Antimicrobial Edible Films and Coatings

Article *in* Journal of Food Protection · May 2004

DOI: 10.4315/0362-028X-67.4.833 · Source: PubMed

3 authors:

Arzu Cagri-Mehmetoglu
Sakarya University
38 PUBLICATIONS **1,215** CITATIONS
SEE PROFILE

Zeynep Ustunol
Michigan State University
75 PUBLICATIONS **3,969** CITATIONS
SEE PROFILE

Elliot T Ryser
Michigan State University
182 PUBLICATIONS **5,993** CITATIONS
SEE PROFILE

Journal of Food Protection, Vol. 67, No. 4, 2004, Pages 833–848

Review

Antimicrobial Edible Films and Coatings

ARZU CAGRI, ZEYNEP USTUNOL, AND ELLIOT T. RYSER*

Department of Food Science and Human Nutrition, 2108 South Anthony Hall, Michigan State University, East Lansing, Michigan 48824-1225, USA

MS 03-147: Received 1 April 2003/Accepted 17 October 2003

ABSTRACT

Increasing consumer demand for microbiologically safer foods, greater convenience, smaller packages, and longer product shelf life is forcing the industry to develop new food-processing, cooking, handling, and packaging strategies. Nonfluid ready-to-eat foods are frequently exposed to postprocess surface contamination, leading to a reduction in shelf life. The food industry has at its disposal a wide range of nonedible polypropylene- and polyethylene-based packaging materials and various biodegradable protein- and polysaccharide-based edible films that can potentially serve as packaging materials. Research on the use of edible films as packaging materials continues because of the potential for these films to enhance food quality, food safety, and product shelf life. Besides acting as a barrier against mass diffusion (moisture, gases, and volatiles), edible films can serve as carriers for a wide range of food additives, including flavoring agents, antioxidants, vitamins, and colorants. When antimicrobial agents such as benzoic acid, sorbic acid, propionic acid, lactic acid, nisin, and lysozyme have been incorporated into edible films, such films retarded surface growth of bacteria, yeasts, and molds on a wide range of products, including meats and cheeses. Various antimicrobial edible films have been developed to minimize growth of spoilage and pathogenic microorganisms, including *Listeria monocytogenes,* which may contaminate the surface of cooked ready-to-eat foods after processing. Here, we review the various types of protein-based (wheat gluten, collagen, corn zein, soy, casein, and whey protein), polysaccharide-based (cellulose, chitosan, alginate, starch, pectin, and dextrin), and lipid-based (waxes, acylglycerols, and fatty acids) edible films and a wide range of antimicrobial agents that have been or could potentially be incorporated into such films during manufacture to enhance the safety and shelf life of ready-to-eat foods.

Quality, safety, and shelf life of ready-to-eat (RTE) foods is dictated by the type and numbers of pathogenic and spoilage bacteria present on the food surface. Approximately two-thirds of all microbiologically related class I recalls in the United States result from postprocessing contamination during subsequent handling and packaging rather than from underprocessing. Most of these recalls are prompted by contamination with *Listeria monocytogenes,* for which the United States has maintained its policy of zero tolerance since 1985. From January 1998 to February 2003, over 130 *Listeria*-related class I recalls involving more than 80 million pounds of cooked RTE meats were issued *(20).* More than 35 million pounds of hot dogs and luncheon meats were voluntarily recalled in 1998 by one Michigan manufacturer in response to an outbreak that resulted in 101 cases of listeriosis (including 21 fatalities) in 22 states *(26).* Two years later, another listeriosis outbreak involving 29 cases in 10 states (including seven fatalities) prompted the recall of approximately 14.5 million pounds of turkey and chicken delicatessen meat; again, the product became contaminated with *L. monocytogenes* after processing *(191).* Most recently, the largest product recall ever issued, 27.4 million pounds of fresh and frozen RTE turkey and chicken products, was linked to another major outbreak of listeriosis emanating from a manufacturer in Pennsylvania. Each year, approximately 2,300 cases of foodborne listeriosis have been reported in the United States at an estimated cost of $2.33 billion (~$1 million per case), making *L. monocytogenes* the second most costly foodborne pathogen after *Salmonella* ($2.38 billion) *(191).*

Product slicing and packaging operations are major points at which both pathogenic and spoilage organisms can be introduced into cooked RTE foods. In commercial manufacturing facilities, slicing of RTE meat products can easily increase microbial populations 100-fold or greater *(150).* Antimicrobial edible films can be used to minimize growth of surface contaminants during refrigerated storage *(39, 139).* Such edible films and coatings also can be used to inhibit microbial growth on the surface of processed fresh produce and nutmeats, thereby extending product shelf life.

Postprocessing pasteurization is one means of inactivating surface contaminants on cooked RTE meat products. Using this strategy, vacuum-packaged foods are individually pasteurized by heat *(106)* or other means, such as high pressure or UV irradiation *(114, 129, 171, 208).* Alternatively, microbial growth can be minimized by the application of various antimicrobial dips and sprays *(142, 157, 162).* However, the effectiveness of these applications over time is limited because of continued diffusion into the food, allowing surface organisms to grow. One strategy for reducing the rate of diffusion is to entrap the antimicrobial or other food additive in an edible film matrix *(71, 80, 81).*

* Author for correspondence. Tel: 517-355-7713 ext. 185; Fax: 517-353-1676; E-mail: ryser@msu.edu.

TABLE 1. *Edible films*

Type	Formulation	Additive or treatment to improve film[a]
Protein based		
Casein	Aqueous solution and glycerol or sorbitol	Lipids, lactic acid, tannic acid, or exposure to UV and γ-irradiation improve WVP *(20, 82, 87, 108, 154)*
Collagen	Dry process: alkaline treatment of hide corium, acidification, shredding, mixing, addition of plasticizing and cross-linking agents, pumping, extrusion, drying, neutralizing *(91)* Wet process: acid or alkaline dehairing of hides, deacidification, grinding, mixing, homogenization, extrusion, addition of plasticizing and cross-linking agent *(91)*	Glyceraldehyde and alkyl diols improve mechanical properties *(18, 97, 113)*; UV irradiation increases strength *(124)*; proteolytic enzymes improve uniformity in diameter and wall thickness *(188)*; formaldehyde and chrome tanning reduce gas permeability *(113)*
Corn zein	Alcohol or acetone solutions and glycerol, oleic acid, or lactic acid	Aldehydes improve WVP and TS *(177)*
Gelatin	Aqueous solution and glycerol	Lactic and tannic acid improve WVP *(79)*
Wheat gluten	Ethanol solution and glycerol, heating under alkaline conditions	Keratin improves OP, WVP, and TS *(199)*; corn zein, soy protein, and cystein improve TS *(69)*
Whey protein	Aqueous solution and glycerol, heating under alkaline conditions	Heat curing improves TS, WVP, OP *(125)*; lipids improve WVP *(164)*
Soy protein	Aqueous solution and glycerin, heating under alkaline conditions	UV or γ-irradiation increases TS *(161)*; heat curing improves WVP, TS, OP *(174)*; calcium chloride and calcium sulfate improve TS *(70)*
Carbohydrate based		
Alginate	Aqueous solutions, calcium ions, glycerol, heating under alkaline conditions	Immersion in multivalent cation solutions improves TS *(102)*
Cellulose	Aqueous solutions, slowly adding, agitation	Lipids improve WVP *(144)*
Chitosan	Acidified aqueous solutions, glycerol	Lipids improve WVP *(200)*
Starch	Aqueous solutions, glycerol, heating	Suspending in NaOH decreases CO_2, O_2, and WVP *(66, 67)*

[a] WVP, water vapor permeability; TS, tensile strength; OP, oxygen permeability.

Definition and historical background of edible film. Edible films or coatings are defined as continuous matrices that can be prepared from proteins, polysaccharides, and lipids. Yuba, the first free-standing edible film, was developed in Japan from soymilk during the 15th century and was used for food preservation *(17)*. Edible coatings for food products date back even further; during the 12th century in China, waxes were applied to oranges and lemons to retard water loss *(86)*. During the 16th century, food products were coated with fat (e.g., lard) to control moisture loss *(110)*. Hot-melt paraffin waxes have been used to coat citrus fruits in the United States since the 1930s, and carnauba wax and oil-in-water emulsions have been used for coating fresh fruits and vegetables since the 1950s *(103)*. Currently, edible films and coatings are used in various applications, including casings for sausages and chocolate coatings for nuts and fruits.

Components of edible films. Edible films typically contain three major components: proteins, polysaccharides, and lipids. Proteins used in edible film include wheat gluten, collagen, corn zein, soy, casein, and whey protein (Table 1) *(104)*. Alginate, dextrin, pectin, and cellulose derivatives are used in polysaccharide-based films *(104)*. Suitable lipids for use in films include waxes, acylglycerols, and fatty acids *(47, 144)*. Composite films containing both lipid and hydrocolloid components also have been developed.

Plasticizers are often added to film-forming solutions to enhance properties of the final film. These film additives are typically small molecules of low molecular weight and high boiling point that are highly compatible with the polymers *(8)*. Common food-grade plasticizers such as sorbitol, glycerol, mannitol, sucrose, and polyethylene glycol decrease brittleness and increase flexibility of the film, which is important in packaging applications. Plasticizers used for protein-based edible films decrease protein interactions and increase both polymer chain mobility and intermolecular spacing *(113)*. The type and concentration of plasticizer influences the properties of protein films *(41, 76)*; mechanical strength, barrier properties, and elasticity decrease when high levels of plasticizer are used *(30, 64, 73)*. Water is another important plasticizer for protein films *(107)*, but moisture content affects film properties.

