

Mini Review

Effect of modified atmosphere packaging on microbial flora changes in fishery products

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Abstract

Modified atmosphere packaging (MAP) has become a popular method for packaging foods as it can extend the shelf life of food with minimal quality defect. Oxygen, nitrogen and carbon dioxide are the common gases used in MAP, Oxygen and carbon dioxide inclusive as only these two gaseous have the preservative effects on the packed food product. Their effect on microbial changes of any food product throughout storage period is highly depend on type of the product and packaging materials, appropriate gas composition, storage temperature, the ratio between gas and product volume, and hygienic manner during processing and packaging. MAP with highest percentage of carbon dioxide is proven to be more effective than vacuum packaging in inhibiting the growth of spoilage and pathogenic bacteria in many fishery products. This article reviews the consequences of MAP towards microbial changes in fishery products.

Keywords

Carbon dioxide
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Introduction

Fish and fishery products are well known nutrition source as they possess high protein content (Masniyom, 2011). Conversely, fishes are easily perishable foods with the presence of unsaturated fatty acids. The products are greatly in risk of spoilage at pH more than 5.2 and water activity beyond 0.95 (Choubert and Baccaunaud, 2006; Ježek and Buchtová, 2007). The rate of quality decline shows a discrepancy in concern with various factors. They include fish species, initial microbial level, and composition in the tissue and actions of microbial enzymes that lead to the alteration of autolytic and proteolytic activities. Bacterial activity leads to prominent unpleasant changes in aroma due to metabolism of amino acids into biogenic amines, sulfides, organic acids and other compounds (Wilhelm, 1982; Gram and Dalgaard, 2002). Additionally, it has been reported that approximately 25% of food products lost during post harvest phase are due to microbial activities (Gram and Dalgaard, 2002). Moreover there are occurrences of rancidity in fishes with high fat content. All of this quality deterioration is determined through microbial, chemical in terms of the end product of metabolites (Alur *et al.*, 1995) and physical methods as well as sensory analysis (Shakila *et al.*, 2003; Özyurt *et al.*, 2009). Nevertheless, the quality deterioration could

be reduced by implementing good and hygienic handling practices since the moment of catch. Ancient preservation methods such as refrigeration storage and icing have been effectively delayed fish spoilage over many years (Ashie *et al.*, 1996). However, the food products tend to be discarded after a short storage phase (Wilhelm, 1982).

As a complement to those ancient preservation techniques, MAP has a tendency to prolong the storage life of foods significantly (Church, 1994; Arritt *et al.*, 2007; Mangaraj and Goswami, 2009). Effects of MAP in extending shelf life of food can be enhanced by combining with low temperature storage (Ohlsson, 1994; Debevere and Boskou, 1996; Arashisar *et al.*, 2004; Choubert and Baccaunaud, 2006; Goulas and Kontominas, 2007; Yilmaz *et al.*, 2009). Nevertheless, the effective gas composition required in MAP method is depending on each type of fishery product. Thus, this review is intended to reveal the consequences of MAP with various gas compositions towards microbial inhibition in a range of fishery products.

What is modified atmosphere packaging (MAP)?

A preservation method by altering atmospheric environment around a perishable food by substituting with single or a mixture protective gases is known as modified atmosphere packaging (MAP) (Arashisar

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et al., 2004; Del Nobile et al., 2009). According to Han (2005), MAP executes the basic goal of packaging which is to protect the food material in terms of microbiological (bacteria, moulds, yeasts, and parasites) and physiochemical (toxic substances, dirt, loss or uptake of moisture) qualities as well as sensory attributes (color, smell and taste). These value aspects that reflect to the appearance of food products are greatly influence the procuring judgments of purchasers as fresh commodities are preferred far more than those frozen or processed commodities (Goulas and Kontominas, 2007). Decline in the quality characters due to microbial actions and various chemical reactions either enzyme or non enzyme catalyzed would lead to consumer's negative response towards the product and subsequently to financial loss for manufacturers and producers (Goulas et al., 2005; Floros and Matsos, 2005).

Moreover, MAP is merely an additional system of vacuum wrapping tools by excluding or optimizing oxygen level in the package in order to inhibit the growth of aerobic microorganisms and delays senescence (Al-Ati and Hotchkiss, 2003). Reduced oxygen level slows down the metabolic rate of food products especially fruits and vegetables and therefore slows down the natural aging process. Conversely, raw material, fish species, appropriate gas composition, storage temperature, the ratio between gas and product volume, hygiene manner during processing and packaging and properties of packaging materials are vital factors contributes to the products life storage in MAP (Sivertsvik et al., 2002; Goulas and Kontominas, 2007). In addition, promotion of high value rated fresh fishes in some European countries has been done through MAP (Baker et al., 1990).

