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Combined Use of Modified Atmosphere Packaging and Natural Compounds for Food Preservation

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Abstract Spoilage of food products is due to activity of microorganisms or biochemical and physical changes. Various food preservation methods have been developed over the years. Traditionally, chemicals are used to control the activity of microorganisms. An increased awareness by the environmental and health agencies and consumers of the harmful chemical residues in food and environment led to a restricted use of chemical preservatives. This trend, known as green consumerism, has resulted, since the beginning of the 1990s, in the increase in consumer demand for natural antimicrobial compounds, i.e. molecules of natural origin, not toxic for humans, environmentally safe, not expensive and easily found on sale. Modified atmosphere packaging (MAP) is one of the most successful preservation techniques suitable for agricultural and food products. With the vast basic and fundamental knowledge available on this subject, the research in this area is taking a new dimension to suit the new consumer trends and demands. The combination of natural antimicrobials to MAP conditions in a sealed packaging system very often represents a strategic solution to prolong food shelf life. The aim of this work is to give an overview on the use of natural compounds combined with modified atmosphere packaging. The effects of this safe and environmentally friendly technology on the improvement of several foods quality and safety will be presented.

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Introduction

Modified Atmosphere Packaging

Food distribution has undergone two major revolutions in the last century, canning and freezing. These gave consumers easy availability to most type of products. However, energy crisis, ecological awareness and demand for healthy and fresh food have created a need for a technology that allows distribution of fresh produce around the year [66].

Modified Atmosphere Packaging (MAP), theoretically, offers a possibility of meeting these requirements. This packaging concept was rapidly grown in the food packaging market. It improves the product quality, freshness and increases the shelf life, as well as provides convenience to consumer and adds value to the product. MAP enables fresh produce or perishable products to be packaged when they are fresh and then maintains them in that condition, thereby, reducing distribution costs and enhancing flavours and nutrition value. Horticultural produce respires even after harvesting. However, the biological processes that cause deterioration get accelerated. This affects the nutritional value, flavour, texture and appearance. In adverse climatic conditions, deterioration happens very quickly. MAP slows the ongoing life processes not by changing the product but by adjusting its environment. MAP maintains this state for a long period of time, during which deterioration is effectively showed down [66].

As stated beforehand, fruit and vegetables are different from other foods as they consume oxygen and produce carbon dioxide while packed, giving rise to a modification of the headspace gas composition [49]. Therefore, MAP can be created either passively by the product (passive MAP) or intentionally introducing gas mixture in the package (active MAP). In passive MAP, the respiring product is placed in a polymeric package and sealed hermetically. Only the respiration of the product and the gas permeability of the film influence the change in gaseous composition of the environment surrounding the product. If the product respiration characteristics are properly matched to the film permeability values, a beneficial modified atmosphere can be passively created within a package. In the case of active MAP, the removal and/or replacement of the atmosphere surrounding the product before sealing in barrier materials was applied. The gas headspace may change during storage in MAP, but there is no additional manipulation of the internal environment [58]. Following, if not explicitly stated the term MAP will be used to refer to active MAP.

Nitrogen, oxygen and carbon dioxide are the three main gases used in MAP, together with water vapour. The role and importance of each gas is related to its properties [61]. In terms of food spoilage, nitrogen is an inert and tasteless gas, without any antimicrobial activity. It is not very soluble in water, and it is primarily used to displace oxygen and prevent package collapse. Oxygen inhibits the growth of anaerobic microorganisms but promotes the growth of aerobic microbes. Additionally, oxygen is responsible for several undesirable reactions in foods, including oxidation and rancidity of fats and oils, rapid ripening and senescence of fruits and vegetables, staling of bakery products and colour changes. Due to its negative effects on food quality, it is generally avoided in realizing MAP for many products. However, its presence in small quantities is necessary, e.g. surface mould-ripened cheeses. Finally, carbon dioxide is soluble in both water and lipids. It has a bacteriostatic effect and slows down the respiration of many products. All these three gases are common and readily available, safe, economical and not considered as chemical additives. However, the optimum level of each gas for each food product must be determined and used in order to optimize the effectiveness [73]. The use of MAP presents several advantages and disadvantages as pointed out by various authors [35, 65]. With the increasing demand for 'fresh' and 'natural' products without addition of 'dangerous' chemicals, MAP seemed to be the ideal method of preservation for many foods, because it could extent significantly the shelf life of product, without affecting its fresh-like characteristics. MAP provides high quality product, reducing economic losses due to longer shelf life. It decreases distribution costs, allowing transportation to remote destinations. The sealed packages are barriers against product recontamination and improve the presentation allowing a clear visibility of the product.

