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Review Article

Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging

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Abstract

Fish and fishery products have been recognized as a nutrition source due to their high protein content. Moreover, they contain considerable amount of unsaturated fatty acids, especially omega-3 fatty acids, which are regarded as preventive compounds. However, shelf-life of seafood is limited by biochemical and microbiological changes. Modified atmosphere packaging (MAP) is widely used for minimally processed fishery products including fresh meat for retarding microbial growth and enzymatic spoilage. CO_2, O_2, and N_2 are most often used in MAP. CO_2 enriched atmosphere inhibits the autolytic degradation of fish muscle during storage. However, high levels of CO_2 negatively affect product quality, especially by increasing drip loss and altering texture. Development of satisfactory methods for shelf-life extension that ensure quality maintenance of products with minimum loss has drawn the attention of food technologists. The application of MAP and combination process in seafood is a promising preservation method to extend the shelf-life of fish and fishery products.

Keywords: deterioration, shelf-life, extension, modified atmosphere packaging

1. Introduction

Packaging technologies are important to protect products against deteriorative effects, which may include microbial, biochemical, and physical activities from environmental influences. This involves retardation of spoilage, extension of shelf-life, and maintenance of quality in packed food. Other functions of packaging include containment, convenience, marketing, and communication (Restuccia *et al.,* 2010). The rate of deterioration during the storage of a product depends on the biochemical compositions of substrates and metabolites in the tissue, the microbial contamination, and the condition of storage. Fresh seafood undergoes spoilage faster than fresh commodities. Thus, the marketing has emphasized on canned and frozen products. Since ready-to-cook or fresh seafood have become increasingly popular, they are available in the market. However, the short shelf-life is a limiting factor for these perishable products. Therefore, certain techniques have been applied to extend the shelf-life of fish products and also safety concern should be taken into consideration.

Over the last years, modified atmosphere packaging (MAP) has received increasing attention as a method of food preservation. MAP is defined as the enclosure of food products in gas–barrier materials, in which the gaseous environment has been changed (Sivertsvik *et al.,* 2002). MAP is used for extending the shelf-life period of fresh or minimally processed foods. Apart from other perishable products, attempts have been carried out to prolong the shelf-life of seafood and fishery products by using MAP. It has been demonstrated that MAP retarded the microbial growth and the enzymatic deterioration. The shelf-life of iced or chilled fish and fishery products stored under MAP increases as a result of lag phase extension of several aerobic spoilage bacteria. The shelf-life of chilled fish is generally limited due to the growth of Gram-negative microorganisms such as

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Pseudomonas, *Shewanella putrefaciens,* and *Aeromonas* under aerobic condition (Ravi-Sankar *et al.,* 2008). Currently, the successful commercial application of MAP for inhibiting spoilage and extending shelf-life of fish products is associated with a number of interrelated factors, such as developments on new food packaging materials including vacuum packaging and gas packaging as a response to consumer demands for products with fresh characteristics, consumer concerns about preservation additives in such products, and favorable consumer perception of MAP technology (Ashie *et al.,* 1996). New developments in MAP systems include active packaging, biopackaging, and biocoating by keeping food products in an atmosphere that is different from the normal composition of air (Lee, 2010). Thus, the objective of this review is to demonstrate the impact of MAP and the combination of several preservation technologies on the shelf-life extension of fish and fishery products. Moreover, safety and quality concerns of products preserved by those techniques have been raised.

2. Biochemical changes of fish and fishery products

Muscles of fish contain protein, lipid (fat), water, carbohydrate, mineral, organic extractive, and nucleic acids, which vary, for example, with species, muscle type, spawning. Fish is one of the most perishable foods. The muscle tissue of fish undergoes faster spoilage than mammalian muscles. The high water and free amino acid content, and the lower content of connective tissue as compared to other flesh foods lead to the more rapid spoilage of fish. Moreover, the shellfish flesh containing high carbohydrate and low nitrogen content can be used as nutrient sources for microbial growth. Immediately after death, several biochemical and enzymatic changes are triggered in seafood muscles, especially with improper handling. Therefore, spoilage in fish and shellfish depends on species and chemical components. Those changes along with enzymatic and microbially induced activities are involved in the degradation of muscles (Pereira de Abreu *et al*., 2010).

2.1 Changes in proteins

The changes in quality of fish and shellfish can be monitored by biochemical or enzymatic freshness and microbial changes during handling and storage. The endogenous proteinases play an important role in protein degradation of postmortem fish and shellfish muscle. The degradation of muscle structure is considered to be caused by proteinases, such as calpain and cathepsin D, B and L (Godiksen *et al.,* 2009). For fish kept in ice, among proteins, myosin heavy chain (MHC) was the most extensively hydrolyzed, followed by troponin-T and α- and β-tropomyosin. Degradation of myofibrils in Pacific whiting fillets occurred at 0°C, possibly caused by cathepsins (An *et al.,* 1994). Apart from endogenous proteases, several microorganisms growing on muscle secrete a wide variety of hydrolytic enzyme, particularly proteinases. *Pseudomonas* spp. is the main organism responsible for deterioration of food proteins (Pantazi *et al*., 2008). Godiksen *et al.* (2009) showed that MHC of rainbow trout muscle was hydrolyzed continuously throughout iced storage for five days and related to firmness of the muscle, whereas changes in actin were observed on SDS-PAGE. The spoilage of fish muscle causes the loss of nutritive values, acceptability as well as the functionality of proteins . In general, functional properties of fish myofibrillar proteins are important for determining and predicting the final quality of fishery products.

