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Innovative Food Science & Emerging Technologies 3 (2002) 113–126

Innovative Food Science & Emerging Technologies

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Review of antimicrobial food packaging

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Abstract

Research and development of antimicrobial materials for food applications such as packaging and other food contact surfaces is expected to grow in the next decade with the advent of new polymer materials and antimicrobials. This article reviews the different types of antimicrobial polymers developed for food contact, commercial applications, testing methods, regulations and future trends. Special emphasis will be on the advantages/disadvantages of each technology. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Food packaging; Antimicrobial(s); Immobilization; Active packaging review

Industrial relevance: The emergence of gentle (non-thermal) process conditions for preservation and shelf life extension of foods makes packaging and packages an integral part of retaining food safety criteria. Antimicrobial packaging is a form of active packaging. This highly interesting review offers a summary of the wide variety of recent antimicrobial packaging materials and of related issues such as testing the effectiveness of antimicrobial packaging, regulatory issues involved and future research recommendations such as the development of 'intelligent' and 'smart' packages.

1. Introduction

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade, and distribution from centralized processing pose major challenges for food safety and quality. Recent food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety. One option is to use packaging to provide an increased margin of safety and quality. The next generation of food packaging may include materials with antimicrobial properties. These packaging technologies could play a role in extending shelf-life of foods and reduce the risk from pathogens. Antimicrobial polymers may find use in other food contact applications as well.

Antimicrobial packaging is a form of active packaging. Active packaging interacts with the product or the headspace between the package and the food system, to obtain a desired outcome (Labuza & Breene, 1989; Rooney, 1995; Brody, Strupinsky & Kline, 2001). Likewise, antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself.

2. Types of antimicrobial packaging

Antimicrobial packaging can take several forms including:

1. Addition of sachets/pads containing volatile antimicorbial agents into packages.
2. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers.
3. Coating or adsorbing antimicrobials onto polymer surfaces.
4. Immobilization of antimicrobials to polymers by ion or covalent linkages.
5. Use of polymers that are inherently antimicrobial.

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1466-8564/02/$ - see front matter
PII: S1466-8564(02)00012-7

3. Addition of sachets / pads containing antimicrobial agents to packages

The most successful commercial application of antimicrobial packaging has been sachets that are enclosed loose or attached to the interior of a package. Three forms have predominated: oxygen absorbers, moisture absorbers and ethanol vapor generators. Oxygen and moisture absorbers are used primarily in bakery, pasta, produce and meat packaging to prevent oxidation and water condensation. Although oxygen absorbers may not be intended to be antimicrobial, a reduction in oxygen inhibits the growth of aerobes, particularly molds. Moisture absorbers can reduce a_w, also indirectly affecting microbial growth. Both oxygen and moisture absorption technologies have been reviewed in detail (Rooney, 1995).

Ethanol vapor generators consist of ethanol absorbed or encapsulated in carrier materials and enclosed in polymer packets. The ethanol permeates the selective barrier and is released into the headspace within the package. Since the amount of ethanol generated is relatively small and effective only in products with reduced water activity ($a_w < 0.92$), applications have been mainly to retard molds in bakery and dried fish products (Smith, Hoshino & Abe, 1995). Commercial examples include Ethicap®, heat sealed packets containing microencapsulated ethanol in silicon dioxide powder, and Fretek®, a paper wafer in which the center layer is impregnated with ethanol in acetic acid and sandwiched between layers of polyolefin films (Rice, 1989). One of the drawbacks is the characteristic off-flavor of ethanol.

Absorbing pads (diapers) are used in trays for packaged retail meats and poultry to soak up meat exudates. Organic acids and surfactants have been incorporated into these pads to prevent microbial growth in the exudates, which are rich in nutrients (Hansen, Rippl, Midkiff & Neuwirth, 1989).

4. Incorporation of antimicrobial agents directly into polymers

Incorporation of bioactive agents including antimicrobials into polymers has been commercially applied in drug and pesticide delivery, household goods, textiles, surgical implants and other biomedical devices. Few food-related applications have been commercialized (Table 1). The number of recently published articles and patents suggest that research on the incorporation of antimicrobials into packaging for food applications has more than doubled in the past 5 years. GRAS, non-GRAS and 'natural' antimicrobials have been incorporated into paper, thermoplastics and thermosets, and have been tested against a variety of microorganisms including *Listeria monocytogenes,* pathogenic *E. coli,* and spoilage organisms including

Table 1
Selected commercial antimicrobial packaging available for food applications[a]

Antimicrobial compound	Tradename	Producer Company	Packaging forms for food applications	Reference
Silver substituted zeolite	AgIon™	AgIon Technologies LLC	Bulk food storage containers, paperboard cartons, plastic or paper food wraps and milk containers.	*http://www.healthshield.com/index*1.*html* Last accessed: 01/25/02
	Novaron®	Toagosei, Co. LTD	Many (Japan)	Toagosei, Co. LTD Brochure
Triclosan	Microban®	Microban Products	Deliwrap, reheatable food containers (UK)	Sherman (1998), Rice (1995)
Allylisothio-cyanate	WasaOuro	Lintec Corporation	Pressure sensitive labels, sheets (Japan)	*http://www.lintec.co.jp/index-e.html* Last accessed: 01/25/02
		Dry Company LTD	Sachets	Anon (1995)
Chlorine dioxide	Microsphere™	Bernard Technologies Inc.	Storage bags for produce, paperboard coating, rigid containers, pressure sensitive labels	Gray (2000)
Carbon dioxide	Freshpax™	Multisorb Technologies	Sachets	Smith et al. (1995)
	Verifrais	SARL Codimer	Sachets (France)	Smith et al. (1995)
Ethanol vapor	Ethicap®	Freund	Sachets	Smith et al. (1995)
	Negamold®			
	Fretek®		Sachets	Rice (1989)
	Oitech™	Nippon Kayaku	Sachets (Japan)	Smith et al. (1995)
Glucose oxidase	Bioka	Bioka LTD	Sachets (Finland)	*http://www.bioka.fi/index.html*
(hydrogen peroxide)				Last accessed: 01/25/02

[a] For additional commercial antimicrobial packaging references, see Brody et al. (2001).