On the other hand, MAP requires different gas formulations for each product type and more specialized and expensive equipment. It requires costs for gases, packaging materials and machinery. Another disadvantage of MAP is the limitation to storage; the increased pack volume affects retail space increasing logistics costs (for instance, no stacking of packages on top of each other). MAP also requires the temperature control to assure the product safety [73].

Main Natural Antimicrobial Compound Groups

As reported by Burt [10], the interest towards health benefits, together with demand for product without chemical preservatives, is increasing around the world. This trend promoted a great interest in natural compounds. A brief description of them is reported in the follows.

One of the most abundant groups of natural compound is represented by the essential oils. Essential oils can extend shelf life of unprocessed or processed foods by reducing microbial growth rate or viability [6]. Some of these substances are also known to contribute to self-defence of plants against infectious organisms [22, 51]. Essential oils occur in edible, medicinal and herbal plants. Essential oils and their constituents have been widely used as flavouring agents in foods, since the earliest recorded history. and it is well established that many of them have wide spectra of antimicrobial action [3, 50, 67]. The composition, the structure, as well as the functional groups of the oils, play an important role in determining their antimicrobial activity. Usually, compounds with phenolic groups are the most effective [23, 33]. Among these, the oils of clove, oregano, rosemary, thyme, sage and vanillin have been found to be very active against microorganisms. They are generally more inhibitory against Gram-positive than Gram-negative bacteria [55, 56, 89]. While this is true for many essential oils, there are some compounds (cinnamon and citral) that are effective against both groups [50, 77, 79]. In addition, some non-phenolic constituents of oils (allyl isothiocyanate, AIT) are more effective against Gram-negative bacteria [85, 88] and Gram-positive fungi [45, 62].

The enzymes represent another group of natural compounds with antimicrobial activity. In particular, the lysozyme is a key enzyme widely distributed in various biological fluids and tissue including avian egg, plant, bacteria and animal secretions such as saliva, tears and milk. It has been used both in the pharmaceutical and food industries. Lysozyme plays a particular role in extending meat shelf life as well as in cheese ageing, through the reduction in butyric fermentation bacteria, which adversely affect cheese quality. In wine making, the bacteriolytic activity of lysozyme has primarily been used to control the malolactic fermentation [14, 30]. It is well known that lysozyme is bactericidal against Gram-positive microorganisms [8], whereas it is essentially ineffective against Gram-negative bacteria, owing to the presence of a lipopolysaccharide layer in the outer membrane. However, its effectiveness could be increased through the use of some chelating agents; in fact the chelation of metal ions apparently destabilizes the lipopolysaccharide layer and increases cell sensitivity, allowing lysozyme to penetrate the lipopolysaccharide layer, resulting in the cell lysis [81].

Another group of natural antimicrobial compounds is represented by the bacteriocins. In 1969, nisin was approved for use as an antimicrobial in food by the Joint FAO/WHO Expert Committee on Food Additives. The suitability of nisin as a food preservative arises from the following characteristics: it is non-toxic, the producer strains of *L. lactis* are regarded as safe (food-grade); it is not used clinically; there is no apparent cross-resistance in bacteria that may affect antibiotic therapeutics; and it is quickly digested by proteolytic enzymes in the human intestinal tract. Nisin works in a concentration dependent manner; thus, more bacteria are present in a food more nisin may be required. Nisin initially forms a complex with Lipid II, a precursor molecule in the formation of bacterial cell walls. The nisin–Lipid II complex then inserts itself into the cytoplasmic membrane forming pores and allows the efflux of essential cellular components resulting in inhibition or death of the bacteria. Gram-negative bacteria are resistant to nisin, because their cell walls are far less permeable than those of Gram-positive bacteria. However, any treatment of Gram-negative bacteria to make their cell walls permeable to nisin makes them susceptible to nisin. Such treatments include exposure to chelating agents, sub-lethal heat, osmotic shock and freezing. Although chelating agents show good in vitro effects, results in food systems are far less pronounced owing to preferred interaction between chelating agents and divalent ions in foods. Nisin action against spores is far less understood. It is predominantly sporostatic rather than sporocidal. Nisin appears to bind to sulphydryl groups on the spore surface [31].