Common covalent cross-linking agents such as glutaraldehyde, calcium chloride, tannic acid, and lactic acid are used to improve water resistance, cohesiveness, rigidity, mechanical strength, and barrier properties *(77, 116)*. Exposure to UV light will increase the cohesiveness of protein films by forming cross-links *(20)*. Alternatively,

TABLE 2. *Application of edible films on foods*

Films	Foods	Benefits
Casein	Peeled carrots	Reduced dehydration and white blush formation *(4, 5)*
Casein, acetylated monoglycerides	Celery sticks	Reduced dehydration *(109)*
Sodium caseinate	Green bell peppers	Reduced oxygen and carbon dioxide permeation *(112)*
Collagen	Hot dogs, sausage	Reduced cost and increased uniformity
Corn zein	Nuts	Delayed rancidity *(176)*
	Tomatoes	Delayed color change, loss of firmness, and weight loss *(143)*
Wheat gluten	Eggshells	Improved shell strength, reduced microbial contamination *(203)*
Whey protein	Frozen King salmon	Reduced moisture loss and oxidation *(175)*
Whey protein, acetylated monoglycerides	Nuts	Delayed rancidity *(117–119)*
Whey protein	Eggshells	Improved shell strength, reduced microbial contamination *(203)*
Soy protein	"Fuji" and "Golden 95 delicious" apples	Retarded changes in firmness, color, and acidity *(112)*
	Eggshells	Improved shell strength, reduced microbial contamination *(203)*
Alginate	Fresh meat, poultry, precooked ground pork patties	Reduced shrinkage, oxidative rancidity, moisture migration, and oil absorption *(104, 120, 197)*
Cellulose	Bell peppers	Reduced oxygen and carbon dioxide permeability *(112)*
	Fried chicken	Reduced oil degradation, moisture loss
	Fried foods	Reduced fat absorption *(89, 90)*
	Eggshells	Improved shell strength, reduced microbial contamination *(203)*
Chitosan	Bell peppers and cucumbers	Reduced respiration, color loss, wilting, and fungal infection *(55)*
	Strawberries	Delayed spoilage *(56)*
	Tomatoes	Retarded ripening and extended shelf life *(57)*
Starch	Prunes	Extended shelf life *(95)*
	Nuts	Delayed rancidity *(65)*
Starch, alginate, stearic acid	Precooked beef patties	Controlled moisture loss *(202)*
Starch, alginate, stearic acid, tocopherol	Precooked beef patties	Controlled lipid oxidation *(202)*
Dextrin	Apples	Reduced oxidative browning *(130)*
Xanthan gum	Carrots	Improved surface color *(122)*
Wax or fatty acids	Fruits and vegetables	Delayed spoilage, reduced water loss *(84, 85)*
	Cheese	Prevent mold growth *(104)*
Acetylated monoglyceride	Frozen King salmon and frozen silver salmon	Reduced moisture loss and lipid oxidation *(68, 175)*

enzymatic cross-linking treatments with transglutaminases or peroxidases can be used to stabilize films.

Film-forming techniques. Several different techniques including solvent removal, thermal gelation, and solidification of melt have been developed for forming edible films. Solvent removal is typically used to produce hydrocolloid edible films. In this process, a continuous structure is formed and stabilized by chemical and physical interactions between molecules. Macromolecules in the film-forming solution are dissolved in a solvent, such as water, ethanol, or acetic acid, that contains several additives (plasticizers, cross-linking agents, solutes). The film-forming solution is then cast in a thin layer, dried, and peeled from the surface.

In preparing some types of protein films (whey protein, casein, soy protein, wheat gluten), the solution is heated for protein gelation and coagulation, which involves denaturation, gelification, or precipitation followed by rapid cooling. Intramolecular and intermolecular disulfide bonds in the protein complex are cleaved and reduced to sulfhydryl groups during protein denaturation *(134)*. When the film-forming solution is cast, reformed disulfide bonds link the polypeptide chains together to produce the film structure, with the aid of hydrogen and hydrophobic bonding.

Melting followed by solidification is another common

TABLE 3. *Selected potential antimicrobial agents*

Agent	pH	Food applications	Microorganisms affected
Benzoate	<6.0	Dairy products, baked goods, vegetables, fruits, meat, fish, beverages *(115)*	*L. monocytogenes (92), Escherichia coli (182), Bacillus cereus, Salmonella* Typhimurium *(182),* yeast and mold *(115)*
Sorbate	<6.0	Dairy products, baked goods, vegetables, fruits (dried fruits, fruit drinks, jams, jellies, wine), fermented sausage, fish, mayonnaise, margarine, salad dressing *(115)*	*Bacillus, E. coli, L. monocytogenes (58, 92), Pseudomonas (170), Salmonella (189), Clostridium botulinum (163), Staphylococcus aureus (111),* yeast and mold *(187)*
Parabens	2–10	Butter, margarine, ices, soy sauce, maple syrup, meats *(10, 156)*	*L. monocytogenes (147), E. coli (127), S. aureus, C. botulinum (53, 150, 151), B. cereus (10), Bacillus megaterium, Lactobacillus lactis, Streptococcus faecalis, Sarcina lutea (156), Salmonella* Typhimurium *(54), Salmonella* Typhosa, *Yersinia enterocolitica (127), Vibrio parahaemolyticus, Pseudomonas (43), Candida albicans, Fusarium oxysporum, Penicillium citrinum, Penicillium chrysogenum, Saccharomyces cerevisiae (94, 99), Torula utilis, Zygosaccharomyces (1), Aspergillus (183)*
Propionate	<6.0	Baked goods, cheese *(156)*	*E. coli (31), S. aureus, S. lutea, Salmonella (32, 33), Proteus vulgaris, Lactobacillus, L. monocytogenes (59, 92), Bacillus subtilis, Aspergillus, Candida, S. cerevisiae (54)*
Fatty acids			*L. monocytogenes (173), E. coli, Salmonella, Clostridium (132),* yeasts and mold *(10)*
NaCl		Meats, fish, vegetables *(19)*	Gram-negative bacteria, gram-positive bacteria *(9, 172),* yeasts and molds *(39)*
Lactic acid		Jam, jellies, sherbet, beverages, pickles, olives, apple slices, fruit, and baked goods *(19)*	*L. monocytogenes, Salmonella, E. coli (32, 50), S. aureus, Clostridium (19)*
Acetic acid		Baked goods, mustard, salad dressing, mayonnaise, pickles, cheeses, dairy product analogs, fats and oils, gravies and sauces, meats, cereals, gelatin, candy, jams, jellies, soup mixes *(10)*	*L. monocytogenes (92), Bacillus, E. coli* O157:H7 *(13, 52), Salmonella, Staphylococcus (2), Salmonella* Newport, *Salmonella* Typhimurium, *Campylobacter jejuni (135)*
Nitrite	<7.0	Meat products *(158)*	*Clostridium (201), S. aureus, E. coli, Achromobacter, Enterobacter, Flavobacterium, Micrococcus (158), Pseudomonas (204), L. monocytogenes (148)*
Nisin	<6.0	Cheese, fermented meat products *(19)*	*L. monocytogenes, Bacillus, Clostridium, Corynebacterium, Lactobacillus, Leuconostoc, Micrococcus, Pediococcus, Streptococcus (35, 133)*
Pediocin	3–9	Fermented meat products *(19)*	*L. monocytogenes (207), B. cereus, S. aureus, Lactobacillus brevis, Lactobacillus plantarum, L. lactis, C. botulinum, Clostridium perfringens, Clostridium sporogenes, Micrococcus luteus, Enterococcus faecalis*
Lysozyme	2–11	Seafood, kimchi, Chinese noodles, potato salad, custard, hard cheeses *(19)*	*L. monocytogenes (207)*

means for producing lipid-based films. Casting molten wax on dried methylcellulose films followed by solubilization of the methylcellulose can also be used to form wax films *(51).*

APPLICATIONS OF EDIBLE FILMS

Heightened consumer demand for enhanced keeping quality and freshness of RTE foods has given rise to the concept of active packaging—a type of packaging that alters conditions surrounding the food to maintain product quality and freshness, improve sensory properties, or enhance product safety and shelf life. Specific functions of active packaging may include scavenging of oxygen, moisture, or ethylene, emission of ethanol and flavors, and the maintenance of antimicrobial activity through controlled diffusion of one or more antimicrobial agents from the packaging material into the product. Many of the edible films listed in Table 1 can potentially serve as active food

TABLE 3. *Continued*

Agent	pH	Food applications	Microorganisms affected
Essential oils		Meat, vegetable products *(15)*	*E. coli (100), B. cereus (190), S. aureus, Salmonella* Typhimurium *(181), Salmonella* Enteriditis, *L. monocytogenes (7, 48), Y. enterocolitica (44), Streptococcus mutans, Lactobacillus viridescens, Leuconostoc mesenteroides, Enterobacter aerogenes, Erwinia carotovora, Pseudomonas aeruginosa, Proteus vulgarus, C. albicans, Rhodotorula glut, Cryptococcus laurentii, Aspergillus niger (178, 179)*
Lactoferrin			*B. subtilis, Bacillus stearothermophilus, L. monocytogenes (195), Micrococcus, Klebsiella, Salmonella* Typhimurium, *Pseudomonas fluorescens, E. coli* O157:H7, *Shigella, Candida, Y. enterocolitica, C. jejuni, S. mutans, Corynebacterium, P. aeruginosa, P. vulgaris, S. aureus, C. perfringens (38, 96, 136, 145, 146, 153)*

packaging materials by altering permeability to water vapor and oxygen. Table 2 lists potential benefits of the protein-, polysaccharide-, and lipid-based films for preserving the quality and safety of nuts, fish, meats, fruits and vegetables, and deep-fried foods.

ANTIMICROBIALS USED IN EDIBLE FILMS AND COATINGS

Edible films can serve as carriers for a wide range of food additives, including various antimicrobials that can extend product shelf life and reduce the risk of pathogen growth on food surfaces. Some of the more commonly used preservatives and antimicrobials (Table 3) include benzoates, propionates, sorbates, parabens, acidifying agents (e.g., acetic and lactic acids), curing agents (e.g., sodium chloride and sodium nitrite), bacteriocins, and natural preservatives (e.g., essential oils, lysozyme, liquid smoke). These agents are discussed here in the context of their use or potential use in protein-, polysaccharide-, and lipid-based edible films.

Benzoic acid and sodium benzoate. Sodium benzoate was among the first chemical preservatives permitted in foods by the Food and Drug Administration. In the United States, benzoic acid and sodium benzoate are generally regarded as safe preservatives *(34)* at levels up to 0.1%. Although typically used as a mold and yeast inhibitor, sodium benzoate and benzoic acid are also inhibitory to pathogenic and psychrotrophic spoilage bacteria (Table 3).

Sodium benzoate is one of the most commonly used antimicrobials in edible films because it is soluble in most film solutions and remains active after film preparation. The antimicrobial activity of sodium benzoate is related to pH. Like many other food antimicrobials, sodium benzoate (pK_a = 4.20) is most effective in its undissociated form; 60% of the compound is undissociated at pH 4.0. Therefore, methycellulose, chitosan, and collagen films, all of which have a relatively low pH, are good candidates for this antimicrobial. Given these restrictions, edible films containing benzoic acid and its sodium salt are best suited for acidic foods such as cheeses and fermented meat products *(115).*

Sorbic acid and potassium sorbate. Sorbic acid is a straight chain α,β-unsaturated monocarboxylic acid with the carboxyl group reacting to form calcium, sodium, or potassium salts and esters *(169).* Potassium sorbate, the commonly used salt of sorbic acid, is highly soluble in water (58.2% at 20°C). Increased antimicrobial activity of potassium sorbate at low pH has been reported for a wide range of microorganisms (Table 3). Therefore, edible films containing potassium sorbate are typically most effective at pH values less than 6.0.

Sorbic acid salts have been among the most studied antimicrobial agents in carbohydrate- and protein-based edible films such as methylcellulose, whey protein isolate (WPI), and chitosan because the sorbates are widely used and remain chemically active in the film matrix. The carboxyl group (the active site in sorbates) forms hydrogen bonds with carbohydrate or protein chains in films. Edible films containing sorbates have been tested against a wide variety of microorganisms (i.e., spoilage bacteria, pathogenic bacteria, yeast and molds) in laboratory media using film disc diffusion assays (Table 4).