Table 2
Antimicrobials incorporated directly into polymers used for food packaging

Antimicrobials	Polymer/carrier	Main target microorganisms	References
Organic acids / anhydrides: Propionic, benzoic, sorbic, acetic, lactic, malic	Edible films, EVA, LLDPE	Molds	Guilbert (1988), Baron & Sumner (1993) Torres & Karel (1985) Devlieghere, Vermeiren, Bockstal & Debevere, (2000) Weng & Hotchkiss (1993)
Inorganic gases: Sulfur dioxide, chlorine dioxide	Various polyolefins	Molds, Bacteria, Yeasts	CSIRO (1994) Wellinghoff (1995)
Metals: Silver	Various polyolefins	Bacteria	Ishitani (1995)
Fungicide: Benomyl, imazalil	LDPE	Molds	Weng (1992)
Bacteriocins: Nisin, pediocins, lacticin	Edible films, cellulose, LDPE	Gram-positive bacteria	Padgett, Han & Dawson (1998) Siragusa, Cutter & Willett (1999) Scanell, Hill, Ross, Marx, Hartmeier & Arendt (2000)
Enzymes: Lysozyme, glucose oxidase	Cellulose acetate, PS Edible films	Gram-positive bacteria	Appendini and Hotchkiss (1997) Padgett et al. (1998)
Chelating agents: EDTA	Edible films	Gram-negative bacteria	Padgett et al. (1998)
Spices: Cinnamic, caffeic, *p*-coumaic acids Horseradish (allylisothiocyanate)	Nylon/PE, cellulose	Molds, yeast, bacteria	Hoshino, Iijima, Hayashi & Shibata (1998) Anon (1995), Nielsen & Rios (2000)
Essential oils (plant extracts): Grapefruit seed extract, hinokitiol, bamboo powder, Rheum palmatum, Coptis chinesis extracts	LDPE, cellulose	Molds, yeast and bacteria	Lee, Hwang & Cho (1998) Imakura, Yamada & Fukazawa (1992) Oki (1998), Chung, Cho, & Lee (1998) Hong et al. (2000)
Parabens: Propylparaben, ethylparaben	Clay-coated cellulose LDPE	Molds	Katz (1998) Dobiaš et al. (1998)
Miscellaneous: Hexamethyl-enetetramine	LDPE	Yeasts, anaerobes and aerobes	Devlieghere et al. (2000)

Abbreviations: EVA (ethylene vinyl acetate); LLDPE (linear low density polyethylene); LDPE (low density polyethylene); PS (polystyrene); PE (polyethylene).

molds (Table 2). Of all the antimicrobials, silver substituted zeolites are the most widely used as polymer additives for food applications, especially in Japan. Sodium ions present in zeolites are substituted by silver ions, which are antimicrobial against a wide range of bacteria and molds. These substituted zeolites are incorporated into polymers like polyethylene, polypropylene, nylon and butadiene styrene at levels of 1–3% (Brody et al., 2001). Silver ions are taken up by microbial cells disrupting the cells' enzymatic activity. Commercial examples of silver substituted zeolites include Zeomic®, Apacider®, AgIon, Bactekiller and Novaron.

In addition to the antimicrobials listed in Table 2, other compounds have the potential to be incorporated into polymers. For example, antimicrobial enzymes such as lactoperoxidase and lactoferrin, antimicrobial peptides such as magainins, cecropins, defensins, natural phenols like hydroquinones and catechins, fatty acid esters, antioxidant phenolics, antibiotics and metals like copper and others may be useful (Hotchkiss, 1997).

Combinations of more than one antimicrobial incorporated into packaging have also been investigated. For example, it is hypothesized that compounds active against Gram-positive bacteria (i.e. lysozyme) combined with chelating agents (i.e. EDTA) can target Gram-negative bacteria. Addition of EDTA to edible films containing nisin or lysozyme, however, had little inhibition effect on *E. coli* (Padgett, Han & Dawson, 2000) and *Salmonella typhimurium* (Natrajan & Sheldon, 2000).

The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods were a large portion of spoilage and contamination occurs. For example, intact meat from healthy animals is essentially sterile and spoilage occurs primarily at the surface. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. The gradual release of an antimicrobial from a packaging film to the food surface may have an advantage over dipping and spraying. In the latter processes, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix. Emulsifiers and fatty acids, for example, are known to interact with nisin reducing the bacteriocin's activity (Henning, Metz & Hammes, 1986; Jung, Bodyfelt & Daeschel, 1992). Vojdani and Torres (1989) found that sorbates are rapidly absorbed from food

surfaces, loosing the protective effect. They incorporated sorbates into polysaccharide films and demonstrated that the films allowed slower diffusion of the sorbates to the surface of a food, which in turn improved surface protection. Films with low diffusion rates were desirable since they maintained higher surface concentrations of sorbate for longer periods. Pectin/gluten/monoglyceride films containing sorbic acid have also been shown to delay the growth of molds in model food systems, as compared to sorbic acid deposited directly into the food's surface (Guilbert, Cuq & Gontard, 1997). When the antimicrobial is released over time, microbial growth kinetics and antimicrobial activity at the product's surface may be balanced.

Many antimicrobials are incorporated at 0.1–5% w/w of the packaging material, particularly films. Antimicrobials may be incorporated into polymers in the melt or by solvent compounding. Thermal polymer processing methods such as extrusion and injection molding may be used with thermally stable antimicrobials. Silver substituted zeolites, for example, can withstand very high temperatures (up to 800 °C) and therefore have been incorporated as a thin co-extruded layer with other polymers (Ishitani, 1995).

