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Modified Atmosphere Packaging of Fish – A Review

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Shelf life and quality of fresh fish can be extended by the application of modified atmosphere packaging using high barrier packaging film and refrigerated storage. Modified atmosphere with high concentration of CO_2 inhibits or slows down the growth of various aerobic spoilage bacteria of fish products by extending the lag phase. It provides conditions for the growth of gram positive bacteria and food pathogens within the package. Major qualiy hazard is the risk of foodborne botulism. This paper reviews the literature relating to the scientific basis of these claims.

Key words: Modified atmosphere packaging, vacuum packaging, Seafood, Microbiological safety, Sensory quality

Fish and other sea products are susceptible to spoilage from postmortem microbial growth, biochemical end products resulting from the microbial growth or combinations of both. Their low body temperature provides a natural environment for psychrotrophic spoilage microflora (Brody, 1989). Presently ice and mechanical refrigeration are the most common means of retarding microbial and biochemical spoilage in freshly caught seafood during distribution and marketing. However as the ice melts it tends to contaminate fresh products, accelerating spoilage and reducing shelf life (Brody, 1989)

Modified atmosphere packaging (MAP), a technologically viable method has been developed as a supplement to ice or mechanical refrigeration to reduce the losses and extend the storage life. According to the definition of Sacks and Gore (1987), MAP is replacement of air in a pack by a different mixture of gases, where the proportion of each component is fixed when the mixture is introduced, but no further control is exercised during storage. Two forms exist, namely vacuum packaging and gas, gas flush or gas exchange packaging. Vacuum packaging (VP) involves placing a product in a film of low oxygen permeability, the removal of air from the package and the application of a hermetic seal (Smith *et al.*, 1990). Gas packaging is an extension of this process involving removal of air from the pack and its replacement with specific gases, either singly or in combination. In vacuum packaging the two major spoilage agents namely aerobic bacteria and oxidative reactions require oxygen. Therefore its unavailability will inhibit spoilage and thus maximize quality and /or storage life. Some deterioration, however will occur due to anaerobic / microaerophillic organisms and nonoxidative reactions. This is usually minimized by chilled storage. Product compression is unavoidable and makes vacuum packaging unsuitable for many products. Controlled atmosphere is packaging in an atmosphere where the composition of gases is continuously controlled throughout storage. The gaseous atmosphere of the vacuum packaging is altered during storage, thus considered modified due to a 10 to 20% increase in the carbon dioxide content produced by microbial activity. This carbon dioxide may inhibit the growth of undesirable organisms (Silliker & Wolfe, 1980).

Development of modified atmosphere packaging for fish

Development started in the 1930s with several workers observing that CO_2 atmosphere prolonged the storage life of fish (Coyne, 1933; Stansby & Griffiths, 1935; Hjorth-Hansen, 1933). Subsequent interest in the use of CO_2 turned to refrigerated seawater systems as an alternative to the use of iced storage for small fish on board or for road transport (Nelson & Barnet, 1971;

Barnett *et al.*, 1978; Hiltz *et al.*, 1976; Bullard & Collins, 1978). Some interest in the technically more difficult task of transporting chilled, whole fish in carbon dioxide atmospheres then appeared. This was a period when all variables associated with manipulation of storage temperatures were keenly examined. The earliest use of CO_2 in retail products exploited the solubility of CO_2 to produce a snugging down effect (Douglas, 1970), regarded as characteristic of CO_2 packs. The vacuum appearance and prevention of movement of the product was seen, ironically in view of the later developments in MAP, as being advantageous but there was only a small amount of kipper fillet packed in this way, with no application to white fish or other fish products (Abbey, 1970). Reductions in rates of deterioration of several species of fish have been recorded in tests using pressure chambers under both hyperbaric (Charm *et al.*, 1977) and hypobaric (Varga *et al.*, 1980; Haard & Lee, 1982) conditions. With unfrozen material, nitrogen flushing for oxygen sensitive products was recommended and applied to shrimps and prawns, but was not recommended for meat and fish which are susceptible to spoilage by anaerobic bacteria (Anon, 1967). As had occurred earlier with CO_2 preservation of whole fish, development and application of controlled gas mixtures for consumer packaging was concerned first with foods other than fish (Schweisfurth & Kalle , 1970; Anon, 1977). According to Kimber (1984), the technology of gas packaging was first perfected and patented in 1963 by Bohme and Kalle Films but it took until 1977 to produce gas flushed packs successfully. The first UK application to fish products was in 1979 in Northern Ireland (Kimber, 1984), with a few specialty products. The technique became more widespread as manufacturers of vacuum packaging equipment adopted their products, and super markets sought alternative ways of presenting fish. With equipment capable of making more precisely designed and practicable products, research workers turned their attention to the demands of prepackaging fish products and the use of gases – mainly CO_2. Trials were conducted with a large range of species and products and many of the results have been published (Martin, 1981; 1982; Cann, 1984; Anon., 1985) There has been an increase in gas packaged food products in the market during the last three decades. This increase has brought improvements to the packaging industry, which has lead to the development of high barrier polymers and thermo mold packaging equipment.

The three main commercially used gases in modified atmosphere packaging are carbon dioxide, nitrogen and oxygen.

Function of carbon dioxide

CO_2 is soluble in water and also in lipids. Its general effects on microorganisms are an intensification of their growth stage and a decrease in the growth rate during the logarithmic stage (Farber, 1991). CO_2 is soluble in water, forming carbonic acid that may lower the pH (Smith *et al.*, 1990). The anti microbial efficacy of CO_2 is greatly enhanced as the storage temperature of the product is reduced due to its increasing solubility with decreasing temperature. Thus to guarantee maximum anti microbial effect, the storage temperature is kept as low as possible (Finne, 1982; Farber, 1991).

