual, sensory/organoleptic rating, though highly subjective, is comparatively more reliable than objective biochemical indices in determining the freshness/quality/acceptability of fish under storage whose history is unknown. This is not to decry the value of the several important biochemical indices of quality worked out the world over by scientists till date, which have proved their worth in particular fishes under particular storage conditions as well as under circumstances where a continuous check is maintained on these variables right from a fairly fresh condition through various stages of storage at room temperatures, refrigerated temperatures or in crushed ice. It is impossible to prescribe levels of these indices which could be applied universally for grading of fresh fish for quality. Nevertheless, in any systematic study meant to follow the changes in quality taking place in fresh fish under storage either at room temperature or under crushed ice, a judicial selection of the objective indices of quality like TMA, TVN, Bacterial counts, Volatile reducing substances, Volatile acids etc is unavoidable, as otherwise the study will be lacking in scientific reasoning or foundation and will be completely based on the highly subjective sensory or organoleptic observations and hence calls for only the skill of a *chef de cuisine* rather than that of a scientist.