For heat-sensitive antimicrobials like enzymes and volatile compounds, solvent compounding may be a more suitable method for their incorporation into polymers. Lysozyme for example, has been incorporated into cellulose ester films by solvent compounding in order to prevent heat denaturation of the enzyme (Appendini & Hotchkiss, 1997). Although bacteriocins and peptides are relatively heat-resistant (Muriana, 1993; Appendini & Hotchkiss, 2001), 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, hydroxypropylmethylcellulose, carrageenan and chitosan (Cha, Park & Cooksey, 2001). In solvent compounding, both the antimicrobial and the polymer need to be soluble in the same solvent. Biopolymers are good candidates for this type of film forming process, due to the wide variety of proteins, carbohydrates and lipids (which act as plasticizers) that form films and coatings. These polymers as well as their combinations are soluble in water, ethanol and many other solvents compatible with antimicrobials. Extensive studies have been focused on sorbic acids and its salts incorporated into zein (Torres & Karel, 1985) and mixtures of fatty acids and cellulose derivatives (Vojdani & Torres, 1989; Coma, Sebti, Pichavant, Pardon & Deschamps, 2001). These films combined with low surface pH have been shown to improve microbial stability in food model systems (Torres & Karel, 1985).

Many antimicrobials are not easily incorporated into or not homogeneously distributed in poly(olefins) and related hydrophobic polymers. Weng and Hotchkiss (1993) addressed the problem of mixing organic acids with LDPE by forming the anhydride of the acid prior to addition to the polymer melt. In the presence of moisture, the anhydride hydrolyzed to the acid form, which led to rapid migration of the free acid from the film's surface to the food where it was effective at retarding mold growth. A similar example is that of hexamethylenetetramine incorporated into LDPE. In acid environments, formaldehyde is formed and released from the films. These films however, failed to show antimicrobial activity in orange juice and formaldehyde has toxic implications (Devlieghere, Vermeiren, Jacobs & Debevere, 2000).

Antimicrobial packaging materials must contact the surface of the food if they are non-volatile, so the antimicrobial agents can diffuse to the surface, therefore, surface characteristics and diffusion kinetics become crucial. The diffusion of antimicrobials from packaging has been the subject of several research papers by Floros, Torres and colleagues (Vojdani & Torres, 1989, 1990; Rico-Pena & Torres, 1991; Han & Floros, 1998a,b) and has been recently reviewed by Han (2000). This work has demonstrated that antimicrobial release from the polymer has to be maintained at a minimum rate so that the surface concentration is above a critical inhibitory concentration. To achieve appropriate controlled release to the food surface, the use of multilayer films (control layer/matrix layer/barrier layer) has been proposed (Floros, Nielsen & Farkas, 2000). The inner layer controls the rate of diffusion of the active substance while the matrix layer contains the active substance and the barrier layer prevents migration of the agent towards the outside of a package.

Packaging systems that release volatile antimicrobials have also been developed. These include chlorine dioxide, sulfur dioxide, carbon dioxide and allylisothiocyanate release systems. The theoretical advantage of volatile antimicrobials is that they can penetrate the bulk matrix of the food and that the polymer need not necessarily directly contact the product. Antimicrobial vapors or gases are appropriate for applications where contact between the required portions of the food and the packaging does not occur, as in ground beef or cut produce. Precursor molecules are incorporated directly into the polymer or into carriers that may be extruded or coated into packaging materials. Allylisothiocyanate for example, has been entrapped in cyclodextrins that are coated to packages or labels. Chlorine dioxide is generated using sodium chlorite and acid precursors which are embedded in a hydrophobic and hydrophilic phases of a copolymer. When moisture from the food contacts the hydrophobic phase, acid is released, which in turn reacts with the sodium chlorite releasing chlorine dioxide.

The reaction of precursors and the diffusion of chlorine dioxide from the polymer are, therefore, moisture- and temperature-dependent (Wellinghoff, 1995). Workers at CSIRO (Australia) have developed materials that gradually release sulfur dioxide from pads containing sodium metabisulfite. The system has been used for table grapes (CSIRO, 1994). Off-odors, especially in the case of allylisothiocyanate, and high volatility of gases are the major drawbacks of the antimicrobial gas release technology. As in MAP, high barrier materials need to be used with volatile antimicrobial to prevent loss from permeation. Control of vapor pressure and stability of the gases are essential to sustain their release and antimicrobial properties through shelf-life.

5. Coating or adsorbing antimicrobials to polymer surfaces

Early developments in antimicrobial packaging incorporated fungicides into waxes to coat fruits and vegetables and shrink films coated with quaternary ammonium salts to wrap potatoes (Shetty & Dwelle, 1990). Other early developments included coating wax paper and cellulose casings with sorbic acid for wrapping sausages and cheeses (Labuza & Breene, 1989).

Antimicrobials that cannot tolerate the temperatures used in polymer processing are often coated onto the material after forming or are added to cast films. Cast edible films, for example, have been used as carriers for antimicrobials and applied as coatings onto packaging materials and/or foods. Examples include nisin/methylcellulose coatings for polyethylene films (Cooksey, 2000) and nisin/zein coatings for poultry (Food Safety Consortium Newsletter, 2000). Proteins have an increased capacity for adsorption due to their amphiphilic structure. Bower, McGuire and Daeschel (1995) demonstrated that nisin adsorbed onto silanized silica surfaces inhibited the growth of *L. monocytogenes*. A similar study showed that surfaces with low hydrophobicity had more nisin activity than those with higher hydrophobicity, even if adsorbed mass values were generally the inverse (Daeschel, McGuire & Al-Makhlafi, 1992). Other examples include: adsorption of nisin on PE, EVA, PP, polyamide, PET, acrylics and PVC (Daeschel & McGuire, 1995; Wilhoit, 1996), pediocin-containing milk-based powders adsorbed onto cellulose casings and barrier bags (Ming, Weber, Ayres & Sandine, 1997) and nisin/EDTA/citric solutions coated onto PVC, nylon and LLDPE films (Natrajan & Sheldon, 2000).

Manipulating the solvents and/or polymer structures can enhance antimicrobial adsorption. Poly(ethylene-co-methacrylic acid) films treated with sodium hydroxide and swollen with acetone showed an increased absorption and diffusion of benzoic and sorbic acids compared to non-treated films. These NaOH-treated films also had the highest inhibitory effect on molds (Weng, Chen & Chen, 1999). The explanation is that the higher polarity of NaOH-treated films enhanced the absorption of the antimicrobials. Binders such as polyamide resins have also been used to increase compatibility between polyolefins surfaces and bacteriocins (An, Kim, Lee, Paik & Lee, 2000). Glucose oxidase has been coated onto moisture proof fabric sheets by using polyvinyl alcohol, starch and casein as adhesives (Labuza & Breene, 1989).