The quality changes of fish associated with degradation and denaturation of muscle leads to the loss in protein properties during handling and storage. Ca^{2+}-ATPase can be used as an indicator for the integrity of myosin and Mg^{2+}-EGTA-ATPase is determining the integrity of the troponin-tropomyosin complex (Benjakul *et al.,* 1997). Mg^{2+}-Ca^{2+}-ATPase activity is indicative of the integrity of the actin-myosin complex in the presence of exogenous Ca^{2+} (Roura and Crupkin, 1995). Masniyom *et al.* (2004a) found that no changes in Ca^{2+}-ATPase, Mg^{2+}-Ca^{2+}-ATPase or Mg^{2+}-ATPase of sea bass (*Lates calcalifer*) actomyosin were observed, but Mg^{2+}-EGTA-ATPase activity gradually increased during refrigerated storage for 12 days. Chomnawang *et al.* (2007) reported that Ca^{2+}-ATPase activities of catfish fillet decreased during extended 4°C storage of 15 days. The changes in ATPase activities and in Ca^{2+}-sensitivity of myofibril were shown to result from proteolysis (Ebashi *et al,* 1968). The loss in Ca^{2+}-sensitivity of myofibrillar protein would be an indicator of the proteolytic degradation of tropomyosin and the modification of actin-myosin interaction by oxidation of sulfhydryl (SH) group of myosin (Benjakul *et al.,* 2003). Benjakul *et al.* (1997) found that total SH content of actomyosin from Pacific white increased slightly after two days of iced storage, followed by a gradual continuous decrease up to eight days. The decrease in total SH content was considered to be due to the formation of disulfide bond via oxidation of SH group or disulfide interchanges (Buttkus, 1970). Chan *et al.* (1995) reported that myosin contained 42 SH groups. Two types of SH groups on the myosin head portion (SH_1 and SH_2) have been reported to involve in ATPase activities of myosin (Benjakul *et al.,* 1997). Another SH groups (SH_a) was localized in the light mermyosin region of myosin molecule and was responsible for the oxidation of MHC and its dimer formation, resulting in an increase in Mg^{2+}-EGTA-ATPase activity of carp actomyosin during iced storage (Sompongse *et al.,* 1996).

Moreover, the increase in surface hydrophobicity indicates an exposure of the interior of molecule due to denaturation or degradation (Multilangi *et al.,* 1996). ANS, a fluorescence probe, has been found to bind to the hydrophobic amino acids containing an aromatic ring, i.e. phenylalanine and tryptophan, when conformational changes occur in the protein (Roura *et al.,* 1992). An increase in surface hydrophobicity was observed with hake stored on ice (Roura *et al*., 1992). An increase in surface hydrophobicity reflects

the loss water holding capacity of muscles, leading to higher exudates in fish; a phenomenon which was observed in cod and haddock muscle kept in ice (Olsson *et al.,* 2003).

Collagen is one of the major constituents of intramuscular connective tissue in fish and shellfish muscle. The characteristic of collagen is normally considered to be a relatively stable protein, which surrounds each muscle fiber and connect to myocommata. The degradation of collagen leads to texture changes in the muscle. The shear force of prawn muscle decreased with increasing cold storage time and was related with decreased firmness of meat (Pornrat *et al.,* 2007). It has been suggested that meat tenderization is due to the disintegration of collagen fiber caused by an enzymatic degradation and by lactic acid production of post-mortem anaerobic glycolysis (Montero and Borderias, 1990). Hernandez-Herrero *et al.* (2003) concluded that post-mortem softening of fish flesh was more affected by the changes of collagen structure than by the changes of the myofibrillar proteins. Sriket *et al.* (2010) reported that the softening of prawn meat during iced storage was probably a result from the degradation of collagen, where enzymes like collagenases cleaved parts of the non-helical region. In intramuscular connective tissue of fish, type I and type V have been identified as major and minor collagens, respectively. Degradation of type V collagen causes disintegration of the thin collagen fibrils in pericellular connective tissue, resulting in post-harvest softening (Sato *et al.,* 1991). Sato *et al*. (1997) proposed that rapid softening of fish flesh during short-term chilled storage is caused by degradation of type V collagen, thereby weakening intramuscular pericellular connective tissue. First, type V collagen was solubilized specifically in the softened muscle by chilled storage. Second, disintegration of thin collagen fibrils and weakening of the intramuscular pericellular connective tissue occurred specifically in the softened muscle. As a consequence, the diameter of the collagen fibrils in the pericellular connective tissue was significantly smaller than those in myocommata (Hallett and Bremner, 1988). Asghar and Henrickson (1982) stated that native collagen in mammals was not very susceptible to be attacked by proteolytic enzymes but lysosomal enzymes could degrade collagen denatured with lactic acid. Cathepsin L and serine protease are capable of hydrolyzing major muscle structure proteins, such as telopeptide collagen (Yamashita and Konagaya, 1991). Extracellular matrix collagenases regarded as initiators of breakdown are active against collagen type I, IV and V (Bremner, 1992).

