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Modified atmosphere packaging of fish and fishery products: A review

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Abstract

The principle of Modified Atmosphere Packaging (MAP) is based on the alteration of gasses composition in the packaging atmosphere; the atmosphere gases introduced consist of O₂, N₂, CO₂. The beneficial effect of MAP during distribution and preservation on quality of fish and shellfish products has been reported by researcher. The MAP works by inhibiting bacterial growth and oxidative reactions in the food, but the extend of preservation depends on species, initial microbial population, fat content, gas mixture and the ratio of gas volume to product volume, and most importantly, storage temperature. CO₂ is the principle inhibiting compound compare to other gases but higher temperatures prevent dissolution of CO₂ in the product and affect the inhibitory properties which will results in higher microbial and enzymatic activity and leads to product damage. Barrier properties of the packaging materials also play a vital role in the preservation of food by MAP. Knowledge on the product properties, pre-treatments with the right combination of gasses, proper packaging and post storage condition are the contributing factors for a successful MAP of fish and shellfish products. This review summarizes scientific data for MAP and discuss in detail about pre-treatments, Specific Spoilage micro-organism, Vacuum Packaging, refrigerated and chilled storage, packaging materials and the application of MAP on fish and shellfish products.

Keywords: specific spoilage carbon dioxide, gas packaging, fish and shellfish

Introduction

Fish and shellfish spoilage from post mortem autolysis and microbial growth is quicker because of the high ambient temperature of our country which favours rising of microorganisms, primarily due to bacterial action ^[1]. Crushed block ice and mechanical refrigeration are common use to retard microbial and biochemical spoilage in freshly caught fish during distribution and marketing having a shelf-life from 2 to 14 days ^[2]. However, as ice melts it reduced the quality because it tends to contaminate the fish thereby accelerating spoilage and reduces period of storage time. Modified atmosphere packaging, a technologically viable method has been developed to scale back the losses and extend the storage lifetime of fish and shellfish products and as a supplement to ice or mechanical refrigeration. Consumer demands for fresh, refrigerated foods with extended shelf-life led to the increasing popularity of modified atmosphere packaged foods. It's a way used for prolonging the shelf-life of perishable products like meat, fish, fruits and vegetables since it slows the natural deterioration of the food ^[3]. In modified atmosphere packaging preservation technique, the air surrounding the food within the package is replaced with different gas mixtures to manage microbial activity and /or retard discolouration of the products. The gases normally employed are greenhouse gas, mixtures of greenhouse gas and nitrogen, dioxide and oxygen and greenhouse emission, oxygen & nitrogen with the only objective to increase the time period of the product beyond that obtained in conventional refrigerated storages. Inhibition by CO₂ manifests in an increased lag phase and a slower rate of growth of microorganisms during logorathmic phase. Inhibitions by carbon dioxide are more effective when the food was stored at refrigerated temperatures ^[4]. MAP is employed with various products and also the mixture of gases within the package depends on the type of product, packaging materials use and storage temperature. Non-respiring products like meat, fish, cheese etc. needs very low gas permeability and high barrier films, fruits and vegetables are respiring products where the interaction of the packaging material with the food product is vital ^[5]. By increasing the CO₂ and reducing the O₂ level respiration and ethylene production rates is reduced, spoilage will be retarded and various compositional changes related to

