Chapter 1

Introduction

Aquaculture brings a variety of high-quality fisheries products. The cultured products are under much greater control than captured items. Hence, the opportunity for increased quality control is now possible. Seabass, a brackish water fish is widely consumed and has received much attention because of its mild taste and white flesh. It has a great potential for being processed into many kinds of products. However, like traditionally caught seafood, aquaculture products are highly perishable when compared with other fresh commodities. Several factors such as sex, age, size, starvation and the manner of death as well as storage conditions are associated with quality changes and deterioration. So the measures for improving the quality have to be based on a clear understanding of factors affecting product quality in the fish trade chain. To maintain the prime quality with high consumer acceptability, the deterioration mechanism of this fish should be investigated to find a proper means to extend its shelf-life.

Modified atmosphere packaging is used to prolong the shelf-life of much fresh produce. Effectiveness of MAP depends on the gas mix ratio, particularly CO_2 levels. Preservatives are available. However, synthetic preservative agents are prohibited in many countries so natural products gain more popularity. There are several published papers reporting that garlic has great antibacterial activity but its odor is a major problem and becomes a limiting factor in its use. So formulation of recipes with preservative agents is very important to consumer preference and food quality.

Recently, some Thai and Japanese researchers found that the main ingredients of Tom-Yum mix, e.g. galangal root, lemon grass and kaffir lime leaves have anticancer properties in a digestive system. In addition, Tom-Yum is considered the best soup in the world, becoming a symbol of Thailand and Thai food. However, no scientific information on the use of Tom-Yum mix as a natural preservative agent or

functional food is available. Generally, spices contain active components and essential oils are good sources of nutrients such as vitamin C, β-carotene, fibers and microelements. In addition, information on the effects of killing methods, time delays for icing, and positioning of fish pieces relating to mechanical, chemical, microbiological and sensory changes of Seabass is still fragmented. Currently, there is no fish product marinated in Tom-Yum mix and packaged under a modified atmosphere is available in the market.

Literature Review

Importance of Aquaculture

Aquaculture fish such as brackishwater and freshwater fish is nothing new. The Chinese, for example, have been farming fish for centuries (Redmayne, 1989). What is new is the significance of aquaculture. While the total world seafood supply is estimated to be approximately 90 million metric tons, live weight, world aquaculture production has been estimated at 10 million metric tons. These numbers are at best educated guesses. Many countries do not report their statistics to the Food and Agriculture Organization of the United Nations, the central clearinghouse for all seafood statistics.

Although agriculture's contribution to the world seafood supply is less than 12 percent, its impact is far greater (Redmayne, 1989). The reason is simple: World seafood demand is growing much faster than wild seafood production. Aquaculture is the source of most of the increased fish production that is needed to meet growing demand.

Seabass (*Lates calcarifer*)

Seabass or Asian seabass, also called Giant sea perch, Cock-up, and Barramundi, is an economically important food fish and sport fish in the tropical and subtropical areas of the Western Pacific and Indian Ocean countries, including India, Burma, Sri Lanka, Bangladesh, Malay Peninsula, Java, Borneo, Celebes, Philippines, Papua New Guinea, Northern Australia, Southern China, and Taiwan (Boonyaratpalin, 1994). It is euryhaline, meaning it can live in both fresh and salt water. However, seabass eggs and larvae will only survive in brackish or salt water (salinities between 22-40 parts per thousand (ppt) (Schipp, 1996).

Seabass become sexually mature between two and three years of age. In the wild, most fish first mature as males and participate in one or more spawning seasons before undergoing a sex change (protany), becoming functional females by the next breeding season (Schipp, 1996). As a general rule, fish less than 80 cm in length are usually male and those greater than 100 cm. are female. However, this is not always

the case, as sexually precocious (fish that mature and change sex at a smaller than usual size) populations of seabass are known to occur in the Northern Territory and Queensland. Bloodstocks held under captive conditions have been found to change sex to females at smaller sizes than wild fish. This may be the result of the captive environment or hormone treatments used during the spawning season (Schipp, 1996).

Seabass are fast growing fish, attaining marketable size within 8 months, generally with a growth rate of 1 kg/year. Because of their white flesh, and mild taste the fish is quite acceptable for making it easy to meet the requirements of any recipe and brings a high market price. In addition, the fish has white flesh and mild taste, In Australia, most production (about 90%) was sold as 400 to 500 g whole fish, with the remainder being larger fish for the premium fillet market (Barlow *et al.*, 1996). An increasing proportion is being sold at around 3 kg. Southeast Asian markets have a strong preference for a live form product of this fish. The major markets identified are Malaysia, Singapore, Hong Kong, Taiwan, Thailand and southern provinces of mainland China. The ideal market size ranges between 600 to 700 g per piece (fish) (Ferdouse, 1995).

Seabass also have many characteristics that are favorable for coastal aquaculture, i.e., they can grow well in water of high turbidity and varying salinities, and can tolerate rough handling and crowded conditions of net-cage culture. They are easily tamed for aquaculture and accept feeding by humans. Therefore, seed production and culture programs are established in many countries (Boonyaratpalin, 1994).

In Thailand, Seabass have been cultured in several provinces such as Samut Prakan, Prachuab Kiri Khan, Songkhla, Pattani and others. Songkhla Province is the main source of the fish cultured in the South, culturing there in cases or pens instead of ponds. Unpublished information indicated that there are few or no problems of a muddy flavor in fish cultured in the case as compared to fish cultured in the pond and it may be one of the reasons why fish cultured in the case have increased over the years. Its price is about 120-180 baht/kg wholesale and up to 240-300 baht/kg

restaurant price. The market value of Seabass from Songkhla Province was about 144,999,000 baht in the year 2000 (Statistics of Marine Fish Farms Survey, 2000).

Post-mortem changes in fish meat

Maintenance of quality in fresh fish is one of the major problems facing the fishery business. It affects fishermen, middlemen, the fishery industry and even consumers throughout the world because of the extremely perishable nature of this type of food. As a whole, the muscle tissue of fish spoils faster than mammalian muscle. The higher water content, the higher free amino acid content, and the lower content of supportive connective tissue, due to the effect of a buoyant environment, make them spoil more rapidly than other foods, such as land animals (Hultin, 1985).

The biology of living skeletal muscle and the processes, which occur during its conversion to meat, are critical to an understanding of quality in muscle–based foods. There is a variety of animal species from which muscle foods are obtained. Although some species–specific differences exist, the changes associated with the conversion of muscle to meat are essentially the same. Onset of rigor mortis varies from 3 to 6 hr in smaller animals such as poultry, and 24-36 hr in large animals such as cattle. Commercial harvesting of fish may be accomplished in a variety of ways. In large operations fish are caught, skinned, scaled or shelled, eviscerated, filleted and stored frozen within 1 hr of harvest. In smaller operations, boats may harvest fish and store these fish on ice until further processing when the boat reaches the port.

Regardless of the species being harvested, death is accompanied by the inability to deliver oxygen within the body, and subsequent anoxia. When normal life processes are halted, many of the biochemical reactions present in the living state retain some degree of activity in the nonliving state. For considerations of conversion of muscle to meat, the nonliving or post-mortem period can be divided into three stages: pre-rigor, rigor and post-rigor. Rigor or rigor mortis describes the contractile process that takes place in post-mortem muscle and results in both shortening and rigidity that persists for several hours or days, depending upon a number of factors (Gill, 2000).

Muscle structure and flesh quality

Flesh quality is becoming of increasing concern to the aquaculture industry as total production increases (Johnston, 1999). It is usually defined in terms of appearance, taste, smell, firmness and juiciness. The desired flesh characteristics for particular species varies between markets and may differ significantly for raw and processed products (cooked, salted, smoked, dried fish etc.) The quality of the flesh is influenced by both intrinsic and extrinsic factors. The extrinsic factors such as feeding regime, diet composition, and environment have significant impact on the condition of the fish and the structure and metabolic characteristics of the muscle tissue. The metabolic and contractile characteristics of the muscle in turn affect post-mortem changes including proteolysis and rigor processes (Dunajski, 1979; Fauconneau *et al.*, 1995 cited by Johnston, 1999). For example, post-mortem pH influences the toughness of fish muscle. pH change is a function of muscle buffering capacity such as histidine (Fennema, 1996), histidine dipeptides such as anserine, carnosine or balein (Szebedniszky and Gilmour, 2002), cystein, and phosphates (Heisler, 1986), glycogen content and the concentrations of metabolites at the point of slaughter (Love *et al.*, 1974). Pre-and post-slaughter procedures and the length and conditions of storage are probably the major determinants of end product quality (Dunajski, 1979; Faergemand *et al.,* 1995). Volatile components of lipid metabolism, amino acid and peptides, probably all make some contribution to flavor. Intrinsic factors of importance in flesh quality include texture, color such as carotenoid pigment, fat content, and in the case of some species such as carp, the presence of inter-muscular bones can affect fish flesh quality (Dunajski, 1979; Faergermand *et al.*, 1995; Fauconneau *et al.*, 1995).

Texture

Fish flesh consists of muscle sheets extending from head to tail on both sides of the body as showed in Fig 1. These long muscles are divided into segments by transverse sheets of uniformly distributed connective tissue, forming a structure very different from that encountered in meat of terrestrial animals. The amount of

connective tissue in fish (about 3%) is much less than in warm-blooded animals. This lack of connective tissue, coupled with the high thermal instability of its collagen, accounts for the delicate, tender texture of cooked fish. Fish collagen liquefies readily on heating, thus causing the connective tissue to lose its binding power. The structure of muscle fibers (i.e., the basic elements of the musculature) of fish, however, is quite similar to that of warm-blooded animals (Dunajski, 1979).