2.2 Changes in lipid

The changes in lipids of fish and shellfish are responsible for the quality deterioration with the extended storage, especially under inappropriate conditions. They involve lipolysis, lipid oxidation, and the interaction of the products of these processes with nonlipid components such as protein. Fish muscles contain an abundance of long chain lipids with a high proportion of polyunsaturated fatty acid that undergoes changes due to oxidation during processing and storage. Apart from the high degree of unsaturated fatty acid, the presence of heme pigments and metallic ions in seafood leads to lipid oxidation. During the advanced stages of lipid oxidation, the breakdown of hydroperoxides generates low molecular-weight carbonyl and alcohol compounds that could lead to the changes in food quality, which affect the color, texture, flavor, and odor characteristics. The resulting products of reaction between protein and oxidized lipid are yellow pigment formation. The color changes of cuttlefish are accompanied with the development of rancid odors during frozen storage (Thanonkaew *et al*., 2006). Thiansilakul *et al.* (2010) reported that the off-odor development in seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus* × O. *niloticus*) correlated with lipid oxidation during 15 days of iced storage. Lipid hydrolysis can occur with the action of enzymes. The majority of lipolysis in most stored fish originates from endogenous enzymes and microorganisms, mainly phospholipase and triacyl lipase (Aryee *et al*., 2007). Lipase, phospholipase A_2, and phospholipase B are believed to be important enzymes in lipid hydrolysis of fish, while phospholipase C is probably derived mostly from microorganisms (Brockerhoft and Jensen, 1974). Hwang and Regenstein (1993) reported that free fatty acid (FFA) and 1, 2- diacylacylglycerol in minced mackerel increased during storage at 2-3^{o}C, particularly after 15 days of storage. Lipase produces FFA that undergoes further oxidation to produce low molecular weight compounds that are responsible for rancid off-flavor and the taste of fish and fish products. Additionally, FFA and products of their oxidation could have an impact on muscle texture and functionality due to their ability to interact with myofibrillar proteins and to promote protein aggregation.

Lipoxygenase and peroxidase are enzymes that oxygenate polyunsaturated fatty acids, converting them to hydroperoxides in fish, which can initiate the auto–oxidation of fatty acids. Other factors that determine lipid deterioration, including oxygen, pH, light, temperature, and water activity, are also important in oxidation. In presence of high O_2 content in a package, rancidity of lipid could be increased, resulting in the generation of fishy and off-odor. Therefore, prevention of lipolysis and lipid oxidation of fish and shellfish during storage can be achieved by incorporation of modified atmosphere packing and combined methods.

3. Microbiological changes of fish and fishery products

Microbial spoilage of fish and shellfish can be caused by the activities of enzymes and microorganisms, resulting in unacceptability for human consumption. Several researchers have observed that the microorganisms associated with seafood directly related to the fishing ground, environmental factor, harvesting method, storage, and transportation. Moreover, each microbial growth during storage will depended on the preservation conditions. Gram and Huss (2000) reported that the microorganisms responsible for spoilage were domi-

