ANTIMICROBIAL FOOD PACKAGING IN MEAT INDUSTRY

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Abstract

Antimicrobial packaging, an active packaging concept, can be considered an extremely challenging technology that could have a significant impact on shelf-life extension and food safety of meat and meat products. Use of antimicrobial substances can control the microbial population and target specific microorganisms to provide higher safety and quality products. Many classes of antimicrobial compounds have been evaluated in film structures, both synthetic polymers and edible films: organic acids and their salts, enzymes, bacteriocins, and miscellaneous compounds such as triclosan, silver zeolites, and fungicides.

The characteristics of some antimicrobial packaging systems are reviewed in this article. The regulatory status of antimicrobial packaging in EU is also examined.

Key words: active packaging, antimicrobial substances, meat, meat products, pathogens

Introduction

Active packaging is one of the innovative food packaging concepts that have been introduced as a response to the continuous changes in current consumer demands and market trends. It has been defined as "*a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food*". This definition of active packaging was chosen for the European FAIR- project CT 98-4170 (Vermeiren, Devlieghere, van Beest, de Kruijf & Debevere, 1999).

In general, active food packaging can provide several functions that do not exist in conventional packaging systems. The active functions may include scavenging of oxygen, moisture or ethylene, emission of ethanol and flavours, and antimicrobial activity.

Microbial contamination reduces the shelf-life of foods and increases the risk of foodborne illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging, and adding antimicrobial agents or salts. Unfortunately, some of these techniques cannot be applied to some food products, such as fresh meats and ready-to-eat products.

Antimicrobial packaging is a promising form of active food packaging, in particular for meat products. Since microbial contamination of these foods occurs primarily at the surface, due to post-processing handling, attempts have been made to improve safety and to delay spoilage by use of antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods have limited benefits because the active substances are neutralized on contact or diffuse rapidly from the surface into the food mass. On the other hand, incorporation of bactericidal or bacteriostatic agents into meat formulations may result in partial inactivation of the active substances by product constituents and is therefore expected to have only limited effect on the surface microflora.

Therefore, the use of packaging films containing antimicrobial agents could be more efficient, by slow migration of the agents from the packaging material to the surface of the product, thus helping maintain high concentrations where they are needed. If an antimicrobial can be released from the package during an extended period, the activity can also be extended into the transport and storage phase of food distribution.

Antimicrobial substances incorporated into packaging materials can control microbial contamination by reducing the growth rate and maximum growth population and/or extending the lag-phase of the target microorganism, or by inactivating microorganisms by contact.

Developing the antimicrobial packaging systems

Most food packaging systems represent either a package/food system or a package/headspace/food system (Figure 1).

A package/food system is a solid food product in contact with the packaging material, or a low-viscosity or liquid food without headspace. Individually wrapped ready-to-eat meat products, "sous-vide" cooked products and deli products are good examples. Diffusion between the packaging material and the food and partitioning at the interface are the main migration phenomena involved in this system. Antimicrobial agents may be incorporated into the packaging materials initially and migrate into the food through diffusion and partitioning (Han, 2000).

Package/headspace/food systems are represented by foods packed in flexible packages, cups, and cartons. Evaporation or equilibrated distribution of a substance among the headspace, packaging material and/or food has to be considered as a part of main migration mechanisms to estimate the interfacial distribution of the substance. A volatile active substance can be used in these systems, as it can migrate through the headspace and air gaps between the package and the food.

Besides diffusion and equilibrated sorption, some antimicrobial packaging uses covalently immobilized antibiotics or fungicides, or active moieties such as amine groups. This case utilises surface inhibition of microbial growth by immobilisation of the non-food-grade antimicrobial substance without diffusional mass transfer.

Figure 2 shows the mass transfer phenomena of an active substance incorporated into a film or coating, with different applications.

The incorporation of an antimicrobial substance into a food packaging system can take several approaches. One is to put the antimicrobial into the film by adding it in the extruder when the film or the co-extruded film is produced. The disadvantage of doing so is poor cost effectiveness since antimicrobial material not exposed to the surface of the film is generally not totally available to antimicrobial activity. An alternative to extrusion is to apply the antimicrobial additive in a controlled matter where the material is needed and not lost; for example, it can be incorporated into the food-contact layer (usually also serving as the inner heat-seal layer) of a multilayer packaging material.