The concentration of CO_2 in the food is dependent on water and fat content of the products, and of the partial pressure of CO_2 in the atmosphere, according to Henrys law (Ho *et al.*, 1987). Devlieghere *et al.* (1998a, 1998b) have demonstrated that the growth inhibition of microorganisms in MAP is determined by the concentration of dissolved CO_2 in the product. After the packaging is opened, CO_2 is slowly released by the product and it continues to exert a preservative effect for a certain period of time, referred to as CO_2's residual effect (Stammen *et al.*, 1990).

The action of CO_2 on the preservation of foods was originally thought to be caused by displacement of some or all of the O_2 available for bacterial metabolism, thus slowing growth (Daniels *et al.*, 1985). However, experiments with storage of bacon and pork showed a considerable increase in shelf life under 100% CO_2 compared with storage in normal air atmospheres (Callow, 1932), but the preservative effect was not because of the exclusion of O_2, as storage in 100% nitrogen (N_2) offered no advantage over normal air storage.

A drop in surface pH is observed in MAP products because of the acidic effect of dissolved CO_2, but this could not entirely explain the bacteriostatic effect (Coyne, 1933). It was shown that CO_2 was more effective at lower temperatures and that the change in pH caused by CO_2 did not account for the retardation of growth. In a study on several pure cultures of bacteria isolated from fish products, CO_2 atmospheres

were found to inhibit the growth of bacteria markedly, whereas normal growth patterns were observed under air or N_2 atmospheres (Coyne, 1932). It was also observed that bacterial growth was inhibited even after the cultures were removed from the CO_2 atmosphere and transferred to an air environment, interpreted as residual effect of CO_2 treatment. Bacterial growth was distinctly inhibited under 25% CO_2 and almost no growth was observed under higher CO_2 concentrations for 4 days at 15°C. The results obtained could neither be explained by the lack of O_2 nor the pH effect. The effect of CO_2 on bacterial growth is complex and four activity mechanisms of CO_2 on microorganisms has been identified (Parkin & Brown, 1982; Daniels *et al.*, 1985; Farber, 1991)

1. Alteration of cell membrane function including effects on nutrient uptake and absorption;
2. Direct inhibition of enzymes or decreases in the rate of enzyme reaction;
3. Penetration of bacterial membranes, leading to intracellular pH changes;
4. Direct changes in physico – chemical properties of proteins

Probably a combination of all these activities account for the bacteriostatic effect. In food having high moisture and/ or fat amounts, the excessive absorption of CO_2 may lead to a phenomenon known as packaging collapse (Parry, 1993). A certain amount of CO_2 will dissolve into the product to inhibit bacterial growth (Gill & Penney, 1988). The ratio between this volume of gas and volume of food product (G/P ratio) should usually be 2:1 or 3:1. This high G/P ratio is also necessary to prevent package collapse because of the CO_2 solubility in wet products. Dissolved CO_2 takes up much less volume compared with CO_2 gas and after packaging a product in CO_2 atmosphere, a drop in pressure develops within the package and package collapse occurs. Increase in dripping is caused by the dissolution of gases on the muscle surface in atmospheres containing high CO_2 levels (>60%), reduced pH and, consequently, low protein water retention ability (Parry, 1993; Randell *et al.*, 1995). As a consequence, high CO_2 concentrations promote organoleptic changes as, for example, texture alterations in meat.

Function of nitrogen

N_2 is an insipid and inert gas, showing low solubility in water and lipids. It is used for displacing the oxygen from the packaging, decreasing oxidative rancidness and inhibiting the growth of aerobic microorganisms (Farber, 1991). Due to its solubility, it is used as a filling gas, preventing the possible packaging collapse caused by the accumulation of CO_2.

Function of oxygen

O_2 generally stimulates the growth of aerobic bacteria and may inhibit the growth of exclusively anaerobic bacteria, although anaerobic microorganisms show different sensitivity levels to oxygen (Farber, 1991). The presence of oxygen may cause oxidative rancidity problems in fish presenting high lipid amounts, promoting the formation of low molecular weight aldehydes, ketones, alcohols and carboxylic acids. The use of oxygen in modified atmospheres is generally avoided with this kind of fish, in order to minimize such effects. The use of O_2 in modified atmosphere packaging for fish is supported by Davis and Slade (1995), who states that there are evidences showing that the use of O_2 reduces the exudation in fish during storage, and suggested that O_2 can be used in low fat fishes. Reddy *et al.* (1992) claim that the use of oxygen associated with N_2 or CO_2 gives a false idea of reducing botulinum risks in fresh packed fish and may lead to illusory safety. However for some products oxygen could or should be used. High levels of oxygen are used in red meat and red fish meat to maintain the red colour of the meat, to reduce and retard the browning caused by formation of metmyoglobin. Oxygen in MAP packages of fresh fish will also inhibit reduction of TMAO to TMA (Boskou & Debevere, 1997).

Examples of some meat and fish products currently gas packaged as well as the composition of gas mixtures used to extend the shelf life of each product are shown in Table 1.