Collagen is not a homogenous protein. Nineteen genetic types of collagen have been isolated so far and marked I, II, III, etc. (Sadowska and Kotlowski, 1999). Collagen may also be considered in the glycoprotein group because it contains small amounts of galactose and glucose. In amino acid composition, glycine predominates (around 30%). All types of collagen contain hydroxyproline and hydroxylysine, which are two amino acids not found in other proteins. These amino acids play important roles in the helical structure that determines collagen strength. The amount of hydroxyproline varies and is dependent upon the genetic type of glycogen, animal species, and kind of tissue. Tryptophan is not present, and the sulfuric amino acids are at a relatively low level. The common characteristic of collagen molecules of all genetic types is that their structure is made of three polypeptide chains, i.e., the α constituents that form the structural unit-tropocollagen. Collagen polypeptide chains are built from tripeptide segments Gly-X-Y, where in many cases, X is proline, while Y is hydroxyproline. Among the connective tissue membranes of skeletal muscles, in the epimysium, collagen of genetic type I predominates, in perimysium, types II and III predominate, while in endomysium, types IV and V predominate (Bailey and Light, 1989).

A quantitative study of the relationship between the collagen content of raw and cooked dorsal muscles of five fish species and instrumentally determined firmness showed that connective tissue contributes to raw fish texture, while the muscle fiber characteristics define the cooked fish texture (Hatae *et al.*, 1986). It should be pointed out that in Far East countries, raw fish is often eaten. Collagen has significant effect on raw and cooked textures of fish and marine invertebrates (Sikorski and Borderias, 1994). The fish raw muscles containing 1.6-2.3% collagen

are tender, whereas those containing 8.8-12.4% collagen are very tough. On the other hand fish containing low amounts of collagen tend to be dry and fibrous after cooking, while fish containing a high content of collagen is very succulent and elastic, possibly due to gelatinization of collagen. It has also been shown for squid mantle that collagen contributes to tensile strength in the longitudinal direction, while muscle cells contribute to it in the transverse direction (Kuo *et al.,* 1991).

Texture is commonly assessed using sensory analysis often in combination with instruments to measure parameters such as the force of resistance to compression (Faergemand *et al.*, 1995) or the breaking strength of isolated muscle pieces (Ando *et al.*, 1991). Muscle cellularity is known to be a major determinant of flesh texture (Hatae *et al.,* 1990; Fauconneau *et al.*, 1993; Hurling *et al.*, 1996). The variations in quality of the fillet along the length of the body are largely due to variations in the size distributions of muscle fiber (Dunajski, 1979), and are also correlated with the amounts of collagen present (Montero and Borderias, 1990). The lipid and water content of the muscle and the geometrical characteristics of the fiber are both thought to contribute to the 'juiciness' of the fish in organoleptic tests (Dunajski, 1979).

Hatae *et al.* (1986) measured collagen content and firmness using texturometer in raw and cooked dorsal muscle samples from five species of fish, skipjack tuna, flying fish, common horse mackerel, and plaice and channel rockfish. Among the different species there was a significant correlation ($r = 0.70$) between collagen content and the firmness of the raw fish but not of the cooked flesh due to the denaturation of the collagen. It was found that species with softer raw meat textures gave a firmer texture on cooking (Hatae *et al.,* 1986). Following cooking the muscle fibers themselves constitute the main clement of resistance to mastication. (Dunajski, 1979).

Besides the collagen content and distribution, lipid content and distribution have consequences for textural properties (Lie, 2001). According to Anderson *et al.* (1997), fillets from rainbow trout fed high lipid diet were evaluated as softer than those from fish fed a low-lipid diet. These results were based on instrumental

measurements (Instron. Mass, USA). High muscle lipid content may not be the only factor involved in palatability of cultured fish but also in its marketability (Wassef *et al.*, 2001).

For cooked flesh sensory firmness, as determined by using a trained panel was negatively correlated with average muscle fiber (cross-section measured in area/diameter) in several species of marine fish (Hurling *et al.*, 1996). Fish with the smallest average cross-sectional area had the highest sensory firmness. Thus dab (*Limanda limanda*) had the smallest diameter muscle fibers and the highest sensory firmness whilst flying fish (Exocoetide) had both the biggest fibers and the lowest firmness (Hurling *et al.,* 1996). Previous studies have also found a relationship between muscle fiber size and firmness of the flesh in which the narrow diameter fibers had a higher intrinsic strength due to scaling effects (Hatae *et al.*, 1990). These authors proposed a model in which sacroplasmic protein is released or squeezed from the contracting muscle during cooking and coagulates in the interstitial spaces affecting the shear properties of the fibers during mastication.

In addition to muscle cellularity, it is important to consider related aspects of muscle structure including the arrangement of the tissue connective matrix and the deposition of lipid stores (Fauconneau *et al.*, 1995). Individual fibers and blocks of fibers are surrounded by a network of collagenous sheets (the myocommata) connected by tubules of collagen (Bremner and Hallett, 1985). Rupture of these tubules where they join the myocommata (Fig.1) is thought to result in slits or holes in the fillet surface, a phenomenon known as gaping. Gaping results in a significant economic loss because such fillets cannot be mechanically skinned or sliced. The causes of gaping are complex and vary among other things, with season of harvest and post-slaughter treatment, particularly with regard to pH. It is likely that changes in muscle cellularity will alter the relative proportions and arrangement of the connective tissue matrix and therefore increase the susceptibility to gaping, although this has yet to be investigated. (Johnston, 1999).

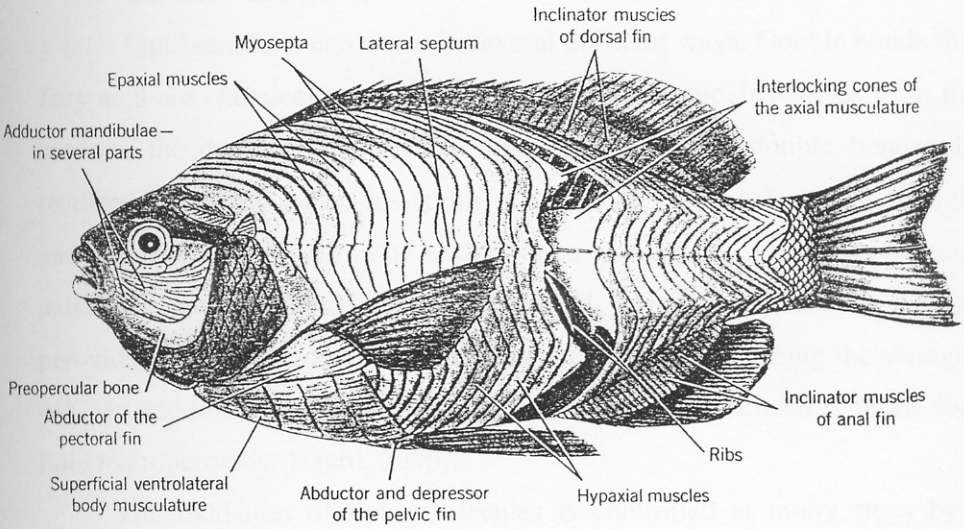

Fig. 1 Musculature of a teleost with two myomeres removed to show the shape of the myosepta

Source: Hildebrand (1988)

Gaping

Gaping occurs when the tissues between the muscle blocks (the myosepta) break and the blocks become separated. Commercially it is a problem as it makes the processing of the flesh much more difficult (Robb *et al.*, 2000). Many reasons for gaping have been discussed, such as high-energy diet, fast growth and changes in muscle collagen structure during chilled storage (Lie, 2001). Jerret *et al.* (1996 a, b) showed that, after rigor, flesh from active fish was more susceptible to the myosepta breaking because of acid condition (lactic acid accumulation) leading to the solubilization of collagen under stain than in flesh from anaesthetized fish. Starved fish become less susceptible to gaping during the seasons of the year when feed is not available (Haard, 2000).

Off odor and flavor

Lipid rancidity can occur in several different ways. Double bonds that occur in fatty acid are chemical reaction centers. Oxidation rancidity arises from the reaction between the double bond systems and oxygen. When double bonds of lipid are oxidized they form peroxides (primary oxidation products), which, though odorless and tasteless, are precursors of a wide range of secondary oxidation products such as anisidine, malonaldehyde, ketone, hexanal, propanal. Loss of the glutathione peroxidase activity (natural antioxidant system) increases during the storage of fish at 4 °C. In addition, loss of the substrate glutathione is associated with the formation of lipid hydroperoxide (Haard, 2000).

The oxidation of lipid molecules is controlled at many steps by enzymes. Lipases hydrolyze the ester linkage between the glycerol molecule and the extended carbon chain, leading to the formation of free fatty acids. Free fatty acids have been shown to be more susceptible to oxidation than their original triacylglycerides (Nawar, 1996). However, some evidence (Shewfelt *et al.*, 1981), suggests that free fatty acids linked to phosphate groups undergo less oxidation than their parent phospholipid. The oxidation of lipids leading to the development of off odor and flavors can occur through autoxidation or may be enzymatic in nature. Enzyme systems that play a role in the oxidation of lipids in fish include lipoxygenase (German and Kinsella, 1985) localized in the skin and gills and microsomal enzyme systems (MacDonald and Hultin, 1987) found in the muscle.

Popular measures of rancidity include acidity, peroxide value, p-anisidine value, 2-thiobarbituric acid value, conjugated dienes, hexanal, propanal (using as headspace technique) and TOTOX value (2 times peroxide value plus anisidine value). However, Kosugi *et al.* (1987) reported that the absorbance of red pigments in TBA methods might not specifically quantify malonaldehyde (MDA) because other substances may also react with TBA and contribute to absorbance. In addition, heating and acidic conditions may cause overestimation of thiobarbituric acid reactive substances. Gray and Monahan (1992) reported that MDA interacted with various compounds such as protein peptides and some oxidized products.