Propionic acid. Propionic acid or its salts are commonly used food preservatives because of their wide spectrum of activity. Propionic acid, a monocarboxylic acid, is produced by *Propionibacterium freudenreichii* subsp. *shermanii.* Swiss cheese contains up to 1% propionic acid from the growth of propionibacteria, which gives it a characteristic flavor and prevents mold growth *(44).*

Antimicrobial activity of the propionates is again pH dependent, with the undissociated form showing 45 times more inhibitory activity than the dissociated form *(54).*

TABLE 4. *Antimicrobial edible films*

Films	Antimicrobial agents	Medium/food	Microorganisms	Reference(s)
MC[a]	Benzoic acid	Taiwanese-style fruit preserves	*Zygosaccharomyces rouxii, Zygosaccharomyces mellis*	*28*
	Benzoic acid, sorbic acid	Culture medium	*Penicillium notatum, Rhodotorula*	*29*
	Potassium sorbate with fatty acid	Culture medium	NT[b]	*194*
	Nisin	Culture medium	*Micrococcus luteus*	*27*
	Potassium sorbate with palmitic acid	Culture medium	NT	*155*
MC/chitosan	Benzoic acid, sorbic acid	Culture medium	*P. notatum, Rhodotorula*	*29*
HPMC[c]	Potassium sorbate with fatty acid	Culture medium	NT	*194*
	Nisin	Culture medium	*M. luteus*	*27*
	Potassium sorbate, acetic acid	Tomatoes	*Salmonella* Montevideo	*210*
	Nisin	Culture medium	*Listeria innocua, S. aureus*	*37*
Cellulose	Pediocin	Cooked meats	*L. monocytogenes*	*126*
Chitosan	Acetic, propionic, lauric acids	Processed meats	*Lactobacillus sakei, Serratia liquefaciens*	*139*
	Acetic, propionic acids	Water	NT	*140*
	Lactic, citric acids	Culture medium	NT	*12*
	Acetic acid	Culture medium	*L. monocytogenes*	*36*
Starch	Potassium sorbate	Chicken breast	*E. coli* O157:H7, *Salmonella* Typhimurium	*11*
Alginate	Lactic acid	Lean beef muscle	*E. coli* O157:H7, *Salmonella* Typhimurium, *L. monocytogenes*	*165*
	Glucose oxidase	Fish	NT	*61*
Casein	Sorbic acid	Papaya cubes	*Staphylococcus rouxii, A. niger*	*78*
WPI	*p*-Aminobenzoic acid with lactic acid, potassium sorbate with lactic acid, acetic acid, lactic acid	Culture media, bologna and summer sausage slices, hot dogs	*L. monocytogenes, E. coli* O157:H7, *Salmonella* Typhimurium DT104	*21–23*
	Nisin	Phosphate buffer	*L. monocytogenes*	*105*
	Nisin with EDTA, lysozyme with EDTA, propyl paraben	Culture medium	*Brochothrix thermosphacta, Salmonella* Typhimurium, *E. coli, L. monocytogenes, S. aureus*	*159, 160*
Soy protein isolate	Nisin	Culture medium	*L. plantarum*	*141*
Corn zein	Nisin	Milk	*L. monocytogenes*	*138*
	Nisin	RTE chicken	*L. monocytogenes*	*93*
	Potassium sorbate	Cheese	*S. aureus*	*185*
	Lauric acid	Culture medium	*L. plantarum*	*46*
	Nisin with lauric acid and EDTA	Culture medium	*Salmonella* Enteritidis, *L. monocytogenes*	*88*
	Sorbic acid	Cooked sweet corn	*L. monocytogenes*	*24*
	Nisin, lysozyme	Culture medium	*L. plantarum*	*141*
	Nisin	Dibasic sodium phosphate	NT	*180*
Wheat gluten	Sorbic acid	Culture medium	NT	*152*
	Sorbic acid	Model food	*P. notatum*	*79*
	Sorbic acid	Cooked sweet corn	*L. monocytogenes*	*24*
	Nisin	Dibasic sodium phosphate	NT	*180*
	Nisin	Phosphate medium	*L. monocytogenes*	*105*

[a] MC, methylcellulose.
[b] NT, not tested for antimicrobial activity.
[c] HPMC, hydropropylmethylcellulose.

Therefore, it is most effective in low pH edible films such as those containing collagen and chitosan. Although this acid is primarily active against molds, some yeasts and bacteria are also inhibited (Table 3). Amounts of propionate used in foods are generally less than 0.4% *(156).*

Parabens. Esterification of the carboxyl group of benzoic acid produces parabens. Because they remain undissociated at pH values up to 8.5, most parabens are active at pH 3.0 to 8.0. The methyl, propyl, and heptyl parabens can be used as food preservatives in most countries, but

the ethyl and butyl esters are more restricted. Parabens can be used effectively in a wide range of foods (Table 3).

Parabens with a longer alkyl chain possess more antimicrobial activity than do those with a shorter alkyl chain *(1)*, which are more inhibitory to gram-positive than to gram-negative bacteria because of their decreased polarity. Methyl, ethyl, propyl, and butyl parabens completely inhibit the growth of gram-positive and gram-negative bacteria at concentrations of 40 to 2,000 and 50 to 4,000 μg/ml, respectively (Table 3). However, parabens are generally more active against molds and yeasts than against bacteria. Using esters of *p*-hydroxybenzoic acid, concentrations of 32 to 1,000 μg/ml are normally needed for complete inhibition of bacteria and fungi (Table 3).

Free fatty acids and their esters. Low concentrations of long-chain fatty acids are inhibitory to microorganisms, especially gram-positive bacteria and yeasts *(101)*. Saturated fatty acids with chain lengths of C_{12} to C_{16} and C_{10} to C_{12} possess the most antimicrobial activity against bacteria and yeasts, respectively *(101)*. Decreasing effectiveness of longer chain fatty acids may be related to increased hydrophobicity and decreased solubility *(196)*. Fatty acids are also more active at low pH (<5.0). Both fatty acids and monoglycerides are inhibitory to many bacterial species (Table 3). Monoesters of glycerols and the esters of sucrose are more antimicrobial than their corresponding free acids.

Monolaurin (lauricin), the most effective of the glycerol monoesters, is inhibitory to various gram-positive bacteria and some fungi at 5 to 100 ppm *(3)* but are most effective at pH values between 5.0 and 8.0. Use of monolaurin as a food preservative is limited by the production of off-flavors and loss of activity from interaction with lipophilic proteins, fat globules, and starch. Fatty acids and polyglycerides are added to edible films and coatings to decrease water vapor permeability. Long-chain alcohols (e.g., stearyl alcohol) and fatty acids (e.g., stearic, palmitic) are commonly used as additives in edible coatings because of their high melting points and hydrophobicity *(83)*. Vojdani and Torres *(194)* developed composite films with methylcellulose and fatty acids of different chain lengths to decrease the diffusion of preservatives such as potassium sorbate from the surface of cheese.

Acetic acid. Acetic acid (CH_3COOH), the primary component of vinegar, is produced by *Acetobacter* species. Acetic acid and sodium diacetate are active against various spoilage and pathogenic bacteria and have been used in many foods (Table 3). Like other organic acids, acetic acid can be used to acidify edible films prepared from chitosan, alginate, collagen, and WPI. Addition of acetic acid also increases the activity of other antimicrobial agents such as sorbic acid and benzoic acid that can be incorporated into edible films.

Lactic acid. Lactic acid ($CH_3CHOHCOOH$), produced naturally by lactic acid bacteria during fermentation, is primarily used for improving and controlling the quality and microbial stability of foods. Lactic acid sprays (1 to 3% solutions) have been widely used to sanitize meat carcass surfaces *(31, 50, 168)*, with gram-negative psychrotrophs generally being more sensitive than gram-positive organisms to this treatment.

Like other organic acids, lactic acid can be used as an acidulant in chitosan and collagen films and can be used to modify both the tensile strength and antimicrobial properties of collagen casings.

Nisin. As the first bacteriocin to be used in the food industry, nisin was recognized as a safe biological food preservative by a joint Food and Agriculture Organization/World Health Organization commission on food additives in 1968 *(62)* and accepted 20 years later by the Food and Drug Administration *(63)*. Nisin is effective against outgrowth of and toxin production by *Clostridium botulinum* in cheese products, particularly processed cheeses and cold-pack cheese spreads *(49)*. Consequently, a legal precedent for use of nisin in U.S. foods was set with pasteurized cheese spreads (900 IU/mg) *(63)*.

Nisin is a protein of 34 amino acids produced by *Lactococcus lactis* subsp. *lactis (98)*. This protein possesses amphiphilic characteristics due to clusters of hydrophobic and hydrophilic residues at the N and C termini, respectively. Nisin is inhibitory to a wide range of gram-positive bacteria, including *L. monocytogenes*, and when combined with a chelating agent it is also effective against some gram-negative organisms (Table 3).

Nisin is one of the most heavily investigated bacteriocins in antimicrobial edible film studies. This protein can be incorporated into the film solution or applied directly to the film surface after casting. Various nisin-containing protein-based films (e.g., whey protein, corn zein, wheat protein, soy protein) have been assessed for antimicrobial activity against gram-positive bacteria such as *L. monocytogenes* and lactic acid bacteria *(88, 93, 180)*. Because nisin is more active in hydrophilic environments, WPI films that contain higher numbers of hydrophilic residues than do zein or wheat protein films reportedly produce larger inhibition zones against *L. monocytogenes*. Teerakarn et al. *(180)* investigated the effects of protein film type (cast corn zein, heat-pressed corn zein, cast wheat gluten, heat-pressed wheat gluten) on nisin diffusivity. Nisin retention was highest in cast corn-zein films, but diffusivities in heat-pressed corn zein, cast wheat gluten, and heat-pressed wheat-gluten films were not significantly different. An active packaging film that releases an additive would address the limitation of rapid loss of preservatives applied directly to food surfaces. Protein packaging films could act as reservoirs and release antimicrobial agents to maintain a relatively high and constant inhibitory effect at the food surface.

Pediocin. The pediocins, produced by *Pediococcus acidilactici,* are another commonly studied group of bacteriocins for edible film use because of their wide spectrum of antimicrobial activity and their effectiveness over a wide range of pH values and temperatures (Table 3). Among the pediocins isolated from different strains, only pediocin PA1 (*P. acidilactici* PAC 1.0) and pediocin AcH (*P. acidilactici* LB42-923) have been well characterized, the latter having

62 amino acids, two disulfide bonds, and a molecular mass of ~2,700 Da *(16)*.