MAP has been used extensively as approach of food conservation where the storage life of the food can be extended as long as possible (Stammen et al., 1990) with minimal losses in terms of quality aspects (Ashie et al., 1996; Amanatidou et al., 2000; Arritt et al., 2007). However, it is acknowledged that the respiration of the enclosed food material, biochemical reactions and slow-moving gases in and out of the packaging materials would lead to the changes in gaseous atmosphere throughout storage period which can affect the expected shelf life. In addition to MAP, several other methods such as chemical preservatives, use of additives, refrigerated storage, freezing, drying, and irradiation are commonly being used to inhibit or slow down decaying rates of food products (Soccol and Oetterer, 2003). On the contrary, concern of consumers' health and high demand on fresh and convenient foods without introduction of

chemical preservatives enabling MAP as a preferable packaging method. Manufacturing firms prefer MAP as they can sell food products that stay fresh longer than the products kept in normal atmosphere condition that spoil more rapidly. MAP products stored in low temperature condition demonstrates an effectual conservation way for shelf life extension (Choubert and Baccaunaud, 2006) and preservation of quality for a range of fresh supplies such as fruits, vegetables, red meat, poultry, fish and fish products, bakery products, cheese, milk powder, snacks, bread and pasta (Lopez-Caballero et al., 2002; Ahn et al., 2003; Goulas et al., 2005; Pantazi et al., 2008).

Gaseous used in MAP

Selection of gas composition in MAP is highly depending on the food product desired to be packed. Single or combination of gases contribute to the extension of shelf life based on the acceptable microbial count and organoleptic properties in terms of colour, odour and feel of the food which being perceived in the course smell, feel and visual inspection. MAP is inclusive of common gases of oxygen, nitrogen and carbon dioxide (Choubert and Baccaunaud, 2006; Ježek and Buchtová, 2007; Masniyom, 2011). Oxygen and carbon dioxide are inclusive as only these two gases have the preservative effects on the packed food product (Soccol and Oetterer, 2003; Gill and Gill, 2005; Ježek and Buchtová, 2007). Oxygen is introduced in MAP to inhibit the growth of anaerobic bacteria and the accumulation of toxin by *Clostridium botulinum* type E (Pantazi et al., 2008). It has a lower degree of solubility in water yet it is being used in very low concentration as to avoid growth of spoilage bacteria and fungi. Nevertheless, it leads to several types of spoilage reactions in the product mainly oxidative rancidity, browning reactions and stimulates growth of aerobic bacteria. On the other hand, MAP with elevated percentage of oxygen, plays role in restraining TMA reduction process but on other words this composition of MAP is preferably limited as it would be desirable for aerobic bacteria ending up with rapid spoilage.

Nitrogen has low solubility in water and has no taste, color and odor. Nitrogen plays a role in delaying oxidative rancidity and inhibiting aerobic bacterial growth. However, it is not capable in inhibiting anaerobic bacterial growth. Due to its low solubility, nitrogen is used to prevent pack collapse in MAP to equilibrate the volume drop of CO₂ during storage time. Carbon dioxide is known as antimicrobial agent (Ohlsson, 1994), soluble in water and lipid (Church, 1994) and gives slight pungent odor at very high concentration. Carbon dioxide is the component

which leads to the effect on microbial growth as it dissolves readily in water and produces carbonic acid (H_2CO_3). Reduction of pH occurs as accumulation of H_2CO_3 raises the acidity of the solution. This phenomenon prolongs the lag phase and therefore lowers the microbial growth rate (Bingol and Ergun, 2011) throughout logarithmic phase (Arashisar *et al.*, 2004). Nevertheless the drop of pH could be minimal and perhaps there would not be any bacteriostatic effect.

Decrease in enzymatic activity or direct inhibition of enzyme system, transmission of membranes that leads to the changes in cell interior pH and alteration in physicochemical parameters of protein are the main theories that have been suggested by researchers for the occurrence of phenomena mentioned earlier (Phillips, 1996; Soccol and Oetterer, 2003). Moreover, solubility of CO_2 declines with rising temperature. Microbial inhibition capacity of CO_2 is noticeably superior at temperatures below $10^\circ C$. Therefore, interaction of storage temperature and concentration of CO_2 would lead to significant implications on modified atmosphere packed foods. In addition, pack collapse is other important factor to be considered in MAP, where reduction of headspace volume occurs as CO_2 (>60%) is highly soluble in the product to impart the bacteriostatic effect on the microbial load. Gill and Gill (2005) revealed that CO_2 has the tendency to readily soluble in muscles and fat tissues of the food.