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ripening is delayed. If the permeability for O₂ and CO₂ of the packaging film is customized to the products respiration, an equilibrium modified atmosphere will establish within the package and therefore the shelf-life of the products will increase [6]. The proportion of every component gas is fixed when the mixture is introduced into the package and there is no control of the composition during the storage period. Composition of the gas mixture changes during storage because of chemical, enzymatic and microbial activity of the food products. Carbonic acid gas enrichment within the storage atmosphere helps within the extension of time period of products because it retards microbial growth. Carbon dioxide lowers the intra and extracellular pH of tissues and possibly that of microorganisms. Further it should affect the membrane potential of microorganisms and influence on the equilibrium of decarboxylating enzymes of microorganisms [7, 2]. Modified atmosphere packaging (MAP) of fishery products has been shown to inhibit the conventional spoilage flora and increase shelf-life significantly. However, microorganism like *Clostridium botulinum* type E and non proteolytic blood type strains will grow and produce toxin in low-oxygen atmospheres at refrigerated temperatures has caused great concern in studies on MAP of seafood [8]. In recent years, there has been debate regarding the utilization of monoxide (CO) within the packaging of pork. While no risk was found within the use of low levels of CO, the actual fact that CO maintains the colour of the meat and might, in this way, hide visual evidence of spoilage was raised. The European Food Information Council (EFIC, 2001) released a report in 2001 reviewing the information. The packaging materials generally employed are flexible films of nylon or surlyn laminates, PVC moulded trays laminated with either polythene, polyester, low density polythene film etc. This review examines the effect of the MAP technology used for fresh fishery products on the spoilage microbiological flora and on the food-borne pathogens that will be present in these products.

Spoilage in fish

Any change in the food which makes it unacceptable for human consumption is known as food spoilage [9]. Fish and shellfish are more susceptible to spoilage due to their high a_w content, neutral pH, and presence of autolytic enzymes. Spoilage in fish and shellfish results from the changes caused by oxidation of lipids, the metabolic activities of microorganisms and reactions caused by activities of enzymes present in fish [10]. The spoilage of fresh fish results in off-odours and flavours and softening of the fish are the primary factors that characterised spoilage. Mostly ice is used to slowdown the rate of spoilage [11]. The rate and type of spoilage depends on the type of processing and preservation techniques. Mostly the spoilage is temperature dependant, whether the fish undergoes biochemical spoilage, microbial spoilage or a combination of both. The spoilage microorganism found in fish correlates with the aquatic environment they live in [12, 1, 10, 13]. The temperate waters microflora is dominated by psychrotrophic, aerobic or facultative anaerobic Gram-negative, rod-shaped bacteria (*Acinetobacter*, *Aeromonas*, *Cytophaga*, *Flavobacterium*, *Moraxella*, *Photobacterium*, *Pseudomonas*, *Shewanella putrefaciens* and *Vibrio* spp.) [14, 15, 9, 2]. Marine fish are mostly dominated by the microflora of *Vibrio*, *Photobacterium* and *S. putrefaciens* that require sodium for growth, whereas freshwater fish dominated by *Aeromonas* spp [16]. Gram-positive organisms are mostly isolated from seafood and

tropical fish carries higher load of Gram-positive bacteria compared with colder waters fish (*Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus*, *Corynebacterium* and *Brochothrix thermosphacta*) [12, 14, 15, 9].

Spoilage in fish starts by the bacteria naturally present which acts on the amino acids and other NPN substrates present. These microorganism mostly *Pseudomonas* actively oxidatively deaminate the amino acids. *Pseudomonas* bacteria selective use amino acids which derepressed proteinase production. Then, an amino acid recruitment to substrate pool by bacterial hydrolysis of protein occur. An increase in ammonia and volatile fatty acid production occur. Specific spoilage organisms (SSO), produces sulphur-containing and other odorous compounds [12, 11, 16]. The fresh fish flesh composition makes it favourable for microbial to grow [1]. There is degradation of trimethylamine oxide (TMAO) in fish muscle to trimethylamine (TMA) by endogenous enzymes, but at chill storage temperatures TMA oxidase an enzyme produce TMA [10]. TMA is responsible for the characteristic 'fishy' odour of spoiled fish. Others odorous low molecular weight sulphur compounds such as CH₃SH and H₂S, with volatile fatty acids and ammonia are produced due to bacterial growth.