Antimicrobial activity of pediocin is retained at 100°C, reduced at 121°C, and most evident at pH values between 4 and 7, with substantial losses at pH <3 or >9. Pediocin remains active following treatment with lipase, phospholipase C, lysozyme, DNase, or RNase, but its activity is destroyed by protease, papain, and α-chymotrypsin *(74)*. Although pediocin activity was reportedly not affected during 6 months of frozen storage, more than 50% of the activity was lost after 12 weeks at ambient temperature *(149)*.

Lysozyme. Another popular choice for the production of antimicrobial films is lysozyme, an enzyme comprising 129 amino acids cross-linked by four disulfide bonds. Dried egg white, the commercial source for lysozyme, contains about 3.5% lysozyme. It is heat stable (100°C) at pH <5.3 but is inactivated at lower temperatures when the pH is increased *(167)*. Lysozyme is most active against gram-positive bacteria.

Plasticizers such as glycerol and sorbitol that are used in edible films help stabilize lysozyme against heat through hydrophobic interactions that reduce the complete transfer of hydrophobic groups from an aqueous to a nonpolar environment *(6, 205)*. Therefore, lysozyme is highly suited for heat-processed films such as those prepared from corn zein *(45, 141)*.

Spices, herbs, and essential oils. Essential oils are responsible for the odor, aroma, and flavor of spices and herbs. These compounds can be added to edible films to modify flavor, aroma, and odor and to introduce antimicrobial properties. Films containing these ethanol-soluble compounds show activity against both gram-negative and gram-positive bacteria (Table 3). Essential oils of angelica, anise, carrot, cardamom, cinnamon, cloves, coriander, dill weed, fennel, garlic, nutmeg, oregano, parsley, rosemary, sage, and thymol are inhibitory to various spoilage or pathogenic bacteria, molds, and yeasts (Table 3). Antimicrobial activities of various plant essential oils have been recognized for centuries. However, the use of these oils as food additives is limited by their strong flavor. These extracts contain mostly phenolic compounds such as abietane diterpenes *(128)*, carnosol, and ursolic acid, which presumably are responsible for their antimicrobial action.

Lactoferrin. Lactoferrin (lactotransferrin), an iron-binding glycoprotein, is present in bovine milk and can bind two iron atoms per molecule *(153)*. Although this protein effectively inhibits the growth of some bacteria (Table 3), other bacteria may be lactoferrin resistant because of the presence of siderophores that aid in adaptation to low-iron environments *(40)*. Bacteria with low iron requirements, such as lactic acid bacteria, would not be adversely impacted by lactoferrin *(153)*.

Lactoferricin B, the active region of lactoferrin, was isolated by acid-pepsin hydrolysis from the N-terminal region of the molecule *(14)* and contains 25 amino acid residues. Bellamy et al. *(14)* and Jones et al. *(96)* determined that lactoferricin was inhibitory to bacteria at concentrations of 0.3 to 150 μg/ml (Table 4). *Pseudomonas fluorescens, Enterococcus faecalis,* and *Bifidobacterium bifidum* strains were highly resistant to this peptide; these results confirm and expand on those of earlier inhibition studies with lactoferricin B *(184, 195)*. Although the mode of action has not been fully elucidated, lactoferricin presumably alters membrane permeability because of its cationic nature *(38, 96)*.

Liquid smoke. Liquid smoke is a solution of natural wood smoke flavors prepared by burning wood (e.g., hickory, maple) and capturing the flavors in water. Commercial liquid smoke products used in processed meats, sausages, and cheeses contain phenols and acetic acid, which are bactericidal at relatively low concentrations. Liquid smoke can inactivate common foodborne pathogens, including *E. coli, Salmonella, Staphylococcus aureus,* and *L. monocytogenes (60, 198)*.

Based on these findings, liquid smoke, which possesses antimicrobial, antioxidant, color, and flavor properties, is a potentially attractive edible film additive. However, incorporation of liquid smoke has only been studied for edible collagen casings, with liquid smoke introduced into the acid-swollen collagen mass before extrusion as a casing or film *(123)*. Because liquid smoke is generally very acidic (pH 2.5 or less), it is compatible with the gel system and can replace a portion of the acid normally added to induce swelling. The resultant edible collagen casings with uniformly dispersed liquid smoke reportedly had increased tensile strength and improved film clarity.

Sodium chloride. Sodium chloride (salt) has been recognized as a food preservative since ancient times and can be used alone or in combination with other preservation techniques such as pasteurization or fermentation. Most bacterial foodborne pathogens are susceptible to elevated concentrations of salt, particularly in the presence of other preservatives. However, the salt tolerance of *S. aureus* is well recognized *(121)*, and *L. monocytogenes* is resistant to 10% NaCl, surviving for months in saturated brine solutions. Yeasts and molds are also more tolerant to low water activity than are bacteria. Xerotolerant fungi can grow at water activity values as low as 0.61 *(39)*.

Incorporation of salt into protein-based films (e.g., whey protein) as an antimicrobial agent is of limited use because physical properties of protein films are altered with increasing ionic strength of the film solution. At high ionic strength, proteins aggregate to form turbid opaque gels with high water-holding capacity *(166)*.

Nitrites. Sodium nitrite ($NaNO_2$) and potassium nitrite (KNO_2) are primarily used to inhibit *C. botulinium* growth and toxin production in cured meats. Nitrite is also inhibitory to other bacteria (Table 3). However, Gibson and Roberts *(72)* found that enteropathogenic *E. coli, Salmonella* spp., fecal streptococci, *Lactobacillus (25)*, and *Bacillus (75)* were resistant to 400 μg/g nitrite when used with 6% salt.

Nitrite has not yet been studied as an edible film additive, although it appears to be suitable for production of

antimicrobial edible films. In this regard, application of films containing nitrite to RTE meat products may help prevent growth of *L. monocytogenes* and spoilage bacteria that can contaminate such products after processing, with the potential benefit of also improving surface color.

POTENTIAL APPLICATIONS OF ANTIMICROBIAL EDIBLE FILMS

Various antimicrobial edible films have been developed to control the growth of spoilage and pathogenic microorganisms that may contaminate the surface of foods after processing. In most solid foods, contamination and microbial growth occurs on the food surface, which leads to a reduction in product shelf life. Edible films containing various antimicrobials such as benzoic acid, sorbic acid, propionic acid, lactic acid, nisin, and lysozyme have been used to retard the growth of bacteria, yeasts, and molds on different product surfaces (Table 4).

The primary advantage of antimicrobial edible films is that the inhibitory agents in these films can be specifically targeted to postprocessing contaminants on the food surface, with the diffusion rate of the antimicrobial into the product partially controlled by agents incorporated into the film. Two teams of researchers *(71, 80)* evaluated diffusivity of sorbic acid from casein films using a different model system. Lactic acid–treated casein films containing sorbic acid were tested on the surface of intermediately moist papaya cubes inoculated with *Staphylococcus rouxii* and *Aspergillus niger (78).* Casein films retained 30% of their original sorbic acid content after 30 days of storage at 95% relative humidity, with no growth of either test organism observed. However, complete diffusion of sorbic acid was observed in the absence of film in control samples after 24 h of storage, confirming that the edible film matrix entrapped the antimicrobial and reduced diffusion during storage.

Diffusion of antimicrobials through an edible film is influenced by the film, (type, manufacturing procedure), food (pH, water activity), hydrophilic characteristics, and storage conditions (temperature, duration). In another study of casein film, low temperatures (10°C) decreased the diffusivity of sorbic acid; however, lower water activity had no effect. The researchers theorized that at higher levels, increased networking within the gel restricted the movement of sorbic acid. In subsequent work, Vojdani and Torres *(192–194)* also assessed permeability of several polysaccharide-based films prepared both with and without various combinations of lipids and potassium sorbate. Using permeability cells, methylcellulose–palmitic acid films appeared most promising, with permeability of the film to sorbic acid decreasing from 10^{-8} to 10^{-10} mg/s/cm^2 as pH increased from 3 to 7 and water activity decreased from 0.8 to 0.65 *(155).* Based on these findings, practical antimicrobial film applications are limited to intermediate moisture food products with high pH at low temperature because of higher retention of sorbic acid. Increased retention of sorbic acid at higher pH also helps balance the lowering of sorbic acid effectiveness as pH is increased.

Controlling the antimicrobial release from edible films is very important. Release of antimicrobial substances from edible film is dependent on many factors, including electrostatic interactions between the antimicrobial agent and polymer chains, ionic osmosis, and structural changes induced by the presence of antimicrobial and environmental conditions. Several studies have provided some insight into the diffusion of antimicrobials. Ouattara et al. *(140)* tested the impact of temperature (4 to 24°C) and pH (5.7 to 7.0) on diffusion of acetic and propionic acid from chitosan films immersed in water. Whereas diffusion was unaffected by pH, a decrease in temperature from 24 to 4°C decreased the diffusion coefficients for acetic and propionic acid from 2.59×10^{-12} to 1.19×10^{-12} m^2/s and 1.87×10^{-12} to 0.91×10^{-12} m^2/s, respectively. The dependency of diffusion on temperature is explained by effects on solubility of the diffusing molecule, the nature of adhesive forces at interfaces, and molecular mobility *(131, 194).*

The effect of the film-forming material such as fatty acids on antimicrobial diffusion also has been investigated for various edible films. For example, Ouattara et al. *(140)* found that addition of lauric acid (1%) or essential oils (0.5%, cinnemaldehyde or eugenol) decreased diffusion of propionic acid because these additives increased film hydrophobicity-based chitosan and modified pore construction of the film, thereby impairing water uptake and molecular transformation. Redl et al. *(152)* also evaluated diffusion of sorbic acid from gluten films immersed in an aqueous medium using high-pressure liquid chromatography. They claimed that the diffusion coefficient of sorbic acid in gluten-based film was 7.6×10^{-12} m^2/s, and the addition of a lipid component such as acetylated monoglyceride reduced diffusivity by 50%.

Methylcellulose, hydroxypropyl methylcellulose (HPMC), hydroxypropylcellulose, and carboxymethylcellulose are water-soluble ethers possessing film-forming properties. Films prepared from methylcellulose, HPMC, and hydroxypropylcellulose are generally tough, flexible, transparent, and good carriers of antimicrobials. Cha et al. *(27)* reported that nisin-containing κ-carrageenan, methylcellulose, and HPMC films prepared by heat press or a casting method were inhibitory to *Micrococcus luteus* in an agar well diffusion assay. Nisin reportedly diffused faster from methylcellulose than from κ-carrageenan or HPMC films. Not surprisingly, the heat-pressed films had lower antimicrobial activity than did the cast films. Coma et al. *(37)* also demonstrated that edible cellulose films with HPMC containing nisin inhibited *Listeria innocua* and *S. aureus* on laboratory media. However, use of stearic acid in film formation reduced the inhibitory activity of nisin against both test organisms because of electrostatic interactions between cationic nisin and anionic stearic acid.

