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Substances with Antibacterial Activity in Edible Films - A Review

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This article is an overview of literature addressing edible films and substances introduced to films in order to impart them the antimicrobial activity. It describes natural polymers applied for the production of food packages and active substances of natural origin added to them, including: bacteriocins, enzymes, oils, and plant extracts. Further discussion refers to chitosan – a polysaccharide used for film formation and characterised by strong antibacterial and antimycotic properties.

INTRODUCTION

The primary task of edible films applied in the food technology is to enable the prolongation of shelf life and improvement of the quality of fresh and processes food products. The films play also protective functions by preventing water loss and taste or colour change in a food product, by cutting off the access of oxygen they additionally inhibit the development of selected microorganisms and fat rancidity processes. Furthermore, the application of novel packages based on natural polymers facilitates the reduction of the use of synthetic packages which contemporarily pose a severe problem of environment pollution.

The most commonly occurring and applied natural polymers include: polysaccharides (starch, cellulose and its derivatives, chitosan, alginate, gellan gum), proteins (collagen, zein, soybean and gluten proteins, milk proteins), and fats (bee wax, candelilla wax, carnauba wax, fatty acids and glycerols) [Ayranci et al., 1997; Bravin et al., 2006; Casariego et al., 2008; Donhowe & Fennema, 1993; Nussinovitch & Hershko, 1996; Park et al., 1994; Pommet et al., 2003; Saucedo-Pompa et al., 2009; Xie et al., 2002]. Such packages have especially been applied in the coating of products with solid and semi-solid consistency, including: meat and meat products, fish, cheeses, fruits and vegetables. An advantage of this type of packages is the fact that they may be consumed together with the food product. This type of films may also be used to separate particular food layers. This has enabled the monitoring of mass exchange processes between particular layers of a food product, e.g. restricted migration of moisture [Rico-Pena & Tor-

* Corresponding author: Tel.: +4822 593 76 63 E-mail: karolinakrasniewska@op.pl (K. Kraśniewska) res, 1990]. In addition, films based on natural polymers may constitute a barrier to lipids, hence they are used to cover food products designed for frying. The best results were achieved with the use of films based on polysaccharides which reduced fat absorption in food products subjected to frying by 50 to 90% [Williams & Mittal, 1999]. Finally, the edible films are carriers of antimicrobial substances, antioxidants, dyes and vitamins, thus improving the sensory properties of food products.

It is assumed that edible films should be characterised by low penetrability of water, gases and water vapour. They should additionally display high adhesiveness, good mechanical resistance, plasticity and cohesiveness of structure. The latter depends on the nature of the polymer used, on the solvent and softening agents applied, as well as on temperature of the production process [Tharanathan, 2003].

FILM FORMING PREPARATION

Different methods of producing edible films are described in scientific literature. The differences relate to the type and form of material as well as additives used *e.g.* plasticizers which are added to film-forming solution to enhance properties of the final film.

One of the most popular methods for edible films production is solvent removal, which was used to make the film-forming solution. The process relies on physical and chemical intermolecular interaction to create and stabilise a continuous structure. Macromolecules in the film-forming solution are diluted in solvent such as water, ethanol or acetic acid, that includes additives. In the next step, film-forming solution is poured out in a thin layer and dried [Cagri, 2004].

If films are produced from purely polymeric ingredients they tend to be fragile and brittle. Addition of a plasticizer

is necessary to overcome the brittleness of film, to improve flexibility and increase toughness. The plasticizers widely used in polymer-based films are water, oligosaccharides, polyols and lipids [Coupland, 2000].

FILMS WITH ANTIBACTERIAL PROPERTIES

In order to impart bactericidal properties to edible films, they are enriched with substances displaying a biological activity. The latter are applied to reduce, inhibit or delay the growth of microorganisms on the surface of food products [Durango et al., 2006]. The selective action of such films consists in the release during food storage of an appropriate active substance that affects microorganisms [Lopez–Rubio et al., 2006]. The active substance applied is supposed to migrate with an assumed rate, i.e. with the rate assuring the effective action of the substance against microorganisms, which means a lower count of bacteria over the entire storage period. A too high rate of active substance migration from the package contributes to the loss of film's antibacterial activity [Quintavalla & Vicini, 2002; Silveira et al., 2007].