nated by psychrotolerant Gram-negative bacteria (*Pseudomonas* spp. and *Shewanella* spp.) grown on chilled fish. MAP with CO_2-enriched atmosphere retards respiratory organisms and dominates *Photobacterium phosphoreum* and lactic acid bacteria (LAB) (Dalgaard, 2000). Spoilage bacteria in temperate water fish are dominated by psychrotrophic, aerobic or facultative anaerobic Gram-negative bacteria usually identified as *Pseudomonas*, *Moraxella, Acinetobacter, S. putrefaciens, Vibrio, Flavobacterium, Photobacterium,* and *Aeromonas* (Sivertsvik *et al.* 2002). Gram-positive bacteria such as *Staphylococcus* spp., *Micrococcus*, *Bacillus*, *Clostridium*, *Cornynebacterium, Brochothric thermosphacta,* and *Streptococcus* were found to be the dominant microflora in tropical marine fish (Al Bulushi *et al.,* 2010). However, the frequencies of microorganism in fish and shellfish depend on geographic location, sampling location within fish habitats, and differentiation techniques. The spoilage of seafood is considered to be caused by specific spoilage organisms (SSOs) such as *Pseudomonas*, *Shewanella,* and *P. phosphoreum* (Gram and Dalgaard, 2002). The seafood SSOs can grow and produce spoilage metabolites, especially volatile bases, hypoxanthine, organic acids and biogenic amines. Some spoilage metabolites would be an indicator of deterioration in fish and shellfish. The microflora of fish and shellfish initially use the low-molecular weight substances in tissue (carbohydrates, free amino acids and small peptide) as a source of energy for further growth. Trimethylamine (TMA) is generally produced by the reduction of trimethylamine oxide (TMAO) possibly by endogenous enzymes in fish, but mainly by the enzyme activity of certain bacteria (*Aeromonas* psychrotolerent enterobacteria, *S. putrefaciens* and *Vibrio* spp.). TMA is associated with the odor of the spoiled fish and is used as an indicator of bacterial activity causing the deterioration (Huss, 1995). The development of TMA in many fish species is accompanied by a production of hypoxanthine. Hypoxanthine, which may cause a bitter off-flavor in the fish, can be formed by the autolysis decomposition of nucleotide, but it can also be formed by bacteria and rate of bacterial formation is higher than the autolysis. Dalgaard *et al.* (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod. Several spoilage bacteria including *Pseudomones* spp. and *S. putrefaciens* produce hypoxanthine from inosine or inosine monophosphate. An increase in pH has been observed with some fish, partially when the storage time was increased during iced or chilled storage. The pH increased throughout the chilled storage of cazon fish and seabass (Masniyom *et al.,* 2002; Ocano-Higuera *et al.,* 2009). The pH increased very quickly in the air atmosphere because of the growth and metabolism of the endogenous or microbial enzymes, which cause the reduction of TMAO to TMA and other basic volatiles (Ruiz-Capillas and Moral, 2001). However, no significant changes in pH, TMA, and TVB for meager (*Argyrosomus regius*) fillets stored in ice were observed throughout the storage up to 18 days, while an increase in mesophile and psychrophillic bacteria was found, especially when the storage time increased (Hernandez *et al*., 2009). The change in pH of mussel in chilled storage was observed to decrease during 15 days of storage, presumably owing to an accumulation of lactic acid generated in anoxic condition from glycogen (Masniyom and Benjama, 2007). According to De Vido *et al.* (2001) the adductor muscle of scallop experienced a decrease in pH after chilled storage.

Biogenic amines, including agmatine, cadaverine, dopamine, histamine, noradrenaline, putrescine, serotonin, spermidine, spermine, tryptamine, and tyramine, are products of bacterial spoilage and their contents are often used as an index to assess the keeping quality and shell-life of fishery products. Thus, an increase in biogenic amines content reflects the decomposition of amino acids during storage of seafood varied with differences in species, concentrations of substrate in tissue, handling procedures, and conditions of storage. The majority of putrescine, cadaverine and histamine formed in horse mackerel originated from amino acid decarboxylation of some microorganisms, such as *Pseudomonas, Vibrio,* and *Photobacterium* (Okuzumi *et al.,* 1990). Furthermore, Al Bulushi *et al.* (2010) found that *Acinetobacter, Aeromomas, Bacillus, Clostridium, Escherichia, Micrococcus, Pseudomonas, Salmonella*, and some LAB were described as prolific biogenic amines-forming bacteria. However, the formation of biogenic amines in seafood was retarded by the MAP due to the inhibition of bacteria by CO_2. The shelf-life of fresh fish is limited in the presence of atmospheric O_2 by the growth and biochemical activities of Gram-negative, psychrotrophic strains of *Pseudomonas* spp., *Flavobacterium* spp., and *Moraxella* sp. These spoilage microorganisms can be inhibited by packaging of products in an impermeable film under a CO_2–enriched atmosphere. As a result of the inhibition of spoilage microorganisms, levels of chemical compounds, for example, TMA, total volatile nitrogen, hypoxanthine and biogenic amines, which are chemical indicators of microbial spoilage of food, are also reduced.

4. Effects of MAP on preservation of fish and fishery products

Enzymatic, biochemical, and microbiological changes are triggered in fish and shellfish mainly due to poor handling practices. In general, a higher storage temperature leads to an increased deterioration in seafood. This is presumably caused by an increase in biochemical and microbial activities. Therefore, low temperature storage effectively retarded the deterioration, but in some case, it could not prevent the chemical deterioration, such as autooxidation or enzymatic hydrolysis of the lipids. Contamination of seafood with psychrotrophic spoilage bacteria results in a non–acceptance by the consumer. Seafood is traditionally stored under refrigeration in air, which gives a shelf-life time ranging from 2 to 10 days, depending upon species, harvest location, season, initial microbiological qualitym and storage temperature. Thus, a combined preservation methods with appropri-

ate storage temperature is effective in reducing deterioration of fish and fishery products.

Shelf-life of fish and shellfish could be extended by using MAP, specifically elevated CO_2 levels, which has been shown to retard the growth of spoilage and pathogenic bacteria. In addition, MAP including vacuum packaging, gas packaging and active packaging can be used to change the atmosphere surrounding fresh product.