6. Immobilization of antimicrobials by ionic or covalent linkages to polymers

A few examples of ionic and covalent immobilization of antimicrobials onto polymers or other materials have been published (Table 3). This type of immobilization requires the presence of functional groups on both the antimicrobial and the polymer. Examples of antimicrobials with functional groups are peptides, enzymes, polyamines and organic acids. Examples of polymers used for food packaging that have functional groups are shown in Table 4. In addition to functional antimicrobials and polymer supports, immobilization may require the use of 'spacer' molecules that link the polymer surface to the bioactive agent. These spacers allow sufficient freedom of motion so the active portion of the agent can contact microorganisms on the food surface. Spacers that could potentially be used for food

Table 3
Antimicrobials covalently/ionically immobilized in polymer supports

Functional support	Antimicrobials	Reference
Ionomeric films	Benomyl	Halek and Garg (1989)
	Benzoyl chloride	Weng et al. (1997)
	Bacteriocin	Dobiaš et al. (1998)
Polystyrene	Lysozyme	Mermelstein (1998)
	Synthetic antimicrobial peptides	Haynie, Crum and Doele (1995)
		Appendini and Hotchkiss (2001)
Polyvinyl alcohol	Lysozyme	Appendini and Hotchkiss (1997)
Nylon 6,6 resins	Lysozyme	Appendini and Hotchkiss (1997)

Table 4
Functional groups in polymers commonly used for food packaging materials

Polymer	Monomer formula
Ethylene vinyl acetate (EVA)	$-(CH_2-CH_2)_m-(CH_2CH)_n-$ O C=O CH_3
Ethylene methyl acrylate (EMA)	$-(CH_2-CH_2)_m-(CH_2CH)_n-$ C=O O CH_3
Ethylene acrylic acid (EAA)	$-(CH_2-CH_2)_m-(CH_2CH)_n-$ C=O OH
Ethylene metacrylic acid (EMAA)	CH_3 $-(CH_2-CH_2)_m-(CH_2C)_n-$ C=O OH
Ionomer	CH_3 $-(CH_2-CH_2)_m-(CH_2C)_n-$ C=O O^-Na^+
Nylon	$-[(CH_2)_5-C-NH]_x$ O
Polyvinylidene chloride (PVdC)- Polyvinyl chloride (PVC) copolymer	Cl Cl $-(CH-CH_2)_m-(CH_2-C)_n-$ Cl
Ethylene vinyl alcohol (EVOH)- polyethylene (PE) copolymer	$-(CH_2-CH_2)_m-(CH_2CH)_n-$ OH
Polystyrene (PS)	$-(CH_2-CH)_n-$ C_6H_5

Adapted from Brown (1992).

antimicrobial packaging include dextrans, polyethylene glycol (PEG), ethylenediamine and polyethyleneimine, due their low toxicity and common use in foods.

The potential reduction in antimicrobial activity due to immobilization must be considered. For proteins and peptides, changes in conformation and denaturization by solvents may result in low activity per unit area. Approaches to increasing activity per unit area include the protection of active sites during film formation and the incorporation of dendrites to increase the surface area of the supports. For example, Soares and Hotchkiss (1998) used the substrate to protect and increase the activity of naringinase immobilized in cellulose acetate films.

Ionic bonding of antimicrobials onto polymers allows slow release into the food. However, diffusion to the product is less of a concern when the antimicrobial is covalently bonded to the polymer unless conditions within the product promote reactions such as hydrolysis. This may occur for example, during the heating of a high acid food.

Lysozyme and chitinase, both active against Gram-positive bacteria, have been covalently immobilized (Appendini & Hotchkiss, 1997; Wang & Chio, 1998). Activity, however, was too low to be practical for packaging commercial applications. Glucose oxidase catalyzes the reaction between glucose and oxygen yielding the antimicrobial hydrogen peroxide. This enzyme has been covalently bound onto insoluble supports that could be compatible with packaging materials (Garcia & Galindo, 1990; Wang & Hsiue, 1993). Beta-galactosidase and glucose oxidase have been co-immobilized with the objective of producing hydrogen peroxide to activate lactoperoxidase in milk (Garibay, Luna-Salazar & Casas, 1995). Other antimicrobial enzymes that could potentially be covalently immobilized for packaging applications include lactoferrin, sulfhydril oxidase and bile-salt stimulated lipase. A major challenge, however, is the incorporation of substrates into the system as well as managing undesirable products from the reactions. For example, glucose oxidase requires glucose as a substrate, which could be provided by the food or added. Lactoperoxidase however, requires hydrogen peroxide and thiocyanate, commonly present in milk but not in many other foods. In both systems, hydrogen peroxide may raise toxicological concerns if amounts exceed FDA regulations.

6.1. Immobilized peptides

Several peptides isolated from animals, plants, mi-

croorganisms, and insects, as well as chemically synthesized analogs, have shown antimicrobial activity against microorganisms including those found in foods (Abler, Klapes, Sheldon & Klaenhammer, 1995; Appendini & Hotchkiss, 2000). Since peptides can be covalently immobilized through amino and carboxylic groups, they may be suitable for attachment to functionalized polymer surfaces. We have studied the potential uses of covalently immobilized peptides for packaging applications. A 14-amino-acid residue peptide was immobilized on polystyrene by solid phase peptide synthesis (SPPS) and tested against several food-borne microorganisms (Appendini & Hotchkiss, 2001). The advantage of SPPS is that the peptide is built directly on the resin by protecting the amino acids functional groups. The resulting surface-modified polystyrene (SMPS) was microcidal in a concentration and time-dependent manner against several bacteria, molds and yeast suspended in buffer (Fig. 1) and growing in nutrient media (Fig. 2). *E. coli* 0157:H7 was among the microorganisms that showed susceptibility against the SMPS. *E. coli* 0157:H7 was also susceptible when tested in apple juice. The study demonstrated the feasibility of attaching peptides with a wide antimicrobial activity spectrum to polystyrene, a polymer commonly used in food packaging. Future technology may allow the controlled immobilization of peptides in polymer films rather than beads and reduce the high costs associated with SPPS.