Accordingly, reduction in pH occurs followed by low protein water retention ability. This phenomenon causes changes in organoleptic properties of the product (Soccol and Oetterer, 2003; Gill and Gill, 2005), where high percentage of CO_2 probably lead to negative effects on other aspects of the product especially on the sensory aspects. According to Phillips *et al.* (1996), efficiency of carbon dioxide as anti microorganism mediator is not entire and is relies on the food product properties and the existence of microbial flora. For instance, recommended gas composition for white fish (low fat fish) is $CO_2/N_2/O_2$: 40/30/30, while for and fatty fish is CO_2/N_2 : 40/60 or CO_2/N_2 : 60/40 (Phillips, 1996; Soccol and Oetterer, 2003). Table 1 present the use of various composition of gas in MAP for various fishery products.

Properties of packaging material

Packaging that made from combination of few polymers has the tendency to impart greater interaction effect on the properties of packaging material. The packaging materials used for MAP are gas proof with multi layer films. Polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP) (Jacobsson

Table 1. Fishery products with various gas proportions in modified atmosphere packaging stored at chilled temperature

No	Fishery products	Temperature ($^\circ C$)	Storage (days)	MAP $CO_2/N_2/O_2$	References
1	Deepwater pink shrimp	1.6 ± 0.4	9	40/30/30; 45/50/5	Lopez-Caballero <i>et al.</i> (2002)
2	Rainbow trout fillets	4 ± 1	14	100/0/0 90/7.5/2.5 40/30/30	Arashisar <i>et al.</i> (2004)
3	Rainbow trout	4	16	40/60/0 40/air	Choubert and Baccanaud (2006)
4	Sardines	4	15	60/40/0	Ozogul <i>et al.</i> (2004)
5	Mussels	4	15	50/50/0; 80/20/0 40/30/30/	Goulas <i>et al.</i> (2005)
6	Gutted farmed bass	3	9	70/30/0; 70/10/20; 60/10/30; 60/0/40; 50/20/30; 0/79/21	Torrieri <i>et al.</i> (2006)
7	<i>Scomber colias japonicus</i>	3	15	50/50/0	Stamatis and Arkoudelos (2007)
8	Grouper fillets	2 ± 2	21	80/20/0; 60/40/0	Siah and Ariff (2007)
9	Red claw crayfish	2	21	80/10/10	Chen and Xiong (2008)
10	Pearlspot	0-2	30	40/0/60; 50/0/50; 60/0/40; 70/0/30; 40/30/30	Ravi Sankar <i>et al.</i> (2008)
11	Swordfish	4	16	40/30/30	Pantazi <i>et al.</i> (2008)
12	Halibut	4	23	50/50/0; 50/0/50	Hovda <i>et al.</i> (2007)
13	Farmed eel	0	37	40/30/30	Arkoudelos <i>et al.</i> (2007)
14	Salmon	5 ± 1	18	50/0/50	Amanatidou <i>et al.</i> (2000)
15	Shrimps	3	5	60/40/0; 5.1/292.9	Arvanitoyannis <i>et al.</i> , 2011)

et al., 2004), polyester (PET), polyamide (PA), and barrier layers of polyvinylidenechloride (PVDC) and ethylenvinyl alcohol (EVOH) are commonly used combination of polymers for the packaging material of MAP (Mexis *et al.*, 2009; Appendini and Hotchkiss, 2002). According to Mangaraj and Goswami (2009), PVC, PE, PP and polyethylene terephthalate are more commonly used for construction of MAP. PA exists with the characteristics of relatively impermeable to oxygen and relatively permeable to water vapour, while other polymers are relatively permeable to oxygen and relatively impermeable to water vapour. Engineers made it with unique permeability properties to control gaseous diffusion in and out of the packaging.

Mould shaped containers can be formed using the rigid films and MAP as well can be applied on daily applicable plastic bags or pouches. These features can boost the products market as they can be customized according to customers' preference. Customers not only prefer to buy food products that stay fresh long, but may also consider their packaging appearance. Several criteria that have to be considered upon selection of the packaging material are as follow:

1. Barrier properties incorporate permeability of various gaseous (oxygen, carbon dioxide, nitrogen, ethylene and others), odour, light and moisture.
2. Anti fogging properties which enable good product visibility.
3. Sealing reliability where the packaging material able to seal itself and the container.
4. Mechanical criteria of the packaging material such as tear and tensile strength, friction, puncture resistance, possibility to be heat-formed and others (Petersen *et al.*, 1999).

How does the gas mixture introduced into the packaging?

The procedure begins by placing the product on trays or inside bags/pouches (specified for MAP) in order to apply vacuum as majority of air will be removed. The ratio between gas and product volume (gas/product) is commonly determined as 2:1 (Arashisar *et al.*, 2004). This step is then followed by injection of gas mixture which varies according to the food products. The tray/bag is then heat sealed to encapsulate the gas mixture and the product stored at preferred temperature condition for a recommended period (Arashisar *et al.*, 2004).

