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Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and its Applications

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ABSTRACT: In response to the dynamic changes in current consumer demand and market trends, the area of Active Packaging (AP) is becoming increasingly significant. Principal AP systems include those that involve oxygen scavenging, moisture absorption and control, carbon dioxide and ethanol generation, and antimicrobial (AM) migrating and nonmigrating systems. Of these active packaging systems, the AM version is of great importance. This article reviews: (1) the different categories of AP concepts with particular regard to the activity of AM packaging and its effects on food products, (2) the development of AM and AP materials, and (3) the current and future applications of AM packaging.

Keywords: active packaging, antimicrobial packaging, antimicrobial additives

Introduction

IN RECENT YEARS, THE MAJOR DRIVING FORCES for innovation in food packaging technology have been the increase in consumer demand for minimally processed foods, the change in retail and distribution practices associated with globalization, new consumer product logistics, new distribution trends (such as Internet shopping), automatic handling systems at distribution centers, and stricter requirements regarding consumer health and safety (Vermeiren and others 1999; Sonneveld 2000). Active Packaging (AP) technologies are being developed as a result of these driving forces. Active Packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product. This is particularly important in the area of fresh and extended shelf-life foods as originally described by Labuza and Breene (1989). Flores and others (1997) reviewed the products and patents in the area of AP and identified antimicrobial (AM) packaging as one of the most promising versions of an AP system. Han (2000) and Vermeiren and others (2002) recently published articles focused on AM systems, but without a detailed discussion of some of the principal AP concepts. The present article reviews the general principles of AP and AM packaging concepts including oxygen scavenging, moisture absorption and control, carbon

dioxide and ethanol generation, and it reviews in detail AM migrating and nonmigrating systems.

Oxygen Scavenging Systems

THE PRESENCE OF O₂ IN A PACKAGED FOOD is often a key factor that limits the shelf life of a product. Oxidation can cause changes in flavor, color, and odor, as well as destroy nutrients and facilitate the growth of aerobic bacteria, molds, and insects. Therefore, the removal of O₂ from the package headspace and from the solution in liquid foods and beverages, has long been a target of the food-packaging scientists. The deterioration in quality of O₂-sensitive products can be minimized by recourse to O₂ scavengers that remove the residual O₂ after packing. Existing O₂ scavenging technologies are based on oxidation of 1 or more of the following substances: iron powder, ascorbic acid, photo-sensitive dyes, enzymes (such as glucose oxidase and ethanol oxidase), unsaturated fatty acids (such as oleic, linoleic and linolenic acids), rice extract, or immobilized yeast on a solid substrate (Floros and others 1997). These materials are normally contained in a sachet. Details on O₂ scavenging can be obtained from other reviews (Labuza and Breen 1989; Miltz and others 1995; Miltz and Perry 2000; Floros and others 1997; Vermeiren and others 1999).

Oxygen scavenging is an effective way to prevent growth of aerobic bacteria and molds in dairy and bakery products. Oxygen concentrations of 0.1% v/v or less in the headspace are required for this purpose

(Rooney 1995). Packaging of crusty rolls in a combination of CO₂ and N₂ (60% CO₂) has shown to be an effective measure against mold growth for 16 to 18 d at ambient temperature. However, the study also revealed that such an "anaerobic environment" is not totally effective without the incorporation of an oxygen scavenger into the package to ensure that the headspace O₂ concentration never exceeds 0.05%. Under such conditions the rolls remain mold-free even after 60 d (Smith and others 1986).

Many researchers have expressed concern about the safety of Modified Atmosphere Packaged (MAP) foods, especially with respect to the growth of psychrotrophic pathogens such as *Listeria monocytogenes* and anaerobic pathogens such as *Clostridium botulinum* (Farber 1991). Lyver and others (1998) monitored the physical, chemical, microbiological, textural, and sensory changes in surimi nuggets inoculated with *L. monocytogenes*, packaged in either air or 100% CO₂ with and without an oxygen scavenger and stored at 4 °C and 12 °C. They found that MAP was not effective in controlling the growth of the pathogen in either raw or cooked nuggets and also that the pathogen overcame competitive inhibition and pH reduction caused by lactic acid bacteria. They concluded that nuggets packaged under these conditions and contaminated with this pathogen could pose a risk to the consumer. More importantly, it was found that the product retained acceptable odor and appearance scores at the above storage temperatures, even though the lev-

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el of the pathogen increased over the storage period. The latter is indeed a cause for concern, since the contaminated product may appear safe from the sensory point of view (Lyver and others 1998).

Oxygen scavenging is advantageous for products that are sensitive to O₂ and light. One important advantage of AP over MAP is that the capital investment involved is substantially lower; in some instances, only the sealing of the system that contains the oxygen absorbing sachet is required. This is of extreme importance to small- and medium-sized food companies for which the packaging equipment is often the most expensive item (Ahvenainen and Hurme 1997).

An alternative to sachets involves the incorporation of the O₂ scavenger into the packaging structure itself. This minimizes negative consumer responses and offers a potential economic advantage through increased outputs. It also eliminates the risk of accidental rupture of the sachets and inadvertent consumption of their contents. A summary of available O₂ scavengers is given in Table 1.

Since the share of polymers in primary packages for foods and beverages increases constantly, they have become the medium for incorporation of active substances such as antioxidants, O₂ scavengers, flavor compounds, pigments, enzymes, and AM agents (Hotchkiss 1997). BP Amoco Chemical (U.S.A.) is marketing Amosorb® 2000 and 3000, which are polymer-concentrates containing iron-based O₂ scavengers. These can be used in polyolefins and in certain polyester packaging applications for wines, beers, sauces, juices, and other beverages. Other recent developments include OS2000™ developed by Cryovac Div., Sealed Air Corporation, U.S.A. (Butler 2002) and ZERO₂™ developed by CSIRO, Div. of Food Science Australia, in collaboration with Southcorp Packaging (now VisyPak), Australia (Brody and others 2001). Both of the latter are organic-based, UV light-activated O₂ scavengers that can be tailored to allow them to be bound into various layers of a wide range of packaging structures. Oxbar™ is a system developed by Carnaud-Metal Box (now Crown Cork and Seal) that involves cobalt-catalyzed oxidation of a MXD6 nylon that is blended into another polymer. This system is used especially in the manufacturing of rigid PET bottles for packaging of wine, beer, flavored alcoholic beverages, and malt-based drinks (Brody and others 2001).

Another O₂ scavenging technology involves using directly the closure lining. Darlex® Container Products (now a unit of Grace Performance Chemicals) has announced an

ethylene vinyl alcohol with a proprietary oxygen scavenger developed in conjunction with Kararay Co. Ltd. In dry forms, pellets containing unsaturated hydrocarbon polymers with a cobalt catalyst are used as oxygen scavengers in mechanical closures, plastic and metal caps, and steel crowns (both PVC and non-PVC lined). They reportedly can prolong the shelf life of beer by 25% (Brody and others 2001).

Oxygen scavengers have opened new horizons and opportunities in preserving the quality and extending the shelf life of foodstuffs. However, much more information is needed on the action of O₂ scavengers in different environments before optimal, safe, and cost-effective packages can be designed. The need for such information is especially acute on O₂ scavenging films, labels, sheets, and trays that have begun to appear in recent years (Miltz and others 1995; Miltz and Perry 2000).

Moisture-Absorbing and Controlling Systems

IN SOLID FOODS, A CERTAIN AMOUNT OF MOISTURE may be trapped during packaging or may develop inside the package due to generation or permeation. Unless it is eliminated, it may form a condensate with the attendant spoilage and/or low consumer appeal. Moisture problems may arise in a variety of circumstances, including respiration in horticultural produce, melting of ice, temperature fluctuations in food packs with a high equilibrium relative humidity (ERH), or drip of tissue fluid from cut meats and produce (Rooney 1995). Their minimization via packaging can be achieved either by liquid water absorption or humidity buffering.

