Modified atmosphere packaging of fish – an impact on shelf life

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Abstract. The shelf life of fresh fish is short. Therefore, the fishing industry has always been willing to explore new technologies for shelf life extension. This paper will focus on one such technique, i.e., changing the nature of the gas surrounding the fish. The main purposes of modified atmosphere packaging of fish and fish products, but also other foodstuffs, are two-fold: to ensure the microbiological shelf life and the sensory quality of the product, including the colour, odour and palatability. During the last two decades, modified atmosphere packaging has become a dominant retail fish packaging technology. The main reasons for the development of modified atmosphere packaging are the continuous increase in fresh fish consumption, increases of urban populations and exhaustion of natural food resources. Developments in packaging materials and technologies have made the application of modified atmosphere packaging on a larger scale for fish and fish products feasible. In this paper, we present the basic principles of modified atmosphere packaging of fish, and the microbiological, sensory, chemical and physicochemical parameters that are important for the shelf life of fish packaged using this technology.

1. Introduction

Proper nutrition is of primary importance for the quality of people’s lives. Hence, fish meat, due to its nutritional value, plays a major role in human nutrition. An increasing number of people are aware of the nutritional value of fish, especially given that fish meat is significantly less burdened with the various additives that are used in modern pig and poultry farming. What makes fish particularly attractive to consumers is that, in addition to favourable contents of proteins, minerals and vitamins, it is a very rich source of essential fatty acids that play an important role in the prevention of numerous human diseases. Due to such properties, fish meat is one of the nutritionally most valuable foods.

The fact that fresh fish is a very perishable food (pH> 6.0; aw> 0.98) has induced producers to focus on finding more optimal methods for fish preservation. In recent years, however, consumers worldwide are increasingly demanding that they have fresh fish at all times, since this fish type has the most acceptable sensory properties. This trend has led to the development of modified atmosphere packaging (MAP) for fish and fish products, thus ensuring fish’s longer shelf life and preservation of the basic parameters associated with fish freshness [1]. The shelf life of a foodstuff, and therefore fresh fish, can be defined as the time after food packaging, during which the product is primarily safe for consumption and during which the sensory properties of the product (colour, odour, flavour, texture) and its nutritional value remain unchanged and acceptable to the consumer.

MAP is used today in the production of fresh and chilled food, including raw and thermally processed meat, poultry, fish, pastries, fruits, and vegetables and, more recently, coffee, tea, and bakery products.
Market demand today, however, requires that the food should be processed to a minimum extent and free of additional preservatives and additives, so MAP food is increasingly present at retail.

2. Principle of MAP

MAP can be defined as removing air from the packaging and replacing it with a particular gas or mixture of gases. The purpose of this technology is to extend the shelf life of food by preventing or slowing down both the biochemical processes (lipid oxidation, reactions caused by activities of the fish’s own enzymes) and the development of bacteria that lead to product spoilage. The most commonly used gases in MAP technology are carbon dioxide (CO\textsubscript{2}), oxygen (O\textsubscript{2}), and nitrogen (N\textsubscript{2}). These gases are used in different combinations, and their roles in the modified atmosphere differ. While N\textsubscript{2} is an inert and tasteless gas with the task of preventing packaging collapse, CO\textsubscript{2} inhibits the growth of several types of microorganisms, especially those that cause deterioration and unpleasant odours in foods stored at refrigerator temperatures. A major advantage of CO\textsubscript{2} is that it is not toxic to humans. CO\textsubscript{2} is highly soluble in water and fat and its solubility increases with decreased temperature. Therefore, the effectiveness of this gas is always conditioned by the food storage temperature, with increased inhibition of bacterial growth as the temperature is decreased. The bacteriostatic effect of CO\textsubscript{2} depends on its concentration and the temperature at which the food is stored, while the mechanisms of action are based on changes in the permeability of the bacterial cell membrane, inhibition of enzymes, change in the physico-chemical properties of the proteins, and change in the bacterial cell pH [2].

O\textsubscript{2} plays an important role in MAP, especially in fresh meat packaging. High levels of O\textsubscript{2} are used in MAP for red meat and red fish meat (tuna, yellowtail) to maintain the myoglobin pigment in the meat in an oxygenated form, thus giving the meat a bright red colour, acceptable to the consumer. However, the content of O\textsubscript{2} in MAP is normally kept as low as possible to inhibit the growth of aerobic spoilage bacteria.

