Review paper
UDC 579.67

CELLULOSE BASED PACKAGING FILMS CONTAINING NATURAL ANTIMICROBIAL AGENTS

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Abstract

Microbial growth on the product surface is the main cause of spoilage of many foods. The application of antimicrobial agents to packaging can create an environment inside the package that may delay or even prevent the growth of microorganisms on the product surface. Hence, it leads to an extension of the shelf life and the improved safety of the product.

In this technology, natural or chemical antimicrobial agents incorporate into the packaging polymers are used to produce antimicrobial materials. Biodegradable packaging materials, made from entirely renewable natural polymers, could contribute to solving environmental pollution and creating new markets for agricultural products. On the other hand, the natural antimicrobial agent incorporation into renewable natural polymers for the development of antimicrobial food packaging materials is a new subject.

This study reviews efficiency of several natural antimicrobial agents against undesirable microorganisms in foods and their usage in cellulose-based packaging materials.

***Key words**: Antimicrobial films/coatings, Biodegradable material, Cellulose derivatives, Natural antimicrobial agents.*

1. Introduction

Microbial growth on the product surface is the main cause of spoilage of many foods. To control undesirable microorganisms in foods during storage, antimicrobial substances can either be coated on food surface or incorporated into the food packaging materials. Antimicrobial packaging is one of active packaging concepts that is an innovative way of inhibiting microbial growth on the foods while maintaining quality, freshness, and safety. It is also an alternative technology to non-thermal process which do not prevent growth of heat-resistant microorganisms and their spores. As antimicrobial packaging films are used for surface preservation, antimicrobial containers and utensils are used for liquid foods [1, 2].

Antimicrobial packaging is gaining interest from researchers and industries due to its potential to provide quality and safety benefits. The reason for incorporating antimicrobial agents into packaging materials is to prevent surface growth of microorganisms in foods where a large portion of spoilage and contamination occurs. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. A controlled release from packaging film to the food surface has numerous advantages over dipping and spraying [2 - 4].

In this technology, natural or chemical antimicrobial agents incorporated into the packaging system/polymer or antimicrobial polymers are used to produce antimicrobial packaging materials. Packaging material having antimicrobial activity limits or prevents microbial growth by reducing growth rate or extending lag phase of microorganisms. Hence, it leads to an extension of the shelf life and the improved safety of the product. Because of the increase in consumer demand for minimally processed, preservative-free products, the preservative agents must be applied to packaging in such a way that only low levels of preservatives come into contact with the food or natural preservative materials should be used. In order to meet this demand, the film or coating technique is considered to be more effective for applications [2, 3]. The natural antimicrobial agent incorporation into a polymeric material for the development of antimicrobial food packaging materials is a new subject. Natural antimicrobial agents such as spice volatile oils (thyme, rosemary, black pepper, sage, garlic etc.), plant extracts (olive leaf extract, grape seed extract etc.), organic acids (acetic, citric, lactic acids etc.), bacteriocins (nisin, lisozim, colisin, pediocin etc.) are used to produce antimicrobial packaging materials [1 - 3].

2. Biodegradable antimicrobial packaging

Synthetic polymers are widely used as packaging materials, because of their excellent mechanical properties, low permeability values and low cost, but they cause a big environmental disposal problem. For this reason, there has been an increasing interest on developing packaging materials alternative to synthetics with not only mechanical and permeability properties but also economical fabrication.

Biodegradable packaging, made from entirely renewable natural polymers, could contribute to solving environmental pollution and creating new markets for agricultural products. Environmental problems can thus result from using non-renewable raw materials and accumulation of such non-biodegradable packaging. One solution is use of biodegradable materials made from polysaccharides, proteins, lipids, polyesters or a combination of these. Moreover, some additives such as colorants, antioxidants and antimicrobial agents can provide to packaging materials some functional properties to prevent or delay microbial or chemical spoilage of food products.

The marketing of environmentally friendly packaging that uses biodegradable materials has the greatest potential in countries where landfill is the main waste management tool. The packaging field is still dominated by petroleum-derived polymers, such as polyethylene and polystyrene, despite global concerns about the environment. This is indicative of problems associated with the performance, processing, and overall cost, which remains a challenge when dealing with these renewable materials. In this content biopolymer-based packaging materials that include both edible films and coatings, along with primary and secondary packaging materials, have been reviewed [1 - 4].