Organic acids are another group of natural compounds used as food additives, but not all of them have antimicrobial activity. The most effective antimicrobials are acetic acid, lactic acid, propionic acid, sorbic acid and benzoic acid. The activity of organic acids is related to pH and to the undissociated form of the acid. The use of organic acids is generally limited to foods with a pH less than 5.5 [32]. Another factor affecting potential activity is the polarity. This relates both to the ionization of the molecule and to the contribution of any alkyl side groups or hydrophobic parent molecules. Antimicrobials must be lipophilic to attach and pass through the cell membrane but also soluble in the aqueous phase [20]. The mechanism of action of organic acids and their esters has some common elements. As already stated, in the undissociated form, organic acids can penetrate the cell membrane lipid bilayer more easily. Once inside the cell, the acid dissociates because the cell interior has a higher pH than the exterior [46]. Bacteria maintain internal pH near neutrality to prevent conformational changes to the cell structural proteins, enzymes, nucleic acids and phospholipids. Protons generated from intracellular dissociation of organic acid acidify the cytoplasm and are extruded to the exterior [21].

Another interesting antimicrobial compound is represented by the chitosan. Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1→4)-*N*-acetyl-D-glucosamine] [63]. It is now widely produced commercially from crab and shrimp shell wastes, with different deacetylation grades and molecular weights and, hence, different functional properties, like emulsification ability, dye binding and gelation [64]. Chitosan has also been documented to possess a film-forming property for use as edible film or coating, thus increasing the shelf life of perishable foods, as well as decreasing transpiration losses [64].

Natural Compounds Combined with Map

Modified atmosphere packaging (MAP) is used as a supplement to low-temperature preservation of food products. An important problem in the commercial application of MAP is that its effect is different for each product. Moreover, MAP may cause a change in the physicochemical parameters and the development of pathogenic microorganisms. Then, the MAP alone may not be sufficient to preserve the quality and safety of food products. Therefore, it is necessary to combine MAP with other preservation techniques. One of them could be represented by the use of natural antimicrobial compounds. The antimicrobial compounds can be incorporated either into the packaging material, or coated on the surface of the packaging film, or added in a sachet into the package to be released during the storage (active packaging). Another possibility to carrier natural compounds is to incorporate the compound into an edible coating, or by dipping or spraying the food or by adding the active compounds directly to the food-making process [13].

This review focuses on the combined effects of natural compounds with MAP on microbiological and physicochemical shelf life and safety of many foods, such as fruit and vegetables, dairy products, meat and fish products. The information is mostly based on studies investigating this preservation technique by monitoring the main detrimental phenomena occurring during storage. Examples of scientific studies are listed in Table 1 and commented on below.

Fruit and Vegetables

As reported above, the use of passive MAP in fruits and vegetables has been found to be effective on quality maintenance, but the carbon dioxide concentration inside the package could not be high enough to act as fungicide or bactericide. In this sense, and taking into account the pressure by consumers about the use of synthetic chemicals, natural compounds have been also used.

One of the first attempts to integrate the use of passive MAP with active compounds was performed on sweet cherry and several cultivars of table grapes such as '*Crimson*', '*Autumn Royal*' and '*Aledo*' [42, 74]. In these studies was evaluated the possibility of improving the overall quality of products by means of the combined use of pure essential oils (eugenol, thymol, menthol or eucalyptol) and passive MAP. The essential oils were placed on a sterile gauze inside the bags avoiding the contact with fruit, and the bags were immediately sealed to minimize vaporization. Loss of weight is one of the most important causes responsible for cherry and table grapes quality deterioration, which increases the fruit susceptibility to fungal decay. Moreover, it is widely accepted that the most important parameters determining products acceptability by consumers are colour, for both fruit and stem. When fruit quality parameters were determined, those treated with eugenol, thymol or menthol showed benefits in terms of reduced weight loss, delayed colour changes and maintenance of fruit firmness compared with control. Stem remained green in treated samples while they became brown in control. However, samples packaged with eucalyptol behaved even worst than control samples, with generation of off-flavours, loss of quality and stem browning. These effects could be attributable to the presence of essential oils, since gas compositions were similar (11–12 O_2 and 2–3 kPa CO_2 for sweet cherry, and 10–14 O_2 and 1.3–2.0 kPa CO_2 for table grapes) in both control bags and treated samples. There is no evidence of the role of these natural compounds on this issue, but the well-known antioxidant activity reported for these essential oils might probably reduce dehydration, chlorophyll degradation and occurrence of browned polymers responsible for stem browning and shrivel (Drake, Kupferman, and Fellman 1988). The antioxidant activities of essential oils are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion-chelation and acting as substrates for radicals such as superoxide and hydroxyl. A direct relationship has been found between the phenolic content and antioxidant capacity of essential oils [2, 19, 72, 83, 87]. Finally, the essential oils show a significant antimicrobial effect, since microbial spoilage increased during fruit storage under MAP conditions. In particular, thymol, eugenol and menthol led to a reduction in mesophillic aerobics, moulds and yeasts.

