Chapter 18

An introduction to biochemical aspects of fish preservation

Suseela Mathew

Email: suseela1962@gmail.com

Biochemistry of foods emphasizes the importance of biochemistry in the rapidly developing field of food science, and to provide a deeper understanding of those chemical changes occurring in foods. The development of acceptable fruits and vegetables on postharvest storage is dependent on critical biochemical transformations taking place within the plant. Meat and fish similarly undergo post-mortem chemical changes which affect their consumer acceptability. In addition to natural changes, those induced by processing or mechanical injury affect the quality of foods. Such changes can be controlled through an understanding of the chemical reactions involved. Increased sophistication in food production has resulted in the widespread use of enzymes in food-processing operations. Biodeterioration of foods by various microorganisms involved in the degradation of proteins, carbohydrates, oils, and fat with special reference to the individual biochemical reactions responsible for food deterioration are important. Meat is basically defined as the flesh of animals used as food. In fish, however, it is the muscle which provides the main nutritional source. While meat is a major source of high- grade protein, fish flesh also provides man with high quality protein; the amount consumed of the latter is increasing annually. Unfortunately, both are expensive foods. The greatest per capita consumption of meat and fish is found in the advanced areas of the world, generally speaking Europe, North America, and Australia. In the developing continents, Africa, Asia, and Latin America, where there is already a deficiency of high-grade proteins, consumption of meat and fish is low, resulting in a high incidence of malnutrition. Such a deficiency of essential amino acids, particularly lysine, methionine, and tryptophan and micronutrients, can now be considered the world's most urgent problem, rather than a shortage of a total quantity of food. With respect to fish muscle, changes occur after death accompanying the decline of pH, including loss of water-holding capacity. This is due to the lactic acid produced, which might be a possible index of freshness in fish. Since lactic acid is known to increase in the post-mortem muscle, at those animals which undergo violent death struggle not only exhibit a reduction of glycogen levels but also ATP and creatine phosphate, which is certainly true for fish muscle.

The development of rigor mortis is highly dependent on temperature. The length of time which elapses between the death of fish and the onset of rigor mortis is ultimately determined by the relative activities of the enzymic systems involved in the synthesis and breakdown of ATP. This, in turn, is controlled by the levels of creatine phosphate, ATP, and glycogen within the muscle tissue at the moment of death. In well-fed, well-rested animals these levels are all high, so that a longer delay period is observed prior to rigor development, producing a meat of low pH and high quality. Any subjection of the animal to starvation or struggling would result inevitably in a much shorter delay period, producing an inferior meat product. The fall of pH to an acidic state accompanied by the various exothermic reactions, such as glycolysis, have a profound effect on the properties of the muscle proteins for fish. The sensitivity of proteins to increase in temperature or marked changes of pH is well established. The sarcoplasmic proteins of fish are generally more stable than the myofibrillar proteins, being unaffected by dehydration or prolonged cold storage. Actin and myosin, the major myofibrillar proteins of fish muscle undergo important changes closely linked with the development of rigor mortis. For instance, in the pre-rigor stages, meat actin and myosin are dissociated, myosin being extractable in solutions of high ionic strengths. Fish actin and myosin are also dissociated during the pre-rigor phase, but are far more labile, associating together at the slightest injury. This has tended to render the isolation of pure fish myosin an extremely difficult operation. As the ATP level decreases, actin and myosin gradually associate to form inextensible actomyosin, an essential criterion for the establishment of rigor. Meat that is cooked during this period is extremely tough in texture. During the development of post-rigor tenderness, however, actomyosin does not dissociate back to actin and myosin, but other subtle changes proceed. A number of studies have been carried out with respect to the denaturation and precipitation of the sarcoplasmic proteins. Fish sarcoplasmic proteins are far more stable than the corresponding myofibrillar proteins. They possess a far greater thermolability and solubility than their counterparts in meat, and do not appear to be involved in fish texture. Comparatively little autolytic enzyme activity appears during the onset of rigor, although some small changes in amino acids are reported in sterile cod muscle. Any deterioration occurring during cold storage is generally attributed to bacterial activity as a result of contamination in the fish samples. A prominent post-mortem change in fish muscle is the loss of fluid or exudation, which is related to the ability of the respective muscle proteins to bind water. In the pre-rigor state, fish muscle possesses a high water-holding capacity that falls within the first few hours following death to a minimal level coincident with the development of rigor mortis. This minimal level corresponds to the final pH of 5.3-5.5, which coincides with the isoelectric point of the principal muscle proteins. These changes are also associated with a decrease in the ATP level. The significance of pH on the water-holding capacity of fish muscle has tended to be overlooked, since the pH is higher than that for meat, hardly ever falling below 6.0 even in full rigor. However, it has since been established that considerable losses of water occurred from excised fish muscle similar to those reported earlier for mammalian skeletal muscle. A rapid rise in expressible fluid is found in cod which increased after storage in ice for a 168-hour period. Although, as mentioned previously, the post-mortem pH in fish muscle hardly ever falls below 6.0, certain species, including halibut and mackerel, have been found to exhibit a post-rigor pH approaching that of meat. A decrease of pH in halibut which resulted in protein insolubility on approaching the isoelectric zone, producing a pale, soft, exudative condition closely resembling a condition found in pork. This condition in halibut is known as chalkiness and has been a particular problem in the fishing industry of the Pacific Northwest, since fish found in this state are generally rejected by the consumer. However, this condition can be alleviated by allowing the fish to remain alive following capture, thus permitting dissolution of the excess lactic acid, resulting in a normal post-mortem pH following death. On dissolution of rigor mortis, a gradual tenderization of fish muscle occurs. Post-rigor fish muscle provides less of a problem in toughness when cooked compared with that cooked in rigor. Meat will generally reach an optimum acceptable tenderness after an aging period of around 10-18 days storage at 0°-5°C.

