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Use of Modified Atmosphere in Seafood Preservation

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ABSTRACT

Fish production has increased in Brazil, mainly the fresh-water aquaculture. However, refrigerated fish presents a relatively short shelf-life (approximately 12 days). In view of the increasing demand for fresh products, there is the need of shelf-life lengthening through a combination of methods in order to minimally expose the product to adverse conditions. The use of the modified atmosphere packaging presents the following advantages: lengthening of the products' shelf-life by 50 to 400%, reduction of economic losses, cost reductions by distributing the product over great distances with the need of fewer shipments and the supply of a better quality product. The purpose of this work was to review and discuss the major effects of the modified-atmosphere packaging, especially carbon dioxide (CO_2), on seafood preservation, as well as on the survival and growth of seafood pathogenic organisms.

Key words: Seafood, freshwater fish, aquaculture, modified atmosphere, vacuum packaging, shelf-life

INTRODUCTION

Fish production in Brazil has been increasing as a result of the expansion in freshwater aquaculture activities. Due to the country's great freshwater potential, fish cannot be regarded only as an excellent source of food, but also as a source of exportation revenues (Oetterer, 1991). Fish has a relatively short shelf-life (12 days) under refrigerated conditions, not presenting hygienic quality hazards when properly packaged (Oetterer, 1999). The shelf-life of many perishable products, such as meat, eggs, fish, poultry, fruit, vegetables and cooked food, is affected by the presence of atmospheric oxygen and is conditioned by three important factors: i) reactions with the atmospheric oxygen; ii) growth of deteriorating aerobic microorganisms; iii) insects attack. Each of these factors, or their combination, leads to alterations in color, flavor, odor and global deterioration of food quality (Smith et al., 1987). There has been a recent interest in lengthening fish shelf-life due to the increase in demand for fresh products, which has led to a greater variety of products being packaged under modified atmosphere, in which air composition is altered or "modified". Such increase in fish shelf-life brings great industrial advantages, once it reduces losses in distribution and display of the product at the retail stores, which may lead to improvements in marketing of fresh products and stabilize the supply at reasonable prices (Lioutas, 1988).

The purpose of this work is to discuss the effects of the modified-atmosphere packaging, especially carbon dioxide (CO_2), on seafood preservation, as well as on the survival and growth of seafood pathogenic organisms.

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LITERATURE REVIEW

Fish and the aquatic environment

Fresh fish is normally considered a safe food however it can be an important source of bacterial food poisoning (Shewan, 1962). Oetterer (1991) described that, due to the presence of excellent quality proteins associated with high water contents, as well as the presence of reasonable amounts of free nitrogen substances that favor deterioration, fish can support great microbial development. Germano et al. (1998) mentioned that fish may be a vehicle for spreading pathogenic microorganisms among human beings. *Vibrio parahaemolyticus* has caused acute gastroenteritis, known for its dysenteric symptoms, especially after the consumption of fresh fish, shrimp and oyster. *V. cholerae* from humans contaminates waters in the sea, rivers and lakes, and may cause deaths in the affected population. *Salmonella* (*S. typhi* and *S. paratyphi*) and *Shiguella* can be found in waters contaminated by sewage or animal waste. As a direct consequence of improper handling, fish can become a favorable environment for the multiplication of *Streptococcus* sp and *Staphylococcus aureus*. Other bacterial agents can contaminate fish, representing health hazard, for example, psychrophilic strains of *Bacillus cereus* can produce enterotoxin in fish products, especially those presenting pH above 6.0, causing diarrhea occurrences.

Dodds (1993) states that waters are frequently contaminated with *Clostridium botulinum* spores and it is expected that fish will be also contaminated. Fish contamination may occur due to exposure to spores before fish capturing, during processing or storage.

An advantage of fresh water aquaculture over seafood production is the possibility of monitoring the water quality in order to ensure the absence of contamination by *V. cholerae*, heavy metals, pesticides and other contaminants (Antunes, 1997).

Lima and Reis (2000) analyzed pacu (*Piaractus mesopotamicus*) commercialized in Cuiabá, MT, Brazil, as to the presence of *Salmonella*, in rivers and production places. The authors compared different methodologies for the isolation of strains, sampling the specimens from fishing areas, production places, street markets and supermarkets. Thirty-five percent of the specimens were found to be contaminated, and 70% of these ones were taken from production tanks.