The incorporation of an antimicrobial into a biodegradable films can take several approaches. Processing techniques used for preparation of edible films are similar to those used in synthetic plastics processing. Factors influencing the choice of the processing techniques when preparing an antimicrobial film are the type and properties of the polymer, the properties of the antimicrobial agent such as heat stability and compatibility with the polymer and the residual antimicrobial activity after manufacturing. Thermal processing methods such as compression molding or extrusion may be used with thermally stable antimicrobial.

An alternative to the incorporation of antimicrobial compounds during extrusion is solvent casting, which is the most commonly used technique to prepare antimicrobial film from biopolymers. Cast edible films have been used as carriers for heat sensitive antimicrobial agents and coated onto the food products. Heat-sensitive antimicrobials like enzymes, spices and essential oils as well as bacteriocins and preservatives may be incorporated into an edible film by solvent compounding [Appendini & Hotchkiss, 2002]. For example, lysozyme has been incorporated into cellulose ester films by solvent compounding in order to prevent heat denaturation of the enzyme [Appendini & Hotchkiss, 1997]. Vojdani & Torres [1990] demonstrate sorbates incorporated into polysaccharides film and show that the film allowed slower diffusion of the sorbates to the surface of the food, which improved surfaced protection.

In the solvent, the compound polymer and antimicrobial need to be soluble in the same solvent. Biopolymers that form films and coating including polysaccharides, proteins and lipids are good candidates because they are soluble in water, ethanol and many other solvents compatible with antimicrobials [Coma, 2008].

Antimicrobial agents can also be immobilised to polymer by ionic or covalent link agents. This way of immobilisation requires the presence of functional groups on both the antimicrobial and the polymer. Antimicrobials with functional groups are for example, enzymes, organic acid, bacteriocins. Immobilisation of antimicrobial agents into polymer causes a slow migration of the agents from the polymer to the surface of the product.

Appendini & Hotchkiss [1997] studied the effectiveness of lysozyme immobilised on cellulose triacetate (CTA) films, polyvinyl alcohol (PVOH) and nylon 6,6 resins. They reported that lysozyme immobilised on CTA films showed the highest antimicrobial activity against *Micrococcus lysodeikticus*. Other antimicrobial agents, such as nisin, were immobilised onto calcium alginate films. Other study reported that films treated with nisin and applied onto raw beef surface reduced *Brochothrix thermosphacta* count [Cutter & Siragusa, 1996].

MIGRATION OF THE ANTIMICROBIAL AGENTS

In recent years research, a growing interest has been observed in a controlled release system in which antimicrobial agents are incorporated into biopolymer materials to provide the slow release onto food surface.

Direct application of the antimicrobial agent onto the food surface causes rapid diffusion from the surface into the food mass. For this reason, more efficient is to use active packaging system the antimicrobial agents are incorporated into. The system slows down the migration of the agents away from the surface and controls the growth of undesirable microorganism on the surface of food [Min *et al.*, 2005a].

The rate of a bactericidal substance penetration into the food product is affected by its storage conditions, pH value, water activity, as well as concentration of the introduced active substance [Quintavalla & Vicini, 2002; Silveira *et al.*, 2007].

The temperature condition during production and distribution have to be predicted to determine their effect on the residual antimicrobial activity of the active compounds. Temperature is a very effective parameter to depict the loss of antimicrobial agents from films. Tunç & Duman [2011] showed that the low storage temperature was more effective to preserve the carvacrol in the methylocellulose film. Concentration of carvacrol was higher in a film stored at 15°C than in a film stored at 45°C. Similar results were obtained for the potassium sorbate release and diffusivity from κ -carrageenan-based film. Choi *et al.* [2005] found that the migration of potassium sorbate decreased at a temperature from 40 to 5°C.

The pH value of a product affects the growth of target microorganisms and changes in the activity of the antimicrobial agents may also affect the retention of antimicrobial agents from a polymer matrix. Rico-Pena & Torres [1991] reported that diffusion of sorbic acid from edible methylocellulose--palmitic acid film decreased with an increase in pH. A recent study by Choi et al. [2005] showed that diffusion of potassium sorbate was not affected by pH. The antimicrobial activity of agents incorporated into a polymer matrix could also be influenced by the water activity of food. Vojdani & Torres [1990] reported that the diffusion of potassium sorbate from polysaccharide films increased with water activity. Similar results were obtained for a methylcellulose film containing potassium sorbate. It was noticed that the diffusion rate of an active agent was much higher at higher values of water activity [Rico-Pena & Torres, 1991].