According to Han (2000), several factors must be taken into account in the design or modelling of the antimicrobial film or package:

1. Chemical nature of films/coatings, casting process conditions and residual antimicrobial activity. The choice of the antimicrobial is often limited by the heat lability of the component during extrusion or by the incompatibility of the component with the packaging material. For example, one per cent potassium sorbate in a LDPE film inhibited the growth of yeast on agar plates. The LDPE resin and potassium sorbate powder can be mixed, extruded and pelletized to produce a master batch. These pellets can be added to LDPE resin. The master batch should be produced at low temperature to prevent heat decomposition of the potassium sorbate (Han & Floros, 1997). Another study (Weng &

Hotchkiss, 1993), however, found the relatively polar sorbate, benzoate and propionate to be incompatible with the apolar LDPE. Acid anhydrides were thought to be more compatible than free acids and their salts because of their lower polarity.

The residual antimicrobial activity is the effective activity of the antimicrobial agents utilized after the casting (extrusion) and converting processes (lamination, printing, drying). The effects of adhesives and solvents should also be characterized quantitatively.

2. Characteristics of antimicrobial substances and foods. Food components significantly affect the effectiveness of the antimicrobial substances and their release. Physico-chemical characteristics of food could alter the activity of antimicrobial substances. For example, the pH of food influences the ionisation (dissociation/association) of most active chemicals, and could change the antimicrobial activity of organic acids and their salts. The antimicrobial activity and chemical stability of incorporated active substances could be influenced also by the water activity of food. Moreover, each food has its own characteristic microflora. The release kinetics of antimicrobial agents has to be designed to maintain the concentration above the critical inhibitory concentration with respect to the contaminating microorganisms that are likely to be present.
3. Storage temperature. Storage temperature can affect the antimicrobial activity of chemical preservatives. Generally, increased storage temperature can accelerate the migration of the active agents in the film/coating layers, while refrigeration slows down the migration rate. The temperature conditions during production and distribution have to be predicted to determine their effect on the residual antimicrobial activity of the active compounds.
4. Mass transfer coefficients. The simplest system is the diffusional release of active substances from the package into the food. A multilayer design has the advantage that the antimicrobial can be added in one thin-layer and its migration and release controlled by the thickness of the film layer or coating. In practice, a matrix of several layers are used to control the rate of release of the active substance. Control of the release rates and migration amounts of antimicrobial substances from food packaging is very important. A mass transfer model of the migration phenomena can be used to describe the concentration profile in the film/coating layer and food over time. Han (2000) summarized traditional mass transfer models and his own proposed models that may be used to describe the migration of active agents through food packaging systems consisting of single, double, or triple layers. By using mass transfer models it is possible to calculate the storage periods that maintain the active agent concentration above the critical effectiveness concentration, so safety shelf-life of the food could be calculated.
5. Physical properties of packaging materials. When antimicrobial activity is added to packaging materials to reduce microbial growth, it may affect the general physical properties of the packaging materials. Han & Floros (1997) found that the transparency of the plastic film under study decreased with the addition of the active agent. The performance of the packaging materials must be maintained after the addition of the active substances, even though the materials contain more heterogeneous formulations.

Examples of antimicrobial packaging concepts

As previously mentioned, approaches to antimicrobial packaging can be classified as either of two types. The first consists of binding an agent to the surface of the package and this would require a molecular structure large enough to retain activity on the microbial cell wall even though bound to the plastic. Such agents are likely to be limited to enzymes or other antimicrobial proteins. The second approach involves the release of active agents onto the surface of the food.

Non-edible packaging systems may contain any type of food grade additives in their packaging materials. Some chemical agents naturally exist in plants or fermented products. However, they mainly are chemically synthetized and categorized as chemical additives sometimes with restricted regulations required.