Table 1. Examples of gas mixtures for selected food products

Product	Temp°C	%O_2	%CO_2	%N_2
Fresh meat	0-2	70	20	10
Cured meat	1-3	0	50	50
Poultry	0-2	60-80	20-40	0
Fatty fish	0-2	0	60	40
White fish	0-2	30	40	30
Cheese	1-3	0	60	40
Baked product	R.T	0	60	40
Pizza	R.T	0	60	40
Dry snacks	R.T	0	20-30	70-80

R.T – Room temperature; (Smith *et al.*, 1990)

Factors influencing shelf life of fish packaged under modified atmosphere

Several interrelated factors influence the shelf life and keeping quality of fish packaged under CO_2 enriched atmospheres. These include concentration of gas mixture, packaging film permeability, storage temperature and microbial contamination.

i) Concentration of gases

Concentration of gases is a very important factor for shelf life of fish packaged under MAP conditions. The bacteriostatic effect of MAP storage increases with increasing CO_2 concentrations (King & Nagel, 1967; Enfors & Molin, 1980; Gill & Tan, 1980). With cod fillets stored in different concentrations of CO_2 and O_2/N_2, Stenstrom (1985) showed that the shelf life increased as the CO_2 concentration increased. Haines (1932) reported that concentrations as low as 10 to 20% CO_2 were sufficient to inhibit the growth of *Pseudomonas* and *Achromobacter* species. High CO_2 concentrations might be used for many types of seafood with little risk of heme protein discoloration because of the low pigment concentration (Parkin & Brown, 1982). Carbon dioxide in sufficient concentrations can inhibit the growth of spoilage molds (Brown, 1922; Moran *et al.*, 1932; Tomkins, 1932; Hintlian & Hotchkiss, 1986). Coyne (1933), in one of the original studies on use of carbon dioxide atmospheres to prolong fish quality, recommended concentrations of 40 and 60% CO_2. Shewan (1949) recommended concentrations between 30 and 40% CO_2 atmospheres and reported superior quality for fish stored in the higher concentration of CO_2. Tarr (1954) recommended a minimum of 40 to 50% CO_2 in the package headspace to derive maximum benefit of CO_2 storage of fresh fish. Based on these studies, approximately 50-60% CO_2 in the package headspace is commonly used, with the remainder comprising of a mixture of O_2 and N_2.

ii) Packaging film permeability

MAP can be effective if used in conjunction with packaging materials of correct O_2/CO_2 permeability characteristics. It is no use having the correct atmosphere if the film allows the atmosphere to change too rapidly. The properties required for a suitable packaging film may not be found in one polymer and individual polymers are laminated to produce films of superior barrier properties. Examples of polymers used in construction of barrier films are: Polyethylene, Polypropylene, Polyvinylidene chloride (PVDC), Ethylene vinylacetate (EVA), Ethylene vinyl alcohol (EVOH), and Metallised polyesters. Examples of high, medium and low barrier films are shown in Table 2.

Table 2. Oxygen permeation rates for packaging films expressed in different units*

Film	cc per 100 in^2 /day	cc per m^2 /day mm Hg	cc per m^2 /day
PP/EVOH/PP	<0.001	<0.00001	0.01-0.02
Foil laminate (mylar/AL/poly)	<0.01	<0.00001	<0.01-0.1
PVDC	1	0.02	15
Acrylonitrile Polymer	1	0.02	15
PET (polyester)	4-6	0.08-0.13	60-100
PVC	10	0.2	150
HDPE	130	2.6	1980
PP	150	3.0	2280
LDPE	400-500	8-10	6000-7000

*For 1 mil flat (unless composite laminate) at 30°C and 50% RH

iii) Storage temperature

The effectiveness of MAP decreases as the storage temperature increases due to the fact that the solubility of CO_2 also decreases at higher temperatures. For respiring products, increasing the temperature also increases the rate of respiration, resulting in a decrease in shelf life. The effects of temperature abuse are particularly important from the standpoint of safety. Temperature abuse of MAP muscle foods may result in the rapid growth of both spoilage and pathogenic bacteria. The minimum reported temperature for *Salmonella* and *Escherichia coli* inoculated in ground meat grew equally well at 12.5 °C when the meat was packaged in low and high permeability film (Goepfert & Kim, 1975). *Staphylococcus aureus* can grow and produce enterotoxin under anaerobic conditions at 10°C or less while *Yersinia enterocolitica* has been reported to grow at temperatures as low as –2°C. Of major concern, with respect to safety of MAP fish, is the growth and toxin production by *Clostridium botulinum* type E (Palumbo, 1986). Proper refrigeration is therefore essential in order to assure the effectiveness of CO_2 as an anti microbial agent and to prevent potential growth of pathogenic organisms.

iv) Microbial contamination

The shelf life extension of fish under modified atmospheres is dependent on the initial

microbial load and the types of organisms. Higher initial counts will accelerate spoilage and shorten the shelf life of fish products. Furthermore, high numbers of aerobic bacteria will also consume rapidly headspace O_2 and may change electronegative potential of product and enhance the growth of anaerobic organism such as *Clostridium botulinum* type E.

Effect of MAP on the shelf life of fish

In vacuum-packed ice stored fish from temperate marine waters an increased development of TMA is seen while the shelf life is unaffected compared to aerobically stored fish. The number of *Pseudomonas* is reduced, but *S. putrefaciens*, which is capable of anaerobic respiration using TMAO, grows to 10^6-10^8 cfu/g (Gram *et al.*, 1987; Jorgensen *et al.*, 1988; Dalgaaard *et al.*, 1993). Jorgensen *et al.* (1988) observed that vacuum-packed cod consisted some very large, almost yeast like cells and suggested that these were involved in spoilage. It was recently shown that these cells are heat sensitive *Photobacterium phosphoreum* (Dalgaard *et al.*, 1993).