Furthermore, polymer additives including plasticizers such as polyols and lipids can negatively affect the antimicrobial activity. These additives may interact with the antimicrobial or change conformation of the polymer which slows down the migration. Redl *et al.* [1996] reported that the addition of a plasticizer-lipid component into a wheat gluten-based film resulted in the reduction of sorbic acid diffusion.

ANTIMICROBIAL SUBSTANCES MOST FREQUENTLY INTRODUCED INTO FILMS: BACTERIOCINS, ENZYMES, OILS, PLANT EXTRACTS, AND PRESERVATIVES

Owing to the excessive chemisation of food, search is underway for mainly substances of natural origin that display the inhibiting effect on the growth of undesirable microflora in food. Such substances may be synthesised microbiologically or from raw materials of animal and plant origin. Also, edible films carrying commonly used preservatives such as benzoates, sorbates. Films with their addition should be characterised by a wide range of activities against both pathogenic microorganisms and those causing food spoilage, including among others: Escherichia coli, Clostridium, Listeria, Salmonella, Pseudomonas and moulds [Quintavalla & Vicini, 2002; Appendini & Hotchkiss, 2002]. The most popular products of microbial metabolism are: nisin - a bacteriocin of Lactococcus lactis bacteria, and pediocin – a bacteriocin of Pediococcus acidilactici bacteria [Chandrapati & O'Sullivan, 1998; Biswas et al., 1991]. So far literature references have been describing attempts of their application in many matrices based on natural polymers. Nisin is commonly applied in films based on proteins of whey, zein, wheat and soybean [Ko et al., 2001; Hoffman et al., 2001]. Satisfactory effects have been achieved in the case of whey-based films that attained the bactericidal properties. They were characterised by a high effectiveness against *Listeria monocytogenes* bacteria [Ko et al., 2001]. A significant antibacterial activity was also achieved in the case of films based on zein with the addition of nisin. They were shown to inhibit the growth of Salmonella Enteritidis and *Listeria monocytogenes* bacteria [Hoffman et al., 2001].

Some works have additionally described attempts of introducing nisin to polysaccharide films, e.g. to cellulose or hydroxypropylmethylcellulose (HPMC) films, thus imparting them activity against L. innocua and S. aureus bacteria [Coma et al., 2001]. Alike investigations were conducted with nisin being introduced into a film from methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC), which are fine carriers of bactericidal substances and additionally are characterised by resistance and elasticity. The films formed were found to well inhibit the growth of *Micrococcus luteus*. Attention was, however, paid to the fact that the diffusion of nisin from the MC film proceeded faster than from the HPMC film [Cha et al., 2003]. Sebti & Coma [2002] presented a concept of applying a mixture of hydroxypropylmethylcellulose (HPMC), stearic acid and an active compound – nisin, in order to produce a film with antibacterial properties and enhanced barrier properties against water vapour. The composition of the HPMC film with nisin was found to effectively inhibit the growth of *L. monocytogenes* and *S. aureus* bacteria. In turn, the addition of fat, in the form of stearic acid, improved the barrier properties of the film against water vapour, but simultaneously diminished its primary antibacterial activity. Another investigated bacteriocin was pediocin which was introduced into the cellulose film. The foil with its addition was demonstrated to completely inhibit the growth of *L. monocytogenes* bacteria [Ming *et al.*, 1997]. Equally promising results were achieved in a study conducted with a pullulan film enriched with bacteriocin, namely with sakacin A. The study demonstrated a significant effect of the film on the reduction of *L. monocytogenes* bacteria [Trinetta *et al.*, 2010].