3. Modified atmosphere packaging of fish and fish product

In the past decade, the attention of researchers engaged in solving problems associated with fish packaging has mostly been focused on gas mixtures with high concentrations of CO\textsubscript{2} and N\textsubscript{2}. The impact of MAP on fresh fish shelf life and the most appropriate mixture of gases, however, depend on the fish species to be packaged, the fat content, the initial microbiological contamination level, fish manipulation following the catch, the volume ratio of gas to fish in the package and, most importantly, the method of packaging and the storage conditions [3]. In some cases, MAP can adversely affect the quality of the packed fish due to CO\textsubscript{2} dissolution in the fish meat, resulting in the formation of carbonic acid. Moreover, at lower pHs, the capacity of fish meat to bind water is reduced, resulting in the separation of fish meat juice from fish in the package, and this juice is an ideal substrate for the development of spoilage microorganisms [3]. It is for these reasons that the optimum ratio of gases in the mixture must be determined. The ratio between the volume of gas and volume of food product (G/P ratio) should usually be 2:1 or 3:1 (volume of gas two or three times the volume of food). This high G/P ratio is also necessary to prevent package collapse, because the CO\textsubscript{2} solubility in the food means the gas volume reduces throughout the shelf life.

The shelf life of fresh, chilled fish is relatively short and at temperatures of 2±2 °C it is about 2 to 3 days. It has been confirmed that the packaging of fish in modified atmosphere significantly extends the shelf life of the product. The effect of MAP on the shelf life of foods in general has been reviewed by several authors in recent decades. In 100 % CO\textsubscript{2} atmosphere, fish kept fresh two to three times longer than control fish in air at the same temperature [4]. Fresh haddock, cod, sole, whiting and plaice were very effectively preserved under 20-100 % CO\textsubscript{2} atmospheres, with optimal conditions under 40-50 % CO\textsubscript{2} [5]. Since these early investigations, numerous research papers have been published on this topic, some reporting a tremendous increase in shelf life, others reporting little or no shelf life extension, but more often, a shelf life extension in the range of 30-60 % for fresh fishery products using atmospheres with high levels of CO\textsubscript{2} is observed. Table 1 summarises some of the more recent published articles concerning MAP and fish.
Table 1. Shelf life of fresh fish and fish products packaged under MAP, vacuum or air

| Type of fish product | Storage temperature (°C) | Atmosphere CO₂:N₂:O₂ | G/P ratio | Shelf life (days) | Reference |
|----------------------|--------------------------|-----------------------|-----------|------------------|-----------|
| Cod fillets (G. morhua) | 0                        | 2:98:0                | 2         | 14               | Dalgaard et al. [6] |
|                       | 0                        | 3:97:0                | 2         | 13               |           |
|                       | 0                        | 29:71:0               | 2         | 16               |           |
|                       | 0                        | 97:3:0                | 2         | 15               |           |
| Chub mackerel (Scomber japonicus) | 2                        | 70:30:0               | 2         | 17               | Goulas & Kontominas [7] |
|                       | 2                        | 50:30:20              | 2         | 11               |           |
|                       | 2                        | vacuum                | -         | 11               |           |
|                       | 2                        | air                   | -         | 8                |           |
| Salmon slices (Salmo salar) | 2                        | 100:0:0              | ns        | 18               | Pastoriza et al. [8] |
|                       | 2                        | air                   | -         | 8                |           |
| Swordfish steaks (Xiphias gladius) | 4                        | 40:30:30             | ns        | 11-12             | Pantazia et al. [9] |
|                       | 4                        | vacuum                | -         | 9                |           |
|                       | 4                        | air                   | -         | 7                |           |
| Sea bass (Dicentrarchus labrax) fillets | 4                        | 60:40:0              | 3         | 14               | Provincial et al. [10] |
|                       | 4                        | 50:50:0              | 3         | 14               |           |
|                       | 4                        | 40:60:0              | 3         | 11               |           |
| Sardine fillets (Sarda pilchardus) | 3                        | 50:50:0              | ns        | 9                | Stamatis & Arkoudelos [11] |
|                       | 3                        | vacuum                | -         | 7                |           |
|                       | 3                        | air                   | -         | 5                |           |
| Tilapia (Oreochromis niloticus) fillets | 4                        | 60:30:10             | ns        | 15               | Masniyom et al. [12] |
|                       | 4                        | vacuum                | -         | 12               |           |
|                       | 4                        | air                   | -         | 6                |           |
| Carp steaks (Cyprinus carpio) | 3                        | 60:40:0              | 2         | 12               | Babić et al. [13] |
|                       | 3                        | 40:60:0              | 2         | 9                |           |
|                       | 3                        | vacuum                | -         | 7                |           |
| Carp steaks (Cyprinus carpio) | 3                        | 100:0:0              | 2         | 15               | Babić et al. [14] |
|                       | 3                        | 40:60:0              | 2         | 9                |           |
| Carp steaks (Cyprinus carpio) | 3                        | 20:0:80              | 2         | 15               | Babić Milijašević et al. [15] |
|                       | 3                        | 90:10:0              | 2         | 17               |           |
|                       | 3                        | air                   | -         | 17               |           |