Effect of MAP on micro flora changes

Gram negative microbia such as *Pseudomonas*, *Psychrobacter*, *Vibrio*, *Shewanella* and *Photobacterium* and gram positive microbes of *Bacillus*, *Clostridium*, *Lactobacillus*, *Micrococcus* and *B. thermosphacta* are found in marine fishes (Debevere and Boskou, 1996). The same microbial groups also present in freshwater fishes except for *Vibrio* and *Photobacterium*. Initial microbial floras of fish which have been dominated by gram-positive microorganisms are generally less susceptible for spoilage. These phenomena would be the reason for extended shelf life of certain fishery products especially for tropical fishes. The mechanism of spoilage occurs in such a way the gram negative spoilage microorganisms such as *Pseudomonads*, *S. putrefaciens* and *Photobacterium phosphoreum* degrade the protein composition, amino acids and other nitrogenous compounds into amines, ammonia, organic sulfides, and hydrogen sulfide. The rate of spoilage is higher towards end of storage as pH of the muscles increased to more than pH 5.2 as this condition not able to inhibit growth of certain spoilage bacteria especially facultative anaerobic bacteria (Jezek and Buchtovz, 2007).

However, application of modified atmosphere with CO₂ has been extensively reported to extend the storage life of food products because it restrains microbial growth (Ozogul, Polat *et al.*, 2004; Chen and Xiong, 2008). Deterioration rate of fish and shrimp are slower when stored in refrigerated sea water with dissolved CO₂ compared to that stored in ice (Reppond *et al.*, 1979). MAP with high intensity of CO₂ able to inhibit certain percentage of spoilage microorganisms which causes off-odor in shrimp, except *Carnobacterium divergens* and *C. maltaromaticum* (Chen and Xiong, 2008). It has been reported that shrimps packed in 35 to 100% CO₂; O₂ or N₂ gas composition and also 50 to 100% CO₂ greatly restrain growth of microbes compared to

air storage of spotted shrimp (Arashisar *et al.*, 2004). Sah and Ariff (2007) report the act of CO₂ in delaying the onset of spoilage in fresh muscle food is due to the inhibition of aerobic, psychrotrophic and gram-negative spoilage bacteria.

Total bacterial count

Total bacterial count of deepwater pink shrimp stored under MAP condition (40-45% CO₂) is two log cycles lower than that stored in ice and ambient temperature (Lopez Caballero *et al.*, 2002). This is mainly due to the restrain effects of CO₂ on the microorganism growth as other researchers have concluded that 50 to 100% CO₂ and 35 to 100% CO₂. Sixty rainbow trout (*Oncorhynchus mykiss* W.) samples kept over four weeks at 4°C in MAP of 60/40 N₂/CO₂ and 60/40 air/CO₂ showed rising of total aerobic plate count from 4 log CFU/g to 7 log CFU/g. Similar results have also been observed in hake (*Merluccius merluccius*) and Jack Mackerel (*Trachurus japonicus*) (Choubert and Baccaunaud, 2006). Arashisar *et al.*, (2004) have studied the consequences of modified atmosphere on microbial changes of rainbow trout fillets. It was found that MAP of CO₂: 100%, CO₂/N₂/O₂: 90/7.5/2.5 and CO₂/N₂/O₂: 40/30/30 each with certain level of inhibition on aerobic bacterial counts. They revealed that MAP of 100% CO₂ exhibited the greater inhibitory effect as the count reached 10⁵ CFU/g on 14th day of storage for mesophilic bacterial count. Arashisar *et al.* (2004) have noted that the effect of CO₂: 90% and CO₂: 40% are lower in comparison with CO₂: 100%. In addition, according to Lopez-Caballero *et al.* (2002), comparable to shrimps stored under iced and air condition, MAP of CO₂/N₂/O₂: 40/30/30 and CO₂/N₂/O₂: 45/50/5 have showed greater inhibition for total bacterial count.

Ozogula *et al.* (2004) had investigated the effect of MAP (CO₂/N₂: 60/40) on total viable count and histamine forming bacteria count in sardines (*Sardina pilchardus*) kept at 4°C. The outcome illustrated the lowest total viable count (TVC) in MAP compared to vacuum packaging and air. Moreover, Goulas *et al.* (2005) has carried out research on microbial changes (total viable count, *Pseudomonas* spp., H₂S producing bacteria and lactic acid producing bacteria) of refrigerated mussels stored under modified atmosphere packaging of CO₂/N₂: 50/50, CO₂/N₂: 80/20 and CO₂/N₂/O₂: 40/30/30. As the results obtained, MAP with composition of CO₂/N₂: 80/20 attained lowest count for total viable count 7.0 logs CFU/g on 15 days of storage which is also similar for vacuum packaging. However control and other MAP conditions reached the microbiological

acceptable limit (7 logs CFU/g) on 8th and 11th day of storage respectively. Goulas *et al.* (2005) reported that this inhibitory effect would be mainly due to high concentration of CO₂ as it inhibits growth of aerobic microorganisms.