Many studies use a marker molecule such as hexanal rather than the total headspace to look for a measure of oxidative deterioration. Hexanal is a popular marker for n-6 polyunsaturated fatty acid (PUFA) oils, while propanal has been used to determine the oxidation of n-3 PUFA oils. However, they are not good indicators of when the oil are deodorized and they can be virtually absent in the oil that still contains significant levels of less volatile bad-tasting oxidation molecules. In the case of triacyglycerols rich in long chain polyunsaturated fatty acid (LCPUFA), sensory evaluation of oil or food products containing the oil is the only reliable method of deciding whether the oil is fit for use (Robards *et al.,* 1988). However, there is strong evidence suggesting that the human nose is good at detecting off-flavors derived particularly from n-3 fatty acid oxidation. It seems that the classical tests for measurement of oil oxidation such as observing at anisidine and peroxide levels are of little value. Moreover, it is no easy task; recruiting, training and maintaining the panel are difficult. Often, there are differences in judgment between the sensory panel of the supplier and the customer because of the difficulties in standardizing panel responses (Frankel, 1998).

In teleosts, free amino acids (FAAs) and creatine are the main components of non-protein nitrogen, they acoount for 50-86% (Konosu *et al.*, 1974) and the most important FAAs from a quantitative point of view are proline, arginine, glycine, alanine, histidine, gluatamic acid and taurine (Suyama and Kobayashi, 1980; Murata and Sakaguchi, 1986). In cooked fish, FAAs are directly responsible for the flavor and taste and they can be precursors of aromatic compounds (Murata and Sakaguchi, 1986; Ababouch *et al.*, 1991; Konosu and Yamaguchi, 1982). During the storage of chilled fish, the first changes in FAAs are caused by muscle autolysis and later by the growth of microorganisms (Sakaguchi *et al.*, 1982; Roura *et al.*, 1992). When fish is stored chilled, FAAs are used as immediate substrates for growing microorganisms and this leads to changes in the fish (Aksnes and Brekken, 1988; Miyagawa *et al.*, 1990; Watanabe *et al.*, 1992).

Ruiz-Capillas and Moral (2001a) addressed the fact that alanine is an amino acid which is also extremely important for giving flavor to hake. An increase in the

concentration of fish FAAs might lead one to believe that the flavor and taste of the hake would improve. However, other deterioration compounds (ammonia, TMA etc.,) would mask the flavor contributed by this FAA (Ruiz-Capillas and Moral, 2001 a).

Some factors affecting fish composition and quality

There are many factors affecting fish composition and quality. They can be classified into two groups, i.e. intrinsic such as sex, size, age and extrinsic factors such as water temperature, killing method and feeding regime etc,. However, in this work only some intrinsic factors are addressed.

1. Anatomy of fish

In round-bodied fish, as distinct from flat fish, no consistent difference has ever been found between constituents in the left side and right side myotomal muscles of the same fish. For example, it was found that no difference between left and right occurred in Atlantic cod (*Gadus morhua*) but there was a decline in protein nitrogen proceeding from the head toward the tail (Brandes and Dietrich, 1970; Hoffman *et al.*, 1994). However, the concentrations of lipids vary enormously in different parts of the body. In fat fish, there is usually a high concentration of fat immediately under the skin and in the belly wall (Hoffman *et al.*, 1994).

Love (1988) noted that the myocommata of cod are most widely spread at around myotome number 12 from the cranial end and that they become steadily more closely packed the nearer they are to the head and tail. The significance of this phenomenon is that a varying proportion of myocomma collagen is found in muscle samples taken from different positions along the body. Thus, the area from which a sample is taken for the purposes of analysis will also influence the biochemical and physical result. If these factors are not taken into account, a distorted image of the product may arise (Hoffman *et al.*, 1994). It is generally known that the organization of the dorsal skeleton muscle in fish is arranged in distinct layers of dark, intermediate muscle and ordinary muscle, and these muscles are composed of red muscle fibers, pink muscle fibers and white muscle fibers, respectively. The method used most frequently to distinguish muscle fiber types in fish muscle is dependent on the

histochemical actomyosin ATPase activity (Carpene *et al.*, 1982; Gill *et al.*, 1982; Gill *et al.*, 1989).

Factors used to defined fiber types (Prestwich, 2003):

1. Speed: how fast can the fiber contract or relax? There are at least two factors that help determine speed of contraction:
   1.1 Velocity of the myosin ATPase reaction. Recall that part of myosin is an enzyme capable of breaking down ATP. The rate at which this process occurs will be an important determinant of how fast cross bridges can cycle, it determines how rapidly the myosin head is reset after letting go of actin and therefore how quickly the myosin can re-attach to actin. The maximum rate of the myosin ATPase from a piece of muscle tissue obtained via biopsy can be measured using biological techniques.
   1.2 The capacity of the muscle fiber to release Ca^{++} and to pump Ca^{++}. The two tend to be related. Muscle fibers that can release more Ca^{++} are also capable of pumping more. However, the maximum rate of release is always greater than the time it takes to gather up the Ca^{++} after a contraction. Clearly the faster that Ca^{++} is released the more quickly the muscle can begin to contract. And the more Ca^{++} that is released, the more forcible the contraction (within limits) occurs. It is a general trend that cells that release Ca^{++} more rapidly tends also to gather it up (pump it into the SR) faster.
2. Strength and ability to get rid of wastes and obtain oxygen:
   2.1 One of the best measures of a strength fiber is the fiber's diameter. Muscle fibers with greater diameters have more sarcomeres packed into the cell (in parallel to each other) and therefore are far stronger. A partial analogy is a rope: to make a rope stronger, it requires more strands in parallel, resulting in a thicker and stronger rope. The force increases as more strands are added (and as the muscle gets thicker).

2.2 On the other hand, muscle cells with greater diameters have more trouble getting nutrients, oxygen and waste in and out of the cell.

Prestwich (2003) reported that metabolic characteristics are related to the types of fuel a muscle can use, the extent to which it can use oxygen, and the types and amounts of waste products produced. These processes are responsible for breaking down complex molecules removing some of the energy stored in them, and then using it to make ATP from ADP and inorganic phosphate (Pi). And the two most important examples of ATP-generating metabolic processes occur in different parts of the muscle.

1. Capacity for oxidative metabolism or aerobic reactions depends on the number of mitochondria and degree of folding of their inner membrane. There are at least two other factors, which tend to go along with mitochondria number in determining the oxidative capacity of muscle. These factors are:
   1.1 Blood capillary density: Mitochondria potentially require large amounts of oxygen. They also produce large amounts of CO_2, which must be removed. Thus, the number of blood capillaries (the vessels that actually exchange gases and nutrients between blood and tissues) is important in determining oxidative capacity. Then, more capillaries usually mean better oxidative ability.
   1.2 Myoglobin content: Myoglobin is a carrier protein. It acts somewhat like an enzyme in the sense that it has a site that a specific molecule (oxygen) can bind to. However, it does not catalyze reactions but instead simply carries the oxygen. Myoglobin is closely related to the red protein hemoglobin found in red blood cells. It is also red.
2. Capacity for glycolysis: Unlike the oxidative reactions, which are fixed in the inner membrane of the mitochondria, glycolysis reactions are spread throughout the sarcoplasm. The enzymes for this metabolic pathway are simply dissolved in the lipid part of the cell that surrounds the sarcomere and all other structures in fiber. The only way to determine the capacity for

glycolysis is to remove the enzymes from the biopsy sample and then use biochemical techniques to see how fast the reactions of glycolysis occur under ideal conditions. The faster this rate, the more enzyme present and the greater the glycolytic capacity. It is worth realizing that glycolysis may be associated with either aerobic or anaerobic processes.

2.1 Glycolysis always starts with glucose or some very closely related sugar.

2.2 It eventually breaks the glucose down into two large molecules; these are the waste products of glycolysis. These waste particles differ according to whether the glycolysis is aerobic or anaerobic:

2.2.1 If glycolysis is aerobic, the waste products (called pyruvic acid) are immediately broken down by the mitochondria. Thus, they do not accumulate in the muscle cell. Furthermore, it was found that a great deal of energy is taken from the pyruvate molecules when they are broken down and much of this energy is conserved for use by the cell as ATP.