Vacuum packaging is used for long-term storage of dry foods and the shelf-life extension of seafood. The product is packed in a vacuum package, which has good barrier properties towards oxygen and water and can be easily sealed. Air is removed under vacuum and the package is sealed. The products kept under a lower O_2 atmosphere, with less than 1% inhibiting the growth of aerobic spoilage microorganisms, particularly *Psuedomonas* spp. and *Aeromonas* spp., compared with the atmosphere packaging. It was reported that psychrotrophic and mesophilic bacteria counts were reduced in rainbow trout packaged under vacuum packaging (Arashisar *et al.,* 2004). Furthermore, vacuum packaging could prevent oxidative rancidity and improve organoleptic quality in seafood. Pantazi *et al.* (2008) found that fresh Mediterranean swordfish kept under vacuum condition had an overall increase of shelf-life of nine days, longer than that of seven days in aerobic packaging. The results were in agreement with those reported by Gimenez *et al.* (2002) who found that vacuum packed rainbow trout had lower malondialdehyde values, compared with those kept in air, as a consequence of the absence of oxygen in the package. Thus, vacuum packaging effectively extends the shelf-life of fishery products by maintaining their odor and flavor.

Gas packaging is commonly used for processing and preserving fresh fish and shellfish (Table 1). MAP has proven to extend the shelf-life and retard the deterioration of seafood under refrigeration. It involves the replacement of air followed by addition of the single gas or gas mixture in package. During storage and distribution, the gas composition in package is not controlled. Nitrogen (N_2), oxygen (O_2) and carbon dioxide (CO_2) are the main gases used commercially in seafood, although trace gases, such as carbon monoxide, nitrous oxide, sulphur dioxide, argon, and xenon are commented as possible gases for MAP in meat, fruits, and vegetables (Zhang *et al*., 2008).

N_2 is an inert, odorless, and tasteless gas. It is used as a filler to prevent package collapse, because of its low solubility in water and fat in gas packaging (Farber, 1991). The use of N_2 results in the reduction in lipid oxidation and the inhibition in the growth of aerobic spoilage microorganisms. O_2 is reduced in gas mixtures for fatty fish. This behavior may be due to the reaction of lipid oxidation, while it was not observed in refrigerated shellfish stored in an atmosphere containing O_2 (Goulas *et al.,* 2005). However, O_2 may be used in low concentrations in fish products to retard the anaerobic conditions and avoid the outbreak of strictly anaerobic pathogens, such as nonproteolytic *C. botulinum*, while the lower O_2 condition may be effective for proliferation of psychrotrophic, facultatively anaerobic include *Listeria monocytogenes* and *S. putrefaciens* (Rutherford *et al*., 2007). The use of O_2 in gas packaging contributes the red color of red fish meat (e.g. tuna), which is associated with the oxygenated form, oxymyoglobin.

CO_2 commonly becomes more effectively as an antimicrobial agent in fish and shellfish because of its bacteriostatic and fungistatic properties. CO_2 is able to dissolve into the liquid phase in muscle, which is associated with the increased carbonic acid. The bicarbonate ion, a dissociation product, changes cell permeability, and affects metabolic processes (Banks *et al.,* 1980). The ratio of the volume of gas and volume of food product (G/P ratio) may need to be twice the volume of meat for adequate microbial retardation, although CO_2 is dissolved and absorbed into the meat surface during storage (McMillin, 2008). Furthermore, the carbonic acid may lower pH, resulting in slight flavor changes in fish, and its absorption by the product may also cause package collapse (Ashie *et al.,* 1996). A high CO_2 content causes a higher exudate loss. This may be due to a greater loss of the water holding capacity of the muscle protein at lower pH values. The shelf-life of fishery products in CO_2-enriched atmosphere can be extended depending on raw materials, gas mixture, storage temperature, and gas concentration.

Table 1. Shelf-life extension of fish and fishery products under MAP.

Fish and fishery products	Storage temperature (°C)	MAP condition CO_2:O_2:N_2	Shelf-life (days)	References
Mediterranean swordfish (*Xiphias gladius*)	4	40:30:30	12	Pantazi *et al.* (2008)
Pearlspot (*Etroplus suratensis* Bloch)	2	60:40:0	10	Ravi-Sankar *et al.* (2008)
Cod (*Gadus morhua*)	-0.9	50:5:45	21	Wang *et al.* (2008)
Sea bass (*Dicentrachus labrax*)	4	60:10:30	13	Kostaki *et al.* (2009)
Atlantic salmon (*Salmo salar* L.)	2	90:0:10	22	Fernandez *et al.* (2009)
Mediterranean swordfish	4	50:5:45	13	Kykkidou *et al.* (2009)
Atlantic salmon (*Salmo salar* L.)	1.2	60:0:40	15	Hansen *et al.* (2009)
Sea bass (*Dicentrachus labrax*)	4	60:0:40	18	Provincial *et al.* (2010)