In Japan, silver-substituted zeolite has been developed as the most common antimicrobial agent incorporated into plastics. Silver-ions, which inhibit a wide range of metabolic enzymes, have strong antimicrobial activity with a broad spectrum. The zeolite, which has some of its surface atoms replaced by silver, is laminated as a thin layer (3-6 μm) in the surface of the food contact polymer and appears to release silver ions as aqueous solution from the food enters the exposed cavities of the porous structure (Ishitani, 1995). Less than 0.001% of the silver is in the coating itself. On June 9th, 2000 the AgION™ Silver Ion Technology received the approval of the Food and Drug Administration for use in all types of food-contact polymers in USA market (http://www.cfsan.fda.gov/~dms/opa-fcn.html).

Several other compounds have been proposed and tested for antimicrobial activity in food packaging including organic acids such as sorbate, propionate and benzoate or their respective acid anhydrides (Table 1), bacteriocins e.g. nisin and pediocin or enzymes such as lysozyme (Table 2), and fungicides such as benomyl (Halek & Garg, 1989) and imazalil (Weng & Hotchkiss, 1992).

An interesting commercial development is the recent marketing of food-contact approved Microban® (Microban Products Co., USA) wide range of products such as cutting boards and dishcloths, which contain triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl-ether, also used in soaps, shampoos, and toothbrushes). Cutter (1999) investigated the effectiveness of triclosan-incorporated plastic (TIP) against populations of foodborne pathogenic bacteria as well as bacteria associated with meat surface. Plate overlay assays indicated that plastic containing 1,500 ppm of triclosan inhibited the growth of *Brochotrix thermosphacta* ATCC 11509, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6051, *Shigella flexneri* ATCC 12022, *Escherichia coli* ATCC 25922 and several strains of *E. coli* O157:H7. However, the same film did not effectively reduce bacterial populations on refrigerated, vacuum-packaged meat surfaces. The presence of fatty acids or other components associated with adipose tissue might diminish the antibacterial activity of TIP on meat surfaces. Recently, the use of triclosan for food-contact applications has been allowed in EU countries by the SCF (Scientific Committee for Food) in the 10th additional list of monomers and additives for food contact materials (SCF, 2000), with a quantitative restriction on migration of 5 mg/kg of food.

In contrast to conventional antimicrobial films, some functional groups that have antimicrobial activity have been immobilized on the surface of polymer films by modified chemical methods to prevent the transfer or migration of antimicrobial substances from the polymer to the food (Haynie, Crum & Doele, 1995).

Modifying surface composition of polymers by electron irradiation in such a way that the surface would contain amine groups has also been shown to exhibit antimicrobial activity that inactivates microorganisms by contact. Cohen et al. (1995) reported that exposure of nylon yarn or fabric to 1-3 J/cm^2 of 193 nm light from an excimer laser in air caused an apparent 10% conversion of amides to amines (resulting in 5 x 10^{12} surface amines/cm^2), which are still bound in the polymer chain.

The surface-bound amine groups proved active in phosphate buffer against *Staphylococcus aureus* ATCC 25923, *Pseudomonas fluorescens* ATCC 13525 and *Enterococcus faecalis* ATCC 19433. The decrease in bacterial concentration was more likely due to bactericidal action rather than surface adsorption of the cells (Paik, Dhanasekharan & Kelly, 1998).

Microbial activity on such nylon films is closely related to the laser wavelength used in the process, because laser irradiation at 248 nm does not change surface chemistry or initiate the conversion of amide moieties on the surface to amine groups.

Shearer, Paik, Hoover, Haynie & Kelley (2000) irradiated a nylon 6,6 film with a 193 nm UV excimer. The irradiated nylon demonstrated antagonistic activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* TV1058 with 4.5 and 6.0 log reductions, respectively, of an initial population of 10^6 cfu/ml. The irradiated nylon was ineffective against *Pseudomonas fluorescens* ATCC 13525 and *Enterococcus faecalis* ATCC 19433 under similar conditions. However, protein and salt inhibited the antimicrobial effect of the irradiated nylon film.