Liquid water absorption

The main purpose of liquid water control is to lower the water activity, a_w , of the product, thereby suppressing the growth of microorganisms on the foodstuff (Vermeiren and others 1999). Temperature cycling of high a_w foods has led to the use of plastics with an antifog additive that lowers the interfacial tension between the condensate and the film. This contributes to the transparency of the films and enables the customer to see clearly the packaged food (Rooney 1995) although it does not affect the amount of liquid water present inside the package.

Several companies manufacture drip-absorbent sheets such as Thermarite® or Peaksorb® (Australia), or Toppán™ (Japan) for liquid water control in high a_w foods such as meat, fish, poultry and fresh produce. Basically, these systems consist of a super-absorbent polymer located between 2 lay-

ers of a micro-porous or nonwoven polymer. Such sheets are used as drip-absorbing pads placed under whole chickens or chicken cuts. Large sheets are also utilized for absorption of melted ice during air transportation of packaged seafood. The preferred polymers used for this purpose are polyacrylate salts and graft copolymers of starch (Rooney 1995).

Humidity buffering

This approach involves interception of moisture in the vapor phase by reducing the in-pack relative humidity and thereby the surface-water content of the food. It can be achieved by means of 1 or more humectants between 2 layers of a plastic film that is highly permeable to water vapor or by a moisture-absorbing sachet. An example of this approach is the Pichit™ film manufactured by Showa Denko (Japan). It is marketed in Japan for wrapping fish and chicken and reduces the ERH in the vicinity of the product, but has not been evaluated experimentally. Pouches containing NaCl have also been used in the US tomato market (Rooney 1995).

Desiccants have been successfully used for moisture control in a wide range of foods, such as cheeses, meats, chips, nuts, popcorn, candies, gums and spices. Silica gel, molecular sieves, calcium oxide (CaO) and natural clays (such as montmorillonite) are often provided in Tyvek™ sachets (Brody and others 2001). Other desiccant systems include the MiniPax® and StripPax® packets, the DesiMax® (United Desiccants, U.S.A.) and the DesiPak®, Sorb-it®, Tri-sorb® and 2-in-1™ sachets (Multisorb Technologies Inc., U.S.A.).

Carbon Dioxide Generating Systems

CARBON DIOXIDE IS KNOWN TO SUPPRESS microbial activity. Relatively high CO₂ levels (60 to 80%) inhibit microbial growth on surfaces and, in turn, prolong shelf life. Therefore, a complementary approach to O₂ scavenging is the impregnation of a packaging structure with a CO₂ generating system or the addition of the latter in the form of a sachet. Table 2 lists the main commercial CO₂ generators. Since the permeability of CO₂ is 3 to 5 times higher than that of O₂ in most plastic films, it must be continuously produced to maintain the desired concentration within the package. High CO₂ levels may, however, cause changes in taste of products and the development of undesirable anaerobic glycosis in fruits. Consequently, a CO₂ generator is only useful in certain applications such as fresh meat, poultry, fish and cheese packaging (Floros

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Table 1—Overview of commercial oxygen scavengers

Format	Trade Name	Manufacturer	References
Card Closure Liner	Ageless® Darex® PureSeal® Smartcap® Amosorb®, 2000, 3000 Oxbar™ Oxyguard™ Oxysorb® Bioka® OS2000® ZERO2™ Ageless® ATCO® FreshMax® Ageless® ATCO® Bioka® Freshilizer® FreshPax® Keplon™ Modulan™ Negamold® ¹ Oxyeater™ Oxysorb® Sanso-cut® Sansoless™ Secule® Sequi® Tamotsu™ Vitalon® ² Oxycap®	Mitsubishi Gas Chemical Co. (Japan) Grace Performance Chemicals (U.S.A.) Advanced Oxygen Technologies Inc. (U.S.A.) Advanced Oxygen Technologies Inc. (U.S.A.) BP Amoco Chemical (U.S.A.) Crown Cork and Seal (U.S.A.) Toyo Seikan Kaisha (Japan) Pillsbury Co (U.S.A.) Bioka Ltd (Finland) Sealed Air Corporation (U.S.A.) CSIRO and VisyPak (Australia) Mitsubishi Gas Chemical Co. (Japan) Standa Industrie (France) Multisorb Technologies Inc. (U.S.A.) Mitsubishi Gas Chemical Co. (Japan) Standa Industrie (France) Bioka Ltd (Finland) Toppan Printing Co. (Japan) Multisorb Technologies Inc (U.S.A.) Keplon Co. (Japan) Nippon Kayaku Co. (Japan) Freund Industrial Co. (Japan) Ueno Seiyaku Co. (Japan) Pillsbury Co. (U.S.A.) Finetech Co. (Japan) Hakuyo Co. (Japan) Nippon Soda Co. (Japan) Dai Nippon Co. (Japan) Oji Kako Co. (Japan) Toagosei Chemical Co. (Japan) Standa Industrie (France)	Teumac (1995), Brody and others (2001) Teumac (1995) Teumac (1995) Brody and others (2001) Butler (2002) Brody and others (2001) Nakamura and Hoshino (1983), Smith and others (1995), Lyver and others (1998) Hurme and Ahvenainen (1996) Ahvenainen and Hurme (1997) Smith and others (1995) Smith and others (1995) Brody and others (2001) Brody and others (2001) Smith and others (1995) Brody and others (2001) Hurme and Ahvenainen (1996) Brody and others (2001) Brody and others (2001) Brody and others (2001) Brody and others (2001) Hurme and Ahvenainen (1996)
Film			
Label			
Sachet			
Thermoformed Tray			

¹Combined actions between O₂ scavenging and ethanol generation

²Combined actions between O₂ scavenging and CO₂ generation

Table 2—Commercial carbon dioxide generators

Trade Name	Manufacturer	References
Ageless® G ¹	Mitsubishi Gas Chemical Co. (Japan)	Nakamura and Hoshino (1983), Smith and others (1995)
Freshilizer® C ¹ and CW ¹	Toppan Printing Co. (Japan)	Smith and others (1995)
FreshPax® M ¹	Multisorb Technologies Inc. (U.S.A.)	
Vitalon® G ¹	Toagosei Chemical Co. (Japan)	Vermeiren and others (1999)
Verifrais®	SARL Codimer (France)	Vermeiren and others (1999)

¹Combined actions between CO₂ generating and O₂ scavenging

and others 1997). In food products for which the volume of the package and its appearance are critical, an O₂ scavenger and CO₂ generator could be used together (Smith and others 1995) in order to prevent package collapse as a result of O₂ absorption.

Nakamura and Hoshino (1983) reported that an oxygen-free environment alone is insufficient to retard the growth of *Staphylococcus aureus*, *Vibrio* species, *Escherichia coli*, *Bacillus cereus* and *Enterococcus faecalis* at ambient temperatures. For complete inhibition of these microorganisms in foods, the authors recommended a combined treatment involving O₂ scavenging with thermal processing, or storage under refrigeration, or using a CO₂ enriched atmosphere. They found that an O₂ and CO₂ absorber inhibited the growth of *Clostridium*

sporogenes while an O₂ absorber and a CO₂ generator enhanced the growth of this microorganism, which is quite a surprising result. This result indicates the importance of selecting the correct scavenger to control the growth of *Clostridium* species in MAP foods.