Torrieri *et al.* (2006) have conducted a study on gutted farmed bass (*Dicentrarchus labrax*) with certain formulation of modified atmosphere packaging at storage temperature of 3±1 °C for 9 days. They used gas composition for MAP are as follows: A (O₂/CO₂/N₂: 0/70/30), B (O₂/CO₂/N₂: 20/70/10), C (O₂/CO₂/N₂: 30/60/10), D (O₂/CO₂/N₂: 40/60/0), E (O₂/CO₂/N₂: 30/50/20) and F (O₂/CO₂/N₂: 21/0/79). In terms of aerobic mesophilic bacteria (AMB), MAP of B and E showed greater inhibition compared to the other MAP compositions. AMB of below 1 log CFU/g at 0 day found to be 2.5 × 10⁶ logs CFU/g and 2.2 × 10⁷ logs CFU/g, respectively in B and E at 9 day which is still under the upper acceptability limit. It has been reported that high levels of AMB observed in D and F was due to high percentage of oxygen in the package headspace. Ahead of that, quality assessment of *Scomber colias japonicas* in modified atmosphere (CO₂/N₂: 50/50) and vacuum packaging at temperatures of 3 and 6 °C have been studied by Stamatis and Arkoudelos (2007). Total viable count (TVC) for MAP samples stored at 3 and 6 °C were 6.6 logs CFU/g and 6.7 logs CFU/g, respectively after 15 days of storage. TVC in Samples stored under vacuum packaging and air for both 3 and 6 °C reached the upper acceptability level (7 logs CFU/g) at day 6 and 10 of storage, respectively.

Another study has also been conducted on fresh grouper (*Epinephelus* sp.) fillets under two different modified atmosphere (MA) condition of CO₂/N₂: 80/20 and CO₂/N₂: 60/40 (Siah and Ariff 2007). They found that aerobic plate count in samples treated with MAP of CO₂/N₂: 80/20 is lower than that treated with CO₂/N₂: 60/40 and stored in 100% air. Total viable count (TVC) on smoked mullet (*Mugil cephalus*) stored at 4 °C in MAP with gas ratio of CO₂/N₂/O₂: 35/60/5 was reported to be lower if compared to the samples packed under vacuum conditions (Ibrahim *et al.*, 2008). Upon storage period, TVC showed increment on both MAP and vacuum condition yet as mentioned the corresponding counts appeared to be lower in MAP (Ibrahim *et al.*, 2008). Precooked red claw crayfish (*Cherax quadricarinatus*) was stored under MAP (CO₂/O₂/N₂: 80/10/10), aerobic polyvinylchloride packaging (PVCP) and vacuum packaging (VP) and shelf enhancement assessments were carried out by Chen and Xiong (2008). Aerobic plate count (APC) remained low during the first three days of storage of all three packaging systems.

However, APC of samples stored in VP and PVCP increased rapidly from day 7 to 21 (end of storage). Moreover, at the end of storage, APC in PVCP, VP and MAP are 9.73, 8.41 and around 3 logs CFU/g, respectively. This is showed that MAP remained the best treatment to keep microbiological count in red claw crayfish under the acceptable limit.

Ravi Sankar *et al.* (2008) have studied the effect of various gas proportions of MAP for pearl spot as each proportion is different for various fishery products. Pearl spot has been packed into MAP of CO₂/O₂ with ratio of 40/60, 50/50, 60/40, 70/30 and CO₂/O₂/N₂: 40/30/30 and stored at 0-2°C for 30 days of storage. Samples treated with MAP of CO₂/O₂: 60/40 exhibited lowest mesophilic aerobic count at the end of storage. While sample with other treatments have reached the upper acceptability limit at around day 30. Swordfish (*Xiphias gladius*) which is well-liked in Greece have been evaluated by Pantazi *et al.* (2008) in MAP CO₂/N₂/O₂: 40/30/30 under storage at 4 °C for 16 days. They revealed that total viable count in MAP samples was significantly lower compared to that in air samples on day 16, although the upper acceptability limit of 7 logs CFU/g has been reached on day 11. Farmed eel (*Anguilla anguilla*) stored at 0 °C under modified atmosphere packaging (CO₂/N₂/O₂: 40/30/30) and vacuum packaging were studied for quality attributes by Arkoudelos *et al.* (2007). Total aerobic plate count increased from 2.8 log CFU/g (day 0) to 7.4 and 7.8 log CFU/g on day 31 and 37 in samples treated with vacuum and MAP storage, respectively. It has been reported that based on upper acceptability limit for marine species, eels stored under air, vacuum and MAP reached 7 logs CFU/g on day 18, 28 and 34, respectively. This clearly demonstrates that eels stored under MAP (CO₂/N₂/O₂: 40/30/30) significantly extends the shelf life longer than air and vacuum condition (Arkoudelos *et al.*, 2007).