Raybaudi-Massilia et al. [71] investigated the combined effects of malic acid and essential oils of cinnamon, palmarosa and lemongrass (0.3 and 0.7%) and their main active compounds (eugenol, geraniol and citral, 0.5%) with passive MAP, on the microbiological, physicochemical shelf life and safety of fresh-cut 'Piel de Sapo' melon (*Cucumis melo* L.). The active compounds were incorporated into an alginate-based edible coating. Melon pieces were inoculated with a *Salmonella enteritidis* (10^8 CFU/ml) culture before applying the coating containing malic acid and essential oils or their active compounds. The combined effect of passive MAP and malic acid was effective to improve shelf life of fresh-cut melon from microbiological (up to 9.6 days) and physicochemical (>14 days) points of view, in comparison with non-coated fresh-cut melon (up to 3.6 days and lower than 14 days for microbiological and physicochemical shelf life, respectively). In addition, the incorporation of essential oils or their active compounds into the edible coating prolonged the microbiological shelf life by more than 21 days. Significant reductions in *S. enteritidis* population in inoculated coated fresh-cut melon were achieved. In particular, palmarosa oil incorporated at 0.3% into the coating with passive MAP appears to be a promising preservation alternative for fresh-cut melon, since it had a good acceptation by panellists, maintained the fruit quality, inhibited the native flora growth and reduced *S. enteritidis* population.

The effectiveness of chitosan (5 mL L^{-1}) immobilized in an edible coating on microbiological shelf life of carrot sticks under two different passive MAP conditions (10 kPa O_2 + 10 kPa CO_2 and 2 kPa O_2 + 15–25 kPa CO_2) was studied by Simões et al. [75]. Two MAP conditions were obtained with two different film permeabilities. The use of the edible coating containing chitosan preserved the overall visual quality and reduced surface whiteness during storage. Microbial populations were very low and not influenced by active compounds or passive MAP. In contrast, the content of total phenolics markedly increased in coated carrot sticks stored under moderate O_2 and CO_2 levels, while it was controlled under low O_2 and high CO_2 levels. The combined application of edible coating containing chitosan and moderate O_2 and CO_2 levels maintained quality and enhanced phenolic content in carrot sticks.

Amanatidou et al. [4] studied the effect of dipping in calcium chloride and alternative disinfectants (citric acid and H_2O_2) in combination with MAP (50% O_2 and 30% CO_2) and passive MAP on the overall quality of minimally processed carrots. The combination of MAP and antimicrobial compounds prolonged the shelf life of sliced carrots compared to storage in passive MAP by 2–3 days. In

Table 1 Overview of studies testing the combined use of MAP and natural compounds in foods