Principle of Modified Atmospheric Packaging (MAP)

The main principle of MAP is replacing the air in the package with a different fixed gas combination. Once the gas mixture is introduced to the package there is no further control of the gas composition and the composition will inevitably change due to interaction of the food materials and respiration of the foods. MAP is a type of non-thermal method of food preservation that uses nitrogen (N₂), Oxygen (O₂) and carbon dioxide (CO₂). N₂ primary function is a filler to prevent pack collapse [17]. N₂ is an inert gas which does not have an antimicrobial effect but it creates an anoxic atmosphere which is effective against anaerobic, aerotolerant *Lactobacilli* [18]. O₂ inhibits the growth of anaerobic bacteria but the principle antimicrobial effect is due to its bacteriostatic and fungistatic properties. N₂ is used as an alternative to vacuum packaging to replace O₂ in packages to slow down oxidative rancidity and inhibit growth of aerobic micro-organisms as O₂ is reported to increase oxidative rancidity [19], but others claim that rancidity caused by presence of O₂ in the atmosphere is no problem [20]. The inhibition effects of CO₂ increase with the increase in concentration in the package. The use of CO₂ for inhibition of bacterial growth is not a new technology, *Bacillus anthracis* could be killed by CO₂ [21, 22] published the first article on the preservative effect of carbon dioxide on food where shelf-life was extended when ox meat placed was inside a cylinder filled with a carbon dioxide atmosphere.

The utilization of high barrier film together with MAP that contains CO₂ effectively inhibits bacterial growth during refrigerated storage of packaged fresh fishery products [23]. Oxygen is important when packaging fresh fruits and vegetables as they still respire after harvesting. The absence of O₂ can cause anaerobic respiration within the package which accelerates spoilage. Too high levels of O₂ don't retard respiration significantly and it's around 12% of O₂ where the respiration rate starts to decrease. Therefore, oxygen was used in low levels of 3-5% to get positive effect. In the case of vegetables and fruits, CO₂ isn't a significant factor since CO₂ levels above 10% are needed to suppress fungal growth significantly. But higher levels of CO₂ beyond 10% will develop phytotoxic in fresh produce. Nitrogen is employed as

filler gas since it neither encourages nor discourages bacterial growth. The composition of the gas mixtures used for MAP of cannon fodder varies, depending upon whether the fish within the package is lean or oily fish. For lean fish, a ratio of 30% Oxygen, 40% dioxide, 30% Nitrogen is usually recommended. Higher values of CO₂ are used for fatty and oily fish with a comparable reduction in level of Oxygen within the mixture resulting in 40% to 60% Nitrogen. By excluding oxygen, the rate of oxidative rancidity in fatty fish is slowed. On the opposite hand, oxygen can inhibit the expansion of strictly anaerobic bacteria like *C. botulinum* although there is a really wide variation within the sensitivity of anaerobes to Oxygen^[24]. There's evidence that inclusion of some Oxygen with Nitrogen or dioxide does not prevent botulism. High levels of oxygen are used in red meat and red fish meat (tunas, yellowtails, etc.) to maintain the red colour, to reduce and retard browning caused by formation of metmyoglobin^[25]. O₂ in Modified Atmosphere packages will also inhibit reduction of TMAO to TMA in fresh fish^[26].