H₂S producing bacteria

The common H₂S producing bacteria species are *Shewanella putrefaciens*, certain *Pseudomonas*, *Vibrio* and *Aeromonas* spp. (Devere and Boskou, 1996). Vogel, Venkateswaran *et al.* (2005) reported *Shewanella baltica* as the main significant H₂S producing species in ice stored Danish marine fish. *Pseudomonas putrefaciens* identified for the species as it revealed the ability to form H₂S in haddock fillets (Levin, 1968).

Lopez-Caballero *et al.* (2002) reported that MAP retarded growth of H₂S producing microorganisms in shrimps. They found that H₂S producing microorganisms count in shrimp kept at MAP of CO₂/

O₂/N₂: 40/30/30 and CO₂/O₂/N₂: 45/5/50 was around 1.1 and 1.6 log CFU/g, respectively after nine days of storage period. However, the particular bacterial count in shrimp treated with ice and air storage was around 3.3 and 3.1 logs CFU/g, respectively within the time period as MAP treatment (Lopez-Caballero *et al.*, 2002). Moreover, Goulas *et al.* (2005) found that H₂S producing bacterial count of refrigerated mussels kept in CO₂/N₂: 80/20 remained under microbiological acceptable limit throughout 15 days storage period. In contrast, H₂S producing bacterial count of refrigerated mussels treated with MAP of CO₂/N₂: 50/50 (7.4 log CFU/g) and CO₂/O₂/N₂: 40/30/30 (8.0 CFU/g) exceed the microbiological acceptable limit on day 15 of storage. Samples of pearl spot has been counted for the H₂S producing bacteria by Ravi Sankar *et al.* (2008) who revealed that the lowest count of H₂S producing bacteria was observed in pearl spot treated with MAP CO₂/O₂: 60/40 compared to other ratio of CO₂ and O₂. In addition, MAP CO₂/O₂/N₂: 40/30/30 significantly inhibited the growth of H₂S producing bacteria in Mediterranean swordfish stored at 4°C for 16 days (Pantazi *et al.*, 2008).

Enterobacteria counts

MAP inhibited the growth of Enterobacteria in shrimp during storage (Lopez-Caballero *et al.*, 2002). Enterobacterial count in shrimp packed with MAP of CO₂/O₂/N₂: 40/30/30 and CO₂/O₂/N₂: 45/5/50 is 2.8 and 3.7 log CFU/g, respectively. The count was lower compared to that of shrimp treated with ice (5.0 logs CFU/g) and air condition (5.6 log CFU/g) at the end of storage (Lopez-Caballero *et al.* 2002). Inhibition of Enterobacterial growth is also observed in rainbow trout fillet treated with MAP of both 100% CO₂ and O₂/N₂/CO₂: 2.5/7.5/90 after 6 days of storage (Arashisar *et al.*, 2004). However, lower percentage of CO₂ is required to delay the growth of Enterobacterial count in gutted farmed bass (*Dicentrarchus labrax*) stored at 3±1 °C for 9 days (Torrieri *et al.*, 2006).

Ravi Sankar *et al.* (2008) revealed that MAP treatment with various compositions of CO₂ and O₂ effectively suppressed the count of Enterobacteria in pearl spot below the upper acceptability limit with least count observed in MAP CO₂/O₂: 60/40. Mediterranean swordfish stored in MAP CO₂/O₂/N₂: 40/30/30 exhibited as low as 4.2-5.6 logs CFU/g of Enterobacteriaceae count on day 16 of storage (Pantazi *et al.*, 2008). Arkoudelos *et al.* (2007) found that Enterobacterial count in farmed eel (*Anguilla anguilla*) treated with modified atmosphere (CO₂/N₂/O₂: 40/30/30), vacuum packaging and air condition were 3.0, 3.4 and 4.5 log CFU/g, respectively, after

stored at 0 °C for about 1 month.