Food group	Food	Natural compounds	Concentration applied	Bacterial species	Notes on experimental set-up	References
Fruit and vegetables	Sweet cherry; table grapes '*Crimson*', '*Autumn Royal*' and '*Aledo*'	Thymol, eugenol, menthol, eucalyptol	0.5 ml l^{-1} placed on sterile gauze inside bags	Natural species	1 °C, passive MAP	[42, 74]
	Fresh-cut 'Piel de Sapo' melon (*Cucumis melo* L.)	Malic acid, essential oils (cinnamon, palmarosa and lemongrass), eugenol, geraniol and citral	0.3, 0.5 and 0.7% incorporated into an alginate-based edible coating	Natural species, inoculated *Salmonella enteritidis*	5 °C, passive MAP	[71]
	Carrot sticks	Chitosan	5 ml l^{-1} incorporated into an edible coating	Natural species	4 °C, passive MAP	[75]
	Minimally processed carrots	Calcium chloride, citric acid and H_2O_2	Dipping in citric acid 0.1–0.5%, H_2O_2 5%, calcium chloride 2%	Natural species	8 °C, passive MAP, active MAP (50% O_2, 30% CO_2)	[4]
	Minimally processed lampascioni (*Muscari comosum*); fresh-cut '*Madrigal*' artichokes	Citric acid, calcium chloride	Dipping in citric acid 1%; calcium chloride 10%; coating with active sodium alginate	Natural species	4 ± 1 °C, passive MAP	[15, 26]
	Sliced strawberries	Chitosan	Dipping in 1% chitosan	Natural species	4, 8, 12 and 15 °C, passive MAP, active MAP (80% O_2, 20% CO_2; 65% N_2, 30% CO_2, 5% O_2)	[11]
Dairy products	'Fior di latte' cheese	Chitosan, lysozyme and EDTA disodium salt	0,012% chitosan added into cheese making, combined with an active coating (0,25 mg ml^{-1} lysozyme, 50 mM EDTA)	Natural species	10 °C, active MAP (30% CO_2, 5% O_2, 65% N_2)	[16, 27]
	'stracciatella' cheese	Chitosan	Chitosan (0.010–0.020%) added into cheese making	Natural species	4 °C, active MAP (75% CO_2, 25% N_2)	[38]
	Cheese	Allyl isothiocyanate (AITC)	1 and 2 labels placed inside the packaging	Inoculated fungi	5 °C, active MAP (<0.5% O_2 and 25–27% CO_2)	[86]
	Caprese salad	Thymol	Dipping (400 ppm)	Natural species	4 °C, MAP (65% N_2, 30% CO_2, and 5% O_2)	[7]
Meat products	Fresh meat	Oregano essential oils	Pure essential oil extract placed inside the bags	Natural species	5 and 15 °C, active MAP (40% CO_2, 30% N_2, 30% O_2; 100% CO_2; 80% CO_2, 20% air; vacuum pack and air)	[78]
	Fresh meat patties	Thymol	Thymol (250, 500, 750 mg/kg) added to the meat mixture	Natural species	4 °C, air and active MAP (40% O_2, 15% CO_2, 45% N_2)	[28]
	Non-conventional poultry patties	Thymol, carvacrol	Thymol (0–300 ppm), carvacrol (0–300 ppm) added to the meat mixture	Natural species	0–18 °C, air and active MAP (40% CO_2, 30%O_2, 30% N_2)	[57]
	Fresh chicken meat	Oregano oil	Oregano oil (0.1% and 1% w/w) added post-production	Natural species	4 °C, active MAP (30% CO_2, 70% N_2; 70% CO_2, 30% N_2)	[12].
	Fresh chicken meat	Nisin, EDTA	Nisin (500-1500 IU/g), EDTA (10-50 mM) added post-production	Natural species	4 °C, active MAP (65% CO_2, 30% N_2, 5% O_2)	[34]

Table 1 continued

Food group	Food	Natural compounds	Concentration applied	Bacterial species	Notes on experimental set-up	References
Fish products	Fresh filleted sea bass	Thyme oil	Thyme oil (0.2% v/w) added to the surface of each fillet	Natural species	4 °C, active MAP (40% CO_2, 50% N_2, 10% O_2; 60% CO_2, 30% N_2, 10% O_2)	[52]
	Fresh Mediterranean swordfish fillets	Thyme oil	Thyme oil (0.1% v/w) added to the surface of each fillet	Natural species	4 °C, active MAP (5% O_2, 50% CO_2, 45% N_2)	[53]
	Cod fillets	Oregano oil	Oregano oil (0.05%) added to the surface of each fillet	Natural species, inoculated *Photobacterium phosphoreum*	2 °C, active MAP (60% CO_2, 40% N_2)	[59]
	Fresh fish burgers	Thymol, lemon extract, grapefruit seed extract	Thymol (110 ppm), lemon extract (120 ppm), grapefruit seed extract (100 ppm) added to the fish mixture	Natural species	4 °C, active MAP (5% O_2, 95% CO_2; 100% CO_2; 30% O_2, 40% CO_2 30% N_2; 50% O_2 50% CO_2)	[18, 29]

particular, when the carrots received a pre-treatment with a citric acid dip (0.1%) and calcium chloride prior to packaging, shelf life was extended by 5–7 days. In fact, the authors observed a reduction of at least 2 log CFU g^{-1} in the natural microbiota of minimally processed carrots.

Use of citric acid (1%) and calcium chloride (10%), both dipping and coating with active sodium alginate, with passive MAP has also been examined in a study of minimally processed lampascioni (*Muscari comosum*) and fresh-cut '*Madrigal*' artichokes in order to prolong the microbiological and physicochemical shelf life. The combined effect of active coating with passive MAP delayed the respiratory activity and the browning process, as well as the microbial growth, thus prolonging the shelf life from 1 to 3 days for the fresh-cut artichokes and from 6 to 13 days for the lampascioni [15, 26].