Besides the influence of the change in water-holding capacity on post-mortem tenderness for meat, insoluble nonprotein nitrogen, namely peptides and amino acids presumably derived from muscle protein by the activity of proteolytic enzymes also are responsible for tenderness. The sarcoplasm within the muscle fibres contains lysozomes, cellular organelles which can be removed by differential centrifugation. These contain hydrolytic enzymes, including cathepsins, proteolytic enzymes active at an acid pH. These enzymes are liberated when the lipoprotein membranes of the lysozomes rupture at pH levels lower than that normally found in vivo, presumably during post-mortem aging. Proteolysis by cathepsins is the most likely theory to account for the increase in tenderness developed during the post-mortem aging of fish muscle. Several theories have been postulated to account for post-rigor tenderness; however, the evidence supporting the cathepsin theory still remains inconclusive. Since the sarcoplasmic proteins are more readily denatured under post-mortem conditions, they would consequently be more susceptible to protease attack. The cathepsins appear to have an optimum pH of around 5.5 and are active at a fairly high temperature, i.e., 37℃. The majority of studies on fish quality have been studied during frozen storage, since fish sold in shops as fresh are actually frozen, but thawed prior to sale. Under such conditions microbial spoilage is arrested, although other changes, chemical and physical, can develop. It is generally accepted that a minimum storage temperature of — 18℃ is necessary in order to retain the desired fish quality, although these conditions are not always adhered to during the commercial distribution of the frozen fish products. Solubility changes of the total extractable proteins is an index of the changes taking place in the frozen muscle proteins due to denaturation. The decrease in tenderness is proportional to the decrease in "actomyosin" extractability in whitefish muscle. These changes in protein are probably due to a decrease in the water-holding capacity of the thawed muscle, resulting in an aggregation of the proteins. In fish muscle the lipids also appear to be linked with the decrease in protein solubility, as well as the production of off-flavours. The lipids located in fish muscle are highly unsaturated. Free fatty acids are found to increase in saltwater fish during frozen storage; the rate is shown to be both temperature and species dependent. In frozen white-fish, free fatty acids are thought to be liberated as a result of enzymic cleavage of phospholipids and triglycerides. The significance of the free fatty acids on the insolubilization of the fish protein is established through interaction studies of lipids with myofibrillar proteins. Either linoleic or linolenic acid is responsible to reduce the solubility of cod actomyosin. In addition to this reaction other reactions such as protein-protein interactions have been demonstrated, which also effect protein insolubilization. Most of the studies concerning fish texture have been related to protein solubility changes, cell fragility, and free fatty acid liberation under specified conditions of temperature and time. With regard to the autolytic processes taking place during the post-mortem changes in fish muscle, these have not been studied to any great extent. It has however, been reported that the cathepsin activity of fish muscle is considerably greater than that of mammalian muscle, but the significance of this with respect to fish tenderization is not known. Changes produced in fish by the naturally occurring microflora inherent to the living animal, as opposed to the post-mortem contamination of the carcass by bacteria from external sources need to be studied. Although fish muscle is generally regarded as sterile, observation of bacteria in muscle have been reported in some seawater fish, as well as in several freshwater fish. However, the latter observation leads to the conclusion that the bacteria isolated from fresh-water fish did not belong to the family of water bacteria and, therefore, might have come from the feed. However, freshly caught marine fish have considerable numbers of bacteria located on their skin as well as on their gill surfaces. Following death, the mechanisms involved in their control are no longer functional, as bacterial growth presumably occurs with movement into the various tissues throughout the vascular system. A particular problem in marine fish is the presence of trimethylamine oxide (TMAO) which is reduced by bacterial and enzymatic action to trimethylamine (TMA), a spoilage product of marine fish. The estimation of TMAO, TMA, and total volatile basic nitrogen might provide a useful index of freshness for marine fish. In short, a number of chemical reactions happen in fish muscle after death and during storage, which directly or indirectly determine the quality of fish. A thorough knowledge in this aspect is absolutely necessary to supply good quality fish to consumers and to address the problem of malnutrition in developing countries. As fish is a highly perishable item, special attention is needed, to keep its quality. However, the presence of essential nutrients and micronutrients in large quantities in fish makes it very special globally in nutrition point of view, hence the preservation of its quality and prevention of post-harvest loss is the need of the hour.

Further Reading

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