7. Use of polymers that are inherently antimicrobial

Some polymers are inherently antimicrobial and have been used in films and coatings. Cationic polymers such as chitosan and poly-L-lysine promote cell adhesion (Goldberg, Doyle & Rosenberg, 1990) since charged amines interact with negative charges on the cell membrane, causing leakage of intracellular constituents. Chitosan has been used as a coating and appears to protect fresh vegetables and fruits from fungal degradation. Although the antimicrobial effect is attributed to antifungal properties of chitosan, it may be that the chitosan acts as a barrier between the nutrients contained in the produce and microorganisms (Cuq, Gontard & Guilbert, 1995). In addition, chitosan-based antimicrobial films have been used to carry organic acids and spices (Ouattara, Simard, Piette, Begin & Holley, 2000). Calcium alginate films reduced the growth of the natural flora and coliform inocula on beef, possibly due to the presence of calcium chloride (Cuq et al., 1995). Bactericidal acrylic polymers made by co-polymerizing acrylic protonated amine co-monomer have been proposed as packaging materials for increased fruit and vegetable shelf life (Pardini, 1987). Polymers containing biguanide substituents also yield antimicrobial activity (Olstein, 1992).

Physical modification of polymers has been investigated as means to render surfaces antimicrobial. For example, the antimicrobial potential of polyamide films

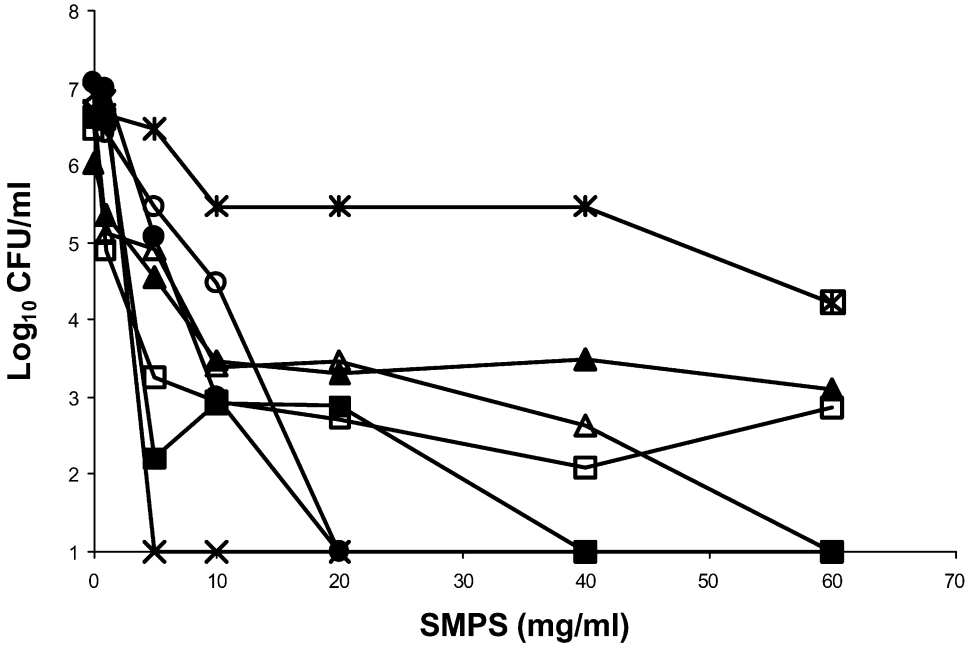

Fig. 1. Effect of surface-modified polystyrene (SMPS) concentration on the viability of *B. subtilis* (×), *E. coli* O157:H7 (■), *K. marxianus* (▲), *L. monocytogenes* (○), *P. fluorescence* (●), *S. typhymurium* (△), *S. liquefasciens* (□) and *S. aureus* (*) suspended in phosphate buffer (pH 7.2) for 10 min at 25 °C (from Appendini & Hotchkiss, 2001).

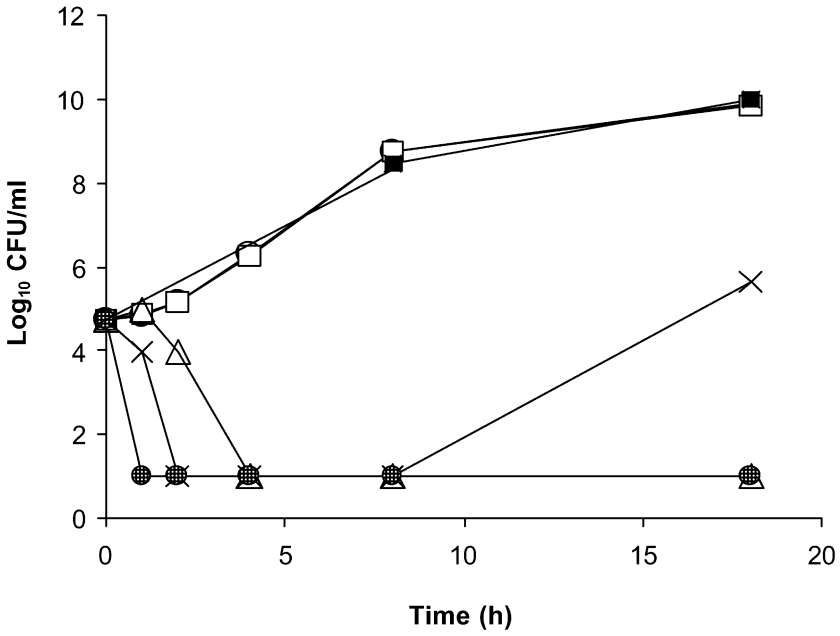

Fig. 2. Effect of surface-modified polystyrene (SMPS) concentration on *E. coli* O157:H7 growth in broth (TSB) at 25 °C. The concentrations of SMPS in TSB were 0 mg ml^{-1} (○), 6 mg ml^{-1}(■), 10 mg ml^{-1} (×), 20 mg ml^{-1}(△) and 40 mg ml^{-1} (●). Resin that had not been treated with peptide served as the control at a concentration of 40 mg ml^{-1} (□) (from Appendini & Hotchkiss, 2001).