ns: not stated in published paper

4. Microbial parameters of importance for shelf life of fish packaged in MAP
There is no doubt the composition of the dominant microbiota of MAP fish and fish products depends on the mixture of gases used in the packaging. Microorganisms that otherwise cause meat spoilage in aerobic conditions are inhibited in MAP conditions by CO₂, and hence their role in fish spoilage in the mixture of MAP gases is insignificant. It is for this reason that the predominant microbiota in fish packaged in MAP is CO₂ resistant. Unlike vacuum packing of fish, where conditions are such as to stimulate the growth of microorganisms that can use trimethylamine oxide (TMAO) as an O₂ source instead of O₂, the mixture of MAP gases inhibits the growth of both trimethylamine (TMA)-producing microorganisms and the hydrogen sulphide producers. Basically, Gram-negative bacteria are much more sensitive to the inhibitory effect of CO₂. This was confirmed in research with carp cuts packaged in different gas mixtures (100% CO₂ and 60% N₂+40% CO₂) and stored at 3 °C; numbers of mesophilic bacteria were lower in the carp packed in MAP with 100% CO₂ than in carp packed in MAP with 60%
CO₂ [16]. Significant reductions in the total number of mesophilic bacteria in fresh sea bass fillets, sardines and trout fillets packaged in MAP gas mixtures with high CO₂ contents were also reported [10,11,17].

According to International Commission on Microbiological Specifications for Foods (ICMSF) recommendations [18], the total number of mesophilic bacteria in fresh fish should not exceed 7 log cfu/g. A good correlation between sensory attributes and number of mesophilic bacteria in carp steaks packaged in MAP was reported by Babić [19]. At the moment when fish was assessed as unacceptable from the sensory point of view, the number of mesophilic bacteria was higher than this recommended limit (7 log cfu/g). The total number of mesophilic bacteria is a good indicator of overall acceptability of salmon fillets packaged in MAP with 40% CO₂ + 60% N₂ and stored at 0 °C [20]. However, in fresh sardines packaged in a MAP gas mixture (60% CO₂+40% N₂), vacuum and air, there was a poor correlation between overall acceptability and the total number of mesophilic bacteria, since the total number of bacteria reached the maximum recommended limit while the sensory attributes of sardines were still acceptable [21].

*Photobacterium phosphoreum*, resistant to CO₂, is most commonly responsible for spoilage of MAP fish. The Gram-positive bacteria, such as lactic acid bacteria, primarily *Lactobacillus* spp. and *Leuconostoc* spp. as well as *Brochothrix thermosphacta* are not sensitive to the effect of CO₂, and hence, these become the dominant genera in fish and fish products packaged in MAP gas mixtures. This has positive effects on fish shelf life, as these microorganisms have less potential to cause spoilage than some others [10]. It should be noted that the low level of carbohydrates in fish meat prevents the occurrence of the sour taste that results from the activity of lactic acid bacteria in the carbohydrate decomposition process. Generally speaking, the initial number of lactic acid bacteria in the fish meat is low and these bacteria are rarely responsible for fish spoilage. However, when growth of aerobic bacteria is inhibited, either by applying low temperatures or by modifying the atmosphere, lactic acid bacteria become the dominant bacterial population, as corroborated by the results of studies on carp cuts [22] and eviscerated trout [23].

During storage at -2°C of Atlantic mackerel fillets packaged in MAP with 100% CO₂, growth in the number of lactic acid bacteria was determined; towards the end of the study, these become the predominant microbiota [24]. The results of numerous studies have shown that high concentrations of CO₂ (70-100%) result in the development of predominantly heterofermentative *Lactobacillus* spp. in MAP fish. Also, lactic acid bacteria constitute 62-85% of the microbiota involved in the spoilage of fish packaged in mixtures with higher concentrations of CO₂ (90-100%) [25].