There is immense diversification of contaminated environments and great microbial variety, being the greatest concentration of microorganisms found in the fish's bowels, gills and skin. Ward (1994) states that a low rate of contaminants in the gills and skin are commonly associated to clean and cold waters, while higher contaminant levels are related to tropical and subtropical waters, as well as to polluted areas. The high contaminants level in the bowels is directly related to feeding, which is intense in artificially fed fish and low in extensively farmed fish.

Intensively farmed fish generally presents sanitary problems under control and are raised in clean and good-quality water, as it is the case of salmon and trout in temperate zones. However, in clay-bottom tanks with little water circulation, there may occur the structuring of non-proteolytic strains of *C. botulinum*, leading to undesirable levels of contamination (ICMSF, 1997).

Fish shelf-live and deterioration

Fish shelf-life is determined by the intensity of enzymatic reactions and by the number and species of microorganisms, affecting the product's perishability. Other determining characteristics on shelf-life are the storage temperature, which must be evaluated along the several production stages, the temperature during capture, delay in refrigeration, variation in the storage temperature and the retail temperature (Huss, 1971).

According to Braga et al. (2000), the determining factor to improve fish quality is the early sanitation of the captured fish using treated water. The authors monitored the water and shrimps (*Xyphopeneaus kroyeri* and *Penaeus schmitti*), evaluating the microbiota associated to the surface of shrimps.

The number of bacteria in fresh fish can be decreased and the shelf-life prolonged through washing the product using high pressure water jets or using a 0.1% chlorine-cetylpyridine solution (Mayer and Ward, 1991). Sprayed chlorine, chlorine dioxide or potassium sorbate can also be used to reduce the bacterial count in fresh fish (Mayer and Ward, 1991; Lin et al., 1996).

Several methods can be used to decrease or inhibit decaying changes, such as refrigerated storage, freezing, drying, heat processing, use of additives and chemical preservatives, irradiation and packaging (Sharp et al., 1986).

Packaging and combined processes

Food preservation is based on combined methods, which can be used for the quality improvement of conventional products or the development of new products. They assure stability and safety, resulting in products presenting adequate sensory and nutritional properties (Leistner, 1992).

Along the latest three decades there has been an increase in gas packaged food products in the market. This increase has brought improvements to the packaging industry, which has lead to the development of high barrier polymers and thermo-mold packaging equipment. Gas packaging is simply an extension of the vacuum packaging technology. Food packaging under modified atmosphere employs different gases, such as CO_2, N_2, and O_2, with CO_2 probably being the most common and effective gas, whether associated with other gases or not (Wolfe, 1980).

There are several techniques through which the atmosphere around a product can be modified and, frequently, there may be some confusion about the terminology used. According to Silliker and Wolfe (1980), the two most relevant techniques applied to fish and its related products are:

- *Modified Atmosphere Packaging – MAP:* the air inside the packaging is replaced by a specific gas or a mixture of gases that differ from the air composition. The proportions of each gas are established, the mixture is introduced into the packaging and no further control is carried out during storage (Silliker and Wolfe, 1980);
- *Vacuum packaging:* the product is placed inside a type of packaging presenting low permeability to oxygen, the air is exhausted and the packaging is sealed. The gaseous atmosphere of the vacuum packaging is reduced, but it is probably altered during storage, thus considered modified due to a 10 to 20% increase in the CO_2 amount produced by microbial activity. This CO_2 may inhibit the growth of undesirable microorganisms (Silliker and Wolfe, 1980).

The three main commercially used gases in modified atmosphere packaging are: carbon dioxide (CO_2), nitrogen (N_2) and oxygen (O_2). CO_2 is soluble, not only in water, but also in lipids, being the main responsible for the bacteriostatic effect in modified atmospheres. Its general effects on microorganisms are an intensification of their latest growth stage and a decrease in the growth rate during the logarithmic stage (Farber, 1991). The bacteriostatic effect is influenced by the CO_2 concentration, the initial bacterial population, the storage temperature and the product being packaged (Reddy et al., 1992).

In food presenting high moisture and/or fat amounts, such as fish, beef and poultry, the excessive absorption of CO_2 may lead to a phenomenon known as "packaging collapse" (Parry, 1993). Increase in dripping is caused by the dissolution of gases on the muscles 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. N_2 can be used as an inert gas in smaller proportions than CO_2. O_2 can also be employed, providing fish does not undergo color alterations (Cann et al., 1983).