Antimicrobial packaging may be an effective way of food preservation, but there are some critical concerns. First of all, antimicrobial agents may be incorporated into the packaging materials initially or coated or immobilized on the surface of packaging material and migrate into through the food diffusion and partitioning for it to be effective. Thus, this antimicrobial agent must be considered as a food additive and it should correspond to the regulatory concerns. Moreover, as much as antimicrobial efficiency, the cost-to-benefit ratio is an important concern. Some antimicrobial systems can be effective, but if produced on a large scale, they might require expenses beyond the benefits. And at last, there are numerous technical challenges related to coating methods, the effects on physical and mechanical properties of film, the effects on color, the texture or flavor of the food, and the ability of the antimicrobial agent to provide effectiveness throughout the package/product life cycle. As researches in this area progress, there is a promise to develop many new systems to meet these challenges [5].

2.1. Cellulose for biodegradable films/coatings

Biodegradable packaging is defined as packaging that contains raw materials originating from agricultural and marine sources. There are three such categories of biopolymers: (a) produced by chemical synthesis from bioderived monomers; (b) produced by microorganisms; (c) extracted directly from natural raw materials, such as cellulose [1].

Cellulose is one of the most abundant biopolymers on earth, occurring in wood, cotton, hemp and other plant-based materials and serving as the dominant reinforcing phase in plant structures. It is one of the most plentiful renewable resources. As a consequence of its chemical structure, it is highly crystalline, fibrous, and insoluble. Cellulose based edible films are very good barriers to aroma, oxygen and oil transfer like other hydrophilic films. Several water-soluble, composite coatings are made commercially from cellulose, carboxymethylcellulose with sucrose-fatty acid esters. Derivatives of cellulose, such as methylcellulose and hydroxypropyl methylcellulose, form strong and flexible water-soluble films. The barrier and mechanical properties of cellulose based edible films have been reported in the literature, with these films also lower in water vapour permeability compared to other hydrophilic edible films [3, 4].

2.2. Natural antimicrobial agents using in cellulose-based films

The natural antimicrobial agent incorporation into a polymeric material for the development of antimicrobial food packaging materials is a new subject. Natural antimicrobial agents such as spice volatile oils (thyme, rosemary, black pepper, sage, garlic etc.), plant extracts (olive leaf extract, grape seed extract etc.), organic acids (acetic, citric, lactic acids etc.), bacteriocins (nisin, lisozim, colisin, pediocin etc.) are used to produce antimicrobial packaging materials. The use of biodegradable polymers (cellulose derivatives, starch, pectin, etc.) may offer a good way for carrying a natural antimicrobial agent.

Antimicrobial agents incorporation into polymers may be a more suitable method for heat-sensitive antimicrobials like enzymes and volatile compounds. Lysozyme for example, has been incorporated into cellulose ester films by solvent compounding in order to prevent heat denaturation of the enzyme. Although bacteriocins and peptides are relatively heat-resistant, their antimicrobial activity may be higher when heat is not used in the process. Studies on nisin show that the activity of the bacteriocin in cast films is three times greater than that of heat-pressed films. The films were made from methylcellulose, hydroxypropyl methylcellulose, carrageenan and chitosan [3].

Chen *et al.* [6] developed antimicrobial methylcellulose films containing chitosan as well as sodium benzoate or potassium sorbate and investigated the effect of the films on *Penicillium notatum* and *Rhodotomla nibra*. The methylcellulose films containing 2% preservatives yielded clear zone at the film/medium interface as well as the area around the disc. However, the chitosan film containing 2 % of preservative did not result in a clear inhibitory zone around the film disc. The complex film comprising of methylcellulose and chitosan, containing 4% preservatives, could release the antimicrobial agents and form clear inhibitory zone during incubation.

Ghosh *et al.* [7] developed fungistatic wrappers with sorbic acid and applied them to bread. This wrapper necessitated heating the wrapped bread at 95 - 100 ^{0}C for a period of 30 to 60 min. The incorporation of an antioxidant in the treated wrapper and also the use of an odor adsorbent inside the bread packs minimized off-flavor development. Sliced bread, based on sensory evaluation, was found acceptable up to 1 month and as a sandwich, up to 3 months. The fungistatic wrappers were made by coating grease-proof paper with an aqueous dispersion of sorbic acid in 2% carboxymethylcellulose solution. Using this sorbic acid-treated paper and then enclosing the food in a polyethylene bag could preserve foods that are generally amenable to spoilage by mold for minimum of 10 days.