5. Chemical and physico-chemical parameters of importance for shelf life of fish packaged in MAP

5.1. Total volatile basic nitrogen (TVB-N)

TVB-N is a chemical indicator of fish freshness. The total volatile N is made up of compounds responsible for the formation of unpleasant odour and flavour in fish meat, among them being ammonia, dimethylamine (DMA), TMA, amines derived from decarboxylation of amino acids, and other nitrogenous compounds that become volatile when converted to their alkaline forms. Ammonia is formed in the process of bacterial disintegration of proteins, peptides and amino acids, as well as in autolytic decomposition of adenosine monophosphate (AMP). Dimethylamine and TMA are produced by the degradation of TMAO, a compound that plays a significant role in osmoregulation and the presence of which has been demonstrated in all marine and in a large number of freshwater fish species. The activity of endogenous fish enzymes results in the decomposition of TMAO and the formation of DMA and formaldehyde. In anaerobic conditions, and using TMAO as the ultimate electron acceptor in anaerobic respiration, the bacteria that cause fish spoilage foster the formation of TMA, a compound responsible for the characteristic odour of spoiled fish [26].

Packaging fish in a modified atmosphere is a very effective method of preventing the creation of TVB-N. A lower average increase of TVB-N was found in carp and trout in MAP with 60% CO₂+40%
N₂, than in MAP with a lower percentage of CO₂ (40% CO₂+60% N₂) or in vacuum [27]. Examination of several different gaseous mixture (10% O₂+50% CO₂+40% N₂, 10% O₂+50% CO₂+40% Ar, 20% O₂+50% CO₂+30% N₂, 20% O₂+50% CO₂+30% Ar, 30% O₂+50% CO₂+20% N₂ and 30% O₂+50% CO₂+20% Ar) on shelf life of trout fillets stored at 1 °C [1] revealed that MAP very effectively prevents the formation of TVB-N, no matter the type of gaseous mixture used. These authors [1] recommended 25 mg N/100 g in trout meat as the highest acceptable limit for TVB-N.

The inhibitory effect of CO₂ on the TVB-N in carp cuts was examined by Milijasović et al. [16], who identified the lowest TVB-N occurred in carp cuts in MAP with 100% CO₂. As concluded by [15], TVB-N in carp steaks was strongly affected by gas atmosphere. During 17 days storage at 3±0.5°C, an increase of TVB-N was observed in all carp steaks, but TVB-N in carp steaks packaged under 90% CO₂+10% N₂ changed to lesser extent than in carp steaks packaged under 80% O₂+20% CO₂ and in carp steaks held on top of flaked ice. The basic role of CO₂, as a gas used for MAP of fish, is to inhibit the growth of microorganisms, in particular bacteria causing the spoilage of food, and even more specifically, those that, with their metabolic activity, give rise to the formation of volatile nitrogenous compounds.

Some researchers have recommended a TVB-N limit from 25 to 35 mg N/100 g as an indicator for rejecting commercial fresh whole fish and processed fish products [28]. However, the European Commission has not established any TVB-N limit for acceptability of common carp. Ježek & Buhtova [29] recommend 20 mg N/100g in carp meat as the highest limit for TVB-N, while [19] recommend the highest limit for TVB-N in carp meat packaged in MAP should be 25 mg N/100 g. Lalitha et al. [30] point out that TVB-N value cannot be considered as suitable indicator of fish muscle freshness, because when fish is assessed as sensorially unacceptable, TVB-N does not necessarily exceed the recommended value of 25 mg N/100 g.

5.2. pH

A significant physicochemical parameter that affects fish quality is the pH. Numerous authors state the pH of fish is impacted by different factors. However, the development of lactic acid bacteria is the main cause of pH reduction in packaged fish. According to the literature data, a reduction of pH occurs in situations when fish and fish products are packaged in CO₂-containing gases. On the one hand, this is a consequence of the solubility of CO₂ in tissues, forming carbonic acid that in turn reduces the pH. On the other hand, pH reductions are caused by the antimicrobial activity of CO₂, which inhibits the growth of microorganisms with metabolic activity leading to the accumulation of base components [31].

pH decreases during storage at 3°C for 21 days were recorded in cleaned trout packaged in MAP with 40%, 60% or 90% CO₂ or under vacuum; at the end of the study period, pH was the lowest in trout with the highest number of lactic acid bacteria, i.e., in vacuum-packed trout [23]. Nevertheless, Milijašević et al. [32] proved moderate increases of pH in carp cuts packaged in MAP with 80% O₂+20% CO₂ after five days of storage; this was explained by the high quantity of basic compounds produced by the activity of fish spoilage bacteria.