The solubility of CO₂ increases with decreased temperature as it is highly soluble in water and fat. The solubility at 0°C and 1 atmosphere is 3.38 g CO₂/kgH₂O, however, at 20 °C the solubility is reduced to 1.73 g CO₂/kg H₂O in water^[27]. Therefore, the effectiveness for inhibition of bacterial growth of the gas is inversely proportional to temperature^[28, 29, 30] and the concentration of CO₂ in the food depends on the product's water and fat content, and the partial pressure of CO₂ in the atmosphere, according to Henry's law^[31]. The solubility of CO₂ leads to dissolved CO₂ in the food product^[27].^[32, 33] have demonstrated that CO₂ inhibits the growth of microorganisms in modified atmosphere. CO₂'s residual effect is that CO₂ is slowly release by the product and exert a preservative effect for a certain period of time after the packaging has been opened^[14]. Experiments with storage of bacon and pork showed an increase in shelf-life under 100% CO₂ compared with storage in normal air atmospheres^[34] and the preservation effect was originally thought due to the displacement of O₂^[4] but a storage in 100% nitrogen (N₂) offered no advantage over normal air storage. The result was similar with that of spoiled pork when pure cultures of microorganisms were isolated. A drop in surface pH is observed in MA products because of the acidic effect of dissolved CO₂, but this could not entirely explain all of CO₂'s bacteriostatic effect^[35]. The change in pH caused by the CO₂ did not relate for the retardation of growth.^[36] Reports that pure cultures of bacteria isolated from fish products show CO atmospheres inhibit the growth of bacteria markedly, whereas normal growth patterns were observed under air or N₂ atmospheres. 25% CO₂ is sufficient to inhibit bacterial growth and with higher CO₂ concentrations no growth was observed for 4 days at 15 °C due to the possibility that intracellular accumulation of CO₂ disturb the normal physiological equilibrium in other ways, i.e. by slowing down enzymatic processes that normally result in production of CO₂ by alteration of cell membrane function including effects on nutrient uptake and absorption; or by penetration of bacterial membranes leading to changes in intracellular pH; lastly by direct changes in the physico-chemical properties of proteins, the combination of all these account for the bacteriostatic effect by CO₂^[37, 38, 39, 40, 41, 14, 42, 23].^[43] Report that some amount of CO₂ depending on the foodstuff needs to dissolve into the product to retard the bacterial growth.

The G/P ratio 2: 1 or 3: 1 of volume of gases two or three times the volume of food is required. Due to CO₂ solubility in

wet foods, higher G/P ratio is also necessary to prevent package collapse. The CO₂ solubility could increase drip as it alter the food and water holding capacity^[44, 45, 46, 47]. A minimum head space of 20-30% is required to achieve bacterial inhibition^[48, 49] with 40-100% CO₂ balanced with N₂ was used in poultry industry. Excess can be reduced by blanching fillets in NaCl prior to packaging^[50, 51]. An acidic, sherbet-like flavour can be observed in fishery products eaten without prior heating, such as crab and cooked fish when high partial pressures of CO₂ is used.

Specific spoilage organisms

The spoilage microflora in spoiled product consists of a mixture of species^[11]. *Shewanella putrefaciens* is main spoilage organism for cod stored under aerobic conditions in ice^[26, 52]. *Shewanella putrefaciens* responsible for very intense and unpleasant off-odours, convert TMAO to TMA and produces H₂S. *Shewanella putrefaciens* together with *Lactobacillus* spp. causes spoilage of cod and sole in MAP^[14]. When cod store at 20°C, strains of Vibrionaceae dominate^[52]. Cod vacuum packs and in MAP with mixtures of CO₂ and N₂, stored at 0 °C the Gram-negative organism *P. phosphoreum* is the responsible spoilage organism^[53]^[54] and an increase in growth rate was observed under anaerobic conditions. *S. putrefaciens* and other spoilage micro-organism are inhibited when packed with CO₂. But *P. phosphoreum* was highly resistant against CO₂^[55]. *Photobacterium phosphoreum* reduces TMAO to TMA at 10 to 100 times compared to *S. putrefaciens* the amount per cell due of the large size of the former (diameter 5 μm) while production of H₂S was little during its growth in fish substrates^[54, 56]. *Photobacterium phosphoreum* is sensitive to freezing when test was done by frozen storage at -20 and -30°C for 6-8 weeks^[50]. Shelf-life of cod increase to 230 days from 11 days when MAP and stored at 2°C^[57], thaw drip was high in frozen storage MAP fish. *Shewanella*-like bacteria was major microflora of gas packed cod fillets stored at 7 °C^[58], and was more resistant to CO₂ compared with *Shewanella putrefaciens*. *S. putrefaciens* was prohibited when CO₂ applied above 50% concentrations O₂ above 70%^[59]. >10% of O₂ is sufficient to restrain the production of TMA by aerobic respiration of *S. putrefaciens*^[26]. Lowered production of TMA in MAP when oxygen was included at (40:40:20 of CO₂: N₂:O₂) compared with atmosphere where O₂ was excluded (40: 60 CO₂: N₂) for cod fillets stored at 2 °C^[57]. Inhibition of *L. monocytogenes* growth occurs below 100% CO₂ atmospheres^[60, 61] or in 50% CO₂ when added with bacteriocins^[62, 61] and/or salt and lowered pH^[63]. MAP of 75% CO₂: 10% O₂, 15% N₂ with 1% lactic acid has 8 days extension of the lag phase of *L. monocytogenes* in samples of crayfish tail meat compared with air or vacuum packaging. There a delayed but did not inhibit growth of *L. monocytogenes* at 100% CO₂ as compared with air in inoculated 10³ cfu g⁻¹ raw and cooked seafood nuggets^[65], and reach 10⁶ cfu g⁻¹ before spoilage occurred in all pack- aging methods^[64].^[66] Demonstrated that 100% CO₂ with increased NaCl level and decreased pH level could have an inhibitory effect on the growth of *C. botulinum* at chill temperatures. Growth and toxin production by *C. botulinum* in raw and cooked surimi are controlled with competitive inhibition by *Bacillus* species nuggets^[67]. *C. botulinum* type B and E (2-log spores g⁻¹) spores inoculated in trout and salmon with of and stored in MAP and vacuum at 10, 15 and 20 °C, spoiled before the food became toxic^[68]. Salmon were less toxigenic