Protein-based edible films are also very good carriers of food additives, including antimicrobial and flavor agents, because of their encapsulated nature. Zein and wheat gluten (the protein fractions from corn and wheat protein, respectively) have been used to produce antimicrobial edible films. Both of these proteins are soluble in aqueous ethanol and insoluble in water because of their high hydrophobicity. Zein films have been used in conjunction with potassium

sorbate to control surface microbial growth. The diffusion barrier properties of zein films were confirmed in microbial tests using a model food system and *S. aureus* as the challenge organism. A reduced preservative diffusion rate due to barrier properties of zein films was identified as the mechanism for product shelf life enhancement *(185, 186)*. Diffusion of sorbic acid from various wheat gluten films into a model food was also measured and modeled. When *Penicillium notatum* was used as the test organism, simple gluten-based films had no fungicidal effect. However, the gluten- and lipid-based films showed strong sorbic acid retention and marked fungicidal activity at 30 and 40°C, delaying *P. notatum* growth for more than 21 days *(82)*.

Chitosan, like other polysaccharides, forms a strong film that can carry high levels of antimicrobials. Chitosan is a good choice for antimicrobial films because of its superior film-forming properties, ability to adsorb nutrients used by bacteria *(42)*, and capacity to bind water and inhibit various bacterial enzyme systems *(206)*. However, neutralized chitosan alone has no effect on bacterial growth when applied to the surface of meat products. Antimicrobial chitosan films have been prepared by dissolving chitosan in solutions of hydrochloric, formic, acetic, lactic, and citric acids. Films containing hydrochloric, formic, and acetic acids were hard and brittle, whereas those containing lactic or citric acids were soft and elastic, making them suitable for use in multilayer films and coatings. The same research group designed antimicrobial chitosan films containing acetic acid or propionic acid, with or without addition of lauric acid or cinnemaldehyde, to improve the refrigerated shelf life of vacuum-packaged processed meats *(140)*. They indicated that film application delayed or completely inhibited enteric bacteria, *Lactobacillus sakei*, and *Serratia liquefaciens* on meat products. Films prepared with propionic acid were more effective than films containing acetic acid for reducing growth of *L. sakei*, with the opposite result observed for *S. liquefaciens*. Diffusion of acetic acid from the film matrix was limited by addition of lauric acid, with 2 to 22% of the acetic acid remaining in chitosan after 7 days of storage at 4°C. However, virtually all propionic acid diffused from the film after 48 h of storage. Coma et al. *(36)* also demonstrated that antimicrobial chitosan films containing 1% acid inhibited *L. monocytogenes* and *L. innocua* on laboratory media and cheese samples, respectively. *L. innocua* populations were 10-fold higher in chitosan-free than in chitosan-coated cheese samples. However, antimicrobial activity of chitosan films decreased over time because of decreased availability of amino groups on chitosan.

Fatty acids, essential oils, sorbate, and benzoate also have been tested in methylcellulose and chitosan films. In one study, Chen et al. *(29)* developed antimicrobial methylcellulose, chitosan, and methylcellulose-chitosan films (3:2) containing 2, 4, or 5% sodium benzoate or potassium sorbate. Methylcellulose films containing 2% sorbate or benzoate yielded clear inhibition zones for *Rhodotorula rubra* and *P. notatum* on potato dextrose agar, whereas chitosan films containing 2% sorbate or benzoate produced no zones of inhibition because high affinity between chitosan and the preservatives prevented diffusion. Incorporating both potassium sorbate and sodium benzoate into methylcellulose-chitosan films did not change the tensile strength or percent elongation. In a glycerol-water model system (water activity of 0.8), 40% and 50 to 60% of both antimicrobial agents were released from the films after 6 h at 4 and 25°C, respectively. Although the rate of preservative release from the film is too high to maintain an effective concentration at the food surface, the remaining preservatives in the film would extend product shelf life. High-moisture foods will increase the release of preservatives from the film because water migration for equilibration helps dissolve preservatives. Thus, such films may only be suitable for low-moisture foods. Another research group from Taiwan evaluated antimicrobial activity of methylcellulose coatings containing benzoic and palmitic or stearic acid against two osmophilic yeasts (*Zygosaccharomyces rouxii* and *Zygosaccharomyces mellis*) on Taiwanese-style fruit preserves made from plums *(28)*. Coatings containing 50 to 100 μg/g benzoic acid inhibited *Z. rouxii* and *Z. mellis* at room temperature, and sensory characteristics of the preserves such as flavor, texture, appearance, and overall acceptability were not affected by the coating.

Antimicrobial soy- and corn protein–based films were developed by Dawson and his lab group at Clemson University. When prepared to contain nisin and lysozyme, these films were inhibitory to gram-positive bacteria in both solid and liquid media *(45, 46, 138, 141)*, and addition of EDTA resulted in inhibition of gram-negative organisms *(137)*. Modifying the water permeability by incorporating short-chain fatty acids (lauric acid) reduced the effectiveness of nisin on a solid medium, whereas films with lauric acid were as effective as nisin against gram-positive bacteria in a liquid medium *(46)*. Padgett et al. *(141)* incorporated nisin and lysozyme into soy protein and corn zein films using the heat press and casting methods. Both antimicrobial films containing lysozyme (10 to 133 mg/g film) or nisin (0.1 to 6.0 mg/g film) inhibited *Lactobacillus plantarum* on deMan Rogosa Sharpe medium. Orr et al. *(138)* found that corn zein films containing 150 mg of nisin reduced *L. monocytogenes* populations in milk by 1.3 to 2.2 log after 72 h at 4°C, with no inhibition observed for nisin-free films. Use of zein film coatings containing nisin (1,000 IU/g) or calcium propionate (1%) also reportedly reduced *L. monocytogenes* populations by 1 to 5 log on RTE chicken during 30 days of refrigerated storage *(93)*. Zein film coatings with nisin show promise in the control of *L. monocytogenes* on the surface of RTE poultry products.

In other work, gluten, corn zein, myvacet, coconut oil, palm oil, or milk stream-based edible film coatings containing sorbic acid (1 mg/g of sweet corn) were tested against *L. monocytogenes* on sweet corn *(24)*. Only zein produced a uniform coat with good adhesion and acceptable sensory properties. Growth of *L. monocytogenes* was 10-fold lower on zein-coated sweet corn. However, when incorporated into zein coatings, sorbic acid had no additional inhibitory effect.

Rodrigues and Han *(159)* also evaluated alternative antimicrobial films produced from WPI against *L. monocy-*

togenes growth. When incorporated into WPI films, lysozyme and nisin effectively inhibited *Brochothrix thermosphacta* but not *L. monocytogenes.* Addition of EDTA increased the inhibitory effect of these films against *E. coli* and *L. monocytogenes* on trypticase soy agar. Subsequently, they showed that antimicrobial WPI films containing nisin inhibited *Bacillus thermosphacta,* nisin-lysozyme inhibited *Salmonella* Typhimurium, nisin and nisin-EDTA inhibited *S. aureus,* and propyl paraben, nisin, and lysozyme–propyl paraben inhibited *L. monocytogenes (160).* Ko et al. *(105)* also tested the antilisterial activity of nisin (200 to 8,000 IU/g film) when incorporated into WPI, soy protein isolate, egg albumin, and wheat gluten films. All of these films inhibited *Listeria,* with greatest activity observed at low pH (2.0 or 3.0). WPI films containing nisin were most effective against *L. monocytogenes* because of their increased hydrophobicity, and their mechanical properties remained unchanged by the addition of nisin. Nisin may interact differently with proteins of different films. Edible films with higher hydrophobicities and added nisin in an acidic environment are more inhibitory to *L. monocytogenes.*

Other antimicrobial films based on WPI containing sorbic acid or *p*-aminobenzoic acid were developed by Cagri et al. *(22).* Both of these films reportedly inhibited *L. monocytogenes, E. coli* O157:H7, and *Salmonella* Typhimurium DT104 on a nonselective plating medium. Subsequently, these films were tested with beef bologna and summer sausage slices that were surface inoculated with the same pathogens at a level of 10^6 CFU/g *(21, 23).* WPI films containing sorbic or *p*-aminobenzoic acid decreased *Listeria, E. coli* O157:H7, and *Salmonella* Typhimurium DT104 populations 3.4 to 4.1, 3.1 to 3.6, and 3.1 to 4.1 log on bologna and sausage after 21 days of aerobic storage at 4°C, respectively. Growth of mesophilic aerobic bacteria, lactic acid bacteria, and mold or yeast on slices was also inhibited with WPI films containing sorbic or *p*-aminobenzoic acid compared with antimicrobial-free control films. In the same study, film tensile strength decreased but percentage of elongation remained unchanged following 72 h of product contact.

The same research group subsequently developed heat-sealed WPI casings containing sorbic acid, *p*-aminobenzoic acid, or sorbic acid–*p*-aminobenzoic acid (1:1) for hot dog manufacture, with these casings compared to commercial collagen and natural casings *(21).* WPI casings containing *p*-aminobenzoic acid inhibited *L. monocytogenes* on hot dogs during 42 days of refrigerated storage; however, films containing sorbic acid or sorbic acid–*p*-aminobenzoic acid were less effective. Sensory (texture, flavor, juiciness, overall acceptability), chemical (thiobarbituric acid, pH, moisture, fat, protein), physical (purge, color), and mechanical (shear force) characteristics of hot dogs with WPI casings containing *p*-aminobenzoic acid were comparable to those of hot dogs prepared with collagen and natural casings. WPI casings containing *p*-aminobenzoic acid may eventually provide a viable alternative to postprocess pasteurization for minimizing the risk of *Listeria* growth on hot dogs.

Various edible antimicrobial films also have been used to prevent growth of spoilage and pathogenic bacteria on vegetables. Zhuang et al. *(209)* investigated the ability of antimicrobial cellulose-based edible films containing citric acid, acetic acid, or sorbic acid (0.2 to 0.6%) to inactivate *Salmonella* Montevideo on inoculated tomatoes. Although coating with an HPMC solution reduced *Salmonella* populations by 4.5 log on the surface of tomatoes; a reduction of only 2.0 log was achieved in core tissue. *Salmonella* Montevideo cells penetrating into the core tissue when tomatoes were dipped in the 30°C bacterial suspension were likely protected from inactivation during coating *(210).* Among the antimicrobials tested in HPMC films, only 0.4% sorbic acid enhanced inactivation of *Salmonella* Montevideo (~1.0 log) on the surface of tomatoes. However, tomatoes coated with HPMC containing 0.4% sorbic acid appeared chalky and less firm and exhibited color changes that may limit possible commercial applications.

The use of edible films or coatings on various food products continues to expand. The many potential benefits of edible films as carriers of antimicrobial agents, flavors, antioxidants, coloring agents, vitamins, probiotics, and nutraceuticals justify continued research in this field of active packaging. Edible films containing antimicrobial agents can effectively inhibit both pathogenic and spoilage organisms on a wide variety of RTE foods. These films have the ability to control the diffusion rate of antimicrobial agents and also serve as strong barriers against oxygen and water vapor transmission. Antimicrobial edible films provide a viable microbial reduction strategy for reducing the incidence of pathogens such as *L. monocytogenes* on RTE foods, even when these packaged products have been opened and recontaminated by the consumer. Although some of these antimicrobial edible films have received consumer acceptance, further research is needed to develop cost-effective production methods for continuous extrusion of these films as flat sheets or casings.