Banks *et al.* (1980); Brown *et al.* (1980); Finne (1982); Layrisse and Matches (1984); Lannelongue *et al.* (1982) and Stenstrom (1985) reported that CO_2 has been shown to delay spoilage of fresh seafood by inhibiting psychrotrophic aerobic Gram negative bacteria. Gill and Molin (1991) attributed the high growth rate of lactic acid bacteria and *S. putrefaciens* under 40% CO_2+30% O_2+30% N_2 to the tolerance of these microorganism to CO_2. Brown *et al.* (1980) noted an atmosphere of CO_2 inhibited microbial growth and extended sensory acceptance of salmon fillets. Ozogul *et al.* (2000a) reported lowest counts in MAP stored herring compared to those in air-stored herring. Ozogul *et al.* (2004) observed significant differences ($P<0.05$) between samples kept in air and in MAP. Dalgaard (1995); Gram and Huss (1996); Drosinos and Nychas (1996) suggested that *P. phosphoreum*, *S. putrefaciens*, lactic acid bacteria and *Brochothrix thermosphacta*, bacteria resistant to carbon dioxide are important for the spoilage of fresh fish stored under either VP or MAP. In similar studies Oberlender *et al.* (1983); Statham *et al.* (1985); Wang and Orgydziak (1986) reported that lactic acid bacteria and *Alteromonas* are the dominant organism in fish stored under MAP conditions. Statham *et al.* (1985); and Fletcher *et al.* (1988) reported that *B. thermosphacta* were the dominant organism in morwong fish and scallops when these were treated with polyphosphates or potassium sorbate and then stored under 100% CO_2. *S. putrefaciens* and some Vibrionaceae produce H_2S from the sulphur containing amino acid L- cysteine (Gram *et al.*, 1987; Stenstrom & Molin, 1990). In contrast, neither *Pseudomonas* nor *P.phosphoreum* produce significant amounts of H_2S. Thus, hydrogen sulphide, which is typical of spoiling iced cod stored aerobically, is not detected in spoiling CO_2 packed cod *(Dalgaard et al., 1993).* Methylmercaptan (CH_3SH) and dimethylsulphide [$(CH_3)_2S$] are both formed from methionine (Herbert & Shewan, 1975).

Quality Changes of fish in MAP storage

i) Physical Changes : Composition of the headspace gas mixtures

When compared to some packs containing meat products with similar concentrations of CO_2 (McMullen & Stiles, 1991), changes in the composition of gas mixtures within MAP fish packs appear to be rapid. The proportion of CO_2 in the headspace falls as CO_2 dissolves in fish tissues (Strasdine *et al.*, 1982 and Davis, 1990). As a consequence, proportions of the diluent components increase and concentrations of oxygen, often above the atmospheric levels to start with, increase further. Later, as the CO_2 solubilisation rate is overtaken by the rate of release caused by bacterial respiration, the curve reverses. Any chemical effects on the fish tissues will be affected by the amount of CO_2, which dissolves, but as the contaminant flora is limited to the fish surfaces, much of the bacteriostatic effect is likely to be influenced by the residual atmosphere. Davis (1990), working with cod fillets packed in a gas mixture containing 40% CO_2 found that the G/P ratio had to be at least 2:1 for maximal benefit. For a given gas mixture, high gas/ fish ratio will present a very different chemical balance from a low ratio. Similarly, any given mass ratio of CO_2 to product will not necessarily confer the same benefits (or harm) when applied via different gas mixtures. Silva and White (1994) in modified atmosphere packed refrigerated channel cat fish observed CO_2 concentration fluctuated between 63% and 87% for higher CO_2 concentration packs and 16 to 33% in low CO_2 concentration packs. Samples stored under air had increased CO_2. The increase in CO_2 in air packed fillets was the result of microbial respiration. Debevere and Boskou (1996) observed in MAP cod fillets a decrease in CO_2 because of diffusion in to the fish muscle.

Table 3. Shelf life of fish products packaged under MAP, vacuum and air

Type of fishery products	Storage temperature (°C)	Atmosphere CO_2:N_2:O_2	Shelf life (days)	Reference
Cod (*G.morhua*)whole	2	100:0:0	10	Jensen *et al*
	2	60:40:0	10	(1980)
	2	40:60:0	9-10	
	2	Vacuum	8-9	
	2	Air	7	
Rockfish (*Sebastes* spp.) fillets	1.7	CA 80:20 air	13	Parkin *et al* (1981)
		Air	6	
Salmon, king	2	Air	8	Stier *et al* (1981)
(*Oncorhynchus tshawytsche*) fillets	4.4	60:15:25	12	
	4.4	Air	6	
	22.2	60:15:25	2	
	22.2	Air	1	
Cod fillets	0	40:30:30	12.5	Cann *et al* (1983)
	5	40:30:30	<7	
	10	40:30:30	3	
	0	Vacuum	9	
	5	Vacuum	<4	
	10	Vacuum	2	
Crayfish	4	80:20air	21	Wang & Brown (1983)
(*Pacifastacus leniusxulus*) whole cooked	4	Air	14	
Swordfish	2	Air	6*	Oberiender *et.al* (1983)
(*Xiphias gladius*) steaks	2	CA 100:0:0	>22*	*Shelf life based
	2	CA 70:0:30	>22*	on microbial data only
	2	CA 40:0:60	14*	
	2	CA 70:30:0	>22*	
	2	CA 40:60:0	20*	
Trout whole	0	60:40:0	8	Cann *et al* (1984)
	5	60:40:0	8	
	10	60:40:0	3.8	
	0	Vacuum	9	
	5	Vacuum	6.5	
	10	Vacuum	3.7	
Snapper	3	100:0:0	6-8	Scott *et al* (1984)
(*Chrysophrys auratus*) fillets	3	Vacuum	3*	*No/medium O_2 barrier mat
	3	Vacuum	6**	**High oxygen
	3	Air	3	barrier material
Cod (*Gadus morhua*)	1	CA 60:40:0	12	Woyewods et.al (1984)
fillets	1	60:40:0	12	
	1	Air	9	
Salmon steaks	0	60:40:0	12.9	Cann *et.al* (1984)
	5	60:40:0	7.1	
	10	60:40:0	3.4	
	0	Vacuum	11.8	
	5	Vacuum	8	
Flounder	26	Air	2	Post *et al* (1985)
(*Limanda ferrugina*) fillets	26	Vacuum	2	
	26	0:100:0	4	
	26	100:0:0	1	
	12	Air	5	
	12	Vacuum	8	
	12	0:100:0	7	
	12	100:0:0	8	
	8	Air	5	
	8	Vacuum	7	
	8	0:100:0	4	
	8	100:0:0	10	
Shrimp, spotted	0	CA100:0:0	>14	Matches &
(*Pandalus platyceros*)	0	Air	7	Layrisse (1985)
whole head on/off	0	Air	7	
Cod (*G.morhua*) fillets	26	100:0:0	2-3	Post *et al.* (1985)
	26	Other atmosphere*	2	*All packaging types at