due lower pH in during storage as compared with trout.

Modified atmosphere packaging of fishery products

The effects of MAP on the shelf-life of foods were studied by [39, 69, 4, 42, 70, 23, 8, 3, 44, 71] and preliminary fish in particular was reported by [30, 41, 1, 72, 73, 74, 14] over the years. The use of CO₂ to store seafood was first reported in the early 1930s in the UK, USA and Russia [59]. Fish has a better keeping quality of 2 to 3 times longer in 100% CO₂ compared to control fish kept in air at the same temperature [75]. Even, MAP of cod at 27 °C was in good condition for several days, CO₂ absorption altered the pH of fish from 6.6 to 6.2, but solved by exposure to air. Fresh haddock, cod, sole, whiting and plaice are well preserved at optimal conditions under 40±50% CO₂ [35]. At 0°C and 80% CO₂, flat fish had better keeping quality as compared with the other fish species. While haddock stored at 25% CO₂ had a shelf-life of twice than products stored by conventional methods [59]. CO₂ storage was beneficial for prolonged storage and maintaining best sanitary conditions during processing and filleting. Most studies concluded that there was an extension of shelf-life for 30±60% for fresh fishery products using an elevated level of CO₂. Herring and trout had an increased shelf-life with optimum G/P ratio of 1 compared with 0.4. It was also reported that 40% CO₂ gave longer shelf-life compared with 20% [76]. Hake shelf-life extended double with G/P ratio of 2 and 50% CO₂ and another more 2 days when prior blanching with NaCl is done [51]. Barrier packaging material is important for MAP and vacuum-packaged products. There is a doubled shelf-life fillets of snapper from 3 to 6 days when vacuum packed in high O₂ transmission barrier bags when compared with no or medium barrier bags. MAP and vacuum packaging with high CO₂ levels 25±100% extends the shelf-life of meat products by several weeks [43, 77]. pH and differences in spoilage microflora are mainly responsible for differences in the shelf-life of fish and meat product [16]. Aerobic Gram-negative organisms like *Pseudomonas* spp., which cause spoilage of meat, can be inhibited by anaerobic conditions and by CO₂. Mostly Gram-positive organisms are responsible for spoilage in MAP meat products which are much resistant to CO₂ and the best effect of MAP storage on shelf-life obtained from warm waters fishes [78, 16]. [79] Summarized 18 years of MAP fish research at the Icelandic Fisheries Laboratories. There is a shelf-life increase of 28-52% for retail packaged and 32-73% for bulk packaged haddock fillets. Cod fillets in retail packaged had a shelf-life of 32-52%, while ocean perch fillets shelf-life increase of 42±74%. Gravad fillets rubbed with 1:2 mixture of salt and sugar have a shelf life of more than six when raw materials are matured for 48 h at 3°C, MAP composition of 25% O₂/60% CO₂/ 15% N₂ is more effective than 40% N₂/60% CO₂ when rainbow trout stored under vacuum at -30°C have a prolonged shelf life up to eight weeks at 3°C [80]. Oregano essential oil acts as a “natural” preservative on fresh, salted rainbow trout fillets when stored under MAP (5% O₂/45% CO₂/50% N₂) at 4°C for 21 days was examined by [81]. Oregano oil limited microbial growth sea bream stored under MAP 60% CO₂/40% N₂ for MAP 1 and 60% CO₂/10% N₂/ 30% O₂ for MAP 2 at 2, 4, and 8°C. lower value were also obtained in the biochemical parameters like TVB-N and TMAN [82].