Coma [8] obtained antimicrobial activity of edible cellulosic films made with hydroxypropyl methylcellulose (HPMC) by the incorporation of nisin into the film-forming solution. The inhibitory activity of nicin was tested for inhibition of *Listeria innocua* and *Staphylococcus aureus*. The use of stearic acid was observed to reduce the inhibitory activity of active HPMC film against both selected strains. This phenomenon may be explained by electrostatic interactions between the cationic nisin and the anionic stearic acid.

Several studies have shown that the use of naturally derived antimicrobial agents in packaging systems for milk products, meat and meat products, fruits, vegetables etc. extends shelf life and maintain product quality (Table 1). Applications of cellulose based films containing natamycin to gorgonzola cheese, methylcellulose based films containing olive leaf extract to kashar cheese, cellulose based films containing nisin, natamycin to mozzarella cheese, lemongrass, oregano oil and vanillin incorporated in apple puree-alginate edible coatings to fresh-cut Fuji apples, methylcellulose-based coatings incorporated with chitosan to melon have retarded the growth rate of microorganisms which cause food spoilage [1 - 5].

Zhuang *et al.* [9] investigated the effect of hydroxypropyl methylcellulose (HPMC) coatings with ethanol to mature-green tomatoes on the survival of *Salmonella montevideo* on the surface and in core tissue. HPMC coating significantly (P ≤ 0.05) reduced the number of viable *S. montevideo* cells on the surface of tomatoes. However, only about a 2 log unit reduction was achieved in core tissue. The addition of citric acid, acetic acid, or sorbic acid to HPMC did not substantially enhance bactericidal activity. Application of HPMC coating also retarded the rate of loss of firmness and change in color of tomatoes stored at 20 ^{0}C for up to 18 days.

Table 1. Applications of some antimicrobial packaging materials to food spoilage microorganisms

Polymer	Antimicrobial agent	Food	Microorganism	Reference
HPMC*	Citric / Sorbic acid	Green tomatoes	*Salmonella montevideo*	[9]
Cellulose	Pediocin	Ham, turkey breast meat and beef	*Listeria monocytogenes*	[10]
Cellulose acetate	Sodium propionate	Bread	Bread mold	[12]
Cellophane	Nisin	Fresh veal meat	Total aerobic bacteria	[13]
Cellulose	Nisin	Cheddar cheese Ham	*Staphylococcus aureus* *Listeria innocua*	[14]
Cellulose	Natamycin	Gorgonzola cheese	*Penicillium roqueforti*	[15]
Cellulose	Nisin	Sausage	*Listeria monocytogenes*	[16]
Methylcellulose	Olive leaf extract	Kashar cheese	*Staphylococcus aureus*	[17]
HPMC	Organic acid salts	Mandarin	*Penicillium digitatum, Penicillium italicum*	[18]
HPMC	Propolis	Grape	Total mesophilic microorganisms, yeast, mold	[19]
Methylcellulose	Propolis	Kashar cheese	*Staphylococcus aureus*	[20]

HPMC*: Hydroxypropyl methylcellulose,

Heat resistant *Pediococcus*-derived bacteriocins inhibit or kill *Listeria monocytogenes* on contact with food. Ming *et al.* [10] applied nisin and pediocin to cellulose casings to reduce *L. monocytogenes* in meats and poultry. Pediocin-coated bags completely inhibited the growth of inoculated *L. monocytogenes* through 12 weeks storage at 4 ^{0}C. Patents taken in this regard are also available. One of them (US Patent 5,573,797) is a patent for the method of employing pediocin-coated cellulose casings on meat for inhibiting the growth of *L. monocytogenes* [1, 11].

Soares *et al.* [12] developed cellulose acetate films with sodium propionate to reduce mould growth during bread storage. The microbiological analyses showed that increased propionate concentration into the film decreased mould growth during storage period.