According to Daniels et al. (1985), several theories can explain the ways that CO_2 influences bacterial cells, the most important being:

- Alterations in cell membranes functions, including effects on nutrients;
- Direct inhibition of enzymes or decrease in enzymatic reactions;
- Penetration in the bacterial membranes, leading to changes in the intra-cell pH;
- Direct alterations in physic-chemical properties of proteins.

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 low solubility, it is used as a filling gas, preventing the possible packaging collapse caused by the accumulation of CO_2.

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 lipids amounts, promoting the formation of low molecular weight aldehydes, ketones, alcohols and carboxylic acids. Thus, the use of O_2 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 (1995), who states that there are evidences showing that the use of O_2 reduces the exudation in fish during storage. The author suggests that O_2 can be used in low-fat fishes. Reddy *et at.* (1992) claim that the use of O_2 associated with N_2 or CO_2 gives a false idea of reducing botulism risks in fresh-packed fish and may lead to illusory safety.

Different types of fish, storages, temperatures and modified atmosphere packaging (MAP) have been used. MAP associated to high CO_2 levels improves the stability of fresh fish, increasing its shelf-life (Baker et al. 1986). Gas mixtures presenting 40% CO_2, 30% N_2 and 30% O_2 have been recommended for low-fat fish and a 40-60% CO_2 mixture, in equilibrium with N_2, has been recommend for fatty fish (Guidelines..., 1985).

Problems related to temperature abuses can occur with all manufactured foods, once the bactericidal and bacteriostatic effects of CO_2 vary with temperature (Wolfe, 1980; Church, 1998). Lak of refrigeration at any period throughout the product's shelf-life may allow the growth of microorganisms that had been inhibited by CO_2 during storage at low temperatures. Facultative anaerobic microorganisms and aerobic pathogens resistant to the antimicrobial effects of CO_2, but which were unable to grow at low temperatures, can also thrive as the result of temperature abuses (Wolfe, 1980).

Ogrydziak and Brown (1982) revised the temperature effects on the solubility and inhibition of CO_2. The authors concluded that, disregarding the synergetic mechanism between temperature and solubility, all evidences show that increases in temperature reduce solubility and increase microbial growth, which is proportionally higher in MAP than under atmospheric air.

The use of high hygienic-sanitary quality raw materials represents an important factor for the successful use of modified atmosphere packaging. Besides initially using high quality raw materials, the use of good hygiene practices during fishing, the selection of the right packaging material and a good temperature control are also necessary (Stammen et al., 1990).

Revisions done by Wolfe (1980); Daniels et al. (1985); Pedrosa-Menabrito and Regenstein (1988); Stammen et al. (1990); Church and Parsons (1995); Álvarez (2000) and Sivertsvik et al. (2002) documented the MAPs' ability to lengthen the shelf-life of several perishable products, such as meat, poultry and fish. Prentice and Sainz (2000) developed a minimally processed product made from grass carp (*Ctenopharyngodon idella*) washing fish fillets using sodium hypochlorite and brine and vacuum packing, achieving shelf-life periods of 30 and 60 days, when the product was kept under refrigeration temperatures of 8°C and 2°C, respectively.

Kosak and Toledo (1981) studied the combination of a chlorine solution (1000 μg/mL free chlorine) with vacuum polyethylene packaging for mullet (*Mugil cephalus*) kept at –2°C. All treatments were organoleptically acceptable up to 14 days of storage.

The effects of carbonic acid on cod (*Gadus morhua*) fillets packed in semipermeable film and kept at 1°C were tested by Daniels et al. (1985; 1986). The carbonic acid increased the shelf-life from 7 to 21 days. However, the organoleptic quality was considered to be poor. Results indicated that the carbonic acid was as effective as the 98% CO_2 controlled atmosphere.

Woyewoda et al. (1984) observed that bacterial growth and organoleptic deterioration in cod (*Gadus morhua*) fillets decreased slightly under 60% CO_2 atmosphere at 1°C. The small differences between samples kept in MAP and those kept in atmospheric air increased along with the storage period.

Matches and Layrisse (1985) studied shrimp (*Pandalus platyceros*) kept under 100% CO_2 controlled atmosphere. The authors observed moderate discoloration, not associated to undesirable smells, differing from the control group, fish exposed to atmospheric air, which was not in an acceptable state. The high CO_2 concentration delayed the appearance of black spots caused by enzymatic action.