treated with UV irradiation has been reported. Antimicrobial activity was presumably the result of an increase in amine concentration on the film's surface (Hagelstein, Hoover, Paik & Kelley, 1995). Positively charged amine groups present in polymer surfaces may enhance cell adhesion but not necessarily death (Lee, Jung, Kang & Lee, 1994). It is possible that in the tests mentioned, simple adsorption occurred, masking the lack of antimicrobial activity of the aminated polymer surface. A subsequent study on UV-treated nylon films showed that the surface amino groups were bactericidal, but that bacterial cells were adsorbed to the surface and diminished the effectiveness of the amine groups (Paik, Dhanasekharan & Kelley, 1998). In many cases, these studies are conducted in buffer. Addition of nutrients could potentially prevent cell membrane damage and bacterial recovery and/or inhibit the adhesion of the cells to the surface due to the interaction of salts and other cations with the surfaces.

8. Applications of antimicrobial packaging in foods

Antimicrobial polymers can be used in several food related applications including packaging (Hotchkiss, 1997). One is to extend the shelf-life and promote safety by reducing the rate of growth of specific microorganisms by direct contact of the package with the surface of solid foods (e.g. meats, cheese, etc.) or in the bulk of liquids (e.g. milk or meat exudates). Second, antimicrobial packaging materials could be self-sterilizing or sanitizing. Such antimicrobial packaging materials greatly reduce the potential for recontamination of processed products and simplify the treatment of materials in order to eliminate product contamination. For example, self-sterilizing packaging might eliminate the need for peroxide treatment in aseptic packaging. Third, at least in concept this could result in self-sterilizing foods, especially liquids. This might be particularly useful for high acid products such as fruit juices. Antimicrobial polymers might also be used to cover surfaces of food processing equipment so that they self-sanitize during use. Examples include filler gaskets, conveyers, gloves, garments, and other personal hygiene equipment.

The target microorganism(s) and the food composition must be considered in antimicrobial packaging. As with any antimicrobial, those to be incorporated into polymers have to be selected based on their spectrum of activity, mode of action, chemical composition, and the rate of growth and physiological state of the targeted microorganisms. The activity of antimicrobials that diffuse from packaging to the food will be determined at least in part by diffusion kinetics (Han, 2000). Antimicrobials attached to the polymer, however, need to be active while attached to the polymer. This activity is related to the mode of action. If, for example, the mode of action is on the cell membrane or wall of the microorganism, it is possible that the attached antimicrobial will act on the cells. This is likely not to be the case if it needs to enter the cytoplasm.

Seldom does microbial growth in synthetic media parallel the growth in the foods, and food components may limit the activity of antimicrobials by inhibiting diffusion from the polymers. Silver-substituted zeolites for example, are not active in nutrient-rich media, since lysine, sulfates, sulfides and other sulfur containing amino acids weaken the antimicrobial activity. The most practical application appears to be for nutrient-poor beverages such as tea and mineral water (Ishitani, 1995). Other examples of polymers with high antimicrobial activity in growth media and low activity in foods include triclosan in plastics (Cutter, 1999).

Polymer additives including fillers, antifog and antistatic agents, lubricants, stabilizers and plasticizers can negatively affect activity of antimicrobial polymers. These additives may change polymer conformation altering diffusion or may interact directly with the antimicrobial. When lysozyme was incorporated into cellulose triacetate for example, addition of a plasticizer (glycerol) was shown to have a negative effect on the enzyme's activity (Fig. 3).

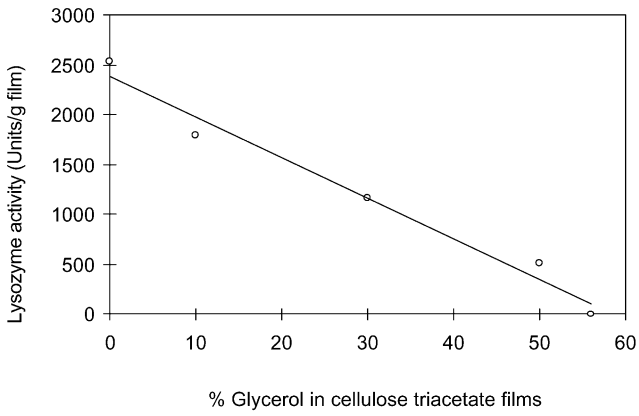

Fig. 3. Effect of plasticizer (glycerol) on cellulose triacetate-immobilized antimicrobial enzyme activity (from Appendini, 1996).

Further considerations in antimicrobial packaging choice are the concentration of antimicrobials in polymer film, the effect of film thickness on activity and the physical and mechanical properties of the polymers after conversion to the final product. For example, antimicrobial activity of compounds coated or immobilized on the surface of polymer films may be independent of film thickness. However, if the antimicrobial is entrapped into the bulk of the material, thickness plays will play a role in the diffusion and concentration at the film's surface.

The effect of the antimicrobial on polymer properties must also be considered. For example, incorporation of particles that carry antimicrobials into the polymer matrix may change the film's mechanical, barrier and optical properties. Plant extracts commonly impart color and opacity to polymers (An, Hwang, Cho & Lee, 1998; Hong, Park & Kim, 2000) and sorbates decrease transparency of LDPE films (Han & Floros, 1997). Tensile, seal strength, and barrier properties usually decrease when additives are incorporated into polymers (Dobiaš, Voldrich, Marek & Derovský, 1998). Oxygen and water vapor transmission rates increase in LDPE containing chitosan but decrease in LDPE containing benzoic acid. Changes in these properties therefore, will be specific for each antimicrobial-polymer pair. Antimicrobials adsorbed or immobilized onto polymers surfaces may alter heat sealing strength, adhesion and printing properties of the plastics.