Other applications of the laser-induced surface modifications may be used to produce antimicrobial packaging systems in the future. UV irradiation, like that produced by an UV excimer laser at an appropriate wavelength, can be used to oxidize O_2, previously absorbed on a modified surface layer, to O_3 by photochemical means (Rooney, 1995). The formed O_3 can then be desorbed or subjected to controlled-release from the polymer matrix to the interior of the package. Even ppm concentrations of O_3 are sufficient to inhibit microbial growth and this approach could significantly overcome the necessity of direct contact between the food and antimicrobial film to be effective.

Not only UV treatments can be used to modify film surfaces. Plasma treatments have gained considerable attention in the food packaging area as methods to improve adhesion, sealability, wettability and other characteristics of polymers (Ozdemir, Yurteri & Sadikoglu, 1999). For example, fluorine-based plasmas can be used to form a fluorinated surface layer on food packaging polymer surfaces, and the antimicrobial properties of halogens have been known for years.

Antimicrobial edible coatings and films

There is a growing interest in edible coatings due to factors such as environmental concerns, need for new storage techniques, and opportunities for creating new markets for under-utilized agricultural commodities with film-forming properties. Edible coatings and films prepared from polysaccharides, proteins and lipids have a variety of advantages such as biodegradability, edibility, biocompatibility, aesthetic appearance and barrier properties against oxygen and physical stress.

Some advantages of using edible coatings and films on meat and meat products have been discussed recently by Gennadios, Hanna & Kurth (1997). Edible coatings could:

- help alleviate the problem of moisture loss during storage of fresh or frozen meats;
- hold juices of fresh meat and poultry cuts when packed in retail plastic trays;
- reduce the rate of rancidity-causing lipid oxidation and brown coloration-causing myoglobin oxidation;
- reduce the load of spoilage and pathogen microorganisms on the surface of coated meats;
- restrict volatile flavour loss and foreign odour pick-up.

As an application of active packaging, edible coatings carrying antioxidants or antimicrobials can be used for direct treatment of meat surfaces.

In the case of edible films and coatings, selection of the incorporated active agents is limited to edible compounds. Because they have to be consumed with edible film/coating layers and foods together, their edibility and safety are essential.

Antimicrobial edible films have received attention as a potential pathogen intervention strategy for various muscle foods. Siragusa & Dickinson (1993) demonstrated that alginate coatings containing organic acids were marginally effective on beef carcasses, reducing levels *Listeria monocytogenes*, *Salmonella* Typhimurium and *Escherichia coli* O157:H7 by 1.80, 2.11, and 0,74 logs, respectively.

Complete inhibition of *Listeria monocytogenes* on ham, turkey breast meat and beef was obtained using pediocin or nisin fixed on a cellulose casing (Ming, Weber, Ayres & Sandine, 1997). Commercial application of this technology is described in U.S. Patent 5,573,797 assigned to Viskase Co. The package is a film, such as a polymer film or a regenerated cellulose film, containing a heat resistant, *Pediococcus*-derived bacteriocin in a synergistic combination with a chelating agent to inhibit or kill *Listeria monocytogenes* on contact with food (Katz, 1999).

Dawson, Han & Padgett (1997) and Padgett, Han & Dawson (1998) used nisin and lysozyme in soy protein and corn zein films to inhibit *Lactobacillus plantarum* and *Escherichia coli* on laboratory media. *L. plantarum* was inhibited by film containing nisin or lysozyme; the addition of EDTA increased the inhibitory effect of films against *E. coli*.

Ouattara, Simard, Piette, Bégin & Holley (2000) prepared antimicrobial films by inclusion of various organic acids and essential oils into a chitosan matrix, and investigated the ability of these films to inhibit the growth of indigenous (lactic acid bacteria and Enterobacteriaceae) or inoculated bacteria (*Lactobacillus sakei* and *Serratia liquefaciens*) onto the surfaces of vacuum-packed cured meat products. Release of organic acids (acetic and propionic acid) was found to be initially fast, when the gradient of ion concentration between the inside of the polymer matrix and the outside environment was high, then decreased as the release of acids progressed. Whereas the antimicrobial films under study did not affect lactic acid bacteria, the growth of Enterobacteriaceae and *S. liquefaciens* was delayed or completely inhibited after a storage of 21 days at 4°C. Strongest inhibition was observed on surfaces with lower water activity value (bologna), onto which acid release was slower, and with films containing cinnamaldeyde, as a result of its greater antimicrobial activity under these conditions.