According to Villemure et al. (1986), gutted filleted cod (*Gadus morhua*) bulk stored at 0 ± 1°C in 25%CO_2/75%N_2 atmosphere maintained reasonable organoleptic quality up to 20 days, outlasting cod stored in atmospheric air. As to fish surface pH, values of 6.6 and 7.5 were observed using MAP and atmospheric air, respectively. The chemical, physical and microbiological alterations in raised catfish (*Silurus glanis*) were evaluated during storage period by Manthey et al. (1988). The acceptable storage time was considered to be 20 days. On the 27th day of storage, fish fillets showed total anaerobic bacteria count of 10^8/cm^2 of fish skin and only 10^5/g of muscle. Ammonia amounts increased from 11.5 to 18.7 mg/100g of muscle, and TBARS (thiobarbituric acid reactive

substances) amounts varied from 0.73 to 1.98 mg of MA/Kg (malonaldehyde). Trimethylamine amounts were low, while dimethylamine was not detected.

The bacteria count, as well as the surface pH of catfish (*Ictalurus punctatus*) fillets packaged in 90%CO_2/7.5%N_2/2.5%O_2 atmosphere and in atmospheric air, stored at 0 and 10°C were evaluated by Silva et al. (1993). The authors observed that pH was not affected by temperature, but by storage time. The bacteria count was reduced in the MAP treatment.

Huang et al. (1993) packed weakfish (*Cynoscion regalis*) in different packaging materials, storing the product in ice for 3 weeks. The results showed that vacuum skin packing reduced fish rancidity and lipidic hydrolysis when compared to the traditional overwrapping packing. Microbiologic acceptability was limited to 14 and 17 days for the overwrapping and vacuum skin packing, respectively.

Reddy et al. (1994; 1995) evaluated the effect of modified atmospheres (75%CO_2/25%N_2; 50%CO_2/50%N_2; 25%CO_2/75%N_2) on the shelf-life of tilapia (*Tilapia* spp) fillets packed in high barrier film at 4°C. The authors observed that tilapia fillets packed in 75%CO_2/25%N_2 showed an increased shelf-life of more than 25 days, presenting acceptable sensory characteristics.

The shelf-life of hake (*Merluccius merluccius*) and salmon (*Salmo salar*) slices stored in ice (2 ± 1°C) under different atmospheres (40%CO_2/50%N_2/ 10%O_2; 60%CO_2/30%N_2/10%O_2; 40%CO_2/ 30%N_2/30%O_2; 60%CO_2/10%N_2/30%O_2), was evaluated by Pastoriza et al. (1996a,b). Hake and salmon slices could be ice stored in MAP for up to 21 and 18 days, respectively, with no quality loss.

The sensory, physical, biochemical and microbial qualities of Atlantic mackerel (*Scomber scombrus*) fillets stored and packed in modified atmosphere at –2°C were evaluated by Hong et al. (1996). MAP storage increased shelf-life to 21 days, causing a slight increase in TVBN and trimethylamine amounts. A number of coliforms, leaven and molds < 10 UFC/g was also detected.

López-Gálvez et al. (1995; 1998) evaluated tuna (*Thunnus alalunga*) slices and sole (*Solea solea*) fillets under different atmospheres (20%CO_2/80% atmospheric air; 40%CO_2/60% atmospheric air and 40%CO_2/60%O_2) stored at 2°C using physical-chemical and microbiological parameters. Sensory results showed that the shelf-life of tuna slices increased from 4 to 8 days under 20% and 40% CO_2, respectively. The 40%CO_2/60% atmospheric air atmosphere was the most effective, microbiologically and biochemically, for the tuna slices. As to the sole fillets, the 40%CO_2/60%O_2 atmosphere was the most effective, regarding microbiological and sensory aspects, increasing shelf-life by one week.

Randell et al. (1997; 1999) evaluated the quality of trout (*Salmo gairdneri*), herring (*Clupea harengus*) and salmon (*Salmo salar*) packaged in polyestirene films, under vacuum and MAP (35%CO_2/32.5%O_2/32.5%N_2; 35%CO_2/65%O_2; 40%CO_2/60%N_2) and stored at 2°C. The authors observed that mesophyll bacteria grew better in polyestirene packed fillets, while the number of coliforms was greater in vacuum packed fillets, which presented faster deterioration than MAP fillets. The sensory quality of trout and herring was similar for the three packaging types. The mixture of gases did not lengthen the fillets shelf-life.