Natural substances of animal origin include lysozyme, protein isolated from hen egg as well as milk enzymes – lactoferrin and lactoperoxidase. Lysozyme is a protein that induces hydrolysis of peptoglycane which constitutes the cell wall of Gram-positive bacteria [Fuglsang *et al.*, 1995]. The injection of lysozyme to the film from whey proteins was reported to inhibit the growth of *L. monocytogenes* bacteria in smoked salmon [Min *et al.*, 2008]. In turn, in the films based on zein lysozyme was demonstrated to inhibit the growth of *L. plantarum* and *B. subtilis* [Mecitoğlu *et al.*, 2006].

In order to improve the bactericidal properties of lysozyme, use is made of its synergistic action with different chemical compounds, including e.g. EDTA. This compound damages the lipopolysaccharide layer of the cell wall of Gram--negative bacteria, which enables extending the bactericidal activity of this enzyme into this group of bacteria as well as allows reinforcing its action [Gill et al., 2000]. Lysozyme was introduced into the film from zein in a combination with EDTA. This combination enhanced the effect of lysozyme action against Gram-negative E. coli bacteria [Güçbilmez et al., 2007]. The effectiveness of lysozyme action in combination with EDTA and nisin was also investigated in a polysaccharide film on the example of a foil from alginate that was imparted strong properties inhibiting the growth of a wide spectrum of bacteria: M. luteus, L. innocua, S. Enteritidis, E. coli, and S. aureus [Cha et al., 2002]. Studies were also conducted to determine the feasibility of introducing lactoferrin and lactoperoxidase to a film from whey proteins. They demonstrated that only the film with the addition of lactoperoxidase displayed the activity against L. monocytogenes, E. coli, Salmonella enterica bacteria and Penicillium commune mould [Min & Krochta, 2005; Min et al., 2005b,c].

In recent years, intensive investigations have been conducted with films enriched with substances of plant origin: essential oils and plant extracts. Strong bactericidal properties are exhibited by among others: thyme, oregano, and clove oils containing phenols: thymol, carvacrol and eugenol [Dorman & Deans, 2000]. Similar properties are displayed by oils containing aldehydes, including cinnamon oil the major compound of which is cinnamic aldehyde, as well as ketones (thujone and camphor) being constituents of sage oil [Matan et al., 2006]. Less active are oils that contain alcohol, i.e. mint (menthol) or lavender (linalool) oils, and ethers present in a rosemary oil (cineol and borneol) [Daferera et al., 2003].

The mechanism of essential oils action on microorganisms may consist in destroying the cell wall and cytoplasmic membrane of bacteria and fungi, which leads to the leakage

TABLE 1. Selected active components incorporated directly into polymer.

Active components	Polymer	Main target microorganisms	References
Bacteriocins:			
nisin	whey protein	L. monocytogenes	Ko <i>et al</i> . [2001] Hoffman <i>et al</i> . [2001]
	zein	S. Enteritidis, L. monocytogenes	
	methylcellulose, hydroxypropylmethylcellulose	L. innocua, S.aureus, M. luteus	Coma et al. [2001]; Cha et al. [2003]
	пускомургоруннескуюскагове	L. monocytogenes	
pediocin	cellulose	L. monocytogenes	Ming et al. [1997]
sakacin A	pullulan	L. monocytogenes	Trinetta <i>et al</i> . [2010]
Enzymes:			
lysozyme	whey protein	L. monocytogenes	Min et al. [2008]
	zein	L. plantarum, B.subtilis,	Mecitoglu et al. [2006]
lysozyme and EDTA	zein	B. subtilis, E. coli	Güçbilmez et al. [2007]
lysozyme, EDTA and nisin	alginate	M. luteus, L. innocua, S. Enteritidis, E. coli, S. aureus	Cha et al. [2002]
lactoperoxidase	whey protein	L. monocytogenes, E.coli, S.enterica, P. commune	Min & Krochta [2005]; Min <i>et al.</i> [2005b, c]
Essential oils: oregano oils garlic oils	whey protein	E. coli, S. aureus, S. Enteritidis, L. monocytogenes, L. plantarum	Seydim & Sarikus [2006]
garlic oils	alginate	S. aureus, B. subtilis,	Pranoto <i>et al</i> . [2005]
cinnamon, lemongrass, palmarosa	alginate	mesophilic and psychrophilic bacteria, yeast, molds	Raybaudi-Massilia et al. [2008]
Plant extracts:			
grape seed extract	soy protein	L. monocytogenes, E. coli, S. Typhimurium	Sivarooban et al. [2008]
grapefruit seed extract	alginian	M. luteus, L. innocua, S. Enteritidis, E. coli, S. aureus	Cha et al. [2002]
Preservatives: sodium benzoate potassium sorbate	methylocelullose, chitosan	P. notatum, R. rubra	Chen et al. [1996]
sorbic acid	zein	L. monocytogenes	Carlin et al. [2001]
	whey protein	L. monocytogenes, E. coli, S. Typhimurium	Cagri et al. [2001, 2002]
	zein	S. aureus	Torres et al. [1985]
potassium sorbate	starch	E.coli	Shen et al. [2010]
	starch	S. Typhimurium, E. coli	Baron & Sumner [1993]