Histamine forming bacteria

Clostridium perfringens, some strains of *Enterobacter cloacae* and *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Vibrio alginolyticus* were identified as histamine producers where these microorganisms possess histidine decarboxylate activity (Lopez-Sabater, Rodriguez-Jerez *et al.*, 1994; Yoshinaga, 1982). Niven *et al.* (1981) concluded that *Proteus morgani* and *Klebsiella pneumonia* are the majority species involved in the production of histamine in scombroid fish and mahi mahi. *Proteus morgani*, *Klebsiella pneumonia* and *Enterobacter aerogenes* were classified as prolific histamine producers, while *Hafnia alvei*, *Escherichia coli* and *Citrobacter freundii* were reported as slow histamine producers (Taylor and Speckhard, 1983). *Morganella morgani*, *Proteus vulgaris*, *Proteus mirabilis*, *Proteus spp.*, *Staphylococcus* genus and *S. epidermidis* are also categorised as prolific histamine producing bacteria (Ababouch *et al.*, 1991; Hernandez-Herrero *et al.*, 1999; Kim *et al.*, 2002).

Histamine forming bacteria was not given much emphasis on studies related to MAP. Ozogul *et al.* (2004) have included this count in sardine samples stored in MAP of CO₂/N₂: 60/40. Histamine forming bacteria has increased in sardines samples (*Sardina pilchardus*) from all treatments (MAP CO₂/N₂: 60/40, vacuum packaging and air) throughout the storage period at 4 °C. However, histamine forming bacterial count was lower compared to other treatments.

Pseudomonas spp.

Goulas *et al.* (2005) found that MAP of CO₂/N₂: 80/20 and vacuum packaging effective to inhibit growth of *Pseudomonas* spp. (6.8±0.3 log CFU/g) in mussels during 15 days of storage. However, the count of *Pseudomonas* spp. in samples treated with MAP of CO₂/O₂/N₂: 40/30/30 and CO₂/N₂: 50/50 reached the microbiological acceptable limit on day 8 and 11 of storage, respectively. Goulas *et al.* (2005) concluded that this inhibitory effect is mainly due to the high concentration of CO₂. *Pseudomonas* and *Shewanella putrefaciens* count is used as spoilage indicator in iced fish and fish products. Stamatis and Arkoudelos (2007) have also reported that *Pseudomonas* count in fresh chub mackerel (*Scomber colias japonicas*) fillets treated with MAP CO₂/N₂: 50/50 was below 7 log CFU/g after stored at 3 and 6 °C for 15 days. According to Arkoudelos *et al.* (2007), farmed eel (*Anguilla anguilla*) stored at 0 °C under modified atmosphere packaging (CO₂/N₂/O₂: 40/30/30) shows

Pseudomonas counts of 4.2 log CFU/g on day 37 of storage. Arkoudelos *et al.* (2007) further stated that *Pseudomonas* count is lower in farmed eel treated with MAP than with air condition (7.3 logs CFU/g at day 18) and vacuum packaging (5.9 log CFU/g at day 31).

Coliform count

Enterobacter, *Citrobacter* and *Aeromonas* are those major strains classified for coliform bacteria. *Aeromonas hydrophila* reported to be more common isolates among the other genera (Niemi and Taipalinen, 1982). Chen and Xiong (2008) reported that samples of precooked and peeled red claw crayfish showed similar trends of packaging effectiveness for coliform counts as observed for aerobic plate count. Coliform count was low in all packaging treated samples until 7 day. Rapid increase in coliform count was observed in both PVCP and VP treated samples, reaching about 8.5 logs CFU/g on day 21 of storage. While samples treated with MAP showed lowest coliform count (below 3 logs CFU/g) at the end phase of storage (21 day). Greater significant difference has been observed for both APC and coliform count between MAP and other packaging system at the end of storage period (Chen and Xiong, 2008).

Halophilic bacterial count

Bacteria in the family of *Halobaculum*, *Halorubrum*, *Natrialba*, *Natronomonas* and *Haloterrigena* were classified as extreme halophilic aerobic Archaea. Other species including *Halobacterium cutirubrum*, *H. halobium*, *H. salinarium*, *H. saccharovororum*, *H. trapanicum*, *H. volcani* (Kamekura 1998). *Halobacterium cutirubrum*, *H. salinarium* and *Pseudomonas salinaria* are some of halophilic bacteria studied by Lanyi (1974) for properties of protein obtained from the particular enzymes.

Ibrahim *et al.* (2008) found that halophilic bacterial count increased in both MAP and under vacuum conditions after two weeks of storage and followed by decline on the third week of storage at 4 °C for smoked mullet samples. However with respect to storage period, halophilic bacterial count showed increment particularly for those smoked mullet samples stored under vacuum condition than the one stored under MAP (Ibrahim *et al.*, 2008).