2.2.2 If the glycolysis is anaerobic, the waste products are molecules of lactic acid. This substance is not broken down further in the muscle. Since it is an acid, it releases large amounts of hydrogen ions (H^+) which have profound effects on the proteins of the muscle and which cause fatigue. In both the aerobic and anaerobic versions of glycolysis, a small amount of ATP is formed from ADP and Pi. This ATP, of course, can be used to fuel the ATP-requiring events of muscle contraction. Table 1 below summarizes the characteristics of different fiber types of fish muscle. It has been reported that red muscle contains more fat than white muscle (Johnston *et al.*, 1975; Gill *et al.*, 1982; Uno *et al.*, 1987). In addition, in rainbow trout (*Salmo gairdneri*), the total fat in the white muscle amounts to 2% but varies in red muscle from 10 to 12% (Kiessling *et al.*,

1989). Moreover, red muscles are also smaller in diameter than white muscles, which implies that there will be more cell wall per volume of tissue in red muscles compared to white muscles (Johnston *et al.*, 1975; Bone, 1978). The increase in percentage of white muscle along the myotomal muscle towards the caudal region will influence the amino acid, fatty acid and mineral profile (Hoffman *et al.*, 1994). Mai and Kinsella (1979) found that dark (red) muscle had a higher phospholipid concentration than the white (light) muscle. Love (1988) noted that phospholipids that make up an integral part of microscopic structures such as cell walls and organelles like mitochondria are rich in docosahexaenoic acid (C 22:6 ω3; DHA). This may explain the high concentration thereof in the tail segment, as it is known that red muscle cells are smaller than white muscle cells and also contain more mitochondria (Johnston and Maitland, 1980; Johnston, 1982). The high concentration of DHA in the tail could have important consideration in the processing industry, since oxidation of this fatty acid is largely responsible for the development of off-flavor during cold-storage (Love, 1988). In addition, the tail segment also has a higher Fe and Zn concentration than other segments (Love, 1970; Hoffman *et al.,* 1994). Thus, ideally, white and dark muscle should be investigated separately, or a carefully specified part thereof, should be analyzed. However, Hoffman and Prinsloo (1990) and Hoffman *et al.* (1995) reported that samples of minced and homogenized whole fillets were analyzed when they studied *Clarias gariepinus* as a "protein" source from a nutritional viewpoint. In this context, the composition of the total fillet is of more value than that of the parts, as often, the whole fillet is consumed. However, it must

be borne in mind that the proportions of the two tissue types will influence the quality of the fillet as dark muscle has more flavor, is more nutritious and is richer in polyunsaturated fatty acids than the white muscle (Hoffman *et al.*, 1994).

Table 1 Summary of characteristic of different fiber types of fish muscles

	Type I	Type II a	Type II b
	Slow oxidative (SO, red) slow twitch Fatigue	Fast oxidative glycolytic (FOG, red) Fast twitch	Fast glycolytic (FG, white) Fast twitch Fatigue prone
Myosin ATPase Reaction velocity	Slow	Fast	Fast
SR Ca^{++} pump and Release (gate) capacity	Moderate	High	High
Diameter(Ralate to strength and diffusion distance)	Moderate surface/volume is high	Moderate surface/volume is intermediate	Large surface/volume is low
Oxidative capacity:	High	High	Low
Mitochondrial content, capillary density, amount of myoglobin	(Mitochondria may occupy as much as 40% of the fiber volume) Large amount of myoglobin. Together with themitochondria This make the fiber reddish in color.		(Mitochondria may occupy as little as 1% the fiber volume) very little Myoglobin
Glycolysis capacity	Moderate	High	High

Source: Adapted from Prestwich (2003)

2. Wild and cultured fish

Fish muscle quality is highly affected by water content and its distribution within the flesh. Ofstad *et al.* (1996) have demonstrated that a relationship between changes in water holding capacity (WHC) and microstructural changes exists, and that changes in muscle pH influence WHC in cod and salmon. Moreover, there was no change in the actomyosin complexes in the white muscle of farmed bass during 5 days of storage, but structural changes, such as sarcolemma detachment may contribute to WHC. It has been proposed that the natural post-mortal degradation of fish involves a time-related proteolytic degradation of myofibrillar structures in early post-mortem followed by degradation of perimysial and endomysial connective tissue networks in the later stages (Ashie and Smith, 1997; Ashie and Simpson, 1998).

It was clearly shown that wild fish tended to have higher pH and greater WHC than their farmed counterparts (Olsson *et al.*, 2003). In the farmed fish, small myofibrillar units probably reflect the different growth history. Intensively farmed fish are provided with a constant supply of nutrient-dense feed, which is formulated to give optimal growth. Wild fish, on the other hand, experience fluctuations in availability and composition of feed (Olsson *et al.*, 2003). The amount of small fiber has been shown to be greater in fish from fast-growing strains than slow-growing strains (Valente *et al.*, 1999). A significant inverse relationship exists between fibre diameter and the textural attributes of firmness and chewiness (Hurling *et al.*, 1996; Johnston *et al.*, 2000). In addition, the endomysial sheath appeared wider in the farmed fish. This may be due to higher fibre density

3) Killing method

Nakayama *et al.* (1999) found a shorter time for ATP depletion (6±0.3 hr) in stressed fish, arranged by holding the fish out of water for 30 min before killing them by insertion of a spike into the medulla oblongata, than in unstressed fish (14±0.9 hr), which were immediately killed in the same manner as the stressed fish. The tension of stressed fish muscle declined more sharply than that of unstressed fish muscle after the peak tension was attained. This phenomenon is probably due to the drastic

structure weakening (both pericellular connective tissue and myofibril such as Z line, A band and I band) induced by the rapid generation of very large peak tension. When the difference between acclimation and storage temperature was greater, the decrease in Ca^{2+} uptake rate of sarcoplasmic reticulum was introduced faster. As a result, the activation of myofibrillar Mg^{2+}-ATPase and the acceleration of rigor mortis progress were introduced. Moreover, in both cases of rigor shortening and cold shortening, muscle contraction was explained by the release of Ca^{2+}ions into myofibrillar space in the presence of adequate levels of ATP.

Loss of freshness and quality

As soon as possible after the catch, fish are placed on ice and stored at that temperature for the fresh market to minimize enzymatic and microbial quality deterioration associated with spoilage. Texture changes in cold storage occur in three consecutive stages: pre-rigor, rigor, and post-rigor. The size, maturity, nutritional status, gross chemical composition, morphological structure, as well as the activity of the fish influence the texture and quality changes following the time of the catch. These changes are also influenced by the condition of death and post-mortem handling. Some of the morphological factors may govern the ratio of connective tissue to myofibrillar proteins.

The early post-mortem textural changes in fish are due to changes in the physicochemical state of the myofibrillar proteins. The muscles of freshly killed fish are soft, plastic, and extensible (Dunajski, 1979). If the fish did not struggle much during the catch and still has some glycogen reserves, the pH may fall in the first several hours post-mortem due to the anaerobic conversion of glycogen to lactic acid.

As the pH drops and approaches the isoelectric point of the myofibrillar proteins, the altered charges on the protein chain lead to the tightening to the protein structure. Fish cooked at that stage will suffer increased cooking losses and will be tough and dry (Dunajski, 1979). When much struggling occurs during the catch, fish will use essentially all of the glycogen in the muscles, which then will be unavailable for the outlined texture altering mechanism. Such fish, culture sturgeon:

Acipenser transmontranus, after a few days of iced storage were shown to be softer after cooking than anaesthetized fish (Izquierdo-Pulido *et al.*, 1992). However, some fish when cooked immediately after the catch will be very tough, because heating accelerates the development of rigor (Dunajski, 1979).

The onset of rigor mortis causes the textural changes in post-mortem fish. It usually reaches its peak in 1-2 days after the catch and is accompanied by a contraction of the muscle leading to toughness. This is caused by the depletion of ATP, which results in formation of bonds between rods of actin and myosin. This reaction prevents myosin and actin filaments from sliding passively past each other and makes the muscle tough, hard, and inextensible (Dunajski, 1979). However, the elevated temperature of cooking brings about a partial resolution of rigor and a decrease in toughness. In contrast to beef, fish muscles do not appear to exhibit cold shortening (Szczesniak, 1998). Iwamoto *et al.* (1987) stated that iced storage actually enhanced nucleotide breakdown in tropical and subtropical fish compared to fish stored at higher temperatures. Moreover, not only the depletion of ATP increased and the onset of rigor shortened, but the K-value also increased more quickly for Japanese scallop tissue stored at -3 and 0 ^{o}C than it did at 5 and 10 ^{o}C (Iwamoto *et al.*, 1991). Therefore, the use of nucleotide as an index of quality was discouraged for this particular spices.

Biochemical changes in the post-rigor period lead to softening and, when excessive, to mushiness and tissue disintegration. It has been reported that the softening is affected more by changes in the structure of muscle tissue than by changes in the protein (Hatae *et al.*, 1986). These are caused by a rise in pH due to formation of basic compounds, such as trimethylamine and ammonia and to bacterial action (Howgate, 1977). Bacteria living on the surface of the fish are accustomed to low temperatures and will grow in refrigerated storage (Dunajski, 1979). Proteolytic breakdown of structural proteins due to endogenous enzymes may be expected and has been postulated to occur. The effect of high pH appears to be reversible upon soaking of the fish in low pH buffer (Love, 1970). However, no conclusive proof is

available (Howgate, 1977) of proteolysis occurring during storage of fish that is still sensorially acceptable.

Kuo *et al.* (1991) reported that, after more than 4 days in refrigerated ($4^{O}C$) storage, the tensile strength of squid mantle decreased significantly in the longitudinal but not in the transverse direction. This also resulted in a greater strength loss on cooking than observed for non-stored fish. It was concluded that refrigerated storage produces changes in collagen that make it more susceptible to high temperature. However, some collagen structure is still believed to remain. Montero and Borderias (1990) showed that in cod stored on ice, degradation of collagen occurs with the development of rigor mortis and continues as the meat ages. A mechanical model has been proposed to characterize the texture of fish in rheological terms. It accounts for the effects of time and temperature and expresses toughening on rigor mortis as well as the post-rigor softening.

Modified atmosphere packaging of fish and fishery products

The deterioration or breakdown of quality in fish products occurs via two main pathways, microbial spoilage and autolytic reactions. Much of the research to date has focused on methods to delay microbial spoilage using modified atmosphere and antimicrobials.