REFERENCES

1. Aalto, R. R., M. C. Firman, and N. E. Rigler. 1953. *p*-Hydroxybenzoic acid esters as preservatives. I. Uses, antibacterial and antifungal studies, properties and determination. *J. Am. Pharm. Assoc.* 42:449–453.
2. Anderson, M. E., H. E. Huff, H. D. Naumann, and R. T. Marshall. 1988. Counts of six types of bacteria on lamb carcasses dipped or sprayed with acetic acid at 25°C or 55°C and stored vacuum packaged at 0°C. *J. Food Prot.* 51:874–877.
3. Andrews, L. S., and R. M. Grodner. 1997. Radiosensitivity of *Listeria monocytogenes* using split dose application of gamma irradiation. *J. Food Prot.* 60:262–266.
4. Avena-Bustillos, R. J., L. A. Cisneros-Zevallos, J. M. Krochta, and M. E. Saltveit. 1993. Optimization of edible coatings on minimally processed carrots using response surface methodology. *Trans. Am. Soc. Agric. Eng.* 36:801–805.
5. Avena-Bustillos, R. J., L. A. Cisneros-Zevallos, J. M. Krochta, and M. E. Saltveit. 1994. Application of casein-lipid edible film emulsions to reduce white blush on minimally processed carrots. *Postharvest Biol. Technol.* 4:319–329.
6. Back, J. F., D. Oakenfull, and M. B. Smith. 1979. Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry* 18:5191–5196.
7. Bahk, J., A. E. Yousef, and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* in the presence of selected spices. *Lebensm. Wiss. Technol.* 22:66–69.
8. Banker, G. S., A. Y. Gore, and J. Swarbrick. 1966. Water vapor

transmission properties of free polymer films. *J. Pharm.* 18:487–502.
9. Banward, G. J. 1989. Basic food microbiology, 2nd ed. Van Nostland, New York.
10. Bargiota, E. E., E. Rico-Munoz, and P. M. Davidson. 1987. Lethal effect of methyl and propyl parabens as related to *Staphylococcus aureus* lipid composition. *Int. J. Food Microbiol.* 4:257–266.
11. Baron, J. K. 1993. Inhibition of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 by an antimicrobial containing edible film. M.S. thesis. University of Nebraska, Lincoln.
12. Begin, A., and M. R. Clastren. 1999. Antimicrobial films from chitosan. *Int. J. Biological Macromol.* 26:63–67.
13. Bell, M. F., R. T. Marshall, and M. E. Anderson. 1986. Microbiological and sensory tests of beef treated with acetic and formic acids. *J. Food Prot.* 49:207–211.
14. Bellamy, W., M. Takase, H. Wakabayashi, K. Kawase, and M. Tomita. 1992. Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. *J. Appl. Bacteriol.* 73:472–479.
15. Beuchat, L. R., and D. A. Golden. 1989. Antimicrobials occurring naturally in foods. *Food Technol.* 43:134–142.
16. Bhunia, A. K., M. C. Johnson, and B. Ray. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. *J. Appl. Bacteriol.* 65:261–268.
17. Biquet, B., and S. Guilbert. 1986. Relative diffusivities of water in model intermediate moisture foods. *Lebensm. Wiss. Technol. Food Sci. Technol.* 19:208–214.
18. Boni, K. A. 27 December 1988. Strength edible collagen casing and method of preparing same. U.S. patent 4,794,006.
19. Brannen, A. L., P. M. Davidson, and S. Salminen. 1990. Food additives. M. Dekker, New York.
20. Brault, D., G. D'Aprano, and M. Lacroix. 1997. Formation of freestanding sterilized edible films from irradiated caseinates. *J. Agric. Food Chem.* 45:2964–2969.
21. Cagri, A., Z. Ustunol, W. Osburn, and E. T. Ryser. 2003. Inhibition of *Listeria monocytogenes* on hot dogs using antimicrobial whey protein-based edible casings. *J. Food Sci.* 68:291–299.
22. Cagri, A., Z. Ustunol, and E. T. Ryser. 2001. Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing *p*-aminobenzoic or sorbic acids. *J. Food Sci.* 66:865–870.
23. Cagri, A., Z. Ustunol, and E. T. Ryser. 2002. Inhibition of three pathogens on bologna and summer sausage slices using antimicrobial edible films. *J. Food Sci.* 67:2317–2324.
24. Carlin, E., N. Gontard, M. Reich, and C. Nguyen. 2001. Utilization of zein coating and sorbic acid to reduce *Listeria monocytogenes* growth on cooked sweet corn. *J. Food Sci.* 66:1385–1389.
25. Castellani, A. G., and C. F. Niven. 1955. Factors affecting the bacteriostatic action of sodium nitrite. *Appl. Microbiol.* 3:15–19.
26. Centers for Disease Control and Prevention. 2000. Multi-state outbreak of listeriosis. *Morb. Mortal. Wkly. Rep.* 49:1129–1130.
27. Cha, D. S., H. J. Park, and D. K. Cooksey. 2001. Preparation and diffusion rate of nisin-incorporated antimcirobial film, p. 170, 73D-8. Abst. Inst. Food Technol. Annu. Meet., New Orleans. Institute of Food Technologists, Chicago.
28. Chen, M., Y. Weng, and W. Chen. 1999. Edible coating as preservative carriers to inhibit yeast on Taiwanese-style fruit preserves. *J. Food Saf.* 19:89–96.
29. Chen, M. X., G. H. C. Yeh, and B. H. C. Chiang. 1996. Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. *J. Food Process. Preserv.* 20: 379–390.
30. Cherian, G., A. Gennadios, C. Weller, and P. Chinachoti. 1995. Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. *Cereal Chem.* 72:1–6.
31. Cherington, C. A., M. Hinton, and I. Chopra. 1990. Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. *J. Bacteriol.* 68:69–72.
32. Cherington, C. A., M. Hinton, G. R. Pearson, and I. Chopra. 1991. Short-chain organic acids at pH 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. *J. Appl. Bacteriol.* 70:161–165.
33. Chung, K. C., and J. M. Goepfert. 1970. Growth of *Salmonella* at low pH. *J. Food Sci.* 35:326–329.
34. Code of Federal Regulations. 1977/1988. Title 21, parts 100–199, Food and drugs. U.S. Government Printing Office, Washington, D.C.
35. Collins-Thompson, D. L., C. Calderon, and W. R. Usborne. 1985. Nisin sensitivity of lactic acid bacteria isolated from cured and fermented meat products. *J. Food Prot.* 48:668–670.
36. Coma, V., A. Martial-Gros, S. Garreau, F. Salin, and A. Deschamps. 2002. Edible antimicrobial films based on citosan matrix. *J. Food Sci.* 67:1162–1169.
37. Coma, V., I. Sebti, P. Pardon, A. Deschamps, and F. H. Pichavant. 2001. Antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin incorporation to inhibit *Listeria innocua* and *Staphylococcus aureus*. *J. Food Prot.* 64:470–475.
38. Conner, D. E. 1993. Naturally occurring compounds, p. 441. *In* P. M. Davidson and A. I. Branen (ed.), Antimicrobials in foods, 2nd ed. M. Dekker, New York.
39. Corry, J. E. L. 1987. Relationships of water activity to fungal growth, p. 51. *In* L. R. Beuchat (ed.), Food and beverage mycology, 2nd ed. Von Nostrand Reinhold, New York.
40. Crichton, R. R., and A. Charloteux-Wauters. 1987. Review. Iron transport and storage. *Eur. J. Biochem.* 164:485–506.
41. Cuq, B., N. Gontard, J. L. Cuq, and S. Guilbert. 1997. Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *J. Agric. Food Chem.* 45:622–626.
42. Darmadji, P., and M. Izumimoto. 1994. Effect of chitosan in meat preservation. *Meat Sci.* 38:243–254.
43. Davidson, P. M., and A. L. Branen. 1980. Antimicrobial mechanisms of butylated hydroxyanisole against two *Pseudomonas* species. *J. Food Sci.* 45:314–316.
44. Davidson, P. M., and A. L. Branen. 1993. Antimicrobials in foods, 2nd ed. M. Dekker, New York.
45. Dawson, P. L., J. C. Acton, I. Y. Han, T. Padgett, R. Orr, and T. Larsen. 1995. Incorporation of antibacterial compounds into edible and biodegradable packaging films. *Rep. Res. Dev. Assoc.* 47:203–210.
46. Dawson, P. L., I. Y. Han, and T. R. Padgett. 1997. Effect of lauric acid nisin activity in edible protein packaging films. *Poult. Sci.* 76: 74–75.
47. Debeaufort, F., and A. Voilley. 1995. Effect of surfactants and drying rate on barrier properties of emulsified edible films. *Int. J. Food Sci.* Technol. 30:183–190.
48. Del Campo, J., M. J. Amiot, and C. Nguyen. 2000. Antimicrobial effect of rosemary extracts. *J. Food Prot.* 63:1359–1368.
49. Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. *Food Technol.* 44:100–108.
50. Dickson, J. S., and M. E. Anderson. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. *J. Food Prot.* 55:133–140.
51. Donhowe, I. G., and O. Fennema. 1993. Water vapor and oxygen permeability of wax films. *J. Am. Oil Chem. Soc.* 70:867–873.
52. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1998. Long-term bacterial profile of refrigerated ground beef made from carcass tissue, experimentally contaminated with pathogens and spoilage bacteria after hot water, alkaline, or organic acid washes. *J. Food Prot.* 61:1615–1622.
53. Dymick, M., and C. N. Huhtanen. 1979. Inhibition of *Clostridium botulinum* by *p*-hydroxybenzoic acid *n*-alkyl esters. *Antimicrob. Agents Chemother.* 15:798–804.
54. Eklund, T. 1985. Inhibition of microbial growth at different pH levels by benzoic acid and propionic acids and esters of *p*-hydroxybenzoic acid. *Int. J. Food Microbiol.* 2:159–163.
55. El-Ghaouth, A., J. Arul, and R. Ponnampalam. 1991. Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. *J. Food Process. Preserv.* 15:359–368.
56. El-Ghaouth, A., J. Arul, R. Ponnampalam, and M. Boulet. 1991.