Table 3 contd.

		Air		26°C (air, vacuum,
	12		6	100% Nitrogen,
		Vacuum		90:8:2 and
	12	0:100:0	10	65:31:4) except
	12	100:0:0	13	100% CO_2 had a
	12	Air	11	shelf life of 2 days
	8	Vacuum	06	
	8	0:100:0	16	
	8	100:0:0	17	
	8	90:8:2	23	
	8	65:31:4	17	
	8	100:0:0	16	
	4		40-53	
Cod fillets	0-2	Air	11	Gopal, 1985
		CAP(50%	19	
		O_2+50%CO_2)		
		Air	5	
		CAP(50%	11	
	5-7	O_2+50%CO_2)		
Whiting	26	All atmosph*	2*	Post *et al* (1985)
(*Merluccius bilinearis*) fillets	12	Air	5	*All packaging
	12	Vacuum	9	types at 26°C (air,
	12	0:100:0	9	vacuum, 100%N_2,
	12	100:0:0	12	100%$C0_2$,90:8:2
	3	Air	4	and 65:31:4) had a
	8	Vacuum	10	shelf life of 2 days
	8	0:100:0	10	
	8	100:0:0	15	
	8	90:8:2	13	
	8	65:31:4	7	
	4	100:0:0	15	
Snapper (*C.auratus*) fillets	-1	40:60:0	9	Scott *et al.* (1986)
	-1	Air	9	
	-1	100:0:0	18	
Cod (*G.morhua*)	0	Air	12-13	Villemure *et al*
whole/fillets	0	25:75:0	20	(1986)
Trout (*Salmo gairdneri*) fillets	1.7	80:20:0	20	Barnett *et al*
	1.7	80:20:0	20*	(1987)
	1.7	Air	10	*Treated with 2%
				potassium sorbate dip
Sardines	5	80:20:0	4	Fujii *et al* (1989)
(*Sardinops melanostictus*)	5	20:80:0	4	
	5	Air	2	
Catla (*Catla catla*),	0-4	Air	12	Gopal *et.al* (1990)
fillets	50%	20		
	O_2+50%CO_2			
	20%	28		
	O_2+80%CO_2			
Cod (*G.morhua*) fillets	0	2:98:0	14	Dalgaard *et al* (1993)
	0	3:97:0	13	
	0	29:71:0	16	
	0	48:52:0	20	
	0	97:3:0	15-16	
Haddock (*Melanogrammus*	0	40:30:30	10	Dhananjaya &
aeglefinus) whole	0	Air	8	Stroud (1994)
	5	40:30:30	7	
	5	Air	7	
	10	40:30:30	4	
	10	Air	4	
Catfish, channel	2	80:20 air	28	Silva & White
(*Catalurus punctatus*)				(1994)
fillet strips				
Cod, blue (*Arapercis colias*)	3	100:0:0	49	Penney *et al* (1994)
fillets	3	Vacuum	14	
commercially smoked	3	Air	14	
	-1.5	100:0:0	113	
	-1.5	Vacuum	35	
	-1.5	Air	28	

Table 3 contd.