Psychrotrophic counts

Pseudomonas, *Vibrio*, *Falvobacterium* and *Moraxella* are some important psychrotrophic Gram negative bacteria which lead to microbial spoilage of seafood (Khan *et al.*, 2005). *Pseudomonas* and *Achromobacter* are rapid growing psychrotrophic

bacteria which cause spoilage in seafood (Montville and Curran, 1993). *Bacillus cereus* is one of the most common psychrotrophic species (Cosentino *et al.*, 1997).

International fish trade organizations used upper acceptability limit of 6 log CFU/g aerobic plate counts for Halibut (*Hippoglossus hippoglossus*), also as an indicator for first-rate shelf life for halibut. Accordingly, Hovda *et al.* (2007) studied the effect of MAP on farmed halibut with gas proportions of CO₂/O₂ and CO₂/N₂ of each 50/50 at storage temperature of 4 °C. On day 23, psychrotrophic counts obtained for both MAP CO₂/N₂ and CO₂/O₂ of 50/50 were below 7.0 logs CFU/g. In short, Hovda *et al.* (2007) concluded that halibuts packed with oxygen fortification shows superior storage life extension as compared to MAP fortified with 50/50: CO₂/N₂. Pearl spot stored in MAP of CO₂/O₂: 40/60, CO₂/O₂: 50/50, CO₂/O₂: 60/40, CO₂/O₂: 70/30 and CO₂/O₂/N₂: 40/30/30 demonstrated similar psychrotrophic counts for all the treatment at day 30 when stored at 0-2 °C (Ravi Sankar, Lalitha *et al.*, 2008). However, MAP of CO₂/O₂: 60/40 which found to be the prominent treatment for other bacteria encountered in the study proves the same trend with lowest count for psychrotrophic bacteria (Ravi Sankar *et al.*, 2008).

MAP and other methods

Traditional preservation methods such as salting, chilling, superchilling, freezing, smoking and others extend shelf life of fishery products as known earlier. The particular shelf life is being lengthened further with introduction of packaging mainly MAP (Phillips, 1996). Superchilled Atlantic Salmon (*Salmo salar*) fillets has shelf life of 10 days based on the aerobic mesophiles (6 log (CFU/g as the microbiological limit). Conversely MAP samples exhibited extended shelf life of 16 and 22 days respectively depending on the CO₂ concentration on the packages. MAP samples remained about 5 logs (CFU/g) for psychrotrophic bacterial counts after 28 days of storage which is about 8 days later than the control sample (Fernandez *et al.*, 2009). Superchilled samples of spotted wolf fish (*Anarhichas minor*) showed lower aerobic and psychrotrophic count compared to samples air samples (Rosnes *et al.*, 2006). CO₂ atmosphere packaged cold smoked salmon samples extended shelf life for five to six weeks by limiting the lactic acid bacteria as only four weeks shelf life in 5 °C (Paludan-Maller *et al.*, 1998).

Lauzon *et al.* (2009) reported total viable psychrotrophic and H₂S producing bacterial count were lower in brined MAP fish than air packed brined fish and unbrined fish samples stored at superchilling

temperatures of -2 and -3.6 °C. Sensory shelf life of cod fillets has been extended in MAP combined with superchilling condition than superchilled air samples. Base on microbiological result, smoked trout has longest shelf life of 47 days in MAP packages of higher composition of CO_2 compare to only 33 days shelf life in vacuum packaged samples. Similarly, extended shelf life has been observed in MAP samples of six days than in air stored maatjes herring samples (Lauzon and Martinsdattir, 2005). Atlantic salmon fillets showed significantly greater shelf life in MAP than samples stored in air (Hansen *et al.*, 2009). Furthermore, it has been reported MAP inclusive of certain composition CO_2 and of O_2 (O_2/CO_2 : 50/50 or O_2/CO_2 : 70/30) retain colour of meat samples for extended period (Li and Xie, 2008).

Conclusion

Application of MAP has increased consumer demand due to its technical opportunity with significant pursue in extending storage life of fishery products, helped in providing good quality food products, avoids or lessens usage of chemical preservatives. MAP also improved other quality characteristics such as stabilizing color of food products and prevent bad odor. MAP in combination with natural preservatives namely antioxidants and antimicrobial inclusive of essential oils tends to extensively prolong the storage life of commodities. Conversely, the use of MAP has its own risk besides the benefits. It may cause number of potential disadvantages which has to be considered such as increase in product cost, requirement of skilled staff during production, and possibly chemical and microbiological hazardous appeared in packed products. MAP has been proved as an effective packaging method to extend the shelf life of a wide range of fisheries products. The use of MAP can also delay the growth of many spoilage and undesired microorganisms as well as inhibit their toxin production to ensure the safety of fisheries products.

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