of cytoplasm, in inhibiting the synthesis of DNA, RNA, proteins and polysaccharides in bacteria and fungi, and in inhibiting the production of enzymes [Burt, 2004]. For instance, eugenol and cinnamic aldehyde are inhibitors of histidine decarboxylase, whilst carvacrol and thymol are known to damage cell walls of bacteria [Helander *et al.*, 1998; Lambert *et al.*, 2001].

Furthermore, essential oils from *e.g.* oregano and garlic were introduced to whey protein-based films, which enabled achieving films active against *E. coli*, *S. aureus*, *S.* Enteritidis, *L. monocytogenes* and *Lactobacillus plantarum* bacteria. Those films have been applied mainly to cover surfaces of cheeses

and cured meat products [Seydim & Sarikus, 2006]. The oil from garlic was, additionally, used in an alginate film, thus imparting it the capability for affecting the growth of *S. aureus* and *Bacillus subtilis* bacteria [Pranoto *et al.*, 2005]. The antibacterial activity was also exhibited by cinnamon, lemongrass and palmarosa oils. The films produced from alginate with the addition of those oils were demonstrated to protect surfaces of melon against the development of native microflora of this fruit, including mesophilic and psychrophilic bacteria as well as yeast and molds [Raybaudi-Massilia *et al.*, 2008].

Sivarooban *et al*. [2008] were investigating the effect of an extract of grape seeds on selected bacterial strains. The extract

examined contained compounds of phenolic origin. Having been introduced into a soybean film, it reduced the growth of *L. monocytogenes* bacteria by 1 logarithmic unit, whereas that of *E. coli* and *S.* Typhimurium – analogously by 0.1. and 0.2 logarithmic units. In turn, a combination of the extract from grape seeds with EDTA and nisin was observed to significantly enhance the antibacterial activity of the investigated film. As a consequence, a film was achieved that enabled reducing the count of *L. monocytogenes* bacteria by 3 logarithmic units, whereas that of *E. coli* and *S.* Typhimurium – by respectively 1.8 and 0.6 logarithmic units. Further on, an extract from grapefruit was successively introduced to an alginate film, which allowed producing a film being active against *M. luteus*, *L. innocua*, *S.* Enteritidis, *E. coli*, and *S. aureus* bacteria [Cha *et al.*, 2002].

The most commonly used preservatives in edible films are benzonic acid, sodium benzoate, sorbic acid and potassium sorbate. Edible films containing these compounds have been tested against a wide variety of microorganisms. Chen et al. [1996] reported that methylocellulose (MC) and chitosan film containing 2% or 4% of sodium benzoate or potassium sorbate inhibited the growth of *Penicillium notatum* and *Rhodotorula rubra*. Sorbic acid and its salts have been among the most studied antimicrobial agents in proteinand polysaccharide-based edible films. A protein film from corn zein with the addition of sorbic acid was shown to inhibit L. monocytogenes growth on sweet corn [Carlin et al., 2001]. Sorbic acid incorporated into whey protein isolate inhibited the growth of *L. monocytogenes*, *E. coli* and *S.* Typhimurium. Furthermore these films were applied onto surface of sliced bologna and summer sausage where they decreased the population of L. monocytogenes, E. coli and S. Typhimurium [Cagri et al., 2001, 2002]. Torres et al. [1985] prepared a corn zein film containing sorbic acid. The film with antimicrobial addition was demonstrated to protect surfaces of cheese against S. aureus. Potassium sorbate was successfully impregnating into starch-based films. For example, starch film incorporated with 15% potassium sorbate resulted in a significant reduction of E. coli [Shen et al., 2010]. Baron & Sumner [1993] showed that starch film combined with potassium sorbate was reducing the growth of S. Typhimurium and E. coli. Furthermore, they found that corn-starch films containing this preservative inhibited the growth of S. Typhimurium and E. coli on poultry products.