Herring (*Clupea harengus*) fillets	0	60:40:0	14	Dhananjaya & Stroud (1994)
	0	Air	12	
Herring (*C.harengus*) whole	0	60:40:0	14	Dhananjaya & Stroud (1994)
	0	Air	12	
Hybrid striped bass (*Morone saxatilis, M.chrysops*) strips	2	60:34:6	13	Handumrongkul & Silva (1994)
	2	Air	7	
Tilapia (*Tilapia* spp.) fillets	4	75:25:0	>25	Reddy *et al* (1995)
	8	75:25:0	13-16	
	16	75:25:0	9-13	
	4	Air	9-13	
	8	Air	6-9	
	16	Air	3-6	
Trout, rainbow (*Oncorhynchus mykiss*) fillets	2	20:80:0	6*	Randell *et al* (1995) *G/P ratio 0.4 **G/P ratio 1.0
	2	20:80:0	9**	
	2	40:60:0	6*	
	2	40:60:0	9**	
	2	Vacuum	6	
Herring, Baltic fillets	2	20:80:0	3*	Randell *et al* (1995) *volume of gas to product ratio 0.4 **volume of gas to product ratio 1
	2	20:80:0	3**	
	2	40:60:0	6*	
	2	40:60:0	8**	
	2	Vacuum	3	
Mackerel (*Scombrus scombrus L*) fillets	-2	100:0:0	>21	Hong *et al* (1996)
Sea bream (*Argyrozona argyrozona*)	0-4	Air	22	Gopal *et al* (1996)
		20% O_2+80%CO_2	40	
Salmon, atlantic (*Salmo salar*) slices	2	100:0:0	18	Pastoriza *et al* (1996)
Catfish fillets	16	Air	3	Reddy *et al* (1997a)
	16	75:25:0	4	
	16	Vacuum	3	
	8	Air	6	
	8	75:25:0	13	
	8	Vacuum	6	
	4	Air	13	
	4	75:25:0	38-40	
	4	Vacuum	20-24	
Salmon fillets	16	Air	4	Reddy *et al* (1997b)
	16	75:25:0	5-6	
	16	Vacuum	3	
	8	Air	13-17	
	8	75:25:0	20-24	
	8	Vacuum	>6<10	
	4	Air	24-27	
	4	75:25:0	55-62	
	4	Vacuum	34-38	
Hake (*Merluccius merluccius*) slices	2	50:45:5	14	Pastoriza *et al* (1998) *5 min 5%Nacl dip
	2	50:45:5	16*	
	2	Air	7-8	
Cod fillets	16	Air	3-4	Reddy *et.al* (1999)
	16	75:25:0	6	
	16	Vacuum	3-4	
	8	Air	13-17	
	8	75:25:0	24-27	
	8	Vacuum	13	
	4	Air	20-24	
	4	75:25:0	55-60	
	4	Vacuum	24-27	
Salmon (*S.salar*) fillets	10	Vacuum	3	Randell *et.al* (1999)
	2	60:40:0	17	
	2	40:60:0	17	
	2	Vacuum	17	
	2	Air	11	

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

Reciprocally to the CO_2 content the proportion of O_2 increased due to respiration of bacteria and the proportion of O_2 decreased later on. Amanatidou *et al.* (2000), in fresh Atlantic salmon using 50% CO_2 + 50% O_2 observed increased O_2 concentration in the packs.

ii) Changes in thaw drip during storage

Exudate or drip in increased amounts is another consequence of treating fish with CO_2. Normally, the small amount released from raw fillets is not a great problem but it becomes a limiting factor for some products in MAP. The problem may be solved by limiting the amount of CO_2 and by placing the fish on absorbent pads within the packs (Tiffiney & Mills, 1982; Cann, 1984). The lower water content of smoked products and fatty fish leaves them less vulnerable to further losses under 60% CO_2 than with raw white fish. The problems are aggravated at the lower storage temperature, perhaps a reflection of the effect on the solubility of CO_2. Pastoriza *et al.* (1996) observed no marked effect of CO_2 on exudation of salmon slices stored in ice under CO_2 atmospheres. Dalgaard *et al.* (1993) observed an increase in drip loss during MAP storage of cod fillets, especially at high CO_2 concentrations. Pastoriza *et al.* (1998) observed increased exudation in air and MAP stored fish showed exudates values higher than control after 7 days of storage. Fey and Regenstein (1982) found increased drip losses for red hake, Chinook salmon and to a lesser extent, Sockeye salmon stored in 60% CO_2, 21% O_2 and 19% N_2 compared to air packed product. Tiffiney and Mills (1982) found that packing in 100% CO_2 increased the rate and quantity of drip loss. In all cases the amount of drip loss of fish stored in high CO_2 concentrations was higher at 0°C than at 5°C.

iii) Changes in pH during storage

Apparent contradictions arise in considering almost every aspect of MAP fish including muscle pH. Fey and Regenstein (1982) reported little or no change whilst others have seen a decrease proportional to CO_2 concentration (Lannelongue *et al.*, 1982a, 1982b; Belleau & Simard, 1987). Fish muscle of relatively high post mortem pH can be expected to be more affected by a given amount of CO_2 than muscle of lower pH but this will be complicated by other variations in the chemical composition affecting the buffering capacity of the tissue. Additional variation in reported measurement may also occur because of differences in method of measurements, mainly because of the gradients, which occur between the product surface and deeper tissue (Tiffiney & Mills, 1982). Initial dissolution of CO_2 (preceding formation of carbonic acid) and bacterial activity (which produces high pH waste products) are surface phenomena, and, therefore, the most rapid and extreme fluctuations in pH occur on the surface. The general pattern seems to be that after any initial fall in pH, surface pH rises whilst internal pH lags behind.

Pastoriza *et al.* (1996) observed an increase in pH in air stored samples throughout the storage period, with a value of 8.03 after 21 days; MAP stored samples showed lower pH values throughout storage. Dalgaard *et al.* (1993) reported a pH increase from 6.6 to 6.8 in low CO_2 concentrations, but it remained almost constant at 6.6 in high CO_2 concentrations. Debevere and Boskou (1996) observed counter effects of CO_2 on the pH increase by TVB production, resulting in a stabilization of the pH around 6.7. Pastoriza *et al.* (1996) observed slight increase of pH in the control during the low temperature storage of salmon slices whereas in MAP, an initial decrease of pH was observed followed by an increase after 6 days of storage. Layrisse and Matches (1984) indicated that CO_2 is rapidly dissolved or absorbed in MAP stored muscle and consequently, pH decreased. Parkin *et al.* (1981) and Lannelongue *et al.* (1982) observed that the decrease of pH could be proportional to the CO_2 content of the package. Banks *et al.* (1980) and Parkin *et al.* (1981) attributed the pH change due to conversion of CO_2 to carbonic acid at the fish muscle surface. Pastoriza *et al.* (1998) observed a pH value of 7.74 in control samples of hake slices after 2 weeks iced storage; fish stored under MAP conditions showed pH values lower than the control after ten days storage. Lopez- Galvez *et al.* (1998) observed a pH increase in refrigerated storage of air packaged sole and only a slight increase was observed in samples stored in 20% CO_2 and no change in pH were observed when samples were stored in CO_2/air (40/60) (v/v). Therefore in general, the dissociation of carbonic acid in fish flesh results in a slight drop in pH. Both the buffering capacity of the fish proteins and the composition of the spoilage flora determine the magnitude of pH change (Cutting, 1953). However Barnett *et al.*