Sivertsvik et al. (1999) studied the quality of refrigerated (≤ 1°C) gutted salmon (*Salmo salar*) stored in plastic bags containing 50% and 100% CO_2 and 60%CO_2/40%O_2, as well as in conventional packaging material (polyestirene) during transport. The authors observed that the microbial growth was greater in the conventionally packed salmon. The MAP salmon presented better sensory quality than the conventionally packed one after 13 days of storage.

Bak et al. (1999) studied the effect of MAP under 100% N_2 on shrimp (*Pandalus borealis*) stored at –17°C. The authors observed that the MAP promoted a 9-month shelf-life period, resulting in better color, lower oxidation and greater fish firmness than the storage in atmospheric air.

Ozogul et al. (2000) compared the quality of Atlantic herring (*Clupea harengus*) stored in ice-free boxes under vacuum and MA at 2 ± 2°C, using ice-stored herring as control. Through chemical and microbiological analyses, the authors observed that the herring shelf-life was extended by 10 and 8 days, for MAP and vacuum packaging, respectively, when compared to the ice stored herring.

Hurtado et al. (2000) evaluated the shelf-life of refrigerated (2 to 3°C) vacuum packaged (400 MPa) hake (*Merluccius capensis*) slices, which were sensory acceptable up to the 43rd day of storage. Low trimethylamine amounts and slight increase in drip were verified after 15 days of storage.

Ordóñez et al. (2000) stored hake (*Merluccius merluccius*) in atmospheres containing 20% and 40% CO_2, as well as in atmospheric air at 2 ± 1°C. The authors observed that the shelf-life increased to 4 and 11 days under 20% and 40% CO_2, respectively. The results showed that the 40% CO_2 atmosphere was more effective, as regards biochemical and microbiological parameters, for refrigerated hake.

Whole gutted hake (*Merluccius merluccius*) was ice stored in boxes under controlled atmospheres (CA) presenting different gas mixtures ($60\%CO_2/15\%O_2/25\%N_2$; $40\%CO_2/40\%O_2/20\%N_2$; $60\%CO_2/40\%O_2$ and $40\%CO_2/60\%O_2$) during 33 days at 0±1°C. Through physical-chemical and sensory analyses, Ruiz-Capillas and Moral (2001) found that the $60\%CO_2/40\%O_2$ CA promoted a better product as to the sensory acceptance, being more effective than the other mixtures.

Effect of MAP on microorganisms

Dalgaard et al. (1997) studied the *Photobacterium phosphoreum* growth in fresh MAP fish by means of 20 experiments conducted in Denmark. The authors did not detect the presence of *P. phosphoreum* in fresh water fish, despite finding great growth (> 10^7 UFC/g) of this microorganism in all marine species.

According to Dalgaard et al. (1993; 1995; 1998), *P. phosphoreum* is the most common bacterium in vacuum and MA (CO_2/N_2 mixtures) packaged cod (*Gadus morhua*) fillets stored at 0°C.

Debevere and Boskou (1996) evaluated the micro-flora behavior in cod (*Gadus morhua*) stored at 6°C under different atmospheres ($60\%CO_2/10\%O_2/30\%N_2$; $60\%CO_2/20\%O_2/20\%N_2$; $60\%CO_2/30\%O_2/10\%N_2$; $60\%CO_2/40\%O_2$). The authors observed that $60\%CO_2/40\%O_2$ MA presented inhibitory effect on the micro-flora growth and on the trimethylamine activity, reducing the number of *P. phosphoreum* cells.

Cod (*Gadus morhua*) fillets kept in atmospheric air at 0°C for 1 to 8 days before MA packing and frozen stored at -20 and -30°C for 6 weeks were evaluated by Boknaes et al. (2000; 2001). The presence of *P. phosphoreum* at 2.3 and 5.8 UFC/g amounts was detected, after 1 and 8 days at 0°C, respectively. Storage at –20 and –30°C reduced the amount of *P. phosphoreum* up to undetectable limits. Only fillets kept for 8 days at 0°C, and at –30°C afterwards, showed significant increase in *P. phosphoreum* growth, as well as in TMA amounts during defrosting at 2°C.