Nisin-adsorbed bioactive cellophane was applied to chopped meat under refrigeration temperatures. To assess the shelf life implications of incorporating bioactive inserts in the packaging of fresh veal meat, total aerobic bacteria counts were determined. The developed bioactive cellophane reduced significantly the growth of the total aerobic bacteria through 12 days of storage at 4 ^{0}C [13].

Olive leaf extract (OLE) is known with its high antioxidant, antimicrobial and antibacterial activity. Antimicrobial efficiency of phenolics extracted from olive, olive oil and olive leaf against some food pathogens, namely *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Salmonella enteritidis, Listeria monocytogenes* have been studied in the literature [21]. The first study on the OLE incorporated antimicrobial packaging materials and application of it performed by Ayana and Turhan in 2009. In the first part of the study, they prepared olive leaf extract (OLE) incorporated antimicrobial methylcellulose films and investigated OLE concentration on the film properties (water vapor permeability and mechanical properties) and the antimicrobial efficiency of these films against *S. aureus*. In the second part of the study, they applied the OLE incorporated methylcellulose films on the *S. aureus* inoculated kasar cheese and investigated the inhibition efficiency of the films. They reported that the *S. aureus* numbers decreased 1.22 log cycle for the slices wrapped with OLE incorporated methylcellulose films after 14 day of storage. On the other hand *S. aureus* count increased 0.64 and 0.60 log cycle at the end of the storage for non-wrapped and methylcellulose film wrapped slices, respectively [17]. A similar study was performed by Scannel *at al.* [14] who immobilized nisin to cellulose-based pouches. Nisin-adsorbed bioactive inserts reduced levels of *Listeria innocua* by ≥ 2 log units in both products, and *S.aureus* by 1.5 log units in cheese, and 2.8 log units in ham in in modified atmosphere packaging at refrigeration temperatures. Similar reductions were observed in cheese vacuum-packaged in nisin-adsorbed pouches [16].

In another study, antimicrobial efficiency of OLE incorporated polylactic acid (PLA) and methylcellulose-PLA films were investigated against *S. aureus* [22]. It was reported that the methylcellulose-PLA films were much successive inhibiting *S. aureus* than the PLA films at all OLE concentrations. It was suggested that the incorporation of hydrophilic methylcellulose into the hydrophobic PLA matrix probably made easy the release of the OLE to the hydrophilic agar medium.

Antimicrobial methylcellulose films containing propolis extract (0 - 6%, w/v) as an antimicrobial agent were produced by Erol [20]. The effect of the amount of propolis extract on the water vapour permeability, the mechanical and the antimicrobial properties of the methyl cellulose based films were investigated. As the concentration of extract increased in methylcellulose films, the water vapour permeability and percent elongation decreased whereas the tensile strength increased. The methylcellulose films containing propolis extract (4,5%, w/v) were applied onto the salami slices inoculated with *S. aureus*. The level of *S. aureus* decreased 0,78 log at the end of the 14th days.

The efficiency of cellulose based packaging materials containing certain antimicrobial agents like nisin, olive leaf extract, propolis, organic acids and others have been widely investigated (Table 1). These studies underline that a proper polymer with an antimicrobial may offer effective food preservation. However, application of plant extracts or essential oils on a foodstuff or a packaging material may not always reflect the results obtained from preliminary in vitro studies of the same compounds. The inhibitory effect of an antimicrobial agent in an antimicrobial package is related with release from the polymer matrix. Foods are complex systems consisting of different connected microenvironments and the antimicrobial additive should release enough from the polymer matrix to show inhibitory effect [2]. Thus, having an inhibition effect against certain group of bacteria of an antimicrobial agent is not sufficient alone; selection of an appropriate polymer should be taken account when developing antimicrobial packaging materials.

3. Conclusions

- Although there has been a rising interest in the researches in this field, availability of natural antimicrobials and new biodegradable polymer materials, regulatory concerns, and appropriate testing methods limit the developments.
- Antimicrobial packaging is highly regulated around the world and researchers must take these regulations into consideration. First of all, the component showing the antimicrobial effect must considered as a food additive and must meet the food additive standards.
- In recent years, studies on usage of antimicrobial plant extracts have gained acceleration since these are generally classified as GRAS (generally recognized as safe).
- The use of biodegradable films for food packaging has been strongly limited because of the poor barrier properties and weak mechanical properties shown by natural polymers. For this reason, further studies are necessary to improve the barrier properties of natural polymers.

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