(1978) found no significant change in pH of salmon flesh stored in 90% CO_2. The extent to which pH decreases is proportional to the concentration of CO_2 in the atmosphere (Lannelongue *et al.*, 1982a; Tiffiney &nd Mills, 1982). Parkin *et al.* (1981) noted a drop in pH of rockfish from pH 6.7 to 6.3, which was maintained throughout the storage period. However Fey and Regenstein (1982) found that after an initial decrease, pH increased and after 27 days storage in a CO_2 enriched environment had reached a level similar to its initial pH.

iv) Changes in Biochemical parameters

a) Changes in Trimethylamine Nitrogen (TMA-N) content

Dalgaard *et al.* (1993) observed that the level of TMA was typically around 10-15 mg TMA-N/100g in aerobically stored fresh fish when rejected by sensory panels. Ababouch *et al.* (1996) observed that the limit of acceptability for sardines was found to be 5-10 mg TMA-N/ 100g of samples according to comparison of sensory and chemical data. Pastoriza *et al.* (1996) observed very low TMA values for salmon slices. Such low values can be consequences of a very low bacterial load leading to a very much-reduced enzymic deterioration of fish muscle (Gerdes *et al.*, 1989). Ozogul *et al.* (2000b) reported a value of 13.5 mg in herring kept in MAP (CO_2:N_2 60/40) for 10 days at 2±2°C. Cann *et al.* (1983) reported a TMA value of 10.4 mg/ 100g in herring fillets stored in MAP with the same gas mixture for 11 days at 0°C. Ozogul *et al.* (2004) reported that significant differences were discovered between sardines held under air and vacuum pack and MAP after 4 days of storage. The concentration of TMA in numerous fatty fishes never reached the limit of 5 mg / 100g although the rejection limit in flesh is usually 5-10 mg /100g.

b) Changes in Total Volatile Base Nitrogen (TVB-N)

The more rapid increase of TVB-N at high microbial numbers indicated the stage of substantial spoilage of the fish. Ababouh *et al.* (1996) reported that the limit of acceptability for sardines was 25-35 mg /100g of flesh. Ozogul *et al.* (2004) reported TVB-N content of sardines stored in air, vacuum pack, and MAP at 4°C. Initial TVB-N value was 5 mg/100g for sardines stored under air. The release of total volatile bases increased up to 15 mg/100g for sardines in VP and 17 mg/100g in MAP at the last day of sensory acceptability for each storage conditions. Pastoriza *et al.* (1996) observed salmon slices stored under CO_2 showed a much slower increase, with values much lower than those considered as limit of acceptability after 20 days storage, and similar to those of control after 10 days storage. They considered 30 mg TVB-N/ 100g as limit of acceptability. Pastoriza *et al.* (1998) observed a reduction in TVB-N values during MAP storage of hake slices, assuming 35 mg TVB-N/100g as the limit of acceptability for consumption of fish.

c) Changes in Thio Barbituric Acid Value (TBA)

Boyd *et al.* (1992) reported very low TBA values in pond raised striped bass and Kyrana and Longovis, (2002) and Papadopoulos *et al.* (2003) also reported very low TBA values for farmed sea bream stored under air in chilled conditions. Pastoriza *et al.* (1996) found increased TBA values in CO_2 modified atmospheres compared to air stored samples in salmon slices and hake. Amanatidou *et al.* (2000) found that salmon samples stored under modified atmospheres and kept under chilled conditions, with TBA value above 1.9 were characterized by unacceptable organoleptic characteristic

d) Changes in K value during storage:

The initial quality loss in fish is primarily caused by autolytic changes. Of particular importance in this respect is the degradation of nucleotides (ATP- related compounds), which is caused by autolytic enzymes.

Boyle *et al.* (1991) studied the adenine nucleotide degradation in modified atmosphere chill stored whitefish and rainbow trout. The results indicated CO_2 atmospheres did not alter the K values compared to those observed for aerobically held fish. However CO_2 atmospheres caused decrease in hypoxanthine concentrations compared to aerobically held samples. Huynh *et al.* (1992) found the same results with sockeye salmon and herring. Randell *et al.* (1995) studied the effect of G/P ratio and CO_2 concentration on the shelf life of MAP fish. The results indicated CO_2 concentration did not affect the K values of Rainbow trout. Reddy *et al.* (1997) also found that at 4,8, and 16°C storage K values of MAP fillets of cat fish increased gradually during early storage time and decreased towards end of

storage period indicating no relationship between sensory spoilage and K value. Lopez-Galvez *et al.* (1998) with sole fillets reported no effect of the atmosphere on K values. Ozogul *et al.* (2000a) studied the effects of modified atmospheres on K values of herring stored at 2°C. They observed 60% CO_2 atmospheres showed lower K values compared to those observed for aerobically stored fish, in addition CO_2 decreased the formation of Hypoxanthine compared to aerobically and vacuum held fish. Ozogul *et al.* (2004) studied the effects of modified atmospheres on K values of sardines at 4°C. The lowest increase in K value was observed for sardines stored in MAP, which was possibly influenced by the presence of CO_2.