Boskou and Debevere (1997; 1998) studied the *in vitro* influence of intrinsic and extrinsic factors on the spoilage by *Shewanella putrefaciens* isolated from cod under $60\%CO_2/30\%O_2/10\%N_2$ MA. The authors observed that the increase in CO_2 amounts (≥ 50%) inhibited the growth of *S. putrefaciens* and, consequently, decreased the trimethylamine production. The authors suggest the 60–$70\%CO_2$/30–$40\%O_2$ combination to prevent the production of trimethylamine by *Shewanella* spp.

Davis and Slade (1995) studied the growth and survival of *Aeromonas* spp and *Yersinia enterolitica* in cod and trout (*Oncorhyncus mykiss*) under different atmospheres (($60\%CO_2/40\%N_2$; $40\%CO_2/30\%N_2/30\%O_2$) and ($60\%CO_2/40\%N_2$; $80\%CO_2/20\%N_2$), respectively, stored at 0, 5 and 12°C. The authors observed greater reductions in the microorganism's growth under the atmosphere richest in CO_2 and at the lowest temperatures.

The development of *Listeria monocytogenes* and *Aeromonas hydrophila* in vacuum packed catfish (*Ictalurus punctatus*) fillets, store at 4°C for 16 days, was studied by Leung et al. (1992). The authors did not observe any increase in the *L. monocytogenes* population, but observed a fast increase in the *A. hydrophila* population in products packed under atmospheric air.

An increase in the *L. monocytogenes* population was observed by Harrison et al. (1991) in vacuum and MA packaged shrimp (*Pandalus platyceros*) after 21 days of storage in ice.

According to Dorsa et al. (1993), *L. monocytogenes* growth was inhibited at 6°C, but temperature-abuse (12°C) conditions for short periods induced a fast growth of this microorganism in lobster (*Procambarus claekii*).

Church (1998) did not verify inhibition of *L. monocytogenes* in cod (*Gadus morhua*) stored under $40\%CO_2/30\%N_2/30\%O_2$ and $60\%CO_2/40\%N_2$ atmospheres.

Lyver et al. (1998a,b) studied the alterations in fresh and cooked surimi nuggets inoculated with 10^3 spores of *L. monocytogenes* and 10^4 spores of E-type *C. botulinum*. All the products were packed under atmospheric air with oxygen absorbent, and stored at 4 to 12°C during 28 days. The authors reported that counts of *Bacillus* spp and lactic acid bacteria in fresh nuggets increased to 10^2 and 10^7 UFC/g, respectively. However, only *Bacillus* spp

in cooked nuggets reached the figure of 10^4 UFC/g on the 28th day. In nuggets packed under 100% CO_2, with or without absorbent, the *L. monocytogenes* count increased to 10^7 UFC/g after 28 days of storage at both temperatures. The authors did not verify the presence of toxin from *Bacillus* spp in any product.

Many researchers are increasingly concerned about the increase in the growth potential of *Clostridium* in MAPs. This worry is justifiable by the pathogenic importance of such microorganism. *Clostridium perfringens* is the causal agent of gastrointestinal diseases. *Clostridium botulinum* produces a neurotoxin that causes facial paralysis classified into A, B, C, D, E, F and G types. The A, B and F types are important to humans (Hintlian and Hotchkiss, 1986).

Post et al. (1985) studied toxin production by E-type *C. botulinum* in cod (*Gadus morhua*), hake (*Merluccius bilinearis*) and flounder (*Limanda ferrugina*) packed in film permeable to atmospheric air, under vacuum and in N_2 and CO_2 packing, kept at 8, 12 and 26°C. The authors observed that flounder deteriorated quickly and was sensorially rejected before toxin production in vacuum and MA packed samples, stored at 12 and 8°C. On the other hand, the toxin production in cod and hake preceded or occurred simultaneously with the sensory rejection.

Baker et al. (1990) inoculated vacuum and MA (100% CO_2) packaged catfish (*Sebastes paucispinis*), salmon (*Oncorhynchus tshawytscha*) and sole (*Microstomus pacificus*) with E-type *C. botulinum* at seven spore concentrations, ranging from 10^{-2} to 10^4 spores/sample, with the products being stored for 60 days at temperatures between 4 and 30°C. The results showed the capability of a few spores, from each type, of growing individually or collectively at 8 and 30°C.