Ethanol Generating Systems

ETHANOL IS USED ROUTINELY IN MEDICAL and pharmaceutical packaging applications, indicating its potential as a vapor phase inhibitor (Smith and others 1987). It prevents microbial spoilage of intermediate moisture foods (IMFs), cheeses, and bakery products. It also reduces the rate of staling and oxidative changes (Seiler 1989). Ethanol has been shown to extend the shelf life of bread, cake and pizza when sprayed onto product surfaces prior to packaging. Sa-

chets containing encapsulated ethanol release its vapor into the packaging headspace thus maintaining the preservative effect (Labuza and Breene 1989).

Many applications of ethanol-generating films or sachets have been patented (Floros and others 1997) and marketed (Smith and others 1995), including an adhesive-backed film that can be taped on the inside of a package to provide AM activity (Labuza and Breene 1989). Mitsubishi Gas Chemical Co. patented a sachet containing encapsulated ethanol, glucose, ascorbic acid, a phenolic compound and an iron salt (Floros and others 1997), thereby achieving the combined effect of O₂ scavenging and ethanol generation. Table 3 lists examples of commercial ethanol generators.

An ethanol-generating technology was

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originally developed in Japan whereby foodgrade ethanol is encapsulated in a fine inert powder inside a sachet. The rate of ethanol vapor release can be tailored by controlling the permeability of the sachet. Several Japanese companies manufacture this type of ethanol generator, the most widely used being Ethicap® or Antimold Mild® produced by the Freund Industrial Co. (Smith and others 1995). These systems, approved for use in Japan, extend the mold-free shelf life of various bakery products.

Smith and others (1987) demonstrated the usefulness of ethanol vapor in extending the shelf life of apple turnovers. The shelf life was found to be 14 d for the product packaged in air or in a CO₂/N₂ gas mixture (60% CO₂) and stored at ambient temperature. Afterwards, visible swelling occurred as a result of *Saccharomyces cerevisiae* growth and additional CO₂ production. When encapsulated ethanol was incorporated in the package, yeast growth was totally suppressed and the shelf life was extended to 21 days. On the other hand, this solution caused the packages to contain 1.5% ethanol at the end of the storage period as compared to only 0.2% when packed without ethanol. Consequently, the final products may be unacceptable to the consumer due to elevated ethanol contents. This problem can be partially resolved by heating the contents of the package prior to consumption, thereby evaporating the ethanol.

Antimicrobial Migrating and Nonmigrating Systems

ANTIMICROBIAL FOOD PACKAGING MATERIALS have to extend the lag phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety (Han 2000). Alternatives to direct additives for minimizing the microbial load are canning, aseptic processing and MAP. However, canned foods cannot be marketed as "fresh". Aseptic processing may be expensive and hydrogen peroxide, which is restricted in level by regulatory agencies, is often used as a sterilizing agent. In certain cases, MAP can promote the growth of pathogenic anaerobes and the germination of spores, or prevent the growth of spoilage organisms which indicate the presence of pathogens (Farber 1991). If packaging materials have self-sterilizing abilities due to their own AM effectiveness, the need for chemical sterilization of the packages may be obviated and the aseptic packaging process simplified (Hotchkiss 1997).

Food packages can be made AM active by incorporation and immobilization of AM

Table 3—Commercial ethanol generators

Trade name	Manufacturer	Reference
Ethicap®	Freund Industrial Co. (Japan)	Smith and others (1995)
Negamold® ¹	Freund Industrial Co. (Japan)	Smith and others (1995)
Oitech™	Nippon Kayaku Co. (Japan)	Smith and others (1995)
ET Pack	Ueno Seiyaku Co. (Japan)	Smith and others (1995)
Ageless® SE ¹	Mitsubishi Gas Chemical Co. (Japan)	Flores and others (1997)
Fretek®	Techno Intl. Inc. (U.S.A.) ²	Brody and others (2001)

¹Combined actions between ethanol-generating and O₂ scavenging

²Under license from Freund Industrial Co (Japan)

agents or by surface modification and surface coating. Present plans envisage the possible use of naturally derived AM agents in packaging systems for a variety of processed meats, cheeses, and other foods, especially those with relatively smooth product surfaces that come in contact with the inner surface of the package. This solution is becoming increasingly important, as it represents a perceived lower risk to the consumer (Nicholson 1998). Table 4 lists a number of substances, which can be bound to polymers to impart AM properties. Such substances can be used in AM films, containers and utensils (Ishitani 1995). Antimicrobial materials have been known for many years. However, antimicrobial packages have had relatively few commercial successes, except in Japan. Table 5 (Brody and others 2001) summarizes some of the antimicrobial systems. Antimicrobial films can be classified in 2 types: (1) those that contain an AM agent that migrates to the surface of the food, and (2) those that are effective against surface growth of microorganisms without migration.

Gas emission or flushing

Gas emission or flushing controls the growth of mold. Typical spoilage molds include *Botrytis cinerea*, *Penicillium*, *Aspergillus* and *Rhizopus* species commonly found in citrus and berry fruits. To extend the storage period of these fruits, fungicides or antimycotic agents can be applied.

Sulfur dioxide (SO₂) is known to be the most effective material in controlling the decay of grapes and is superior to the gamma irradiation and heat-radiation combination methods (Smilanick 1990). However, a SO₂-releasing material entails a number of problems, including bleaching and SO₂ residues.

Thomas and others (1995) studied the effect of SO₂ generating pads on the decay and quality of table grapes. In Australia, 2 different SO₂ release sheets were tested for packaging of the white "Thompson Seedless" and the purple "Red Globe" grapes (Christie and others 1997). SO₂ in the surrounding air is absorbed into the grapes and initially converted to sulfite and then me-

tabolized into the sulfate form. At the end of the experiment (after 4 d at 21 °C), the sulfite levels in the "Red Globe" were found to be lower than those in the "Thompson Seedless", even though the former was subjected to a higher SO₂ level. This reflects the different metabolic rates of the 2 varieties (Christie and others 1997). The authors suggested development of a controlled release polymer that would apply the fungicide at a sufficient level to retain satisfactory fungistatic action, while minimizing undesirable effects.

Opperman and others (1999) considered controlling the decay of table grapes with monolithic-type polymer structures that release SO₂ at a constant rate over an extended period. Two different systems containing either 2 or 4 SO₂-containing polymer discs were tested. In the 4-disc system, a disc was placed in the corner of each carton box whereas in the 2-disc system they were placed in a central location, approximately 10 cm from the edges of the carton. In 50% of the monolithic device treatments, the discs were placed directly on top of the grapes, while in the other treatments, they were placed on top of a corrugated paper liner. It was found that the liner acted as a physical barrier between the grapes and the SO₂ generator and that the carton absorbed much of the free SO₂. The controlling effect was vastly improved by raising the level of sodium metabisulfite (Na₂S₂O₅) impregnated in the polymer structure, but the product suffered from SO₂ damage. The optimum range for the Na₂S₂O₅ concentration was found to be 10 to 20% w/w.