v) Changes in colour during storage

Discoloration can occur via bleaching action of cut surfaces (Cann, 1984) probably due to low pH precipitation of sarcoplasmic proteins (Statham & Bremner, 1989). There is also risk of discoloration of haem pigments (Przybyski *et al.*, 1989). Brown *et al.* (1980) demonstrated that inclusion of 1% CO in the gas mix could help retain a red color, unlike Fey and Regenstein (1982) who observed a negative effect. Inclusion of O_2 as a means of maintaining more attractive red color of haem pigments is recommended for red meats but high concentrations are necessary. With fatty fish, Tiffiney and Mills (1982) found that the fresh appearance was retained for longer in O_2- free packs. High concentrations of CO_2 cause discoloration of meats, especially red meats. The degree of redness depends upon the amount of pigment present in the meat and the availability of O_2. Myoglobin can be oxidized in an atmosphere rich in CO_2 to form metmyoglobin, thus reducing the amount of redness to a light brown discoloration. Since fish are low in myoglobin, the discoloration induced by elevated concentrations of CO_2 is not as noticeable as in red meat. Colour changes in fish include graying of the cornea (Coyne, 1933), bleaching of the skin and damage to bloom at high CO_2 concentrations (>60%) (Stansby & Griffiths, 1935). Goodfellow (1982) found it necessary to restrict CO_2 levels to 25% or less to prevent discoloration problems.

Silva and White (1994) in modified atmosphere packaged refrigerated channel cat fish observed increased Hunter L* values in all packaging environments over time. Hunter a* value had an initial decrease in fish held in high CO_2 concentration but increased as time progressed. Hunter b* values showed no differences among any treatments. Pastoriza *et al.* (1996) observed increased Hunter L* and b* values and decreased Hunter a* value in both air and MAP stored samples during the storage period. Amanatidou *et al.* (2000) in modified atmosphere packaging of Atlantic salmon observed a product with lightness above the threshold value of 70 or red colour below 13 is unacceptable.

vi) Changes in texture during storage

Texture of the fish is very important, since a poor texture can result in bad visual appearance of the product. Springiness can be used to simulate finger feel of the raw fillets/ portions, and shear value as an index of tenderness/firmness of the raw fish. The most common types of measurements are based upon rheological principles of shear strength, puncture and compression.

Amanatidou *et al.* (2000) observed a lower cutting strength in MA packed fresh Atlantic salmon, indicating an increase in the softness upon storage. Textural characteristics were retarded in MAP compared to control. Fagan *et al.* (2004) tested effects of MAP with freeze chilling on some quality parameters of raw whiting, mackerel and salmon portions. The results indicated that storage time had no effect on the springiness of whiting, mackerel or salmon portions. MAP had no effect on the shear values of whiting fillets or salmon portions. However mackerel fillets stored in 100% CO_2 had higher shear values than samples from other treatments.

Effect of MAP on microbiological hazards

Fish stored under MAP may not increase the risks from Salmonella, Staphylococcus, *C.perfringens, Yersinia, Campylobacter, Vibrio parahaemolyticus* and Enterococcus above those expected for air stored products (Silliker & Wolfe, 1980: Reddy *et al,* 1992). Growth of *L.monocytogenes* is apparently not significantly affected by MAP (Rocount & Cossart, 1997). Pothuri *et al* (1996) observed an 8 days extension of the lag phase of *L.monocytogenes* in samples of crayfish tail meat treated with 1% lactic acid and packaged under MA conditions (75% $C0_2$:10% 0_2, 15% N_2) as compared with that in air or vacuum packaging. 100% CO_2 delayed, but did not inhibit the growth of *L.monocytogenes* as

compared with air in inoculated raw and cooked seafood nuggets (Lyver *et al.*, 1998) and was able to reach 10^6 cfu/g before spoilage occurred in all packaging methods. Packaging under enhanced CO_2 atmospheres will delay the growth of *L.monocytogenes*, but the MAP and chilled storage alone are not sufficient to control the growth of the pathogen in some products. To ensure the safety, especially for value added sea food products with extended shelf life, additional hurdles must be applied. Modified atmosphere packaging conditions create an environment, which supports the growth of psychrotrophic, anaerobic bacterial pathogens, including non-proteolytic types of *C. botulinum*.

Genigeorgis (1985); Baker and Genigeorgis (1990); Stammen *et al.* (1990); Reddy *et al.* (1992) have reviewed the risks from botulism in MAP fish. Post *et al.* (1985); Garcia and Genigeorgis (1987); Taylor *et al.* (1990) reported that toxin has been detected in MAP fish prior to the product being considered spoiled. Cann *et al.* (1984); Reddy *et al.* (1996; 1997a; b; 1999) and Cai *et al.* (1997) reported that MA or vacuum packed fish spoiled prior to or in coincidence with toxin production. Gibson *et al.* (2000) demonstrated that 100% CO_2 could have an inhibitory effect on the growth of *C.botulinum* at chill temperatures, and an inhibitory effect was observed when combining 100% CO_2 with increased NaCl level and decreased pH level. Reddy *et al.* (1999) reported the fat content might influence the margin of safety of MA packaged aqua cultured fresh fillets during storage. The safety margin being less for fatty fish when compared with lean fish. Lilly and Kautter (1990) reported in a study of 1074 test samples of commercial, vacuum packaged fresh fish, none of the marginally organoleptic acceptable samples was positive for *C. botulinum* after 12 days at 12°C. The authors concluded that either the fish did not contain *C.botulinum* spores, or the spores were unable to grow out and produce toxin before the spoilage made the product marginally unacceptable. However, an increased use of modified atmosphere and other minimal processing technologies combined with improper cold chains and abuse of temperature may represent an increased risk of botulism.

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