Active packaging is a technique used for extending the shelf-life of seafood or fresh foods by addition of active agents that absorb or release a compound in the gas phase. Compounds in packaging include CO_2, O_2, water vapor, or volatiles. Active agents can be useful in a package, such as oxygen or carbon dioxide scavengers, moisture absorber and oxygen or carbon dioxide emitter. Moreover, active packaging systems have also been studied, in which specific bioactive substances are combined with the packaging material or within the package resulting in the retardation of the microbial growth and lipid oxidation (Lee, 2010). Carbon dioxide emitters release CO_2 in the packages during storage. It was reported that the use of carbon dioxide emitters in fish can control the G/P ratio and volume reduction compared with traditional MAP (Hansen *et al*., 2009). MAP that contained a CO_2 emitter remained stable or showed an increased CO_2 content in the packages, whereas the CO_2 content in packages without CO_2 emitter decreased 40% during storage. Bacterial growth of Atlantic cod used with CO_2 emitter and kept under MAP increased more slowly than those stored with MAP without CO_2 emitter, indicating that a CO_2 emitter might show synergistic effect on the inhibition of bacterial growth (Hansan *et al.,* 2007). The O_2 scavengers were used for improving the effect of shelf-life extension of catfish (Mohan *et al.,* 2008). It can also be used to reduce O_2 in high fat products to prevent chemical deterioration e.g., oxidative rancidity and aerobic microbial spoilage. Seer fish (*Scomberomorus commerson*) packed in aerobic condition was sensorially acceptable only up to 12 days compared to 20 days in O_2 scavenger packs (Mohan *et al.,* 2009). Similar results were reported by Goncalves *et al.* (2004) for seabream packaged with O_2 scavenger at 4°C. Furthermore, packaging with O_2 scavenger could reduce the use of vacuum packaging equipments and it is cost effective.

Refrigerated sardine and mussel packed with CO_2 had a 60-80% increase in stability, mainly due to an extension in the lag phase of organisms and their reduced growth rate in the logarithmic phase (Ozogual *et al*., 2004; Goulas *et al*., 2005). Arashisar *et al.* (2004) found that a 100% CO_2 enriched atmosphere could lower microbial counts of rainbow trout. CO_2 commonly becomes more effective as an antibacterial agent when its concentration is increased. King and Nagel (1975) reported that CO_2 enters into mass-action equilibrium for enzymatic decarboxylation, leading to the inhibition of the metabolic activity of the microbial flora. CO_2 retards the microbial growth of spoilage bacteria such as *Pseudomonas* spp. and *Shewanella* spp. Although, *P. phosphoreum* is more resistant to CO_2 but microbial growth is generally inhibited by higher carbon dioxide concentration (Emborg *et al.,* 2005). Goulas *et al.* (2005) found that the growth of microorganism in mussel was inhibited by higher CO_2 concentration (80%). TVB and other compounds associated with seafood spoilage of seafood kept under atmosphere with high CO_2 concentration increased more slowly than that of seafood stored under that with low CO_2, indicating that a higher CO_2 concentration potentially inhibited the growth of aerobic Gram negative bacteria, including volatile compounds producing microorganisms. LAB counts were increased in chub mackerel packaged under vacuum packaging and MAP (Stamatis and Arkoudelos, 2007). The growth of LAB was concomitant with the increase in lactic acid concentration and bacteriocin, which is natural antimicrobial agent. The formation of lactic acid may have led to the inhibition of other bacteria in seafood products. Generally, spoilage flora was replaced, probably to a large extent, by CO_2 resistant organisms, e.g. LAB and *Brochothrix thermosphacta* (Asensio *et al.,* 1988).

However, an increase in thiobarbituric acid reactive substances (TBARS) was observed in hake slices when CO_2 concentration increased, indicating that lipid oxidation still occurred under MAP (Pastoriza *et al.,* 1996). Masniyom *et al*. (2002) also reported the increased lipid oxidation in seabass slices stored under CO_2 enriched packaging. It was postulated that the carbonic acid formed in the sample kept under MAP may induce the denaturation of muscle protein, leading to the release of free haem iron, a potential pro-oxidant in the muscle system. Moreover, an increase in TBARS was observed in seafood when the proportion of O_2 concentration increased (Gimenez *et al.* 2002). Lipid oxidation was also postulated to cause changes in color and flavor. However, Arashisar *et al.* (2004) found no significant changes in TBARS for rainbow trout fillets stored at 100% CO_2 throughout the storage up to 14 days. As reported by Fagan *et al.* (2004), drip loss was increased in mackerel and salmon packaged under the higher content of CO_2. Similar results were reported by Pastoriza *et al.* (1998) in hake packaged in MAP at 2°C. This might be due to a greater loss of water holding capacity of the muscle protein at lower pH values, in which higher dissolution of CO_2 in the aqueous phase of fish muscle took place. CO_2-enriched packaging effectively inhibited the spoilage caused by microorganisms, but it could not prevent the chemical deterioration, especially lipid oxidation and physical changes. To maximize the use of MAP, techniques should be applied to minimize these problems. Thus, some technique would have to be applied in combination with MAP to achieve an extended shelf-life in terms of both microbiological and chemical aspects.