Another volatile compound exhibiting AM effects is allyl isothiocyanate (AIT), the major pungent component of black mustard (*Brassica nigra*), brown mustard (*Brassica juncea*) and wasabi (*Eutrema wasabi* Maxim.). Isshiki and others (1992) compared the minimum inhibitory concentration (MIC) of AIT vapor against microorganisms on agar. In the experiments, a mixture (500 mg) of AIT and beef fat (2:98, w/w) was placed on top of a perforated cellophane film, and packed in the bag with the sample food. It was claimed that at such

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Table 4—Examples of antimicrobial agents for potential use in food packaging materials

Class	Examples	References
Acid Anhydride	Benzoic anhydride	Weng and Hotchkiss (1993), Huang and others (1997), Dobias and others (2000)
	Sorbic anhydride	Weng and Chen (1997)
Alcohol	Ethanol	Luck and Jager (1997)
Amine	Hexamethylenetetramine (HMT)	Luck and Jager (1997), Devlieghere and others (2000b)
Ammonium Compound	Silicon quaternary ammonium salt	
Antibiotic	Natamycin	Luck and Jager (1997)
Antimicrobial Peptide	Attacin	Dillon (1994)
	Cecropin	Dillon (1994)
	Defensin	Dillon (1994)
	Magainin	Abler and others (1995)
Antioxidant Phenolic ¹	Butylated hydroxyanisole (BHA)	Hotchkiss (1997)
	Butylated hydroxytoluene (BHT)	Hotchkiss (1997)
	Tertiary butylhydroquinone (TBHQ)	Hotchkiss (1997)
Bacteriocin	Bavaricin	Nettles and Barefoot (1993)
	Brevicin	Nettles and Barefoot (1993)
	Carnocin	Nettles and Barefoot (1993)
	Lacticin	Nettles and Barefoot (1993), An and others (2000), Scannell and others (2000)
	Mesenterocin	Nettles and Barefoot (1993)
	Nisin	Luck and Jager (1997), An and others (2000), Natrajan and Sheldon (2000a, b), Scannell and others (2000)
	Pediocin	Barnby-Smith (1992), Nettles and Barefoot (1993)
	Sakacin	Nettles and Barefoot (1993)
	Subtilin	Barnby-Smith (1992)
Chelator	Citrate	Hotchkiss (1997)
	Conalbumin	Conner (1993)
	EDTA	Luck and Jager (1997), Rodrigues and Han (2000)
	Lactoferrin	Conner (1993)
	Polyphosphate	Shelef and Seiter (1993)
Enzyme	Chitinase	Fuglsang and others (1995)
	Ethanol oxidase	Fuglsang and others (1995)
	β-Glucanase	Fuglsang and others (1995)
	Glucose oxidase	Fuglsang and others (1995)
	Lactoperoxidase	Conner (1993), Fuglsang and others (1995)
	Lysozyme	Conner (1993), Fuglsang and others (1995), Appendini and Hotchkiss (1997), Luck and Jager (1997), Rodrigues and Han (2000)
	Myeloperoxidase	Fuglsang and others (1995)
Fatty Acid	Lauric acid	Ouattara and others (1997; 2000b)
	Palmitoleic acid	Ouattara and others (1997)
Fatty Acid Ester	Monolaurin (lauricidin®)	Luck and Jager (1997)
Fungicide	Benomyl	Halek and Garg (1989)
	Imazalil	Hale and others (1986), Weng and Hotchkiss (1992)
	Sulfur dioxide	Thomas and others (1995), Christie and others (1997), Luck and Jager (1997), Opperman and others (1999)
Inorganic Acid	Phosphoric acid	Hotchkiss (1997)
Metal	Copper	Ishitani (1995)
	Silver	Ishitani (1995), Luck and Jager (1997), An and others (1998), Chung and others (1998)
Miscellaneous	Reuterin	Helander and others (1997)
Natural Phenol	Catechin	Walker (1994)
	p-Cresol	Hotchkiss (1997)
	Hydroquinones	Hotchkiss (1997)

(continued on next page)

low concentrations, only slight odors were perceived, which suggests that AIT can be employed in MAP. The shelf life of various foods (such as fresh beef, cured pork, sliced raw tuna, cheese, egg sandwich, noodles, and pasta) packaged in barrier plastic

bags was enhanced when the package was flushed with AIT.

The AM effectiveness of AIT inside a package depends on its interaction with the particular packaging materials. Lim and Tung (1997) determined the vapor pressure

of pure AIT and that of AIT above AIT-canola oil mixtures. Canola oil is effective in depressing the vapor pressure of AIT, and may be used as a controlling diluent for this purpose in MAP applications. It was found that the diffusion, solubility and permeability

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Table 4—continued

Class	Examples	References
Oligosaccharide	Chitooligosaccharide	Cho and others (2000), Hong and others (2000)
Organic Acid	Acetic acid Benzoic acid Citric acid Lactic acid Malic acid Propionic acid Sorbic acid Succinic acid Tartaric acid	Doores (1993), Ouattara and others (2000a, b), Luck and Jager (1997) Luck and Jager (1997), Weng and others (1997), Chen and others (1999), Weng and others (1999) Doores (1993), Luck and Jager (1997) Doores (1993) Doores (1993), Ouattara and others (2000a, b), Luck and Jager (1997) Luck and Jager (1997), Weng and others (1999) Doores (1993) Doores (1993)
Organic Acid Salt	Potassium sorbate Sodium benzoate	Chen and others (1996), Han and Floros (1997, 1999), Devlieghere and others (2000a) Chen and others (1996)
Paraben	Ethyl paraben Methyl paraben Propyl paraben	Davidson (1993), Luck and Jager (1997), Dobias and others (2000) Davidson (1993), Luck and Jager (1997) Davidson (1993), Luck and Jager (1997), Dobias and others (2000)
Plant-Volatile Component	Allyl isothiocyanate (AIT) Carvacrol Cineole Cinnamaldehyde Citral <i>p</i> -Cymene Estragole (methyl chavicol) Eugenol Geraniol Hinokitiol (β -thujaplicin) Linalool Terpineol Thymol	Isshiki and others (1992), Luck and Jager (1997), Brody and others (2001) Ouattara and others (1997), Scora and Scora (1998) Lis-Balchin and others (1998), Scora and Scora (1998) Ouattara and others (1997; 2000b) Lis-Balchin and others (1998), Scora and Scora (1998) Scora and Scora (1998) Scora and Scora (1998), Suppakul and others (2002) Ouattara (1997), Scora and Scora (1998) Scora and Scora (1998) Fallik and Grinberg (1992), Brody and others (2001) Lis-Balchin and others (1998), Scora and Scora (1998), Suppakul and others (2002) Scora and Scora (1998) Ouattara and others (1997), Scora and Scora (1998)
Polysaccharide	Chitosan Konjac glucomannan	Sudarshan and others (1992), Begin and Calsteren (1999), Hong and others (2000) Xiao and others (2000)

¹Although generally used as Antioxidants, they have shown also Antimicrobial activity (Hotchkiss, 1997).

Table 5—Trade names and manufacturers of commercial antimicrobial materials

Format	Trade Name	Manufacturer	References
Concentrate	AgION™	AgION Technologies LLC (USA)	www.agion-tech.com
	Apacider-A®	Sangi Co. (Japan)	Brody and others (2001)
	MicroFree™	DuPont (U.S.A.)	Brody and others (2001), Vermeiren and others (2002)
	Microban®	Microban Products (U.S.A.)	Brody and others (2001), Vermeiren and others (2002)
	Novaron®	Milliken Co. (U.S.A.)	Vermeiren and others (2002)
	Sanitized®	Sanitized AG / Clariant (Switzerland)	Vermeiren and others (2002)
	Surfacine®	Surfacine Development Co. (U.S.A.)	Vermeiren and others (2002)
	Ultra-Fresh®	Thonson Research Associates (Canada)	Vermeiren and others (2002)
	Zeomic®	Shinanen New Ceramics Co. (Japan)	Brody and others (2001)
	Citrex™ (Grapefruit seed)	Quimica Natural Brasileira Ltd. (Brazil)	Lee and others (1998)
Extract	Nisaplin® (Nisin)	Integrated Ingredients (U.S.A.)	Scannell and others (2000), Brody and others (2001)
	Take Guard (Bamboo)	Takex Co. (Japan)	Brody and others (2001)
	WasaOuro® (Mustard)	Green Cross Co. (Japan)	Brody and others (2001)
	MicroGard™	Rhone-Poulenc (U.S.A.)	Brody and others (2001)
Film	Piatech	Daikoku Kasei Co. (Japan)	Brody and others (2001)

coefficients of AIT in polyvinylidenechloride (PVDC) / polyvinylchloride (PVC) polymer films are concentration and temperature dependent. At a fixed vapor activity, the diffusion and permeability coefficients increased whereas the solubility

coefficient decreased with an increase in temperature.