Chitosan coating effect on storability and quality of fresh strawberries. *J. Food Sci.* 56:1618–1620, 1631.
57. El-Ghaouth, A., R. Ponnampalam, F. Castaigne, and J. Arul. 1992. Chitosan coating to extend the storage life of tomatoes. *Hortic. Sci.* 27:1016–1018.
58. El-Shenawy, M. A., and E. H. Marth. 1988. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. *J. Food Prot.* 51: 842–847.
59. El-Shenawy, M. A., and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* in the presence of sodium propionate. *Int. J. Food Microbiol.* 8:85–94.
60. Faith, N. G., A. E. Yousef, and J. B. Luchansky. 1992. Inhibition of *Listeria monocytogenes* by liquid smoke and isoeugenol, a phenolic componenet found in smoke. *J. Food Saf.* 12:303–314.
61. Field, C. E., L. F. Pivarnick, S. M. Barnett, and A. Rand. 1986. Utilization of glucose oxidase for extending the shelf-life of fish. *J. Food Sci.* 51:66–70.
62. Food and Agriculture Organization/World Health Organization. 1970. Specifications for the identity and purity of food additives and their toxicology evaluation: some antibiotics. 20th report of the Joint FAO/WHO Expert Committee on Food Additives. *FAO Nutr. Meet. Rep. Ser.* 45:49.
63. Food and Drug Administration. 1988. Nisin preparation: affirmation of GRAS status as a direct human food ingredient. *Fed. Regist.* 53: 11247.
64. Galietta, G., L. Di-Gioia, S. Guilbert, and B. Cuq. 1998. Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and crosslinking agents. *J. Dairy Sci.* 81:3123–3130.
65. Ganz, A. J. 1969. CMC and hydroxypropylcellulose—versatile gums for use. *Food Prod. Dev.* 3:65–69.
66. Garcia, M. A., M. N. Martino, and N. E. Zaritzky. 2000. Lipid addition to improve barrier properties of edible starch-based films and coatings. *J. Food Sci.* 65:941–947.
67. Garcia, M. A., M. N. Martino, and N. E. Zaritzky. 2000. Microstructural characterization of plasticized starch-based films. *Starch* 52:118–124.
68. Gennadios, A., M. A. Hanna, and L. B. Kurth. 1997. Application of edible coatings on meats, poultry and seafoods: a review. *Lebensm. Wiss. Technol.* 30:337–350.
69. Gennadios, A., C. L. Weller, and R. F. Testin. 1993. Modification of physical and barrier properties of edible wheat gluten-based films. *Cereal Chem.* 70:426–429.
70. Ghorpade, V. M., H. Li, A. Gennadios, and M. A. Hanna. 1995. Chemically modified soy protein films. *Trans. Am. Soc. Agric. Eng.* 38:1805–1808.
71. Giannakopoulus, A., and S. Guilbert. 1986. Determination of sorbic acid diffusivity in model food gels. *J. Food Technol.* 21:339–342.
72. Gibson, A. M., and T. A. Roberts. 1986. The effect of pH, sodium chloride, sodium nitrite, and storage temperature on the growth of *Clostridium perfringens* and faecal streptococci in laboratory media. *Int. J. Food Microbiol.* 3:195–199.
73. Gontard, N. G., S. Guilbert, and J. L. Cuq. 1993. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J. Food Sci. Technol.* 58:201–211.
74. Gonzalez, C. F., and B. S. Kunka. 1987. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. *Appl. Environ. Microbiol.* 53:2534–2538.
75. Grever, A. B. G. 1974. Minimum nitrite concentration for inhibition of clostridia in cooked meat products, p. 103. *In* B. J. Tinbergen and B. Krol (ed.), Proceedings of the International Symposium on Nitrite in Meat Products. Pudoc, Wageningen, The Netherlands.
76. Gueguen, J., G. Viroben, P. Noireaux, and M. Subirade. 1998. Influence of plasticizers and treatments on the properties of films from pea proteins. *Ind. Crop Prod.* 7:149–157.
77. Guilbert, S. 1986. Technology and application of edible protective films, p. 371. *In* M. Mathlouthi (ed.), Packaging and preservation—theory and practice. Applied Science, Elsevier, New York.
78. Guilbert, S. 1988. Use of superfacial edible layer to protect intermediate moisture foods: application to the protection of tropical fruit dehydrated by osmosis. *In* Proceedings of the International Symposium, Penang, Malaysia.
79. Guilbert, S., and B. Biquet. 1996. Edible films and coating, p. 120. *In* G. Bureau and J. L. Multon (ed.), Food packaging technology. VLH, New York.
80. Guilbert, S., A. Giannakopoulos, and J. C. Cheftel. 1985. Diffusivity of sorbic acid in food gels at high and intermediate water activities, p. 343. *In* D. Simatos and J. L. Multon (ed.), Properties of water in foods in relation to quality and stability. Nijhoff, Dordrecht, The Netherlands.
81. Guilbert, S., N. Gontard, and L. G. M. Gorris. 1996. Prolongation of the shelf life of perishable food products using biodegradable films and coatings. *Lebensm. Wiss. Technol.* 29:10–17.
82. Guilbert, S., A. Redl, H. De-Regh, C. Baron, H. Mujica Paz, B. Cuq, and N. Gontard. 1998. Gas and solute barrier properties of wheat gluten films: application to the design of "active biopackaging." *Biopolymer Sci. Food Non-Food Appl.* 28–30:313–320.
83. Hagenmaier, R. D., and P. E. Shaw. 1990. Moisture permeability of edible films made with fatty acid and (hydroxypropyl)methylcellulose. *J. Agric. Food Chem.* 38:1799–1803.
84. Hagenmaier, R. D., and P. E. Shaw. 1991. Permeability of shellac coatings to gases and water vapor. *J. Agric. Food Chem.* 39:825–830.
85. Hagenmaier, R. D., and P. E. Shaw. 1992. Gas permeability of fruit coating waxes. *J. Am. Soc. Hortic. Sci.* 117:105–109.
86. Hardenberg, R. E. 1967. Wax and related coatings for horticultural products—a bibliography. *Agric. Res. Bull.* 965:1–123.
87. Ho, B. 1992. Water vapor permeabilities and structural characteristics of casein films and casein-lipid emulsion films. M.S. thesis. University of California, Davis.
88. Hoffman, K. L., I. Y. Han, and P. L. Dawson. 2001. Antimicrobial effects of corn zein coatings impregnated with nisin, lauric acid, and EDTA. *J. Food Prot.* 64:885–889.
89. Holownia, K. I., M. S. Chinnan, M. C. Erickson, and P. Mallikarjunan. 2000. Quality evaluation of edible-coated chicken strips and frying oils. *J. Food Sci.* 65:1087–1090.
90. Holownia, K. I., M. C. Erickson, M. S. Chinnan, and R. R. Eitenmiller. 2001. Tocopherol losses in peanut oil during pressure frying of marinated chicken strips coated with edible films. *Food Res. Int.* 34:77–80.
91. Hood, L. L. 1987. Collagen in sausage casings. *Adv. Meat Res.* 4: 109–129.
92. Islam, M., J. Chen, M. P. Doyle, and M. Chinnan. 2002. Control of *Listeria monocytogenes* on turkey frankfurters by generally-recognized-as-safe preservatives. *J. Food Prot.* 65:1411–1416.
93. Janes, M. E., S. Kooshesh, and M. G. Johnson. 2002. Control of *Listeria monocytogenes* on the surface of refrigerated, ready-to-eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. *J. Food Sci.* 67:2754–2757.
94. Jermini, M. F. G., and W. Schmidt-Lorenz. 1987. Activity of Na-benzoate and ethyl-paraben against yeasts as different water activity values. *J. Food Prot.* 50:920–926.
95. Jokay, L., G. E. Nelson, and E. L. Powell. 1967. Development of edible amylaceous coatings for foods. *Food Technol.* 21:12–14.
96. Jones, E. M., A. Smart, G. Bloomberg, L. Burgess, and M. R. Millar. 1994. Lactoferricin, a new antimicrobial peptide. *J. Appl. Bacteriol.* 77:208–214.
97. Jones, H. W., and R. A. Whitmore. September 1972. Collagen food coating composition and method of preparation. U.S. patent 3,694,234.
98. Jung, G. 1991. Lantibiotics—ribosomaly synthesized biologically active polypeptides containing sulphide bridges and a,b-didehydro amino acids. *Angew. Chem. Int. Ed. Engl.* 30:1051–1068.
99. Jurd, L., A. D. King, K. Mihara, and W. L. Stanely. 1971. Antimicrobial properties of natural phenols and released compounds. I. Obtusastyrene. *Appl. Microbiol.* 21:507–511.
100. Juven, B. J., J. Kanner, F. Schved, and H. Weissllowicz. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J. Appl. Bacteriol.* 76:626–631.
101. Kabara, J. J., and T. Eklund. 1991. Organic acids and esters, p. 23.

In N. J Russell and G. W. Gould (ed.), Food preservatives. Blackie and Son, Glasgow, UK.