9. Testing the effectiveness of antimicrobial packaging

There are a variety of official test methods to determine the resistance of plastic materials to microbial degradation (Table 5). There is, however, no agreement upon standard methods to determine the effectiveness of antimicrobial polymers. In Japan, a method referred to as 'Film Contact Method' (SIAA, 1998) is used as a standard to assess the ability of products containing antimicrobials to impart antimicrobial properties to products. The method was developed for inorganic antimicrobials such as silver substituted zeolites. It is suitable for films and sheets and consists of inoculating bacteria on the test specimen and incubating and counting the bacteria under specified conditions. The intent is to determine the resistance of the plastic to microbial growth, but it may also serve to determine if polymers are 'self-sterilizing'.

To assess if antimicrobial packaging have an effect on microorganisms present in the foods, agar plate methods, minimally inhibitory concentrations (MIC), and dynamic shake flask tests have been used using similar methods to those used to evaluate antimi-

Table 5
Standard methods for testing plastic materials resistance to microbial attack

Method	Description
IEC 68-2-10	Basic environmental testing procedures
EN ISO 846	Plastics-Evaluation of the action of microorganisms
ASTM G21-90	Standard practice for determining resistance of synthetic polymeric materials to fungi
ASTM G22-76	Standard practice for determining resistance of synthetic polymeric materials to bacteria

From Ochs (2000).

crobials alone (Ochs, 2000; Davidson and Parish, 1989). MIC can indicate the antimicrobial strength of the polymer and allows the comparison of the polymer's antimicrobial activity to that of the antimicrobial alone. The method consists in seeding a series of tubes containing growth medium with the target microorganism and with polymers containing different concentrations of antimicrobial. The tubes are incubated for a predetermined period of time and visually inspected for microbial growth (turbidity). MIC is the lowest concentration of an antimicrobial in a polymer resulting in the complete inhibition of growth of a test microorganism. Results should include polymer dimensions, composition and other relevant characteristics that vary from specimen to specimen.

In the agar plate test, antimicrobial film is placed on a solid agar medium containing the test microorganism. The agar plates are incubated until growth is visible. A clear zone surrounding the film indicates antimicrobial diffusion from the film and subsequent growth inhibition (Fig. 4). Lack of growth under a film may indicate inhibition, but appropriate controls must be included this may be due to simple restriction to oxygen. The agar plate tests method simulates wrapping of foods and may suggest what can happen when films contact contaminated surfaces and the antimicrobial agent migrates from the film to the food. The method can be quantitative if the diameter of the clear zones around the films is measured.

Shake flasks tests provide more detailed information on antimicrobial kinetics. Liquid media (buffer, growth media or foods) are seeded with the target microorganisms and the antimicrobial polymer. The flasks are incubated with mild agitation. Samples are taken over time and enumerated Unlike the MIC test, this method measure reduction in growth rate even if substantial grow occurs. Tests in buffer provide information on the microcidal properties of the polymers while tests in broth provide information on microbial growth kinetics and the antimicrobial mode of action of the polymers. Tests in buffer may be misleading since cells susceptible in nutrient-poor media may recover if nutrients are present. When testing antimicrobial films by the shake flask test, the ratio of film surface area to volume (of product or media) must be considered. Previous examples show that increasing the surface area/volume ratio increases the activity of bioactive molecules incorporated into polymer films (Fig. 5). From an antimicrobial standpoint, high surface/volume ratios may seem adequate. But in real packaging applications, surface area/volume ratios of one are considered optimal, and values higher than that may be impractical. By accounting the area/volume ratio, the feasibility of such films for practical applications may be assessed. As its name implies, the shake flask test includes agitation, which enhances the contact between the antimicrobial polymer and the cells. The test may not be indicative of the degree of agitation that packaged foods receive and therefore studies should simulate agitation during storage and transportation.

10. Regulatory issues

Food packaging is highly regulated around the world

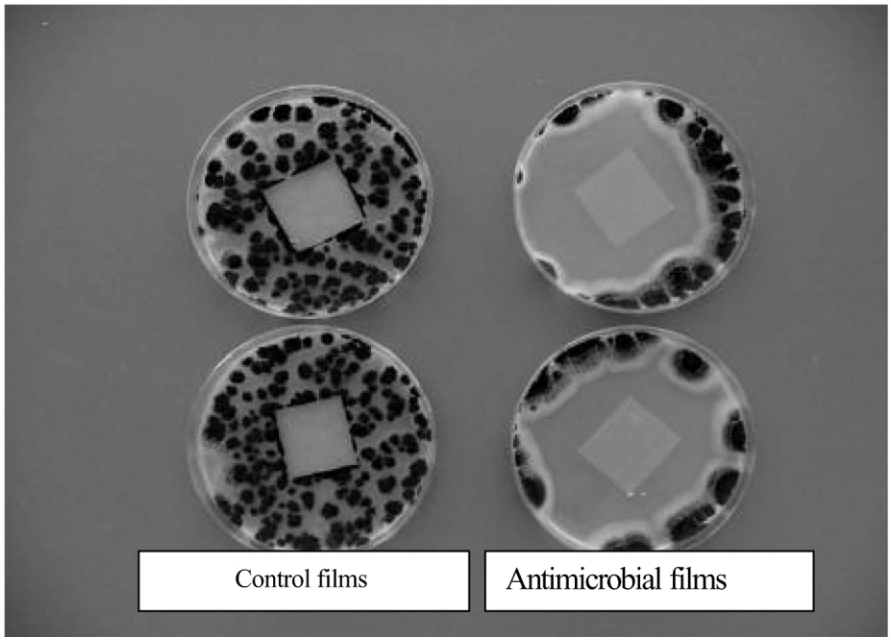

Fig. 4. Effect of antimicrobial plastic film on *Aspergillus niger*. Agar diffusion method (Photograph).