Regulatory status of antimicrobial packaging in EU

The general problem arising from the use of food contact materials depends on their content of substances capable of migrating into the contacted food. Therefore, to protect the consumer, an assessment of the potential hazards from oral exposure to those constituents that migrate into the food must be made.

Gergely (2001) provided a clear vision of the regulatory status regarding the use of antimicrobials in food applications in EU.

In general, antimicrobial substances fall under the definition of "biocidal products":

active substances (and preparations containing one or more active substances, put up in the form in which they are supplied to the user), intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect of any harmful organism by chemical or biological means.

provided in Directive 98/8/EC, called Biocide Directive (EU, 1998). This Directive excludes many product categories, falling under other EU directives; hence, on the basis of this exclusion, the category "film preservatives", listed in one of the Annexes of the directive, would

only cover those antimicrobial products used for the preservation of plastic materials in general, but not antimicrobials used in food contact plastic applications. Also finished packaging materials containing antimicrobial substances would not be covered by the Biocide Directive, even for general applications, because the directive limits the definition of biocides to cover substances that are "*put in the form in which they are supplied to the user*".

As a consequence, antimicrobial additives are permitted in food packaging under the "Plastics Directive", Directive 90/128/EEC as last updated by Directive 2002/17/EC (EU, 2002) and, for substances that are not covered by a listing in the Plastics Directive, the national legislation in place in each of the individual Member Sates must be consulted.

However, the use of biocides is only compliant with the requirements of these laws if they are required to manufacture a finished product, without having a direct impact on the quality of the packaged food.

In practice, the producer presenting an application for safety assessment of a substance to be used in food contact materials should provide evidence (i) that any migration into food is not intentional but only incidental; (ii) that its use does not exert any preservative effect on the food; (iii) that it does not allow the selection of non-sensitive organisms on the surface of the food contact materials and that it does not allow the development of biocide resistance in sensitive microorganisms. The petitioner should also provide evidence that the substance is not used to reduce the normal hygienic measures required in handling foodstuffs (SCF, 2001).

If the second criterion is not met, the product must be considered a "direct food additive" (*a preservative that prolongs the shelf-life of foodstuffs by protecting them against deterioration caused by microorganisms*) and would fall under the Miscellaneous Additives Directive (EU, 1995).

It is quite difficult to draw a strict line where the technical function of an additive is solely its antimicrobial effect on the packaging without an impact on the food itself. Most of the materials used in antimicrobial packaging systems only act as a reservoir for the active substance and the target effect is on the food in contact with the package. This is clearly in conflict with the Plastics Directive.

The EU- wide regulatory instruments covering antimicrobial substances differ distinctively in scope, depending upon both the intended application and the actual effect of the active substances. So far, it is not the active product itself, but its use conditions that determine which directive applies in regulating the marketing of food packaging products with antimicrobial activity in the European Union.

Conclusions

Consumers continue to demand foods that are minimally processed and posses fresh-like quality, while modern distribution systems require an adequate shelf-life. Numerous type of food packaging can be used in combination with food preservation techniques in order to extend the effectiveness of food preservation chain. The idea of combining antimicrobials with packaging films to control the growth of microorganisms in food could have a significant impact on shelf-life extension and food safety.

The application of antimicrobial films might allow for migration of the antimicrobial to the film surface and therefore a continued antimicrobial effect at the food surface during extended exposure. Direct addition of antimicrobials to food will result in an immediate reduction of bacterial population but this technique may not account for the recovery of injured cells or the growth of cells that were not destroyed by direct addition.

The use of antimicrobial packaging materials in food packaging can minimize the microbial contamination of food product surfaces during storage, transportation and handling. The main action of these films is based on the release of antimicrobial substances into food products. Some of these agents could pose a safety risk to consumers if the release is not tightly controlled by some mechanism within the packaging material itself. An interesting innovation could be the use of polymers which surfaces have been modified by electron irradiation or plasma treatment to generate antimicrobial activity without any transfer or migration of substances to the food.