MAP on Micro-organism and Biochemical Parameters

Combination of O₂ with CO₂ was preferable to N₂ as a filling gas for these lean fishes, as it provide longer shelf-life. Shelf-

life of cod (*Gadus morhua*) under MAP conditions at 4 °C up to 40-60 days [83, 84], homofermentative *Lactobacillus* spp. was the dominant spoilage microflora in high CO₂ concentrations accounting for 80, 62 and 85% of cod fillets stored in 100% CO₂, 90% CO₂: 10% N₂, and 90% CO₂:10% O₂, respectively [85]. In addition, *S. putrefaciens*, *Vibrionaceae* and *Micrococcus* spp. and *B. thermosphacta* were observed. Active modified atmosphere (10% O₂/80% CO₂/10% N₂) was applied to evaluate the effect on chemical parameters of seabass stored at 4°C. Ca₂C, Mg₂C, and Mg₂C–Ca¹⁺ ATPase activity of natural actomyosin remained unaffected that is 0.31, 0.36, and 0.4 mmol Pi mg/protein minutes, respectively, on the 21st day on samples stored under MAP, while there was a slight increase in Mg-EGTA ATPase (0.1 mmol Pi mg/protein minutes). The sulfhydryl content dropped at a lower rate compared to that of control sample (5.5 mol/10.5. g/protein) [86]. Study on MAP (10% O₂/80% CO₂/10% N₂) with three different pre-treatments trisodium phosphate (TSP), sodium pyrophosphate (PP) and sodium tripolyphosphate (STPP) were used for sea bass preservation at 4°C for 21 days. All pre-treatments are effective in reducing microorganism numbers of TPC (6 log CFU/g) and psychrophiles (5.8–6 log CFU/g) whereas there was no reduction incase of LAB (3.8 log CFU/g). TMA and TVB were controlled on pre-treated MAP samples with all treatments (0.19 and 0.045 and 0.29 and 0.17 mg/g muscle for control and MAP samples, respectively) [87]. Thyme essential oil added to sea bass samples stored at two different atmosphere modifications (10% O₂/40% CO₂/50% N₂ for MAP 1 and 10% O₂/60% CO₂/30% N₂ for MAP 2) at 4 ± 0.5°C for 21 days. Samples under MAP 2 with thyme oil displayed prolonged shelf life by 12 days [88]. [89] Studied on shelf life extension of sea bass samples stored under MAP (40% CO₂/60% N₂ for MAP 1, 50% CO₂/50% N₂ for MAP 2 and 60% CO₂/40% N₂ for MAP 3) at 4°C for 21 days. MAP samples have no variation in TBARS values whereas differed significantly with control samples (0.11–0.21 mg MDA/kg) after 21 days. APC and psychrotrophic growth were both limited by the application of MAP (6.05, 6.5, and 7.28 log CFU/g for APC and 7.06, 7.62, and 8.39 log CFU/g for psychrotrophs for MAP 3, 2, and 1, respectively). There is an extension of shelflife up to 11 days for MAP 1 and 14 days for samples stored at MAP 2 and 3. Study on mussels for 14 days at 3 ± 0.5°C vacuum-packed or stored under MAP1 (20% O₂/60% CO₂/20% N₂ and MAP 2 40% CO₂/60% N₂. Sensory attributes were better preserved under MAP 1 (10–11 days) while MAP 2 also led to a shelf life prolongation of the products (seven to eight days). TMA and TVB-N of samples stored under MAP 1 were the lowest (11 and 36 mg N/100 g, respectively) but TBA was significantly higher than the other treatments (1.38 mg MDA/kg). The Total Viable Count does not exceed the acceptability limit of 7 log CFU/g in samples of MAP 1 which was 6.7 log CFU/g [90]. Wild mussels were packaged under MAP (50% CO₂/50% N₂ for MAP 1, 80% CO₂/20% N₂ for MAP 2, 65% CO₂/35% N₂ for MAP 3) and VP and kept under refrigeration (2 ± 1°C) for 12 days. MAP 2 gave the best microbiological results 6.8 (TPC), 6.78 (psychrophilic bacteria) and 5.42 (LAB log) CFU/g respectively. Chemical parameters remained acceptable until the eighth day of storage (30.6 mg/100 g for TVB-N, 3.3 mg malondialdehyde/ kg for TBA and 4.2 mg/100 g for TMA-N respectively [91]. Live mussels of 33 and 44 units/kg kept at various MAP of 75% O₂ and 85% O₂ at 2 ± 1°C shows that survival rate was more than 20% exceeded for small mussels