Modified atmosphere (MA) packaged foods have become increasingly more acceptable, because it helps food manufacturers to meet consumer demand for both fresh and refrigerated foods with extended shelf-life. The main effect of MA in food quality control is on changes in CO_2 level as a result of retarding microbial growth (Stiles, 1991). It is also one means of delaying oxidation. Fish and shellfish are highly perishable and their deterioration is co-operated of bacteria action. Typical shelf-life under current icing and refrigerated storage conditions ranges from 2 to 14 days (Stammen *et al.*, 1990). MAP is addressed to inhibit the normal spoilage flora and increase shelf-life significantly. However, the possibility that *Clostridium botulinum* type E and non-proteolytic type B strain will grow and produce toxin in a low-oxygen

atmosphere at refrigerated temperatures has caused a great concern in studies on MAP of seafood (Church, 1994).

Microbial spoilage of fresh fish

Food spoilage can be considered as any change that renders the product unacceptable for human consumption (Huis in't Veld, 1996). Fish and shellfish spoilage results from the oxidation of lipids, the reactions caused by activities of the fish's own enzymes and the metabolic activities of microorganisms (Ashie *et al.*, 1996). The reasons for high deterioration of fish are their high a_W, neutral pH, and presence of autolytic enzymes. In addition, the rate of spoilage is highly temperature dependent so it can be inhibited by the use of chilled storage. Generally, the spoilage of fresh fish is caused by microorganisms, however, chemical changes, such as auto-oxidation or enzymatic hydrolysis of the lipid also play important roles and may result in off-odor and off–flavor and even tissue enzyme activity can lead to unacceptable softening of fish flesh (Huss *et al.*, 1997). The degree of processing and preservation, together with storage temperature, will determine whether the fish undergoes microbial spoilage, chemical spoilage or a combination of both.

Several investigators have concluded that microorganisms can reflect the environmental sanitation (Liston, 1980 a, b; Colby *et al.*, 1993; Ashie *et al.*, 1996; Gram and Huss, 1996). The temperate fish is dominated by psychrotrophic, aerobic or facultative anaerobic Gram-negative, rod-shaped bacteria, particularly by *Pseudomonas, Moraxella, Acinobacterium, Shewanella putrefaciens, Flavobacterium, Cytophaga, Vibrio* spp., *Photobacterium and Aeromonas* (Stammen *et al.*, 1990; Gram and Huss, 1996; Huis in't Veld, 1996). *Vibrio, Photobacterium* and *S. putrefaciens* require sodium for growth and are typical of marine waters, whereas *Aeromonas* spp. are typical of fresh water fish. However, *S. putrefaciens* has been isolated from freshwater environments (Huss, 1995). Varying proportions of Gram-positive organisms (*Bacillus, Micrococcus, Clostridium, Lactobacillus, Cornynebacterium* and *Brochothix thermosphacta*) have been isolated from seafood (Stammen *et al.*, 1990; Gram and Huss, 1996; Huis in't Veld, 1996). The flora on

tropical fish often carries a slightly higher load of Gram-positive bacteria compared with fish from colder waters (Liston, 1980 a, b).

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total numbers of organisms vary enormously and Liston (1980 a, b) states a normal range of 10^2-10^7 cfu cm^{-2} on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu g^{-1} (Huss, 1995). The fish muscle is sterile at the time of slaughtering/catch. But it becomes quickly contaminated by surface and intestinal bacteria and from equipment and humans during handling and processing. During chilled storage, there is a shift in bacterial types. Psychrotrophic *Pseudomonas* and *Shewanella* dominate the microflora after 1-2 weeks of storage. At higher temperature (25°C), the microflora at the point of spoilage is dominated by mesophilic *Vibrionaceae,* particularly if the fish are caught in polluted waters, *Enterobacteriaceae* (Huss, 1995). The part of the microflora which will ultimately grow on the products, is determined by intrinsic factors (for example, post-mortem pH in the flesh) and the poikilothermic nature (environmental, cold temperature adaptation of proteins and enzymes to low low temperature) of the fish, as well as the presence of trimethylamine oxide (TMAO) and other non-protein-nitrogen (NPN) components) and extrinsic parameters (for example, temperature, processing and packaging atmosphere) (Huss *et al.*, 1997).

The first extensive research on seafood stored in CO_2 was first reported in the early 1930s in the UK, USA and Russia (Stansby and Griffiths, 1935). In a 100% CO_2 atmosphere, fish keep fresh 2-3 times longer than control fish in air at the same temperature (Sivertsvik *et al.,* 2002). Even at 27°C, MA-packaged cod was found to be in good condition after several days. The absorption of CO_2 altered the pH of fish from 6.6 to 6.2, but it was reversed on subsequent exposure to air. Fresh haddock, cod, sole, whiting and plaice were very effectively preserved under a 20-100% CO_2 atmosphere, with the optimal conditions under 40-50% CO_2 (Coyne, 1933). The flat fish had better keeping quality at 0°C and 80% CO_2 compared with the other fish species. Haddock stored in the atmosphere of lower than 25% CO_2 had a shelf-life of approximately twice that of products handled by conventional methods (Stansby and

Griffiths, 1935). CO_2 storage was most beneficial during prolonged storage and when the best sanitary conditions during filleting were used. Since these early investigations, numerous articles have been written on this topic, some reporting a tremendous increase in shelf-life, others reporting little or no shelf-life extension. More often an extension in the range of 30-60% for fresh fishery products using atmospheres with elevated levels of CO_2 is observed. Table 2 summarizes some of the more recent published articles about MAP and fish.

From Table 2, and as reported by others (Schvester, 1990; Stammen *et al.*, 1990), many of the publications about MAP do not state the Gas/Package (G/P) ratio. Changes in the CO_2 and O_2 levels inside the package headspace, or the amount of dissolved CO_2, during storage are also seldom measured. This makes comparison between different studies difficult. Various qualities of the raw material, species, different storage conditions, type of atmosphere (CA vs. MA), packaging material, analytical methods used for shelf-life assessments and criteria for endpoint of shelf-life, further increase the difficulties of comparing results from different experiments (Skura, 1991).