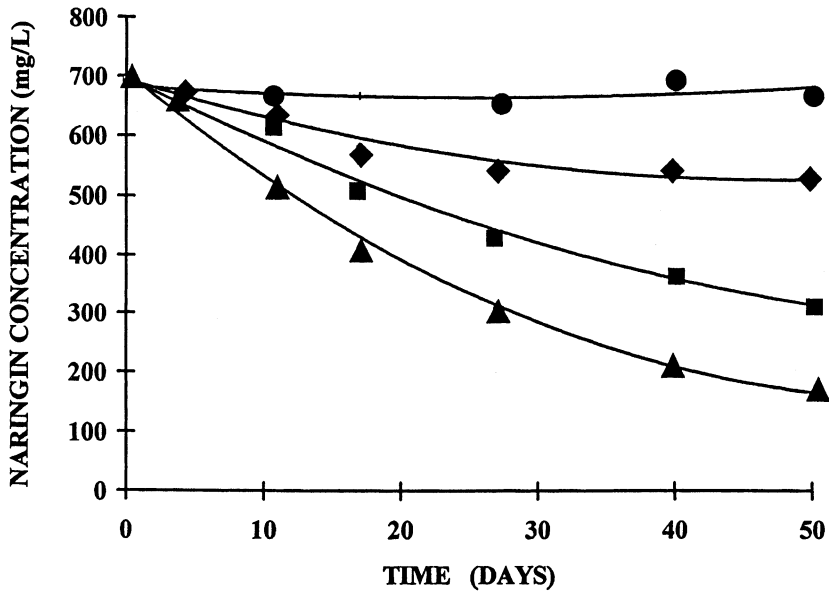

Fig. 5. Naringin hydrolisis in grapefruit juice using increasing ratios of film area to juice volume during 6 weeks storage at 7 °C. Ratios of film area/juice volume were 3.6 cm^2/ml (▲), 2.1 cm^2/ml (■), 1 cm^2/ml (♦) and 0 cm^2/ml (●) (from Soares & Hotchkiss, 1998).

including active and antimicrobial packaging and development projects must take these regulations into consideration. For example, Actipak, a project supported by the European Commission was started with the 'aims of initiating amendments to European legislation for food contact materials in order to establish and implement active and intelligent concepts within the current relevant regulations for packaged food in Europe' (DeKruijf, 2000). In the US, no specific regulation exists for active packaging (CFR, 2001). Antimicrobials in food packaging that may migrate to food are considered food additives and must meet the food additive standards. Packaging forms include bulk food storage containers, paperboard cartons, plastic or paper food wraps, jars and bottles. Examples of antimicrobial uses include surface sanitizing solutions for milk containers, hydrogen peroxide uses in aseptic packaging, and antimicrobials impregnated into food packaging to protect either the package, or to extend the shelf-life of the food. To date, the only FDA approved materials for direct food contact are Zeomic®, a silver substituted zeolite (FCN No. 47) and chlorine dioxide generated from particles (GRN No. 62). For Zeomic, the maximum use level permitted is 5% by weight of the polymer and its approval is granted for preventing microbial growth on plastic surfaces. Particles that release chlorine dioxide are approved for use in unprocessed meats and produce at levels not exceeding 2.71 $\mu g/cm^2$ of chlorite in finished LDPE packaging films. It is possible that compounds that are not approved food additives could be transformed into approved additives during the migratory process. For example, benzoic anhydride is not approved but when released from LDPE hydrolyzes into benzoic acid, which is FDA approved for foods. If the released compound is approved and precursors not, it is likely these precursors will need to be incorporated in middle layers of laminated structures and not on the food contact layer (sealant).

Several studies have focused on the use of plant extracts and oils as antimicrobial additives for polymers since these are generally classified as GRAS (i.e. generally recognized as safe). The concentrations that are required for antimicrobial packaging applications are much higher than the concentrations found in nature, which may raise regulatory concerns.

Antimicrobial packages where the antimicrobial does not detach from the surface of the packaging materials hold long-term promise as a means of inhibiting microorganisms in foods. Such polymers would maintain their antimicrobial efficacy and the regulatory hurdle faced by food additives and contact migrants could be minimized.

11. Future research

Antimicrobial packaging is gaining interest from researchers and industry due to its potential to provide quality and safety benefits. Currently, development is limited due to availability of antimicrobials and new polymer materials, regulatory concerns, and appropriate testing methods. With the advent of new materials and more information this may change. New coating/binder materials compatible with polymers and antimicrobials, functionalized surfaces for ionic and covalent links and new printing methods combined with encapsulation are examples of the technologies that

will play a role in the development of antimicrobial packaging. Antimicrobials that can be attached or coated to films and rigid containers after forming to avoid high temperature and other processing issues will allow a wide range of compounds to be incorporated into polymers. These developments will require surfaces containing functional groups available for attachment. Physical methods to modify polymer surface (electron beam, ion beam, plasma and laser treatments) are emerging and pose potential for functionalizing inert surfaces such as those of PE, PET, PP and PS (Ozdemir, Yurteri & Sadikoglu, 1999). HDPE and LLDPE have already been functionalized by graft polymerization with amide, amino and carboxyl groups in order to immobilize proteins and enzymes (Hayat, Tinsley, Calder & Clarke, 1992; Sano, Kato & Ikada, 1993; Wang & Hsiue, 1993). It has been suggested also that cross-linking edible films like calcium caseinate by gamma irradiation will find applications as supports for the immobilization of antimicrobials and other additives (Lacroix & Ouattara, 2000).

Future work will focus on the use of biologically active derived antimicrobial compounds bound to polymers. The need for new antimicrobials with wide spectrum of activity and low toxicity will increase. It is possible that research and development of 'intelligent' or 'smart' antimicrobial packages will follow. These will be materials that sense the presence of microorganism in the food, triggering antimicrobial mechanisms as a response, in a controlled manner.

Antimicrobial packaging can play an important role in reducing the risk of pathogen contamination, as well as extending the shelf-life of foods; it should never substitute for good quality raw materials, properly processed foods and good manufacturing practices. It should be considered as a hurdle technology that in addition with other non-thermal processes such as pulsed light, high pressure and irradiation could reduce the risk of pathogen contamination and extend the shelf-life of perishable food products. Participation and collaboration of research institutions, industry and government regulatory agencies will be key on the success of antimicrobial packaging technologies for food applications.

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