Coating of films with antimicrobial agents

Appropriate coatings can sometimes im-

part AM effectiveness. An and others (2000) claimed that a polymer-based solution coating would be the most desirable method in terms of stability and adhesiveness of attaching a bacteriocin to a plastic film. It was found that low-density polyethylene

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(LDPE) films coated with a mixture of polyamide resin in *i*-propanol/*n*-propanol and a bacteriocin solution provided AM activity against *Micrococcus flavus*. The migration of bacteriocins reached equilibrium within 3 d, but the level attained was too low to affect several bacterial strains spread on an agar plate media. When the films were in contact with a phosphate buffer solution containing strains of *M. flavus* and *L. monocytogenes*, a marked inhibition of microbial growth of both strains was observed.

LDPE film was successfully coated with nisin using methylcellulose (MC)/ hydroxypropyl methylcellulose (HPMC) as a carrier. Nisin was found to be effective in suppressing *S. aureus* and *L. monocytogenes* respectively (Cooksey 2000). Natrajan and Sheldon (2000a) studied the efficacy of nisin-coated polymeric films such as PVC, linear low-density polyethylene (LLDPE), and nylon, in inhibiting *Salmonella typhimurium* on fresh broiler drumstick skin. As anticipated, the more hydrophobic LLDPE film repelled the aqueous nisin formulations to a greater extent than the other films and caused coalescence of the treatment solution droplets. The repulsion between the LLDPE film and the treatment solution may have affected the overall inhibitory activity of the formulations by causing more localized inactivation of the target. An agar-based film containing nisin was also studied. It was found that in this film, the degree of cross-linking depends on the agar concentration, which may affect the migration of nisin to the surface of a broiler drumstick skin (Natrajan and Sheldon 2000b). Thus, 0.75% w/w compared with 1.25% w/w gels formed a more open and elastic network, allowing greater migration of the treatment components over time. The respective levels of bacterial inhibition exhibited by the films, especially after 96 h, appeared to support this postulation.

Incorporation of antimicrobial additives

The direct incorporation of AM additives in packaging films is a convenient means by which AM activity can be achieved. Several compounds have been proposed and/or tested for AM packaging using this method. Han and Flores (1997) studied the incorporation of 1.0% w/w potassium sorbate in LDPE films. A 0.1-mm thick film was used for physical measurements, while a 0.4-mm thick film was used for AM effectiveness tests. It was found that potassium sorbate lowered the growth rate and maximum growth of yeast, and lengthened the lag period before mold growth became apparent. The results of this study, however, con-

tradict those obtained by Weng and Hotchkiss (1993) with LDPE films (0.05-mm thick) containing 1.0% w/w sorbic acid. In the latter case, the films failed to suppress mold growth when brought into contact with inoculated media. Devlieghere and others (2000a) studied these contradicting results. Their results confirm that ethylene vinyl alcohol/linear low-density polyethylene (EVA/LLDPE) film (70- μ m thick) impregnated with 5.0% w/w potassium sorbate is unable to inhibit the growth of microorganisms on cheese and to extend its shelf life. As suggested by Weng and Hotchkiss (1993), very limited migration of potassium sorbate into water as well as into cheese cubes occurs, probably because of the incompatibility of the polar salt with the nonpolar LDPE. The choice of an AM agent is often restricted by the incompatibility of that agent with the packaging material or by its heat instability during extrusion (Weng and Hotchkiss 1993; Han and Flores 1997).

While polyethylene (PE) has been widely employed as the heat-sealing layer in packages, in some cases the copolymer polyethylene-co-methacrylic acid (PEMA) was found to be preferable for this purpose. Weng and others (1999) reported a simple method for fabricating PE MA films (0.008- to 0.010-mm thick) with AM properties by the incorporation of benzoic or sorbic acids. The experimental results suggest that sodium hydroxide and preservative-treated films exhibit dominantly AM properties for fungal growth, presumably due to the higher amount of preservatives released from the films (75 mg benzoic acid or 55 mg sorbic acid per g of film) than hydrochloric acid and preservative-treated films. Chen and others (1996) found that chitosan films made from dilute acetic acid solutions block the growth of *Rhodotorula rubra* and *Penicillium notatum* if the film is applied directly to the colony-forming organism. Since chitosan is soluble only in slightly acidic solutions, production of such films containing the salt of an organic acid (such as benzoic acid, sorbic acid) that is an AM agent is straightforward. However, the interaction between the AM agent and the film-forming material may affect the casting process, the release of the AM agent and the mechanical properties of the film.

Begin and Calsteren (1999) showed that films containing AM agents with a molecular weight larger than that of acetic acid are soft and can be used in multi-layer systems or as a coating. Acetic acid diffusion was, however, not as complete as that of propionic acid when chitosan-containing films were used in contact with processed meats (Ouattara and others 2000a) in spite of the fact that in

an aqueous medium, acetic acid diffused out of chitosan more rapidly than propionic acid (Ouattara and others 2000b). These results suggest that the release of organic acids from chitosan is a complex phenomenon that involves many factors such as electrostatic interactions, ionic osmosis, and structural changes in the polymer induced by the presence of the acids.

According to Weng and Hotchkiss (1993), anhydrides are more compatible with PE than their corresponding free acids or salts, due to the lower polarity and higher molecular weight of the former compared to the latter. Hence, anhydrides may serve as appropriate additives to plastic materials for food packaging. LDPE films impregnated with benzoic anhydride completely suppressed the growth of *Rhizopus stolonifer*, *Penicillium* species and *Aspergillus toxicarius* on potato dextrose agar (PDA). Similarly, LDPE films that contained benzoic anhydride delayed mold growth on cheese (Weng and Hotchkiss 1993). PE films (0.010- to 0.015-mm thick) containing benzoic anhydride (20 mg benzoic anhydride per g of PE in the initial preparation) alone or in combination with minimal microwave heating, were effective in controlling microbial growth of tilapia fillets during a 14-d storage at 4 °C (Huang and others 1997). Shelf-life studies of packaged cheese and toasted bread demonstrated the efficiency of LDPE film containing benzoic anhydride against mold growth on the food surface during storage at 6 °C (Dobias and others 2000). Dobias and others (2000) also studied the migration of benzoic anhydride, ethyl paraben (ETP) and propyl paraben (PRP) in LDPE films. It was found that the incorporation of these parabens in the polymer was more difficult than that of benzoic anhydride due to their higher volatilities.

No single AM agent can cover all the requirements for food preservation. Weng and Chen (1997) investigated a range of anhydrides for use in food packaging. It is known that for mold growth inhibition, the effectiveness of sorbic anhydride (10 mg sorbic anhydride per g of PE initial concentration) incorporated in PE films (0.10- to 0.12-mm thick) is much better with slow-growing (*Penicillium* species) than with fast-growing mold (*Aspergillus niger*). This is due to the time required for the PE to release sorbic acid to an inhibitory concentration.