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air

Type of fishery product*	Storage temperature (°C)	Atmosphere+ C $O_2 : N_2 : O_2$	*G/P* ratio	Shelf-life" (day)	Reference (comments)
Catfish, channel (*Icatalurus punctatus*) fillet strips	2	80 : 20 air	ns	28	Silva and White (1994)
Catfish (ns) fillets	16	air	ns	3	Reddy *et al.* (1997a)
	16	75 : 25 : 0	ns	4	
	16	vacuum	-	3	
	8	air	ns	6	
	8	75 : 25 : 0	ns	13	
	8	vacuum	-	6	
	4	air	ns	13	
	4	75 : 25 : 0	ns	38-40	
	4	vacuum	-	20-24	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere+ $CO_2 : N_2 : O_2$	*G/P* ratio	Shelf-life″ (day)	Reference (comments)
Cod (*Gadus morhua*) fillets	1	CA 60 : 40 : 0	-	12	Woyewoda *et al.* (1984)
	1	60 : 40 : 0	ns	12	
	1	air	ns	9	
Cod (*G. morhua*) fillets	2	40 : 60 : 0	2	11	Guldager *et al.* (1998) ″Thawed shelf-life after 2 months –20°C storage
	2	40 : 60 : 0	2	20″	
	2	40 : 40 : 20	2	13	
Cod (ns) fillets	16	air	ns	3-4	Reddy *et al.* (1999)
	16	75 : 25 : 0	ns	6	
	16	vacuum	-	3-4	
	8	air	ns	13-17	
	8	75 : 25 : 0	ns	24-27	
	8	vacuum	-	13	
	4	air	ns	20-24	
Cod (ns) fillets	4	75 : 25 : 0	ns	55-60	Cann *et al.* (1983)
	4	vacuum	-	24-27	
	0	40 : 30 :30	3	12.5	
	5	40 : 30 :30	3	< 7	
	10	40 : 30 :30	3	3	
	0	vacuum	-	9	
	5	vacuum	-	< 4	
	10	vacuum	-	2	
Cod (*G. morhua*) whole	2	100	ns	10	Jensen *et al.* (1980)
	2	60 : 40 : 0	ns	10	
	2	40 : 60 : 0	ns	9-10	
	2	vacuum	-	8-9	
	2	air	ns	7	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere+ $CO_2 : N_2 : O_2$	*G/P* ratio	Shelf-life″ (day)	Reference (comments)
Cod (G. morhua) Whole/fillets	0	air	ns	12-13	Villemure *et al.* (1986)
	0	25 : 75 : 0	ns	20	
	0	3 : 97 : 0	2	13	
	0	29 : 71 : 0	2	16	
	0	48 : 52 : 0	2	20	
Cod (*G. morhua*) fillets	0	97 : 3 : 0	2	15-16	Post *et al.* (1985) ᵀAll packaging types at 26°C (air, vacuum, 100% N_2, 90 : 8 : 2 and 65 : 31 :4) Except 100% CO_2 had a shelf-life of 2 days
	26	100 : 0 : 0	ns	2-3	
	26	air	ns	2	
	12	air	ns	6	
	12	vacuum	-	10	
	12	0 : 100 : 0	ns	13	
	12	100 : 0 : 0	ns	11	
	8	air	ns	6	
	8	vacuum	-	16	
	8	0 : 100 : 0	ns	17	
	8	100 : 0 : 0	ns	23	
	8	90 : 8 : 2	ns	17	
	8	65 : 31 : 4	ns	16	
	4	100 : 0 : 0	ns	40-53	
Cod, blue (*Arapercis colias*)fillets commercially smoked	3	100 : 0 : 0	2	49	Penney *et al.* (1994)
	3	vacuum	2	14	
	3	air	2	14	
	-1.5	100 : 0 : 0	2	113	
	-1.5	vacuum	2	35	
	-1.5	air	2	28	
Crayfish (*Pacifastacus leniusxulus*) whole cooked	4	80 : 20 air	ns	21	Wang and Brown (1983)
	4	air	ns	14	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere+ $CO_2 : N_2 : O_2$	*G/P* ratio	Shelf-life″ (day)	Reference (comments)
Flounder (*Limanda ferrugina*) Fillets	26	air	ns	2	Post *et al.* (1985)
	26	vacuum	-	2	
	26	0 : 100 : 0	ns	4	
	26	100 : 0 : 0	ns	1	
	12	air	ns	5	
	12	vacuum	-	8	
	12	0 : 100 : 0	ns	7	
	12	100 : 0 : 0	ns	8	
	8	air	ns	5	
	8	vacuum	-	7	
	8	0 : 100 : 0	ns	4	
	8	100 : 0 : 0	ns	10	
Haddock (*Melanogrammus aeglefinus*) whole	0	40 : 30 : 30	ns	10	Dhananjaya and Stroud (1994)
	0	air	ns	8	
	5	40 : 30 : 30	ns	7	
	5	air	ns	7	
	10	40 : 30 : 30	ns	4	
	10	air	ns	4	
Haddock (*M. aeglefinus*) fillets	0	60 : 20 : 20	ns	14	Dhananjaya and Stroud (1994)
Haddock (*Melanogrammus Aeglefinus*) slices	0	air	ns	10	Pastoriza *et al.* (1996)
	2	50 : 45 : 5	2	14	
	2	50 : 45 : 5	2	16[π]	[π]5 min 5% NaCl-dip
	2	air	2	7-8	
Herring, Baltic (ns) fillets	2	20 : 80 : 0	0.4	3	Randell *et al.* (1995)
	2	20 : 80 : 0	1	3	
	2	40 : 60 : 0	0.4	6	
	2	40 : 60 : 0	1	8	
	2	vacuum	-	3	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere+ $CO_2 : N_2 : O_2$	*G/P* ratio	Shelf-life" (day)	Reference (comments)
Herring	0	60 : 40 : 0	ns	14	Dhananjaya and
(*Chupea harengus*) fillets	0	air	ns	12	Stroud (1994)
Herring	0	60 : 40 : 0	ns	14	Dhananjaya and
(*C. harengus*) whole	0	air	ns	12	Stroud (1994)
Herring	0	60 : 40 : 0	ns	14	Dhananjaya and
(*C. harengus*) whole	0	air	ns	12	Stroud (1994)
Hybrid striped bass	2	60 : 34 : 6	ns	13	Handumrongkul and
(*Morone saxatilis x* M. chrysops) strips	2	air	ns	7	Silva (1994)
Mackerel (*Scombrus scombrus* L.) fillets	-2	100 : 0 : 0	3	> 21	Hong *et al.* (1996)
Rockfish (*Seabastes* spp.) Fillets	1.7	CA 80 : 20 air	-	13	Parkin *et al.* (1981)
	1.7	air	-	6	
Salmon, atlantic	2	100 : 0 : 0	1.5	18	Pastoriza *et al.*
(*Salmosalar*) slices	2	air	1.5	8	(1996)
Salmon, king (*Oncorhynchus Tshawytscha*) fillets	4.4	60 : 15 : 25	ns	12	Stier *et al.* (1981)
	4.4	air	ns	6	
	22.2	60 : 15 : 25	ns	2	
	22.2	air	ns	1	
Salmon (ns) fillets	16	air	ns	4	Reddy *et al.* (1997b)
	16	75 : 25 : 0	ns	5-6	
	16	vacuum	-	3	
	8	air	ns	13-17	
	8	75 : 25 : 0	ns	20-24	
	8	vacuum	-	> 6, < 10	
	4	air	ns	24-24	
	4	75 : 25 : 0	ns	55-62	
	4	vacuum	-	34-38	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere+ $CO_2 : N_2 : O_2$	*G/P* ratio	Shelf-life" (day)	Reference (comments)
Salmon (ns) steaks	0	60 : 40 : 0	3	12.9	Cann *et al.* (1984)
	5	60 : 40 : 0	3	7.1	
	0	vacuum	-	11.8	
	5	vacuum	-	8	
	10	vacuum	-	3	
Salmon (*S. salar*) fillets	2	60 : 40 : 0	1	17	Randell *et al.* (1999)
	2	40 : 60 : 0	1	17	
	2	vauum	-	17	
Sardines *(Sardinops melanostictus)*	2	air	-	11	Fuji *et al.* (1989)
	5	80 : 20 : 0	ns	4	
	5	20 : 80 : 0	ns	4	
	5	air	ns	2	
Shrimp, spotted (*Pandalus platyceros*) Whole Head On/off	0	CA 100 : 0 : 0	-	> 14	Matches and Layrisse (1985)
	0	air	-	7	
	0	air	-	7	
Snapper (*Chrysophrys Auratus*) fillets	3	100 : 0 : 0	ns	6-8	Scott *et al.* (1984) [π]No/medium O_2-barrier mat. [π]High O_2-barrier mat.
	3	vacuum	-	3[π]	
	3	vacuum	-	6[π]	
	3	air	ns	3	
Snapper (*C. auratus*) fillets	-1	40 : 60 : 0	5	9	Scott *et al.* (1986)
	-1	air	ns	9	
	-1	100 : 0 : 0	ns	18	
Swordfish (*Xiphias gladius*) Steaks	2	air		6[π]	Oberlender *et al.* (1983) [π]Shelf-life based on Microbial data only
	2	CA 100 : 0 : 0	-	> 22[π]	
	2	CA 70 : 0 : 3	-	> 22[π]	
	2	CA 40 : 0 : 60	-	14[π]	
	2	CA 70 : 30 : 0	-	> 22[π]	
	2	CA 40 : 60 : 0	-	20[π]	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere^{+} $CO_2 : N_2 : O_2$	*G/P* ratio	Shelf-life″ (day)	Reference (comments)
Tilapia (*Tilapia* spp.) fillets	4	75 : 25 : 0	ns	> 25	Reddy *et al.* (1995)
	8	75 : 25 : 0	ns	13-16	
	16	75 : 25 : 0	ns	9-13	
	4	air	ns	9-13	
	8	air	ns	6-9	
	16	air	ns	3-6	
Trout (ns) whole	0	60 : 40 : 0	3	8	Cann *et al.* (1984)
	5	60 : 40 : 0	3	8	
	10	60 : 40 : 0	3	3.8	
	0	vacuum	-	9	
	5	vacuum	-	6.5	
Trout (*Salmon gairdneri*) Fillets	10	vacuum	-	3.7	Barnett *et al.* (1987)
	1.7	80 : 20 : 0	ns	20$^{\pi}$	$^{\pi}$Treated with 2% Potassium-sorbate dip
	1.7	air	ns	10	
Trout, rainbow (*Oncorhynchus mykiss*) fillets	2	20 : 80 : 0	0.4	6	Randell *et al.* (1995)
	2	20 : 80 : 0	1	9	
	2	40 : 60 : 0	0.4	6	
	2	40 : 60 : 0	1	9	
	2	vacuum	-	6	

Table 2 Shelf-life of fresh fishery products packaged under MA, vacuum or air (continued)

Type of fishery product*	Storage temperature (°C)	Atmosphere^{+} CO_2 : N_2 : O_2	*G/P* ratio	Shelf-life″ (day)	Reference (comments)
Whiting (*Merluccius Billinearis*) fillets	26	all atmosphere$^{\pi}$	ns	2^{π}	Post *et al.* (1985) $^{\pi}$All packaging types at 26°C (air, vacuum, 100% N_2, 100% CO_2, 90 : 8 : 2 and 65 : 31 : 4) had a shelf -life of 2 days
	12	air	ns	5	
	12	vacuum	-	9	
	12	0 : 100 : 0	ns	9	
	12	100 : 0 : 0	ns	12	
	3	air	ns	4	
	8	vacuum	-	10	
	8	0 : 100 : 0	ns	10	
	8	100 : 0 : 0	ns	15	
	8	90 : 8 : 2	ns	13	
	8	65 : 31 : 4	ns	7	
	4	100 : 0 : 0	ns	15	

Source; Adapted from Sivertsvik *et al.* (2002)

ns = not stated in article.

*Type of fish (species).

$^{+}$Initial atmosphere in percent CO_2: N_2: O_2. If mixtures of CO_2 and air are used this is reported as %CO_2: %air (e.g. 80: 20 air), if initial atmosphere is maintained during storage either in controlled atmosphere tank or by reflushing, this is indicated with CA.

G/P ratio = Volume of gas to volume of product (assuming density ~ 1 kg L^{-1} for fish); (-) not relevant (vacuum/CA). ″ Shelf-life in days after packaging as determined by sensory analysis is not otherwise noted in comments.$^{\pi}$ See comments in reference field to the right of asterisk for information on additional treatments, etc.

Natural active components in plants, spices and herbs

Definition of spices

According to the American Spice Trade Association (ASTA), Englewood Cliffs, NJ, (http;//ataspice.org) the proper definition of spices, as opposed to herbs or botanicals, is " any dried plant product used primarily for seasoning purposes". This definition includes a wide range of tropical plant aromatics, leafy herbs, spice seeds, roots, dehydrated vegetables and spice blends. In the past, herbs were leaves and seeds of temperate-zone plants, while the term spice denoted tropical aromatics only. Over time, this classification shifted, so that in general, the term spice now covers a whole range of elements–spices, herbs, blends and dehydrated vegetables. The FDA's definition of spices, however, does not include dehydrated vegetables, so these require separate labeling in products, as do any color-contributing spices such as paprika, tumeric or saffron. The USDA has much the same rules as the FDA, but also requires that onion and garlic be listed as " flavors" (Deis, 1999. Food Product Design: Cover Story - -The Secret World of Spices (http://www.foodproduct design.com/archive).