Campaniello et al. [11] evaluated the possibility to prolong the microbiological and physicochemical shelf life of sliced strawberries treated with a solution of 1% chitosan and packaged in passive and active MAP with high (80% O_2 and 20% CO_2) and low (65% N_2, 30% CO_2 and 5% O_2) percentage of oxygen. A chitosan coating inhibited the growth of microorganisms and affected significantly and positively the microbiological stability of the products, above all when the samples were packaged under MAP. Besides, the presence of high percentage of oxygen, combined with a chitosan coating, seemed to affect positively the colour.

Dairy Products

The potential of MAP and natural compounds to extend the shelf life of different dairy products has been also widely demonstrated [36, 68, 69]. Several authors stated that the success of a cheese packaging is dependent on several important parameters, such as the type of cheese, the use of starter cultures during production, the microbial contamination and the storage conditions [60]. There are a number of potential methods of incorporating naturally occurring antimicrobials into cheese. They may be added to the milk or applied to cheese by spraying, immersing or dusting the products. Antimicrobials may be spread onto the packaging material that comes in contact with the cheese or incorporated into the plastic films used for packaging [44, 80, 84].

The addition of chitosan into cheese making, combined with an active coating (lysozyme and EDTA disodium salt) and MAP (30% CO_2, 5% O_2 and 65% N_2), was used to prolong the microbiological shelf life of 'Fior di latte' cheese [16, 27]. This combination represented a strategic solution to prolong the shelf life of 'Fior di latte' cheese to more than 3 days, compared to the control sample in the traditional brine solution that record a storability less than 1 day.

The integrated approach of MAP and active compounds was also studied in a consecutively study by Gammariello et al. [38]. The addition of different amounts of chitosan during cheese making combined with MAP (75% CO_2 and 25% N_2) was also used to prolong the microbiological stability of 'stracciatella' cheese. In fact, the traditional stracciatella showed a short shelf life limited to more or less 3 days, whereas the combination of chitosan and MAP allowed to obtain a significant shelf-life prolongation that accounted to about 7 days.

Allyl isothiocyanate (AITC), a major antimicrobial component in mustard and horseradish oil, has been used in a number of foods against a variety of organisms. Winther and Nielsen [86] studied the effectiveness of AITC and MAP (<0.5% O_2 and 25–27% CO_2) against cheese-related fungi, both on laboratory media and cheese. The AITC labels were placed inside the packaging prior to sealing. The shelf life of the cheese was estimated from the non-inoculated cheese. The shelf life of cheese stored in MAP without AITC labels was 18 weeks, while the presence of AITC labels extended the shelf life from 18 to 28 weeks.

Bevilacqua et al. [7] evaluated the possibility to improve the microbiological shelf life of caprese salad by means of the combined use of thymol dip (400 ppm) and MAP (65% N_2, 30% CO_2 and 5% O_2). This combined approach decreased the coliform populations from 5.65 to 4.23 log CFU g^{-1} and extended the microbiological shelf life from 3.77 to 12 days. It also decreased the concentration of *Pseudomonadaceae* from 7.03 to 5.09 log CFU g^{-1}, prolonging the lag phase to approximately 3 days. This combination did not affect the growth kinetics of lactic acid bacteria and enterococci, thus preserving the function of mozzarella cheese in the salad.

Meat Products

Fresh meat is one of the most perishable foods in commerce. There are many factors influencing meat shelf life, such as pH, water content, availability of oxygen and food composition [1]. During storage, these factors could promote spoilage bacterial growth and oxidative processes which, in turn, provoke meat deterioration in flavour, texture and colour [25, 47]. Several strategies have been used to improve fresh meat quality, such as the hygienic conditions of animal at slaughter, the control of spread of contamination during meat handling, the improvement of storage conditions in terms of temperature and gas composition of package headspace, the vacuum and package parameters, the light exposition and the treatments with antioxidant and/or antimicrobial additives [48, 54].

Skandamis and Nychas [78] evaluated the combined effect of volatile compounds (oregano essential oil) with MAPs (40% CO_2/30% N_2/30% O_2, 100% CO_2, 80% CO_2/20% air, vacuum pack and air) on sensory, microbiological and physicochemical attributes of fresh meat stored at 5 and 15 °C. Whatman paper No. 6 was immersed into pure oregano essential oil for 10 s, drained and then it was placed inside the bags avoiding the contact with the meat. It was found that the extension of shelf life of meat samples depended on the packaging conditions and increased in the order: air < vacuum pack < 40% CO_2/30% N_2/30% O_2 < 80% CO_2/20% air < 100% CO_2. Longer shelf life was observed in samples supplemented with volatile compounds of oregano essential oil and stored under MAPs respect to the control samples. The sensory evaluation did not reveal any effect of investigated antimicrobial compound on meat flavour. The presence of essential oil contributed to the maintenance of visual appearance of meat for quite a long time with the exception of samples packaged in 100% CO_2, where fresh colour of meat disappeared immediately after packaging.