Apart from organic acids and anhydrides, Imazalil has also been used with LDPE film. Weng and Hotchkiss (1992) showed that an Imazalil concentration of 2000 mg/kg LDPE film (5.1 μ m thick) delayed *A. toxicarius* growth on potato dextrose agar, while LDPE film containing 1000 mg/kg Imazalil

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substantially inhibited *Penicillium* sp. growth and the growth of both of these molds on cheddar cheese.

Little published data exist on the incorporation of bacteriocins into packaging films. Siragusa and others (1999) highlighted the potential of incorporating Nisin directly into LDPE film for controlling food spoilage and enhancing product safety. Devlieghere and others (2000b) were probably the first to use hexamethylene-tetramine (HMT) as an AM packaging agent. The AM activity of the latter is believed to be due to the formation of formaldehyde when the film comes into contact with an acidic medium (Luck and Jager 1997). It was found that a LDPE film containing 0.5% w/w HMT exhibited AM activity in packaged cooked ham and therefore this agent is a promising material for food packaging applications.

In Japan, the ions of silver and copper, quaternary ammonium salts, and natural compounds such as Hinokitiol are generally considered safe AM agents. Silver-substituted zeolite (Ag-zeolite) is the most common agent with which plastics are impregnated. It retards a range of metabolic enzymes and has a uniquely broad microbial spectrum. As an excessive amount of the agent may affect the heat-seal strength and other physical properties such as transparency, the normal incorporation level used is 1 to 3% w/w. Application to the film surface (that is increasing the surface area in contact with the food) is another approach that could be investigated in the future (Ishitani 1995).

Another interesting commercial development is Triclosan-based antimicrobial agents such as Microban®, Sanitized® and Ultra-Fresh®. Vermeiren and others (2002) reported that LDPE films containing 0.5 and 1.0% w/w triclosan exhibited antimicrobial activity against *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *Salmonella enteritidis* and *Brochothrix thermosphacta* in agar diffusion assay. The 1.0% w/w Triclosan film had a strong antimicrobial effect in *in vitro* simulated vacuum-packaged conditions against the psychrotrophic food pathogen *L. monocytogenes*. However, it did not effectively reduce spoilage bacteria and growth of *L. monocytogenes* on refrigerated vacuum-packaged chicken breasts stored at 7 °C. This is because of ineffectiveness towards microbial growth.

Other compounds with AM effects are natural plant extracts. Recently, Korean researchers developed certain AM films impregnated with naturally-derived AM agents (An and others 1998; Chung and others 1998; Lee and others 1998; Hong and others 2000; Ha and others 2001; Suppakul and others 2002). These compounds are perceived to

be safer and were claimed to alleviate safety concerns (Lee and others 1998). It was reported that the incorporation of 1% w/w grapefruit seed extract (GFSE) in LDPE film (30 µm thick) used for packaging of curled lettuce reduced the growth rate of aerobic bacteria and yeast. In contrast, a level of 0.1% GFSE yielded no significant effect on the rate of microbial growth in packaged vegetables, except for lactic acid bacteria on soybean sprouts (Lee and others 1998). Ha and others (2001) studied GFSE incorporated (by co-extrusion or a solution-coating process) in multilayered PE films and assessed the feasibility of their use for ground beef. They found that coating with the aid of a polyamide binder resulted in a higher level of AM activity than when incorporated by co-extrusion. A co-extruded film (15 µm thick) with 1.0% w/w GFSE showed AM activity against *M. flavus* only, whereas a coated film (43 µm of LDPE with 3 µm of coating layer) with 1.0% w/w GFSE showed activity also against *E. coli*, *S. aureus*, and *Bacillus subtilis*. Both types reduced the growth rates of bacteria on ground beef stored at 3 °C, as compared with plain PE film. The 2 investigated GFSE levels (0.5 and 1.0% w/w) did not differ significantly in the efficacy of the film in terms of its ability to preserve the quality of beef.

Chung and others (1998) found that LDPE films (48 to 55 µm thick) impregnated with either 1.0% w/w *Rheum palmatum* and *Coptis chinensis* extracts or silver-substituted inorganic zirconium retarded the growth of total aerobic bacteria, lactic acid bacteria and yeast on fresh strawberries. However, the study of An and others (1998) showed that LDPE films (48 to 55 µm thick) containing 1.0% w/w *R. palmatum* and *C. chinensis* extracts or Ag-substituted inorganic zirconium did not exhibit any AM activity in a disk test (Davidson and Parish 1989) against *E. coli*, *S. aureus*, *Leuconostoc mesenteroides*, *S. cerevisiae*, *A. niger*, *Aspergillus oryzae*, *Penicillium chrysogenum*. A film containing sorbic acid showed activity against *E. coli*, *S. aureus*, and *L. mesenteroides*. The reasons for this unusual result are not clear. During diffusion assays, the AM agent is contained in a well or applied to a paper disc placed in the center of an agar plate seeded with the test microorganism. This arrangement may not be appropriate for essential oils, as their components are partitioned through the agar due to their affinity for water (Davidson and Parish 1989). Accordingly, broth and agar dilution methods are widely used to determine the AM effectiveness of essential oils (Davidson and Parish 1989). According to Hong and others (2000), the AM activity of 5.0% w/w Propolis extract, Chitosan polymer and oligomer, or Clove extract in LDPE films (0.030- to

0.040-mm thick) against *Lactobacillus plantarum*, *E. coli*, *S. cerevisiae*, and *Fusarium oxysporum* is best determined through viable cell counts. Overall, LDPE films with incorporated natural compounds show a positive AM effect against *L. plantarum* and *F. oxysporum*. Preliminarily studies by Suppakul and others (2002) with LLDPE films (45 to 50 µm thick) containing 0.05% w/w linalool or methyl chavicol showed a positive activity against *E. coli*.

Edible films and various AM compounds incorporated in edible food packages have also been investigated recently (Rodrigues and Han 2000; Coma and others 2001). Rodrigues and Han (2000) investigated edible AM materials produced by incorporating Lysozyme, Nisin and Ethylenediamine tetraacetic acid (EDTA) in whey protein isolate (WPI) films. Such Lysozyme or Nisin-containing films are effective in inhibiting *Brochothrix thermosphacta* but fail to suppress *L. monocytogenes*. The incorporation of EDTA in WPI films improved the inhibitory effect on *L. monocytogenes* but had a marginal effect only on *E. coli* O157:H7.

Coma and others (2001) studied the moisture barrier and the AM properties of HPMC-fatty acid films (30-50 µm thick) containing Nisin (10^5 IU/mL) as the AM agent and its efficacy against *Listeria innocua* and *S. aureus* growth in food products. Stearic acid was chosen as the fatty acid because of its ability to reduce the rate of water vapor transmission. However, it impaired the effectiveness of the film against both strains. This may be explained by electrostatic interaction between the cationic Nisin and the anionic stearic acid.

Immobilization

Besides diffusion and sorption, some AM packaging systems utilize covalently immobilized AM substances that suppress microbial growth. Appendini and Hotchkiss (1997) investigated the efficiency of Lysozyme immobilized on different polymers. It is known that cellulose triacetate (CTA) containing Lysozyme yields the highest AM activity. The viability of *Micrococcus lysodeikticus* was reduced in the presence of immobilized Lysozyme on CTA film (Appendini and Hotchkiss 1997). Scannell and others (2000) showed that PE/polyamide (70:30) film formed a stable bond with Nisin in contrast to Lacticin 3147. Nisin-adsorbed bioactive inserts reduced the level of *L. innocua* and *S. aureus* in sliced cheese and in ham.