6. Sensory parameters of importance for shelf life of fish packaged in MAP

Consumers rate fish based on a number of parameters, the most important of which are safety, nutritional characteristics, flavour, odour, colour, texture, convenience for culinary processing and preservation.

Changes in fish meat begin at the moment fish dies, or even earlier, at the time of the catch, and are the result of the activities of the fish’s own enzymes, the metabolism of microorganisms and the oxidation of lipids. Changes in the sensory characteristics of fish usually result from proliferation of microorganisms. The decomposition of fish and the growth of microorganisms cause an unpleasant odour and flavour as well as the production of visible pigmented or unpigmented colonies. The synthesis of polysaccharide extracellular materials and diffuse pigments results in sensory changes in the form of mucous formation and discoloration [26]. On the other hand, chemical changes such as auto-oxidation or enzymatic hydrolysis of fats can cause the rise of unpleasant odour and flavour or, in the latter case, the activity of tissue enzymes can lead to unacceptable softening of the fish meat.
The results of numerous studies show that fish packaged in various gas mixtures is, overall, more acceptable from the sensory point of view and, hence, has a longer shelf life than does fish in air or vacuum packed fish. Babić et al. [13] showed the highest average rates of overall sensory acceptability, which also proved to be statistically significantly higher, were established for carp cuts packed in an atmosphere consisting of 60% CO\textsubscript{2}+40% N\textsubscript{2}. Carp cuts with somewhat smaller average ratings of overall acceptability were packed in a gas mixture of 40% CO\textsubscript{2}+60% N\textsubscript{2}; the lowest average ratings of overall acceptability were those of vacuum packed carp cuts. Statistically significantly higher sensory ratings during storage were established by Masniyom et al. [33] for sea bass fillets packaged in various mixtures of gases than for sea bass held in air, and similar results were obtained by Goulas and Kontominas [7] who examined MAP and vacuum packed mackerel. Such results can also be a confirmation of Murcia et al. [34], that food packed in a modified atmosphere retains a more natural and better appearance than does vacuum packed food.

7. Perspective of modified atmosphere packaging of fish

The growing need for fresh fish is stoking the development of new technologies to extend fish’s shelf life. The use of active packaging and hurdle technology provide more potential for improving the safety and shelf life of fish packed under MAP. The use of active packaging has recently become increasingly present in the food industry. In active food packaging, packaging materials and the environment interact, including different types of gas emitters and gas absorbers in the process, which results in an extended shelf life [35]. Of the greatest importance for the majority of food types are O\textsubscript{2} absorbers and CO\textsubscript{2} emitters, used either for obtaining a modified atmosphere in the package, or for maintaining a constant atmospheric composition throughout the storage period. O\textsubscript{2} absorbers can be used to maintain a low concentration of O\textsubscript{2} in the package even when materials that are not completely O\textsubscript{2}-impervious are used for packaging [31].

Hurdle technology involves a combination of preservation techniques to create conditions in the food that inhibit the growth of microorganisms. These hurdles can be the storage temperature, water activity, pH, the redox potential, but also newer technologies such as MAP, bioconservation, use of bacteriocins, high-pressure treatment, and the use of edible coatings. Potassium sorbate is used as a preservative to extend the shelf life of fish in combination with modified atmosphere [36].

The use of smart packaging such as time and temperature indicators (TTIs) is a technology with great potential, especially when it comes to products packaged in MAP that require storage in a strictly defined temperature regime. In order to achieve the microbiological adequacy of the product, very rigorous control of the temperature regime is required. The TTI monitors the temperature during storage along the food chain and detects packages that are not kept under the strictly defined cold chain conditions for a certain period of time [37].

8. Conclusion

The shelf life of fresh fish can be significantly extended using MAP, but only when the fish is produced with proper control of its hygienic condition and stored at appropriate temperatures. Also, the appropriate selection and use of preservative methods is a very important prerequisite. Only the highest quality fish should be selected to benefit from the extended shelf life advantages of MAP.

Overall, we believe that MAP, if used properly for the right commercial reasons, offers sufficient benefits to both the fishing industry and to consumers, and we strongly advise this is one of many alternatives that the fish industry should consider using as part of a high quality fish marketing program.

Because research data on MAP fresh fish and fishery products has raised safety concerns, additional studies are needed. It is anticipated that future research will help shed light on many unanswered questions and help determine ways to optimise the shelf life extension of fish and fish products, while still maintaining their safety.
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