The combined effect of oregano oil (0.1% and 1% w/w) and MAP (30% CO_2/70% N_2 and 70% CO_2/30% N_2) on sensory and microbiological shelf-life extension of fresh chicken meat was also investigated by Chouliara et al. [12]. Microbial populations were reduced by 1–5 log CFU g^{-1} for a given sampling day, with the more pronounced effect being achieved by the combination of MAP and oregano oil. On the base of the sensory evaluation, oregano oil at a concentration of 1% imparted a very strong taste to the product. On the contrary, a shelf-life extension of breast chicken meat by 3–4 days for samples containing 0.1% oregano oil, 2–3 days for samples under MAP and 5–6 days for samples under MAP containing 0.1% of oregano oil was attained. Thus, oregano oil and MAP exhibited an additive preservation effect.

In a study, Del Nobile et al. [28] evaluated the possibility of extending the microbiological shelf life of packed fresh meat patties by a combination of thymol and other extrinsic factors, such as storage temperatures and MAP. Fresh minced beef was supplemented with thymol at levels of 250, 500 and 750 mg per kg of ground beef. Treated samples were packed using a high barrier film and stored under ordinary and modified atmosphere (40% O_2, 15% CO_2 and 45% N_2) conditions for 16 days. Results of this study showed that thymol, working alone, was effective on coliforms and *Enterobacteriaceae*, whereas it did not seem to inhibit, to a great extent, the growth of the other microbial populations. On the other hand, an increased amount of thymol, under MAP conditions, had better effects on the product quality, with a consequent prolongation of the shelf life from 2 to 5 days respect to the control samples.

Mastromatteo et al. [57] also studied the combined effect of thymol (0–300 ppm), carvacrol (0–300 ppm) and temperature on the microbiological quality of non-conventional poultry patties packaged under ordinary and modified atmosphere (40% CO_2, 30% O_2 and 30% N_2). The results showed that the final total viable count (TVC) for patties packaged in air decreased with the increase in carvacrol up to 150 ppm, for higher carvacrol concentration an increase was observed. The effect of thymol was less evident than carvacrol. The combination of MAP and thymol had a sinergic effect on TVC. Similar evolution was observed for the growth of the other microbial groups (*Enterobacteriaceae*, lactic acid bacteria and *Pseudomonas* spp.). The same trend was observed also for the patties packaged under MAP. However, the combined use of antimicrobial compounds and MAP caused the higher log reduction for *Pseudomonas* spp., respect to the control during the entire storage time, limiting the off-odours associated with spoilage of this microbial group.

Economou et al. [34] evaluated the effect of nisin (500–1,500 IU/g) and EDTA (10–50 mM) treatments on the microbiological shelf life of fresh chicken meat stored under MAP (65% CO_2, 30% N_2 and 5% O_2). The antimicrobial treatments affected populations of mesophilic bacteria, *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria and *Enterobacteriaceae*, respect to the control samples. Moreover, the use of MAP in combination with nisin–EDTA treatments resulted in an organoleptic extension of fresh meat by approximately 1–2 and 13–14 days, according to the EDTA and nisin concentration.

Fish Products

Fresh fish is a highly perishable product due to its biological composition. The main cause of deterioration is the activity of typical spoilage seafood microorganisms [40, 43], which provokes loss of essentially fatty acids, fat-soluble vitamins and protein functionality, production of biogenic amines and formation of off-odours [37, 40, 41].

The use of refrigerated temperatures represents a useful mean to achieve lower rates of microbial growth, but it is not a sufficient mild procedure to control the microbial spoilage and extend the shelf life of seafood products. The most suitable and extensively reviewed technology for perishable seafood product is the use of MAP. Several combinations of oxygen, carbon dioxide and nitrogen were used to package fish products with different effects on shelf life [5, 9, 17, 24, 39, 70, 76, 82]. Essential oils are potentially able to also extend the storability of seafood when used alone or in combination with other preservation techniques.