on day 13 for both modified atmospheres [92]. Oregon oil limited microbial growth at the rate 7.2 and 5.7 for TVC, 5.3 and 4.5 for H₂S producing bacteria, 4.59 and 4.33 for Pseudomonads, 5.7 and 5.6 for LAB, 5.4 and 4.9 log CFU/g for Enterobacteriaceae respectively when stored under two MAP atmosphere 60% CO₂/40% N₂ for and 60% CO₂/10% N₂/30% O₂ when study was conducted on sea-bream.

MAP and storage temperature

Increased in solubility of CO₂ at lower temperatures relatively increase the effect of MAP. Studies shows there is no increase in keeping quality when that haddock packed in MAP of 40 or 60% CO₂ atmosphere compared with air at 5 and 10 °C, but have a longer shelf-life of 204 days when kept at 0°C [93]. MAP combined with superchilling to extend the shelf-life of fresh fish as the fish temperature is reduced below 1-2 °C (initial freezing point) and some ice is formed inside the product [20, 99]. Freezing points of foods depend on the water content in the products [95]. Freezing points in fish vary from about -1 to -2.5 °C. For example, salmon, shrimp, and mackerel are frozen at about -2.2 °C and carps about -1.0 °C [96]. High CO₂ and superchilled atmosphere for bulk salmon (*Salmo salar*) maintained a high sensory quality for more than 3 weeks [97]. Salmon fillets (*Oncorhynchus kisutch* and *O. keta*) packed in air-tight have an acceptable sensory shelf-life of 21 days in 90% CO₂ atmosphere at 0 °C [98]. The same shelf-life was observed for mackerel in 100% CO₂ at -2 °C [28] and a doubling of shelf-life was observed for smoked blue cod when the storage temperature was lowered from 3 to -1.5 °C [99]. Crustacean shellfish keep up to 30% longer at 0 °C in a MA than in other types of packaging, and the onset of blackspot in shell-on products is delayed [100]. Spotted wolf-fish (*Anarhichas minor*) portions were packaged and stored at superchilled (-1.0°C ± 0.2°C) or chilled (+4.0°C ± 0.2°C) temperatures in air and in Modified Atmosphere of CO₂:N₂ (60%:40%) with a gas:product (approx1:1) improved odour and flavour scores (p < 0.05) were observed in MAP with shelf life of 15 days at -1°C but higher drip loss than fish stored in air (8-10 days) [101]. There is a shelflife extension of 2 days when the combination for salmon fillets are used; stored freeze-chilled -35°C for 2.5 hours and -30°C for three days and subsequent storage at 2-4°C under MAP (40% CO₂/60% N₂) for seven days [63].