Surface modification

Ozdemir and others (1999) introduced (by chemical methods) functional groups

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possessing AM activity into polymer films with the purpose of preventing the transfer of the AM agents from the polymer to the food. Cho and others (2000) synthesized a new biopolymer containing a chito-oligosaccharide (COS) side chain. The COS was introduced on polyvinylacetate (PVA) by cross-linking with the bifunctional compound, *N*-methylolacrylamide (NMA). It was found that the growth of *S. aureus* was almost completely suppressed by this means.

Surface amine groups formed in polymers by electron irradiation were also shown to impart AM effectiveness (Cohen and others 1995; Ozdemir and others 1999). Another AM film has recently been developed using a UV excimer laser. Nylon 6,6 films irradiated in air by a laser at 193 nm exhibited AM activity, apparently due to a 10% conversion of the amide groups on the nylon surface to amines bound to the polymer chain (Cohen and others 1995). By contrast, irradiation at 248 nm did not change the surface chemistry or initiate conversion of the amide (Ozdemir and others 1999).

Paik and others (1998) and Shearer and others (2000) observed a decrease in all bacterial cells, including *S. aureus*, *Pseudomonas fluorescens*, and *E. faecalis* in bulk fluid when using an AM nylon film. The results indicate that this decrease is more probably to be due to the bactericidal action than to surface adsorption (Paik and others 1998). Although the mechanism of the reduction in the bacteria population remained uncertain, electrostatic attractive forces between the positively charged film surface and the negatively charged *E. coli* and *S. aureus* were presumed to be the reason for this effect (Shearer and others 2000). Further research is needed to characterize the AM active groups on the irradiated film surface and the mechanism of AM action.

Ionomers, with their unique properties such as a high degree of transparency, strength, flexibility, stiffness and toughness, as well as inertness to organic solvents and oils, have also drawn much attention as food packaging materials. Halek and Garg (1989) successfully incorporated the Benomyl fungicide into ionomer films via its carboxyl groups. Unfortunately, Benomyl is not an approved food preservative.

Weng and others (1997) investigated application of AM ionomers combined with approved food preservatives. Anhydride linkages in the modified films were formed by reaction of acid/or base-treated films with benzoyl chloride. The AM activity was characterized in terms of the release of benzoic acid, which was higher in the base-

treated version indicating the superiority of the latter. The AM effect of modified ionomer films was further demonstrated by their ability to inhibit the growth of *Penicillium* species and *A. niger*.

Factors to Consider in the Manufacturing of Antimicrobial Films

IT IS CLEAR THAT THE SELECTION OF BOTH THE substrate and the AM substance is important in developing an AM packaging system. Furthermore, when an AM agent is added to a packaging material, it may affect the inherent physico-mechanical properties of the latter.

Process conditions and residual antimicrobial activity

The effectiveness of an AM agent applied by impregnation may deteriorate during film fabrication, distribution and storage (Han 2000). The chemical stability of an incorporated AM substance is likely to be affected by the extrusion conditions, namely, the high temperatures, shearing forces and pressures involved (Han and Floros 1999). To minimize this problem, Han (2000) recommended using master batches of the AM agent in the resin for preparation of AM packages. Also, all operations such as lamination, printing and drying as well as the chemicals used (adhesives and solvents) in the process may affect the AM activity of the package. In addition, some of the volatile AM compounds may be lost during storage. All these parameters should be evaluated.

Characteristics of antimicrobial substances and foods

The mechanism and kinetics of growth inhibition are generally studied in order to permit mathematical modeling of microbial growth (Han 2000). Foods with different biological and chemical characteristics are stored under different environmental conditions, which, in turn, may cause different patterns of microflora growth. Aerobic microorganisms can exploit headspace O₂ for their growth. The pH of a product affects the growth rate of target microorganisms and changes the degree of ionization of the most active chemicals, as well as the activity of the AM agents (Han 2000). Weng and Hotchkiss (1993) reported that LDPE film containing benzoic anhydride was more effective in inhibiting molds at low pH values. Rico-Pena and Torres (1991) found that the diffusion of sorbic acid decreased with an increase in pH. The food a_w may alter the microflora, AM activity, and chemical stability of active ingredients applied by impregnation. Vojdani and Torres (1989a) showed

that the diffusion of potassium sorbate through polysaccharide films increases with a_w ; this has a negative impact on the amount available for protection. Rico-Pena and Torres (1991) found that potassium sorbate diffusion rates in MC/HPMC film containing palmitic acid were much higher at higher values of a_w .

Chemical interaction of additives with film matrix

During incorporation of additives into a polymer, the polarity and molecular weight of the additive have to be taken into consideration. Since LDPE itself is nonpolar, additives with a high molecular weight and low polarity are more compatible with this polymer (Weng and Hotchkiss 1993). Furthermore, the molecular weight, ionic charge and solubility of different additives affect their rates of diffusion in the polymer (Cooksey 2000). Wong and others (1996) compared the diffusion of ascorbic acid, potassium sorbate, and sodium ascorbate in calcium-alginate films at 8, 15, and 23 °C. They found that ascorbic acid had the highest and sodium ascorbate the lowest diffusion rate at all studied temperatures. These findings were attributed to the different ionic states of the additives.

Storage temperature

The storage temperature may also affect the activity of AM packages. Several researchers found that the protective action of AM films deteriorated at higher temperatures, due to high diffusion rates in the polymer (Vojdani and Torres 1989a, b; Wong and others 1996). The diffusion rate of the AM agent and its concentration in the film must be sufficient to remain effective throughout the shelf life of the product (Cooksey 2000). Weng and Hotchkiss (1993) stated that low amounts of benzoic anhydrides in LDPE might be as effective at refrigeration temperatures as high levels at room temperature.

Mass transfer coefficients and modeling

Mathematical modeling of the diffusion process could permit prediction of the AM agent release profile and the time during which the agent remains above the critical inhibiting concentration. With a higher diffusivity and much larger volume of the food component compared to the packaging material, a semi-infinite model in which the packaging component has a finite thickness and the food component has infinite volume could be practical (Han 2000). The initial and boundary conditions that could be

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used in mass transfer modeling have been identified.

Physical properties of packaging materials

AM agents may affect the physical properties, processability or machinability of the packaging material. Han and Flores (1997) reported no significant differences in the tensile properties before and after the incorporation of potassium sorbate in LDPE films, but the transparency of the films deteriorated as the sorbate concentration increased. Weng and Hotchkiss (1993) reported no noticeable differences in clarity and strength of LDPE film containing 0.5 and 1.0% benzoic anhydride. Similar results were reported for naturally-derived plant extracts such as propolis at 5.0% (Hong and others 2000), clove at 5.0% (Hong and others 2000), *R. palmatum* at 1.0% (An and others 1998; Chung and others 1998), and *C. chinensis* at 1.0% (An and others 1998; Chung and others 1998). On the other hand, LDPE film coated with MC/HPMC containing Nisin was difficult to heat-seal (Cooksey 2000).

Dobias and others (2000) found statistically significant differences between the physical properties of films without AM agents and with different agents at concentrations of 5 g/kg and 10 g/kg. It was found that the tensile and sealing strengths were lower in all samples containing AM agents including benzoic anhydride, ethyl paraben (ETP) or propyl paraben (PRP). In all studied cases, the coefficient of friction increased with the addition of AM substances, water vapor permeability declined by the incorporation of PRP, and oxygen permeability decreased by the impregnation of benzoic anhydride or PRP.

Cost

There are no published data on the cost of films impregnated with AM agents, but they can be expected to be more expensive than their basic counterparts. Commercialization of such films could therefore become viable for high-value food products only (Cooksey 2000).