Silva e White (1994) studied color and pH changes in catfish (*Ictalurus punctatus*) fillets kept under 25% CO_2 and 80% CO_2 atmospheres, as well as in atmospheric air at 2 and 8°C during 4 weeks. The authors observed that there was a decrease in pH during the 4th week in the 80% CO_2 treatment. *C. botulinum* was not observed in any atmosphere, temperature or storage period. The best treatment was the 80% CO_2 at 2°C.

Reddy et al. (1996; 1997a,b) evaluated the E-type *C. botulinum* toxin production potential in farm tilapia (*Tillapia* spp), catfish (*Ictalurus punctatus*) and salmon (*Salmo* spp) fillets packed in high barrier film under 100% atmospheric air, 75%CO_2/25%N_2 MA and vacuum, stored at 4, 8 and 16°C. The authors observed a decrease in the fillets shelf-life packed in all atmospheres as a function of an increase in temperature from 4°C to 16°C. At 16°C, the toxin production preceded the sensory rejection for salmon fillets and was coincident with the sensory rejection for catfish. Yet, at 8°C, the toxin production coincided with the sensory rejection for salmon fillets. At 4°C, the sensory rejection preceded the toxin production.

Cai et al. (1997) inoculated catfish (*Ictalurus punctatus*) packaged in O_2-permeable film, in 80% CO_2 and 20% N_2 MA and stored at 4°C with a mixture of 4 E-type *C. botulinum* strains. The toxin production was detected after 9 and 18 days in the O_2-permeable film and MA packaging, respectively. The deterioration preceded the toxin production in all packing methods.

Pre-packing treatment with acids and salts

Some studies have been conducted on the possibility of combining MAPs with preservative methods, such as ice (Fey and Regenstein, 1982) or immersion in salty solutions (Regenstein, 1982).

Shaw et al. (1983) reported that the addition of potassium sorbate to fresh fish inhibited the growth of deteriorating microorganisms, such as *Pseudomonas fluorescens* and *P. fragi*, as well as the production of trimethylamine by *Altermonas putrefaciens*. The authors also observed the potassium sorbate effect on the pathogens inhibition such as *Clostridium botulinum* and *Staphylococcus aureus*.

The shelf-life of 2.5 and 5% potassium sorbate-treated herring fillets packed in low, intermediate or high barrier bags, under 100% CO_2 MA and kept under refrigeration below 3°C, was evaluated by Sharp et al. (1986). The 5% potassium sorbate treatment increased the fillets shelf-life, exceeding 14 days. As to the films' permeability, all packing methods enabled a shelf-life of 15 days; low barrier packaging promoted a shorter shelf-life.

The use of 50 to 100% CO_2 modified atmosphere has successfully increased the shelf-life of morwong (*Nemadactylus macropterus*) (Statham et al., 1985). The combination of treatments leads to a synergistic effect. Vaporized or ice-combined potassium sorbate, when used together with 60% CO_2, effectively increased the shelf-life of hake (*Merluccius merluccius*) and salmon (*Salmo* spp) at 1°C, to 4 weeks (Fey and Regenstein, 1982; Regenstein, 1982). Pathogens such as *Salmonella*

enteriditis and *Staphylococcus aureus* treated with potassium sorbate associated with storage in CO_2-enriched atmospheres showed to be more effectively inhibited than when isolated treatments were used (Elliott and Gray, 1981).

Williams et al. (1995) treated fresh catfish (*Ictalurus nebulosus*) fillets with 0.1 and 2.0% sodium lactate solution adjusted to 5.5 pH, then vacuum packed and stored the product at 1.11 ± 1°C. The shelf-life of the fillets treated with 2% sodium lactate was increased from 4 to 7 days.

Gibson et al. (2000) evaluated the growth of a spores mixture containing non-proteolytic B- and E-type *C. botulinum* isolated at 5 and 10°C. The mixture was used to evaluate the combined effects of NaCl (0.5–4.5%), pH (5.5–6.5) and different atmospheres ($10\%H_2/90\%N_2$; $5\%CO_2/10\%H_2/85\%N_2$ and 100% CO_2). The authors observed that, considering all the tested atmospheres, *C. botulinum* grew at low rates only when exposed to 100% CO_2. The results evidenced that CO_2 decreased the *C. botulinum* growth at refrigeration temperature and that the prevention of growth depends on the concentration of NaCl and pH.