Kostaki et al. [52] studied the combined effect of MAP, using two different gas mixtures (40% CO_2/50% N_2/10% O_2 and 60% CO_2/30% N_2/10% O_2), and thyme oil (0.2% v/w) as natural preservative, on the microbiological quality of fresh filleted sea bass during a refrigerated storage period. Thyme essential oil was added to the surface (two sides) of each fillet. Aerobically packaged sea bass fillets were used as control samples. Total viable counts for fresh sea food in air exceeded 7-log CFUg^{-1} after 7 days, while fillets packaged under MAP with thymol reached the same microbial level later.

The combined effect of thyme essential oil (0.1% v/w) and packaging on microbial and sensorial characteristics of fresh Mediterranean swordfish fillets was also investigated by Kykkidou et al. [53]. In particular, air, MAP (5% O_2/50% CO_2/45% N_2), air with thyme oil and MAP with thyme oil were used. The MAP and the combination of MAP and thyme oil were the most effective solutions for inhibiting pseudomonads and H_2S-producing bacteria. Based primarily on sensory data, the shelf life of fresh refrigerated Mediterranean swordfish were 8 and 13 days under aerobic and MAP conditions, respectively. Addition of 0.1% thyme essential oil extended the product shelf life under aerobic conditions by 5 days, whereas the combination of MAP and thyme oil resulted in a significant shelf-life extension of the swordfish fillets, i.e. by approximately $7^1/_2$ days, when compared to the control sample. Odour, taste and overall acceptance of swordfish samples stored both under aerobic and MAP condition, either with or without thyme essential oil, showed a similar trend: the sample quality decreased with storage time.

Mejlholm and Dalgaard [59] observed that oregano oil (0.05%) reduced the growth of *Photobacterium phosphoreum* and extended the microbiological shelf life of cod fillets kept under MAP; this preservation technique caused 2-log CFU g^{-1} and 1-log CFU g^{-1} reduction in aerobic viable count and of *P. phosphoreum* cell load, respectively.

The combined use of essential oils (thymol, lemon extract and grapefruit seed extract) with MAP was also revealed effective in improving the microbiological shelf life of packed fresh fish burgers [18, 29]. The essential oil solutions were added to the fish mixture and then homogenized to have a homogeneous distribution of the active compound. In particular, these studies highlighted the possibility to improve the microbial quality of fish burgers by using very small amount of thymol (110 ppm), GFSE (100 ppm) and lemon extract (120 ppm), in combination with MAP characterized by a high CO_2-concentration (5% O_2, 95% CO_2; 100% of CO_2). This combined approach was able to guarantee the microbial acceptability of fish burgers until 28th day of storage at 4 °C.

Conclusion and Future Trends

The development of the combined approach based on natural compounds added to active or passive MAP was designed to respond to a number of issues related to food quality deterioration during refrigerated storage. This new approach amplifies the benefits related to the MAP. In fact, it guarantees the maintenance during storage of organoleptic attributes and reduces the risk of microbial spoilage.

In particular, in the case of fruit and vegetables, the use of active compounds such as essential oils or their constituents, organic acid and chitosan in combination with MAP improves the product overall quality in terms of maintenance of organoleptic and functional properties together with microbial growth. Moreover, the low pH and fat content of fruits and vegetables contributes to the success obtained with essential oils.

The use of active compounds, dissolved in brine or added during cheese making, combined with MAP allows to obtain a significant shelf-life prolongation of dairy products. Moreover, an interesting approach is to use edible coating, which is an interesting alternative to the product brine, as a carrier of natural antimicrobials.

Modified atmosphere packaging in combination with active compounds has proven to be an effective preservation method for the extension of meat and fish products shelf life. Moreover, active compounds, used as preservatives in foods, often impart a strong, often unpleasant, flavour to products. In particular, if active compounds such as essential oils are used as the only preservative in foods, more than 1% (w/w) is generally required to extend the packaged food shelf life. These high levels often impart to the food a very strong and bad flavour. A very small amount of these active compounds used in combination with MAP yielded a distinctive but pleasant flavour to food products, and it significantly delayed spoilage reactions extending the packaged food shelf life.

Research is required on integrating MAP and active packaging to make it a commercially successful technology. Future work will focus on the use of new antimicrobials with wide spectrum of activity and low toxicity. Although not discussed extensively, food safety concerns will continue to impact packaging choices by processors, retailers and consumers. Therefore, the combined effect of active compounds and MAP on the safety of the food products should be investigated. Moreover, the design of specific active packaging able to slowly release in the package headspace the selected molecules should be also addressed.

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