Food contact approval

Some organic acids, bacteriocins and volatile compounds derived from plants have FDA approval as additives for certain foods (see Table 6). AIT is currently not approved by the FDA for use in the U.S.A. (Brody and others 2001) due to a safety concern that this *synthetic* compound may be contaminated with traces of the toxic allyl chloride used in the manufacturing process (Clark 1992). In Japan, the use of AIT is allowed

Table 6—List of permitted food additives that could be used as antimicrobial agents in packaging materials.

Additive	Australia/New Zealand ¹	Code Assigned by Legislative Authority	U.S.A. ³
		Europe ²	
Acetic acid	260	E260	GRAS
Benzoic acid	210	E210	GRAS
Butylated hydroxyanisole (BHA)	320	E320	GRAS
Butylated hydroxytoluene (BHT)	321	E321	GRAS
Carvacrol			FA
Citral			GRAS
Citric acid	330	E330	GRAS
p-Cresol			FA
EDTA			FA
Estragole (methyl chavicol)			GRAS
Ethanol		E1510	GRAS
Ethyl paraben		E214	GRAS
Eugenol			GRAS
Geraniol			GRAS
Glucose oxidase	1102		GRAS
Hexamethylenetetramine (HMT)		E239	
Konjac glucomannan		E425	GRAS
Lactic acid	270	E270	GRAS
Lauric acid			FA
Linalool			GRAS
Lysozyme	1105	E1105	GRAS
Malic acid	296	E296	GRAS
Methyl paraben	218	E218	
Natamycin	235	E235	FA
Nisin	234	E234	GRAS
Phosphoric acid	338	E338	GRAS
Polypophosphate		E452	GRAS
Potassium sorbate	202	E202	GRAS
Propionic acid	280	E280	GRAS
Propyl paraben	216	E216	GRAS
Sodium benzoate	211	E211	GRAS
Sorbic acid	200	E200	GRAS
Succinic acid		E363	GRAS
Sulfur dioxide	220	E220	GRAS
Tartaric acid	334	E334	GRAS
Tertiary butylhydroquinone (TBHQ)	319		FA
α-Terpineol			FA
Thymol			FA

Source: CFR (1988); Davidson and Branen (1993); Maga and Tu (1995); Lück and Jager (1997); Saltmarsh (2000); Taubert (2000).

¹Assignment of a number signifies that additive is approved by the Australian and New Zealand Food Authority (ANZFA) and The Australian New Zealand Food Standards Council (ANZFSC) as being safe for food use.

²Assignment of an "E" number signifies that additive has been approved by the European Communities (EC) Scientific Committee on Food (SCF).

³Classification in accordance with Food and Drug Administration (FDA) Title 21 of the Code of Federal Regulations (21 CFR) wherein substances intended for use in the manufacture of foodstuffs for human consumption are classified into 3 categories: food additives (FA), prior-sanctioned food ingredients and substances generally recognized as safe (GRAS).

only when this compound is extracted from a natural source (Isshiki and others 1992). Weng and Hotchkiss (1993) pointed out that the rapid hydrolysis of benzoic anhydride to benzoic acid should not pose a safety concern, although at the time of their study benzoic anhydride did not have FDA approval. The use of Ag-zeolite as an acceptable food additive in Europe has not been clarified (Brody and others 2001). However, recently, Ag-zeolites such as AgION™ and Zeomic® received the approval of the FDA for use in food-contact materials. Triclosan is also not accepted by US regulatory authorities for food contact materials (Brody and others 2001). In Europe, the legislative status of Triclosan is unclear. Triclosan does not

appear on the EU directive list of approved food additives that may be used in the manufacturing of plastics intended for food contact materials (Vermeiren and others 2002).

No European regulations exist currently on the use of active and intelligent packaging. Packages intended for food contact applications are required to belong to a positive list of approved compounds, and an overall migration limit from the material into the food or food simulant was set at 60 mg/kg. This is incompatible with the aim of active packaging, especially when the system is designed to release active ingredients into the foods. Consequently, as was also stated by van Beest (2001), a new approach in food packaging regulations is needed. The cur-

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Table 7—Current and future applications of antimicrobial packaging

System	Beverage	Minimally Processed			Food group		
			Meat/Poultry	Seafood	Dairy	Bakery	Produce
O ₂ Scavenging	Wine Beer Fruit juice		Fresh meat Processed meat Sausages Meat Chicken		Cheese	Bread	Vegetables
H ₂ O absorb/control			Fresh meat Poultry	Fish			Vegetables Fruit
CO ₂ generating				Fish	Cheese		
Ethanol generating				Fish	Cheese	Bread Cake	
Antimicrobial ¹	Fruit juice ² Tea	Noodles ² Pasta ² Sandwiches ²	Meat Ham Pastrami Bologna Chicken	Fish	Cheese	Bread Cake ²	Vegetables Fruit

¹Including both migrating and nonmigrating systems.

²Possible future applications.

rent applications of AM food packaging are rather limited, although promising. This is because of the legal status of the tested additives (Vermeiren and others 2002). The major potential food applications of AM films include meat, fish, poultry, bakery goods, cheese, fruits and vegetables (Labuzza and Breene 1989). Table 7 lists the current and potential future applications of AM packaging technologies.

The Future

AM PACKAGING IS A RAPIDLY EMERGING TECHNOLOGY. The need to package foods in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient, and safe food products presages a bright future for AM Packaging (Floros and others 1997). However, more information is required on the chemical, microbiological and physiological effects of these systems on the packaged food especially on the issues of nutritional quality and human safety (Floros and others 1997). So far, research on AM packaging has focused primarily on the development of various methods and model systems, whereas little attention has been paid to its preservation efficacy in actual foods (Han 2000). Research is essential to identify the types of food that can benefit most from AM packaging materials. It is likely that future research into a combination of naturally-derived AM agents, bio-preserved and biodegradable packaging materials will highlight a range of the merits of AM packaging in terms of food safety, shelf-life and environmental friendliness (Nicholson 1998; Rodrigues and Han 2000; Coma and others 2001). The reported effectiveness of natural plant extracts suggests that further research is needed in order to evaluate their antimicrobial activity and potential side effects in

packaged foods. An additional challenge is in the area of odor/flavor transfer by natural plant extracts to packaged food products. Thus, research is needed to determine whether natural plant extracts could act as both an antimicrobial agent and as an odor/flavor enhancer. Moreover, in order to secure safe food, amendments to regulations might require toxicological and other testing of compounds prior to their approval for use (Vermeiren and others 2002).

Abbreviations

AIT	Allyl isothiocyanate
AM	Antimicrobial
AP	Active Packaging
a _w	Water activity
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
COS	Chito-oligosaccharide
CTA	Cellulose triacetate
EDTA	Ethylenediaminetetraacetic acid
ERH	Equilibrium relative humidity
EVA	Ethylene vinyl alcohol
ETP	Ethyl paraben
FDA	Food and Drug Administration
GFSE	Grapefruit seed extract
GRAS	Generally recognised as safe
HDPE	High density polyethylene
HMT	Hexamethylenetetramine
HPMC	Hydroxypropyl methylcellulose
IMF	Intermediate moisture foods
LDPE	Low density polyethylene
LLDPE	Linear low density polyethylene
MAP	Modified atmosphere packaging
MC	Methylcellulose
MIC	Minimum inhibitory concentration
NMA	N-methylol acrylamide
OSP	Oxygen scavenging packet
OTR	Oxygen transmission rate
PDA	Potato dextrose agar
PE	Polyethylene
PEMA	Polyethylene-co-methacrylic acid
PRP	Propyl paraben

PVA	Polyvinylacetate
PVC	Polyvinylchloride
PVDC	Polyvinylidenechloride
TBHQ	Tertiary-butyl-hydroquinone
WPI	Whey protein isolate

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