As the growth and death rates of bacteria will vary for each growth medium, conclusions on how antimicrobial films will perform with a food product must be determined for each food application.

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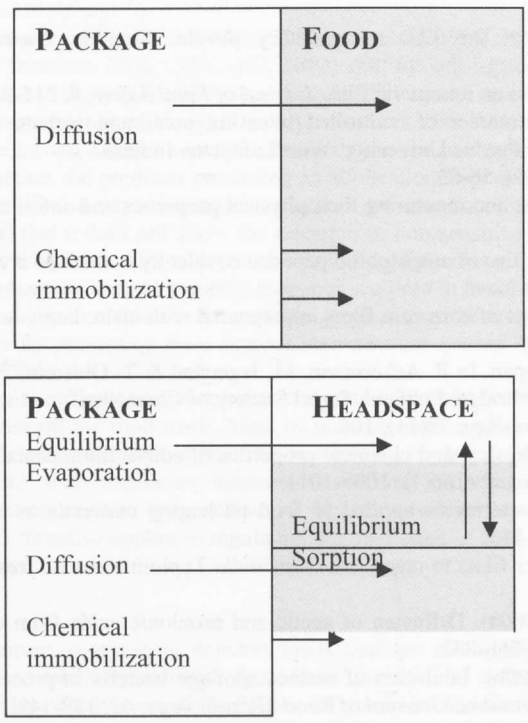

Figure 1. Food packaging systems and relative behaviour of active substances. (Han, 2000)

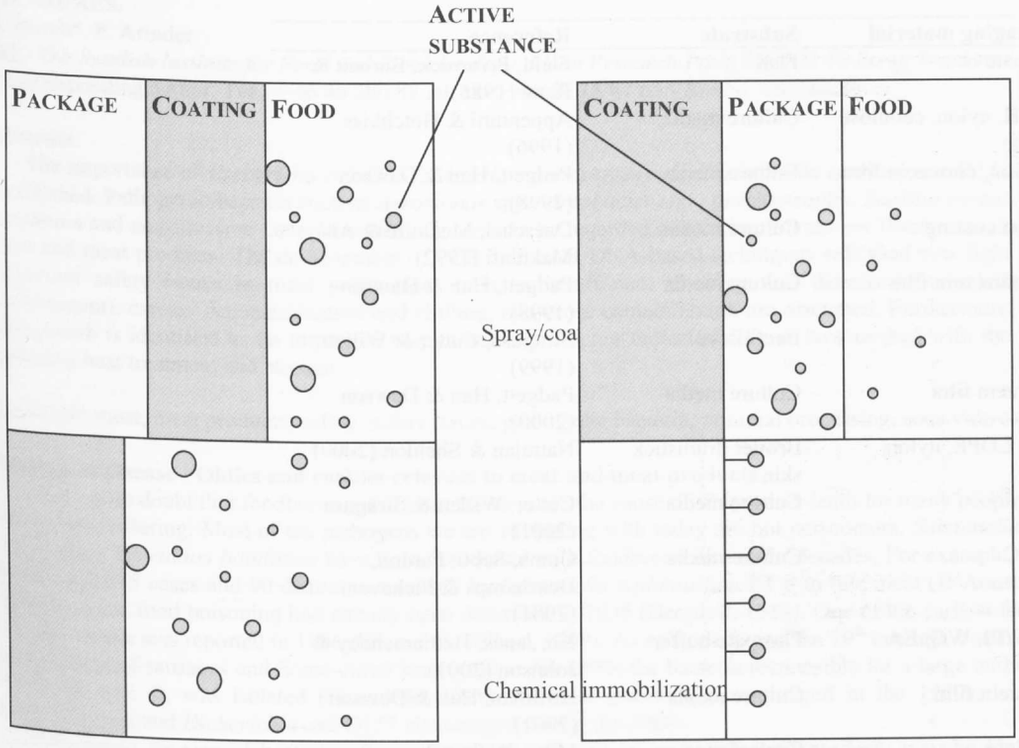

Figure 2. Migration of active substance in different applications of antimicrobial packaging systems (Han, 2000)

Table 1. Applications of antimicrobial food packaging. Incorporation of organic acids and their salts in plastic or edible films.