5. Combined effects of MAP and other treatments

Generally, high levels of CO_2 negatively affect product quality, especially by inducing physical and sensory changes. Lalitha *et al.* (2005) found that CO_2 flush packed pearl spot (*Etroplus suratensis* Bloch) had less desirable odor, texture and flavor. Additionally, high level of CO_2 results in the increase in exudate, leading to the less acceptability of products. To expand the benefits of CO_2 and to provide more comprehensive preservation, adjunct treatments have been explored to reduce CO_2 content needed. Quality changes in fish under high concentration of CO_2 can be reduced significantly by combining other treatments with MAP. The exudate losses of fish kept under MAP were reduced with the

pretreatment of sodium chloride (NaCl) prior to packaging (Pastoriza *et al.,* 1998). Moreover, the use of dips containing polyphosphates was able to prevent drip loss and to reduce cooking losses of refrigerated fish fillets stored under MAP (Masniyom, *et al.,* 2004b). Polyphosphates and polyanion might interact with the positive charges of the protein molecules to increase the net negative charge, resulting in the increased water uptake ability. As a result, the repulsive forces between protein molecules may increase, leading to the increased water retention. Therefore, soaking the fish in NaCl or phosphate solution prior to MAP might extend the shelf-life of fish with high acceptability during storage.

The use of antimicrobial agents in the combination with MAP has also been investigated. Preservatives, such as lactic acid, sorbate, phosphate acid and essential oils (EOs), which have significant antimicrobial effects on various food borne microorganisms can be used to retard the growth of microorganisms. Lactate was believed to lower the a_w of the environment and thus exert its antimicrobial effect. Pothuri *et al.* (1995) reported that lactic acid combined with MAP was effective in controlling *L. monocytogenes* in crayfish tail meat. The lag phase was extended by eight days in sample treated with 1% lactic acid and modified atmosphere, compared to that in air. The antimicrobial properties of acid are attributed to the undissociated molecule and to a reduction of pH below the level at which the growth of many bacteria is inhibited (Masniyom *et al.,* 2007). Low temperature (0-1°C) and the presence of both O_2 and potassium sorbate presumably serve as protection against botulism development (Fey and Regenstein, 1982). Masniyom *et al.* (2004b) reported that the synergistic effect between polyphosphate treatment could improve quality of fish in CO_2 at 4°C. Phosphates act as growth inhibitors of certain food spoilage microorganism, owing to their ability to chelate calcium, magnesium and iron needed for microbial growth (Dziezak, 1990). Zaika *et al.* (1997) reported that growth inhibition by sodium polyphosphate increased with decreasing temperature and increasing NaCl concentration. Growth inhibition induced by sodium polyphosphate was accompanied by changes in cellular morphology. EOs from herbs and spices were used to extend the shelf-life of fresh fishery products. EOs including clove, cinnamaldehyde, cinnamon, garlic, lemongrass, rosemary, thyme, etc., or their extracts have been shown to possess antimicrobial activity and antioxidant for shelf-life extension of fish and shellfish. *P. phosphoreum* and foodborne pathogens are effectively inhibited by EOs such as cinnamaldehyde and oregano (Goulas and Kontominas, 2007). The MAP in combination with oregano oil was used for improving the effect of preservation of cod, prolonging their shelf-life to 21-26 days whereas air-stored salmon was sensorially rejected after 11 days of storage. Similar results were reported by Fernandez *et al.* (2009) in Atlantic salmon fillets packaged in MAP combined with rosemary extract and superchilling. However, practical application of EOs is limited due to color and flavor consideration.

6. Safety concern of fish and fishery products stored under MAP

MAP is used to extend the shelf-life of perishable foods. Elevated CO_2 and modified atmospheres reduce microbial growth and enzymatic activity. In general, Gram-negative aerobic bacteria are more resistant in MAP. Recently, *L. monocytogenes*, *E. coli, S. aureus* and *C. botulimun* outbreaks have attracted worldwide attention. The storage of products under modified atmosphere may give rise to safety concerns because MAP suppresses the growth of pathogens such as *L. monocytogenes, Salmonella* spp., and *E. coli* (Kimura *et al.,* 1999). Hendricks and Hotchkiss (1997) reported that CO_2 level of 80% significantly reduced the growth rate of *L. monocytogenes* in buffered nutrient broth at 7.5°C. Rutherford *et al.* (2007) reported that CO_2 modified atmosphere (100% CO_2) reduced growth of *L. monocytogenes* on ready-to-eat shrimp stored at 3°C compared to air or vacuum packaging. Levels of 70 to 100% CO_2 cause a significant increase in lag phase as well as a reduction in growth rate at chilled temperature (Hendricks and Hotchkiss, 1997). The growth of *L. monocytogenes* and *E. coli* O157 were retarded in seabass packaged under combination with MAP and pyrophosphate, but did not inhibit microbial growth (Figure 1) (Masniyom *et al,* 2006). Therefore, low temperature in combination with MAP and other treatment was the promising means to prevent or retard the growth of pathogen. However, proper sanitation and processing practices that prevent and reduce contamination on fish are needed.