Pastoriza et al. (1998) studied the effect of a mixture of gases ($50\%CO_2/45\%$ $N_2/5\%O_2$), combined with the sprinkling of sodium chloride, on hake (*Merluccius merluccius*) slices. The sprinkling of sodium chloride showed better biochemical and microbiological inhibition, while decreasing the sensory deterioration of MA packed hake slices. Exudation was also reduced and the shelf-life of the hake slices increased to 2 and 8 days, when packed under MA and when submitted to the sodium chloride solution treatment, respectively.

Boskou and Debevere (2000) studied the effect of 10% acetic acid on cod fillets packed under $50\%CO_2/45\%O_2/5\%N_2$ MA stored for 12 days at 7°C. The authors observed a reduction in aerobic microorganisms, H_2S-producing bacteria and enterobacteria, as well as a consequent inhibition of TMA and TVBN.

Taylor et al. (1990) studied the nisin inhibitory effect on the production of toxin by E-type *C. botulinum* in cod, herring (*Clupea harengus*) and smoked mackerel (*Scomber japonicus*) packed under 100% CO_2 and stored at 10 and 26°C. The authors observed that there was no shelf-life lengthening for the studied species. Toxin production had been verified for both temperatures and in all species before the products were considered improper for human consumption.

Nilsson et al. (1997) evaluated the bacteriostatic and bactericidal effect of nisin (500 – 1000 UI/mL) combined with 100% CO_2 and NaCl (0.5 – 5.0%) on the *in vitro* survival of *L. monocytogenes* in cold-smoked salmon (*Salmo* spp) stored at 5 and 10°C. The authors found that the addition of nisin to CO_2 led to a reduction of 1 to 2 log of *L. monocytogenes* followed by a lag-phase of 8 and 20 days, when 500 and 1000 UI/g of nisin were used, respectively. *L. monocytogenes* amounts remained below 10^3 UFC/g during 27 days of storage for both nisin concentrations.

In another study, cold-smoked salmon (*Salmo salar*) was vacuum packed under $60\%CO_2/40\%N_2$ atmosphere at 5°C. Nisin associated with CO_2 was used to inhibit the acid lactic-producing bacteria, as well as Gram-negative bacteria. Paludan-Müller et al. (1998) observed an inhibition in the Gram-negative bacteria growth and a 4-week shelf-life lengthening under the same conditions.

Mitsuda et al. (1980) related the effects of formic, succinic, acetic, malic, citric, propionic and lactic acids, at concentrations of 1%, on color alteration in *Seriola aurevettata* slices. All acids were greatly effective, except for the formic acid, in preventing color alteration during storage; the succinic acid was especially efficient in maintaining the product's color. The color maintenance effect rank for the tested acids was: succinic > malic > acetic > citric > propionic > lactic. As to the firmness of the slices treated with such acids, after one-week storage, the formic, succinic, malic, lactic, citric and propionic acids promoted softening during storage; the acetic acid promoted firmness level similar to the control.

FINAL CONSIDERATIONS

The success of the MAP depends on various factors such as: good initial quality of the product, good hygiene practices during fishing, selection of the right packaging material, a safe packing equipment, good maintenance and control of temperature, a proper gas mixture for the product and the gas/product ratio.

The ideal CO_2 concentration depends on the fish species, initial microbial population, gas/fish ratio and on the packing method. The most used CO_2 concentrations are between 40 and 60%.

RESUMO

A produção de pescado vem crescendo no Brasil, principalmente a piscicultura de água doce, porém o pescado refrigerado tem uma vida útil relativamente pequena, cerca de 12 dias, e a demanda de produtos frescos é crescente, havendo, portanto, a necessidade de aumentar a vida útil destes, através da utilização de processos combinados, que exponham o mínimo possível o produto a condições adversas.

O uso de embalagens com atmosfera modificada têm como vantagens potenciais: aumento da vida útil de 50 a 400%; redução de perdas econômicas; distribuição dos produtos a longas distâncias e com poucas remessas, diminuindo os custos; além do fornecimento de produtos de qualidade.

Esta revisão teve como objetivo discutir os efeitos da atmosfera modificada na conservação do pescado, principalmente CO_2, sobre o crescimento e sobrevivência de patógenos de origem alimentar.

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