Antimicrobial agent	Packaging material[a]	Substrate	Reference
Acetic acid	Chitosan	Water	Ouattara, Simard, Piette,
	Chitosan	Bologna, cooked	Bégin & Holley (2000a)
		ham, pastrami	Ouattara, Simard, Piette,
			Bégin & Holley (2000b)
Benzoic acid	PE-co-MA	Culture media	Weng et al (1997)
Benzoic anhydride	LDPE	Culture media	Weng & Hotchkiss (1993)
Sodium benzoate	MC/chitosan	Culture media	Chen, Yeh & Chiang (1996)
p-aminobenzoic acid	WPI	Culture media	Cagri, Ustunol & Ryser
			(2001)
Lactic acid	Alginate	Lean beef muscle	Siragusa & Dickinson (1992)
Lauric acid	corn zein film	Culture media	Padgett, Han & Dawson
			(2000)
	corn zein film	Culture media	Hoffman, Han & Dawson
			(2001)
Propionic acid	Chitosan	Water	Ouattara, Simard, Piette,
	Chitosan	Bologna, cooked	Bégin & Holley (2000a)
		ham, pastrami	Ouattara, Simard, Piette,
			Bégin & Holley (2000b)
Sorbic acid	WPI	Culture media	Cagri, Ustunol & Ryser
			(2001)
Sorbic acid anhydride	PE	Culture media	Weng & Chen (1997)
Potassium sorbate	MC/HPMC/fatty acid	Culture media	Vojdani & Torres (1990)
	MC/palmitic acid	Culture media	Rico-Pena & Torres (1991)
	Starch/glycerol	Chicken breast	Baron & Summer (1993)
	MC/chitosan	Culture media	Chen, Yen & Chiang (1996)
			Han (1996)
	LDPE	Cheese	Han & Floros (1997)
	LDPE	Culture media	

[a] LDPE = low-density polyethylene; MC = methyl cellulose; HPMC = hydroxypropyl MC; CMC = carboxyl MC; PE = polyethylene; MA = methacrylic acid ; SPI = soy protein isolate ; WPI = whey protein isolate

Table 2. Applications of antimicrobial food packaging. Incorporation of pediocins and enzymes in plastic or edible films.

Antimicrobial agent	Packaging material	Substrate	Reference
Glucose oxidase	Alginate	Fish	Field, Pivarnick, Barnett & Rand (1986)
Lysozyme	PVOH, nylon, cellulose acetate	Culture media	Appendini & Hotchkiss (1996)
	SPI film, corn zein film	Culture media	Padgett, Han & Dawson (1998)
Nisin	Silicon coating	Culture media	Daeschel, McGuire & Al-Makhlafi (1992)
	SPI, corn zein film	Culture media	Padgett, Han & Dawson (1998)
	PE	Beef tissue	Siragusa, Cutter & Willett (1999)
	Corn zein film	Culture media	Padgett, Han & Dawson (2000)
	PVC, LDPE, nylon	Broiler drumstick skin	Natrajan & Sheldon (2000)
	PE	Culture media	Cutter, Willett & Siragusa (2001)
	HPMC	Culture media	Coma, Sebti, Pardon, Deschamps & Pichavant (2001)
	SPI, WPI, WG, EA	Phospate buffer	Ko, Janes, Hettiarachchy & Johnson (2001)
	Corn zein film	Culture media	Hoffman, Han & Dawson (2001)
Pediocin	Cellulose	Cooked meats	Ming, Weber, Ayres & Sandine (1997)

[a] LDPE = low-density polyethylene; MC = methyl cellulose; HPMC = hydroxypropyl MC; PE = polyethylene; PVOH = polyvinyl alcohol; PVC = polyvinyl chloride; SPI = soy protein isolate; WPI = whey protein isolate; WG = wheat gluten; EA = egg albumen

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