MAP of fish products may pose a potential danger. The major concern in relation to safety of MAP fish products is the potential for growth and toxin production by *C. botulinum* (Ravi-Sankar *et al.,* 2008). Thus, the facultative anaerobic conditions often encountered in MAP low acid foods (pH>4.6) can be favorable to growth and toxin production by the bacteria if the suitable storage temperatures prevail. *C. botulinum* type E, nonproteolytic, and psychrotrophic strain, can grow and produce toxin at low temperatures. It is found in marine and fresh water environments; thus fish products have the potential of being contaminated. Cann *et al.* (1984) found that trout and salmon inoculated with spore of *C. botulinum* and kept in MAP and vacuum at 10, 15, and 20°C spoiled before they became toxic. However, toxin has been found in MAP or vacuum packed flounder fillets prior to the fish being determined spoilage (Arritt *et al.,* 2007). Peck *et al.* (2008) reported that storage temperature played a major role in the extension of shelf-life, the margin of safety between sensory spoilage and onset of *C. botulinum* growth, and the toxin production in retail type packages of fresh salmon fillets packed under MAP. Prolonged storage of MAP salmon at abuse temperature (16°C) may present a public health hazard because toxin formation preceded sensory spoilage. Wilhelm (1982) reported some evidence that acids and peroxides produced by *Lactobacillus* favored in MAP may have an inhibitory effect on *C. botulinum*. Low

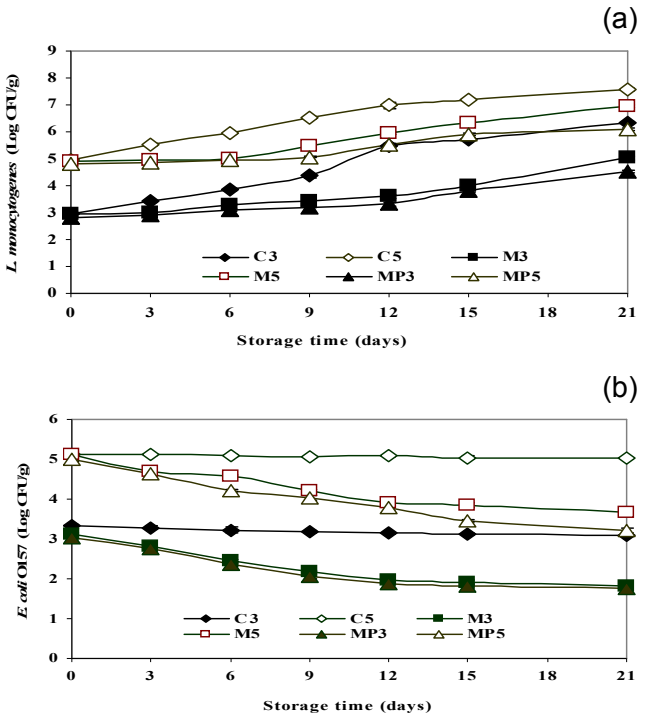

Figure 1. Changes in *L. monocytogenes* counts (a) and *E. coli* 0157 counts (b) in seabass slices inoculated with *L. monocytogenes* and *E. coli* 0157 at different levels (10^3 and 10^5 cfu/g) and stored under different conditions at 4°C: air (C3=10^3, C5=10^5), MAP (80%CO_2, 10%N_2, 10%O_2) (M3=10^3, M5=10^5) and MAP in combination with pyrophosphate pretreatment (MP3=10^3, MP5=10^5) (Masniyom *et al.,* 2006).

storage temperature and presence of O_2 in the headspace are very important in preventing botulinal hazard in MAP products. However, storage temperature at 3.3°C or below is necessary to prevent development of *C. botulinum* and *L. monocytogenes* (FDA, 2001; Rutherford *et al.,* 2007). Kimura *et al*. (1996) reported that no increase in the *C. perfrigens* population in inoculated mackerel fillets with *C. perfrigens* stored in CO_2 atmosphere (40% CO_2, 60% N_2) at 15°C within three days, but samples were spoiled rapidly at the abuse temperature (30°C) after 4 hrs. At low temperature, activity of CO_2 was increased due to its greater aqueous and lipid solubility as temperature decreased in MAP. Carbonic acid from dissolved CO_2 retards microbial growth. Packaging in an atmosphere containing >2% O_2 and storage at close to 0°C provides adequate safety against their growth and toxin production (Phillips, 1996). The use of gas packaging, specifically elevated CO_2 levels, has been shown to inhibit normal spoilage bacteria including *Pseudomonas* spp., *Shewanella* spp., *Moraxella* spp., and *Acinetobacter* spp. in fish and shellfish. These include some pathogens such as *L. monocytogenes, S. aureus, Salmonella* spp., *E. coli*., and *Clostridium* spp. However, the effectiveness of CO_2 inhibition is strongly related to raw material (species, fat content, microbial loading) storage temperature, gas mixture, G/P ratio, and packaging material.

7. Conclusion

Shelf-life of fish and fishery products can be extended by using modified atmosphere packaging. Microbial growth in fish and fishery products was retarded, leading to the delayed spoilage as well as decreased deteriorative compounds. However, chemical and physical changes still occurred. A combined application of MAP and other treatment is effective in quality maintenance of fish and fishery products during the extended storage in concert with good manufacturing practice.

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