CHITOSAN AS A NATURAL POLYMER WITH STRONG ANTIBACTERIAL PROPERTIES

A group of substances of animal origin includes chitosan. This compound is isolated from exoskeleton of marine crustacea or from the cell wall of filamentous fungi [Hirano, 1999]. It displays the capability for film formation, and its additional advantage are strong antibacterial properties. Its activity was confirmed against, *i.a.*, Gram-positive bacteria: *L.monocytogenes, B. megaterium, B. cereus, S. aureus, L.plantarum, L.brevis, L. bulgaricus*; Gram-negative bacteria: *E. coli, P. fluorescens, S. Typhimurium, Vibrio parahaemolyticus*, and also fungi: *Fusarium* and *Alternaria* [Hirano 1999; No *et al.*, 2002]. The mechanism of chitosan action consists

in the electrostatic interaction between its positively-charged amine groups and a negatively-charged surface of the cell wall of microorganisms. This leads to permeability of the cell membrane of bacteria and leakage of intracellular components, which finally leads to cell apoptosis [Shahidi *et al.*, 1999].

Chitosan has been applied as a base matrix for the production of films; it may also be used as an additive (owing to its bactericidal activity) in edible packages [Shahidi et al., 1999]. The antibacterial activity of chitosan depends on the degree of its acetylation, its molecular weight and solvent applied [Zheng & Zhu, 2003; Dutta et al., 2009]. The minimal inhibitory concentration (MIC) of chitosan varies between 0.01 and 1%. The increasing degree of its acetylation is accompanied by its enhancing activity. The latter may additionally by increased by applying acetic acid as a solvent [No et al., 2002; Beverlya et al., 2008]. A parameter which greatly determines the antibacterial properties of chitosan is also its molecular weight. According to Zheng & Zhu [2003], the mechanism of action of chitosan with a low molecular weight involves penetration to a cell, which induces changes in cell metabolism and finally its apoptosis. In the case of chitosan with a higher molecular weight, the impossibility of penetrating into the cell contributes to the formation of a film onto its surface, which hinders nutrients availability

Chitosan is a compound soluble in water, and films containing it are applied to cover food products characterised by a high water content [Kittur et al., 1998]. They are used primarily in order to extend the shelf life of such food products as: fruits, meat, fish and sea fruits [Suyatma et al., 2004]. In addition, chitosan films exert a positive effect on the texture of a food product, mitigate processes of enzymatic browning, reduce the production of ethylene, display poor antioxidative potential, and play a role of a carrier of active substances [Devlieghere, 2004; Zhang & Quantick, 1997]. For example, chitosan films were used to protect meat products against the development of L. monocytogenes, E. coli and S. aureus bacteria, and to cover surfaces of Ementaler cheese in order to inhibit the growth of *P. aeruginosa* bacteria [Beverlya et al., 2008; Zheng & Zhu, 2003]. In a study carried out by Coma et al. [2003], chitosan films were active against S. aureus and L. monocytogenes bacteria. Other works describe also attempts of introducing substances with antibacterial activity to chitosan films. For instance, chitosan films enriched with organic acids (propionic, acetic) were applied to protect meat products against the development of Enterobacteriaceae and Lactobacillus sakei bacteria. Good effects were reported for chitosan films enriched with oleoresins of rosemary and chili pepper. The foil thus produced was found to inhibit the growth of L. monocytogenes [Ponce et al., 2008].

CONCLUSIONS

Traditional packages do not assure completely safe foods, for many food products are subject to secondary infections at the final stages of the production process, which is one of the causes of their more rapid spoilage. It seems advis-

able, therefore, to cover surfaces of food products with novel packages, namely edible films, which by being additionally enriched with active substances with antibacterial and antimycotic properties protect food products against the development of detrimental microflora.

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