Formulation of spices

Prior to the early 1800s, spices were available in whole form only, and it was up to the user to grind them. Today, there are whole spices, ground spices, seasoning blends, which may be a combination of several spices and several forms, and spice extracts. Spice extracts include essential oil (volatile aromatic fractions); oleoresins (derived by solvent extraction of the whole spice, including volatile and non-volatile fractions); liquid soluble; (oleoresins plus solubilizing agents to create a liquid seasoning); dry soluble (oleorasins plated on a dry carrier); encapsulated spices; standardized oleoresins; and WONFs (wonderful oil and natural flavor; essential oils plus other natural flavoring materials) (Deis,1999;http://www.foodproductdesign.com /archive).

Importance of spices

Spices do much more than impart flavor. In fact, they have also been used for centuries as preservatives, colorants and medical remedies.

Antimicrobials from plants

Concerns about the use of antimicrobial agents in food products have been debated in the public domain for decades. Both increasing demand for reduced-additive (including antimicrobial preservatives) and more "natural" foods and the increasing demand for greater convenience have provoked in the food industry and researchers the search for alternative antimicrobial agents or combinations. In this search, a wide range of natural systems from animals, plants, and microorganisms is being studied (Lopez-Malo and Guerrero, 2000). However, mainly economic aspects originated in the strict requirements to obtain approval, and efforts to get the product onto the market restrict the specter of new chemical compounds that help in the preservation of foods; furthermore, the approval process is very long (10-12 years) These obstacles have originated the search for emerging preservatives by examining compounds already used in the food industry, perhaps for other purposes, but with potential as antimicrobials, approved and not toxic in the used levels, many of them classified as generally recognized as safe (GRAS). Within these compounds are, for example, the so-called green chemicals present in plants that are utilized as mainly flavor ingredients (Nychas, 1995).

The antimicrobial compounds in plants material are commonly contained in the essential oil fraction of leaves (rosemary, sage), flowers and flower buds (clove), bulbs (garlic, onion), rhizomes (asafetida), fruit (pepper, cardamon) or other parts of the plant as shown in Table 3.

Table 3 Some components with antimicrobial activity found in plants, herbs and spices

Plants, Herbs or Spices	Major component(s)	Other component(s)
All spices (*Pimenta dioica*)	eugenol	methyl ether, cineol
Basil (*Ocimum basilicum*)	d-linalool, methyl chavicol eugenol, cineol,	eugenol, cineol, geraniol
Black pepper (*Pipper nigrum*)	monoterpenes, sesquiterpenes oxygenated compounds	
Bay (*Laurus nobilis*)	cineol	l-linalool, euginol, geraniol
Caraway seed (*Carum carvi*)	caravone	limonene
Celery seed (*Apium graveolens*)	d-limonene	
Cinnamon (*Cinnamomum zeylanicum*)	cinnamic aldehyde	l-linalool, p-cymene, eugenol
Clove (*Syzygium aromaticum*)	eugenol	cariofilene
Coriander (*Coriandum sativum*)	d-linalol	d-α-pinene
Cumin (*Cumin cyminum*)	cuminaldehyde	p-cymene
Fennel (*Foeniculum vulgare*)	anethole	
Garlic (*Allium sativum*)	diallyl disulfide, diallyl trisulfide	
Lemongrass(*Cymbopogon citrates*)	citral	geraniol
Majoram (*Origanum majorana*)	linalool, cineol, eugenol, terpinieol	methyl chavicol
Mustard (*Brassica hirta*) *B. juncea, B. nigra*	allyl-isothiocyanate	
Onion (*Allium cepea*)	d-n-propyl disulfide, methyl-n-propyl disulfide	
Oregano (*Origanum vulgare*)	thymol, caracrol	
Parsley (*Petroselinum crispum*)	borneol, cineol	camphor, α-ene,bornyl acetate, terpinol
Sage (*Salvia officinalis*)	thujone, cineol, boreol	thymol, eugenol
Tarragon (*Artemisia dracunculus*)	methyl chavicol	anethole
Thyme (*Thymus vulgaris*)	thymol	caracrol, l-linalool, geraniol, p-cymene
Vanilla (*Vanilla planifolio*)	vanillin	
V. tahilensis	hidroxibenzoic and	p-coumaric acid

Source: Adapted from Lopez-Malo *et al.* (2000)

Essential oils of a large number of plants possess useful biological and therapeutic activities, and the oils are extensively utilized in the preparation of pharmacological drugs. They are commercially recovered from plant materials primarily by steam distillation, and their use in the food industry is influenced by the nature of their constituents.

Antimicrobial activity of essential oils and extracts from plants, herbs, and spices depends not only on the extraction method, but also on the initial quantity of essential oil in the plant. Within the same spice or plant, the levels of compounds and therefore, active antimicrobial groups, can substantially vary. Also, the geographic zone of the cultivation may influence the extract composition. Mishra and Dubey (1994) reported that lemon grass essential oil varied in its effectiveness as an antimicrobial, depending on the time of harvest. During May to December, it was more effective, inhibiting 100% of the evaluated microbial strains, but the essential oil from plants collected during February to April was only 73-80% effective. Therefore there is a necessity to establish methods to fix or standardize essential oil purity or concentration of active components.

Antioxidant activity

Antioxidants are very important in disease prevention in both plants and animals, inhibiting or delaying the oxidation of biomolecules by preventing the initiation or propagation of an oxidizing chain reaction. In foods, lipid oxidation (Belitz and Grosch, 1999), protein oxidation (yielded protein hydroxides, the degradation of which can generate the carbonyl moiety (Simpson *et al.,* 1992), and enzyme oxidation cause shelf-life problems. Rancidity development is an oxidative process that can be inhibited by antioxidants, which block formation of free radicals by donating electron or hydrogen ions to halt the oxidative process. While synthetic phenolic compound such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), or tertiary butylhydroquinone (TBHQ) are very effective, economical ingredients, but they are not appropriate for some natural products, and also health concern problems. On the other hand, commercial rosemary products

might be a better fit. For those reasons, there is interest in the use of naturally occurring antioxidants (Frankel, 1998). Active compounds in these herbs are diphenolic diterpene, which are highly effective antioxidants. The main constituents include: carnosol, carnosoic acid and its esters, and lesser amounts of rosemanol, rosmaridiphenol and rosmarinic acid. Sage and thyme also contain natural antioxidant compounds.

Recently, the positive effects of antioxidants have not been limited only to food preservation. Many scientific papers reported that vitamin E, a strong antioxidant, can limit the death of brain cells exposed to a free-radical-stimulating protein in the brains of Alzheimer's patients(Deis,1999; http://www.foodproduct design.com/archive).

Spices may also provide some of the same effects. The consequent search for natural replacements for synthetic antioxidants has led to the evaluation of number of plant sources (Heinonen *et al.*, 1998). The concentration of lycopene and the various phenolic compounds as well as the antioxidant activity are significantly influenced by tomato variety. In addition, agronomic, geographical and seasonal factors also determine the concentrations of individual phenolic compounds and lycopene. Individual compounds found to be significantly related to antioxidant capacity are lycopene and ferulic and caffeic acids, but not quercetin and chlorogenic acid (Martinez-Valverde *et al.*, 2002).

Colorant

Spices also improve the appearance of food. At 4-16% carotenoids, chili peppers provide a range of red to green color, for example. Paprika, turmeric and their oleoresins, as well as saffron, are approved in the United States as color additive in 21 CFR section 73, and may be used with no restrictions.

Saffron, produced from the stigma of *Crocus sativas,* a member of the iris family, has a very intense water-soluble, yellow-orange color. This color is caused by carotenoids, particularly crocetin and crocin, as well as P-and L-carotene, lycopene and zeazanthin. Saffron may be unique in its color and fragrance, but it is the most

expensive spice in the world, at a cost of over $300 per lb. Saffron is permitted as a natural color in the United States, but economy limits its use. Safflower, *Carthmus tinctorius*, is a frequent saffron substitute overseas, but it is not permitted in the United States of America.

Tumeric, *Curcumar longa* a member of the ginger family, is also a very effective natural colorant. The name Curcuma is actually derived from the Persian word "kirkum" meaning saffron. When the roots of this plant are dried and ground, the powder produced is yellow with orange tinge. The powder is often blended with paprika and annatto to produce the desired color. Its largest use is in prepared mustard, but it is also widely used in curry powder, pickles, relish, and sausages. The extractable color in turmeric comes from curcumin, which is also a natural antioxidant.

Paprika provides a brilliant red powder derived from carotenoids-capsanthrine, capsorubin, β-carotene and others. Paprika is produced from the pods of *Capsicum annum*, a mild bell pepper. Formulators usually prefer to use paprika oleoresin due to its better light stability. Product applications include blends for curry powder, cereals, sauces and baked goods.

Cinnamon can also provide a range of colors, depending on the type chosen. Cassia-type cinnamons, native to China and Indonesia, have a range of essential oil contents (cinnamic aldehyde), and provide a selection of aroma, flavors and color intensities. Ceylon-type cinnamon is very low in essential oils, and so is weak in color, flavor and aroma (Deis, 1999; http://www.foodproductdesign.com/archive).

Microorganisms in plants

Most botanicals are either cultivated or gathered in the wild and are usually contaminated with fungi, bacteria, or other microoganisms and thus should be cleaned or sterilized before use. However, heat sterilization can have a detrimental

effect on some active constituents of the plant. Ethylene oxide (ETO) treatment is one form of sterilization. However, it was found to cause certain changes in the compounds due to a reaction with ETO. The other alternatives would be gamma-radiation, which is very effective, especially in inactivating larvae. CO_2 also seems to work well as well as cold filtration and pasteurization (D' Amelio, 1999).