Shellfish and cooked fish in MAP

80% CO₂ and 20% air in MAP was found to be the optimum atmosphere for storage of freshwater crawfish (*Procambaris clarkii*) tail meat when compared with 100% CO₂ or air [102]. CO₂ enriched atmosphere increase shelf-life by 200% of whole cooked shrimps when compared with stored on ice or exposed to air [103]. In MAP shrimps are fresh after 16 days of storage, CO₂ atmospheres is also beneficial for storage of fishcakes [104], chilled storage of raw squid and white octopus [105]. With 100% CO₂ atmosphere there is no change in K-value (degradation of adenine nucleotides) in chill stored whitefish (*Coregonus clupeaformis*) and rainbow trout (*S. gairdneri*) during 26days storage when compared with air storage [106]. Though K-values of tilapia fillets increased during Modified Atmosphere 75: 25 CO: N₂ and air storage at 4 °C the MAP fillets were still sensory acceptable even at high K-values compare to those stored with air [107]. Hot smoked trout accepted for 30 days when hot smoking combined with MAP 10% CO₂/90% N₂ and nitrogen flush for 30 when inoculated with *L. monocytogenes* stored at 3 and

7°C for 30 days [108]. The microbiostatic effect of carbon dioxide was proved when there is an inhibiting in growth of when hot smoked rainbow trout fillets were inoculated with *L. monocytogenes* strains in an MAP of 100% CO₂ and 50% CO₂/50% N₂ and stored under vacuum. The two MAP conditions have a shelflife of 4 and 5 weeks respectively [109]. Cold-smoked salmon fillets stored under vacuum shows an increase in storage temperature resulted in shelf life reduction [110]. The combination of thymol (110 ppm), lemon extract (120 ppm) and grapefruit seed extract (100 ppm) and MAP (30% O₂/40% CO₂/30% N₂ for MAP 1, 50% O₂/50% CO₂ for MAP 2 and 5% O₂/95% CO₂ for MAP 3) for blue fish burgers storage at 4°C was assessed by [111] shows an increased shelflife where the combination of oil and MAP 3 gave the best microbiological results. MAP of 5% O₂/35% CO₂/60% N₂ and 30% CO₂/70% N₂ is used for preservation of fish salad samples of rainbow trout [112]. Marinated fish salad of boiled squid, mussels, surimi, octopus and shrimp under MAP;70% CO₂/30% N₂ for and 50% CO₂/50% N₂ and stored at 2 ± 2°C was studied by [113], results of TBA and TVB-N show that up to seventh month the result were below rejection level.

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Conclusion

After reviewed of the scientific reports it can be concluded that for beneficial effect of MAP only fresh and high grade quality fish and shellfish should be used. It will further largely depend on the fat content, species, initial microbial load, gas mixture, the ratio of G/P, packaging material used and most importantly temperature of storage. Details about the specific spoilage organism (*P. phosphoreum*, *Listeria*, *Clostridium*, etc) for a particular product, the environment they are present and the right combination of the gases (CO₂/N₂/O₂) required. The main inhibitory effect is reported by CO₂ and its beneficial effect depends on the storage temperature. The increase in storage temperature will have a negative effect as it leads to loss of dissolved CO₂ in the product and reduced its inhibitory effects, an increase in microbial and enzymatic activity would result in product collapse. Therefore, a proper storage temperature should be maintained during a modified atmosphere packaging. Important points to remember to maintain quality assurance in MAP, is to maintain tight through package seal testing. Incorrect gases levels, empty gas cylinders and bad sealing bars can cause inaccurate gas mixture can results in product spoilage. A proper routine package testing; headspace gas analysers and leak detectors will assure package quality, thereby ensuring the shelf life.

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