Application of some active plant compounds in foods

Unfortunately, many of the published reports about the applications of phenolic compounds (antioxidants and constituents of extract and essential oils) as antimicrobials have been accomplished in model and laboratory systems, and there are few studies that have been carried out in the real food (Board and Gould, 1991).

The essential oils of spices, and plants, as well as their major components, are more effective in microbiological media than when evaluated in real food (Zaika, 1988). In most cases, the inhibitory concentrations found in model systems increase significantly when evaluated with the same microorganisms in actual food. In consequence, few of the applications of phenolic compounds as antimicrobials have been successful (Kabara and Eklund, 1991). The reduction in the effectiveness observed in vivo represents an important limitation to the use of essential oils and phenolic antioxidations as antimicrobial agents in foods (Juven *et al.*, 1994). The interactions among phenolic groups and proteins, lipid and aldehydes could explain, at least partially, the reduction of the antimicrobial effect of essential oil where the major constituents are phenols. Tassou and Nychas (1994) demonstrated that inoculated size, oleuropein concentration, and pH significantly influenced *S. aureus* growth and lag time, and proved that the efficiency of phenolic compound antimicrobial action was reduced in foods with relatively low protein content. Robach *et al.* (1977) reported that the reduction in BHA antimicrobial capacity in a crab homogenate was due to a partial inhibition of antioxidant properties by the presence of lipids and oxidation stage. However, Rico-Munoz and Davidson (1983), studying casein and corn oil effects in the phenolic antimicrobial activity, reported that casein did not have an effect on *Saccharomyces cerevisiae* or *S. aureus* growth and slightly

reduced growth of *P. fluorescens*. The activity of BHA in the presence of protein depended on the species studied. Aureli *et al.* (1992) found that essential oil antilisteric efficiency of thyme decreased when tested in ground pork meat (in vivo) in comparison with the behavior in laboratory media (solid media). Also Robach *et al.* (1977) reported a decrease in antimicrobial activity of BHA in foods. For example, it was found that *Vibrio parahaemolyticus* growth was inhibited with 50 ppm BHA in trypicase-soy broth, whereas up to 400 ppm were required to achieve the same effect in the crab homogenate. Shelef and Liang (1982) reported an increase in the inhibitory concentration of BHA for several strains of *Bacillus*. More than 100-fold was required in chicken meat, in comparison with laboratory media. Cornell *et al.* (1971) explained that BHA was bound to casein through hydrophobic interactions and should probably decrease its antimicrobial activity.

Spencer *et al.* (1988) also reported that the interaction or complex between phenols and proteins depends partially on protein characteristics, on pH and on phenolic groups. This interaction takes place by hydrogen bridges between phenolic groups and peptides as well as by hydrophobic interaction. Moreover the antimicrobial activity of BHA and TBHQ was affected by the presence of casein or corn oil in the laboratory media because TBHQ is less effective as an antimicrobial agent in the presence of fats or protein, as compared with BHA (Rico-Muco and Davidson, 1983). The principle loss could probably be the solubilization of these compounds in the lipidic phase of the medium, reducing its availability to act as antimicrobial. BHA is a lipophilic antioxidant with a low hydrophilic-lipophilic balance (HL), whereas TBHQ is more anphipatic with greater HL balance. Thus these differences in fat solubility could explain, at least partially, the greater decrease in antimicrobial activity of BHA in fat presence than that observed for TBHQ. Ahmad and Branen (1981) demonstrated that small amount of lipid (0.25%) could reduce the antimyotic activity of BHA. They evaluated BHA activity in real foods and demonstrated that BHA inhibited *P. expansum* and *A. flavus* growth inoculated in spread cheese and apple pulp. Concentrations needed to inhibit mold growth were greater than those found in laboratory media. In apple pulp, 200ppm was necessary,

and in spread cheese, 400 ppm was required. These authors explained the activity reduction on solubility in the lipid phase and/or on protein interactions.

The interactions of aldehydes with proteins have been extensively studied, because protein addition to aldehyde solutions can decrease the effective concentration of these groups (Cha, 1988; Tateo *et al.,* 1988). Citral (lemon flavor component) concentration was reduced almost 100% when 5% casein or soy protein isolate was added in aqueous solutions. Sixty-eight percent initial vanillin concentration, measured by HPLC, was lost after 26 hrs in drinks containing aspartame (Hussein *et al.,* 1984). The reduction in vanilla flavor by the reduction of vanillin concentration had been reported when adding faba-bean proteins, sodium caseinates, or milk whey protein concentrate (Hussein *et al.,* 1984; Ng *et al.,* 1989 a,b; Barr, 1990).

Herbs and spices used in Thai cuisine

Many herbs and spices used in Thai cuisine have beneficial medicinal properties (10 Thai Dish: Spicy and Hot cooking Recipes of Thailand, http://www.10Thaidish.com/herb/HerbSpice.htm). These are some examples.

Chilli: "Phrik" in Thai
Chilli is an erect, branched, shrub-like herb with fruits used as garnishing and flavouring in Thai dishes. There are many different species. All contain capsaicin, a biologically active ingredient beneficial to the respiratory system, blood pressure and heart. Other therapeutic uses include being a stomachic, carminative and antiflatulence agent, and digestant.

Garlic: "Kra-thiam" in Thai
Garlic is an annual herbaceous plant with underground bulbs comprising several cloves. Dried mature bulbs are used as a flavouring and condiment in Thai cuisine. The bulbs contain a 0.1-0.36% garlic oil and organic sulfur compounds. Therapeutic uses are as an antimicrobial, diaphoretic, diuretic, expectorant, antiflatulence and cholesterol lowering agents.

Galanga: "Kha" in Thai
Greater Galanga is an erect annual plant with aromatic, ginger-like rhizomes, and is commonly used in Thai cooking as flavouring. The approximately 0.04 volatile oil content has therapeutic uses as carminative, stomachic, antirheumatic and antimicrobial agents.

Kaffir: "Ma-krut" in Thai
The leaves, peel and juice of the Kaffir Lime are used as a flavouring in Thai cuisine. The leaves and peel contain a volatile oil. The major therapeutic benefit of the juice is as an appetiser.

Lemon Grass: "Ta-khrai" in Thai
This erect annual plant resembles a coarse grey-green grass. Fresh leaves and grass are used as flavouring. Lemongrass contains a 0.2-0.4 volatile oil. Therapeutic properties are as a diurectic, emmanagogue, antiflatulence, antiflu and antimicrobial agent.

Shallot: "Hom, Hom-lek, Hom-daeng"in Thai
Shallots, or small red onions, are annual herbaceous plants. Underground bulbs comprise garlic-like cloves. Shallot bulbs
contain a volatile oil, and are used as flavouring or seasoning agents. Therapeutic properties include the alleviation of stomach discomfort, and as an antihelmintic, antidiarrhoeal, expectorant, antitussive, diuretic and antiflu agents.

Tom-Yum

Thai food has gained popularity, and Tom-Yum is an important ethnic food consumed worldwide. Tom-Yum is the most delicious soup in the world, according to Book of the World Records (Annon, 2003). This is due to its tastes, colors and health effect. In Japan and Thailand, researchers have discovered that some components found in galagal root, lemon grass and kaffir lime leaves, which are major ingredients of the soup, are effective in inhibiting tumors in the digestive tract (Division of Health Statistics, 1989; Murakami *et al.,* 1993, 1994 and 1995). In addition, the ingredients of Tom-Yum also include chili, shallot (red onion) and garlic, which are natural antimicrobial, antioxidant compounds with health benefits (Nishimura *et al.,* 2000). Garlic and onion have generally been found to be a great antibacterial (Shelef, 1983), antidiabetic, hypocholesterolemic and cancer preventive agent (Nishimura *et al.,* 2000). Allicin, one of the active components of freshly crushed garlic homogenates, has a variety of antimicrobial, antifungal, antiparasitic as well as antiviral activities. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, such as alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase (Ankri and Mirelman, 1999).

Chili pepper is a good source of β-carotene as a protector against photooxidation, other carotenoids, vitamin A, C and E (Osuna-Garcia *et al.,* 1998). Carotenoids are one of the most abundant groups of natural pigments because most living plants synthesize them as a protector against photo-oxidative processes and they are constituents of chromoplasts (Deli *et al.,* 2001). However, content of carotenoids in vegetables greatly varies in amount, depending on species, variety, time of the year, and degree of ripeness (Deli *et al.,* 2001; Osuna-Garcia *et al.,* 1998). Essential oil of lemon grass is mainly comprised of citral which exhibited a broad antifungal sprectrum (Adeoke and Odesola, 1996; Schaneberg and Khan, 2002) while galangal root and kaffir lime leaves extracts failed to inhibit *Bacillus cereus, B. megaterium, E. coli, Pseudomonas aeruginosa, Aspergillus ochraceous,* and *Cryptococcus neoformans* (Mackeen *et al.,* 1997). As researchers and consumers are increasingly concerned about health problem from synthetic additives, they are continually focusing on the use of

plant products as alternatives to synthetic ones. However, no scientific study using whole Tom-Yum mix instead of individual active components as functional foods and natural preservatives is available.

Research objectives

1. To investigate the rigor mortis stage and sensory profile in fresh and ice stored fish to find the definite sensory indicator in each step of quality loss
2. To study the effect of killing method, icing delay and fish position on storage life
3. To investigate the potential use of Tom-Yum mix as a natural preservative
4. To study the shelf-life extension of marinated cut fish with selected Tom-Yum mix packaged under various modified atmospheres
5. To develop a functional product that may have potential for patent application