102. Kaletunc, G., M. D. Normand, E. A. Johnson, and M. Peleg. 1991. "Degree of elasticity" determination in solid foods. *J. Food Sci.* 56:950–953.
103. Kaplan, H. J. Washing and color adding, p. 379. *In* W. F. Wardowdki, S. Nagy, and W. Grierson (ed.), Fresh citrus fruits. AVI, Westport, Conn.
104. Kester, J. J., and O. R. Fennema. 1986. Edible films and coatings: a review. *Food Technol.* 40:47–59.
105. Ko, S., M. E. Janes, N. S. Hettiarachchy, and M. G. Johnson. 2001. Physical and chemical properties of edible films containing nisin and their action against *Listeria monocytogenes*. *J. Food Sci.* 66: 1006–1011.
106. Kozempel, M., N. Golberg, E. R. Radewonuk, O. J. Scullen, and J. C. Craig, Jr. 2000. Rapid hot dogs surface pasteurization using cycles of vacuum and steam to kill *Listeria innocua*. *J. Food Prot.* 63:457–461.
107. Krochta, J. M. 2002. Proteins as raw materials for films and coatings: definitions, current status, and opportunities, p. 1. *In* A. Gennadios (ed.), Protein-based films and coatings. CRC Press, Boca Raton, Fla.
108. Krochta, J. M., and C. De Mulder-Johnston. 1997. Edible and biodegradable polymer films: challenges and opportunities. *Food Technol.* 51:61–74.
109. Krochta, J. M., A. E. Pavlath, and N. Goodman. 1990. Edible films from casein-lipid emulsions for lightly processed fruits and vegetables, p. 329. *In* W. E. L. Spiess and H. Schubert (ed.), Engineering and food. Elsevier, New York.
110. Labuza, T. P., and W. M. Breene. 1989. Applications of active packaging for improvement of shelf life and nutritional quality of fresh and extended shelf-life foods. *J. Food Process. Preserv.* 13:1–69.
111. Lahellec, C., D. Y. Fung, and F. E. Cunningham. 1981. Growth effect of sorbate and selected antioxidants on toxigenic strains of *Staphyloccoccus aureus*. *J. Food Prot.* 44:531–536.
112. Lerdthanangkul, S., and J. M. Krochta. 1996. Edible coating effects on postharvest quality of green bell peppers. *J. Food Sci.* 61:176–179.
113. Lieberman, E. R., and S. G. Gilbert. 1973. Gas permeation of collagen films as affected by cross-linkage, moisture, and plasticizer content. *J. Polymer Sci.* 41:33–43.
114. Lucore, L. A., T. H. Shelhammer, and A. E. Yousef. 2000. Inactivation of *Listeria monocytogenes* Scott A on artificially contaminated frankfurters by high-pressure. *J. Food Prot.* 63:662–664.
115. Lueck, E. 1976. Sorbic acid as a food preservative. *Int. Flavors Food Addit.* 7:122–138.
116. Marquie, C., C. Aymard, J. L. Cuq, and S. Guilbert. 1995. Biodegradable packaging made from cottonseed flour: formation and improvement by chemical treatments with gossypol, formaldehyde, and glutaraldehyde. *J. Agric. Food Chem.* 43:2762–2767.
117. Mate, J. I., E. N. Frankel, and J. M. Krochta. 1996. Whey protein isolate edible coatings: effect on the rancidity process of dry roasted peanuts. *J. Agric. Food Chem.* 44:1736–1740.
118. Mate, J. I., and J. M. Krochta. 1996. Whey protein coating effect on the oxygen uptake of dry roasted peanuts. *J. Food Sci.* 61:1202–1206, 1210.
119. Mate, J. I., M. E. Saltveit, and J. M. Krochta. 1996. Peanut and walnut rancidity: effects of oxygen concentration and relative humidity. *J. Food Sci.* 61:465–468, 472.
120. McCormick, R. D. 1975. Edible coating isolates oxygen and moisture, controls structure-seals in flavor. *Food Prod. Dev.* 9:14–16.
121. McLean, R. A., H. D. Lilly, and J. A. Alford. 1968. Effects of meat-curing salts and temperature on production of staphylococcal enterotoxin B. *J. Bacteriol.* 95:1207–1210.
122. Mei, Y., Y. Zhao, J. Yang, and H. C. Furr. 2002. Using edible coating to enhance nutritional and sensory qualities of baby carrots. *J. Food Sci.* 67:1964–1968.
123. Miller, A. T. 1975. Manufacture of edible collagen casings using liquid smoke. U.S patent 3,894,158.
124. Miller, A. T., and R. B. Marder. October 1998. Process for strengthening collagen casings using ultraviolet irradiation. U.S. patent 5,820,812.
125. Miller, K. S., M. T. Chiang, and J. M. Krochta. 1997. Heat curing of whey protein films. *J. Food Sci.* 62:1189–1193.
126. Ming, X., G. H. Weber, J. W. Ayres, and W. E. Sandine. 1997. Bacteriocin applied to packaging materials to inhibit *Listeria monocytogenes* on meat. *J. Food Sci.* 62:413–415.
127. Moir, C. J., and M. J. Eyles. 1992. Inhibition, injury, and inactivation of four psychrotrophic foodborne bacteria by the preservatives methyl *p*-hydroxybenzoate and potassium sorbate. *J. Food Prot.* 55:360–366.
128. Moujir, L., A. M. Gutierriz-Navarro, L. San-Andres, and J. G. Luis. 1993. Structure–antimicrobial activity relationships of abietane diterpenes from salvia species. *Phytochem. Oxford* 34:1493–1495.
129. Murano, A. E., P. S. Murano, R. E. Brennan, K. Shenoy, and R.G. Moreira. 1999. Application of high hydrostatic pressure to eliminate *Listeria monocytogenes* from fresh pork sausage. *J. Food Prot.* 62:480–483.
130. Murray, D. G., and L. R. Luft. 1973. Low D.E. corn starch hydrolysates: multi-functional carbohydrates aid in food formulation. *Food Technol.* 2:32–34, 36, 38, 40.
131. Myint, S., W. R. W. Daud, A. A. Mohamad, and A. A. H. Kadhum. 1996. Temperature-dependent diffusion coefficient of soluble substance during ethanol extraction of clove. *J. Am. Oil Chem. Soc.* 73:603–610.
132. Notermans, S., and J. Dufrenne. 1981. The effect of glyceryl monolaurate on toxin production by *Clostridium botulinum* in meat slurry. *J. Food Saf.* 8:82–88.
133. Ogden, K., and R. S. Tubb. 1985. Inhibition of beer-spoilage lactic acid bacteria by nisin. *J. Inst. Brew.* 91:390–392.
134. Okamoto, S. 1978. Factors affecting protein film formation. *Cereal Foods World* 23:256–262.
135. Okrend, A. J., R. W. Johnson, and A. B. Moran. 1986. Effect of acetic on the death rates at 52°C of *Salmonella* Newport, *Salmonella* Typhimurium and *Campylobacter jejuni* in poultry scald water. *J. Food Prot.* 49:500–505.
136. Oram, J. D., and B. Reiter. 1968. Inhibition of bacteria by lactoferrin and other iron chelating agents. *Biochim. Biophys. Acta* 170: 351–365.
137. Orr, R. V., I. Y. Han, J. C. Acton, and P. L. Dawson. 1996. Comparison of the zone of inhibition assay and enumeration assay for evaluation of effectiveness of antimicrobial packaging films, p. 29–30. *In* Proceedings of the 42nd Annual International Congress on Meat Science & Technology.
138. Orr, R. V., I. Y. Han, J. C. Acton, and P. L. Dawson. 1998/1999. Effect of nisin in edible protein films on *Listeria monocytogenes* viability in milk. *Act. Rep. Res. Dev. Assoc. Mil. Food Packag. Syst. Inc.* 50/51:300–305.
139. Ouattara, B., R. E. Simard, G. Piette, A. Begin, and R. A. Holley. 2000. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *Int. J. Food Microbiol.* 62:139–148.
140. Ouattara, B., R. E. Simard, G. Piette, A. Begin, and R. A. Holley. 2000. Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *J. Food Sci.* 65:768–773.
141. Padgett, T., I. Y. Han, and P. L. Dawson. 1998. Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *J. Food Prot.* 61:1330–1335.
142. Palumbo, S. A., and A. C. Williams. 1994. Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatment. *J. Food Sci.* 11:293–300.
143. Park, H. J., M. S. Chinnan, and R. L. Shewfelt. 1994. Edible cornzein film coatings to extend storage life of tomatoes. *J. Food Process. Preserv.* 18:317–331.
144. Park, J. W., R. F. Testin, H. J. Park, P. J. Vergano, and C. L. Weller. 1994. Fatty acid concentration effect on tensile strength, elongation, and water vapor permeability of laminated edible films. *J. Food Sci.* 59:916–919.
145. Payne, K. D., P. M. Davidson, and S. P. Oliver. 1994. Comparison

of EDTA and lactoferrin with lysozyme on the growth of foodborne pathogenic and spoilage bacteria. *J. Food Prot.* 57:62–65.
146. Payne, K. D., P. M. Davidson, S. P. Oliver, and G. L. Christen. 1990. Influence of bovine lactoferrin on the growth of *Listeria monocytogenes*. *J. Food Prot.* 53:468–472.
147. Payne, K. D., E. Rico-Munoz, and P. M. Davidson. 1989. The antimicrobial activity of phenolic compounds against *Listeria monocytogenes* and their effectiveness in a model milk system. *J. Food Prot.* 52:151–153.
148. Pelroy, G., M. Peterson, R. Paranjpye, J. Almond, and M. Eklund. 1994. Inhibition of *Listeria monocytogenes* on cold-process (smoked) salmon by sodium nitrite and packaging method. *J. Food Prot.* 57:114–119.
149. Ray, B. 1996. Characteristics and applications of pediocin(s) of *Pediococcus acidilactici*: pediocin PA-1/AcH, p. 155. *In* T. F. Bozoglu and B. Ray (ed.), Lactic acid bacteria, current advances in metabolism, genetics and applications. Springer, New York.
150. Reddy, N. R., and M. D. Pierson. 1982. Influence of pH and phosphate buffer on inhibition of *Clostridium botulinum* by antioxidants and related phenolic compounds. *J. Food Prot.* 45:925–927.
151. Reddy, N. R., M. D. Pierson, and R. V. Lechowich. 1982. Inhibition of *Clostridium botulinum* by antioxidants phenols and related compounds. *Appl. Environ. Microbiol.* 43:835–839.
152. Redl, A., N. Gontard, and S. Guilbert. 1996. Determination of sorbic acid diffusivity in edible wheat gluten and lipid based films. *J. Food Sci.* 61:116–120, 136.
153. Reiter, B., and J. D. Oram. 1986. Iron and vanadium requirements of lactic acid streptococci. *J. Dairy Res.* 35:67–69.
154. Ressouany, M., C. Vachon, and M. Lacroix. 1998. Irradiation dose and calcium effect on the mechanical properties of cross-linked caseinate films. *J. Agric. Food Chem.* 46:1618–1623.
155. Rico-Pena, D. C., and J. A. Torres. 1991. Sorbic acid and potassium sorbate permeability of an edible methycellulose–palmitic acid film: water activity and pH effects. *J. Food Sci.* 56:1991–1995.
156. Robach, M. C. 1980. Use of preservatives to control microorganisms in food. *Food Technol.* 34:81–86.
157. Robach, M. C., and J. N. Sofos. 1982. Use of sorbates in meat products, fresh poultry and poultry products: a review. *J. Food Prot.* 45:374–378.
158. Roberts, T. A., L. F. J. Woods, M. J. Payne, and R. Cammack. 1991. Nitrite, p. 89. *In* N. J. Russell and G. W. Gould (ed.), Food preservatives. Blackie and Son, Glasgow, UK.
159. Rodrigues, E. T., and J. H. Han. 2000. Antimicrobial whey-protein films against spoilage and pathogenic bacteria, p. 30, 78E. Abstr. Inst. Food Technol. Annu. Meet., Dallas, Tex. Institute of Food Technologists, Chicago.
160. Rodrigues, E. T., J. H. Han, and R. A. Holley. 2002. Optimized antimicrobial edible whey protein films against spoilage and pathogenic bacteria, p. 252, 100B-35. Abstr. Inst. Food Technol. Annu. Meet., Anaheim, CA. Institute of Food Technologists, Chicago.
161. Sabato, S. F., B. Ouattara, H. Yu, G. D'Aprano, C. Le-Tien, M. A. Mateescu, and M. Lacroix. 2001. Mechanical and barrier properties of cross-linked soy and whey protein based films. *J. Agric. Food Chem.* 49:1397–1403.
162. Samelis, J., J. N. Sofos, M. L. Kain, J. A. Scanga, K. E. Belk, and G. C. Smith. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork stored at 4 degrees C in vacuum packages. *J. Food Prot.* 64:1722–1729.
163. Seward, R. A., R. H. Deibel, and R. C. Lindsey. 1982. Effect of potassium sorbate and other antibotulinal agents on germination and outgrowth of *Clostridium botulinum* type E spores in microcultures. *Appl. Environ. Microbiol.* 44:1212–1217.
164. Sherwin, C. P., D. E. Smith, and R. G. Fulcher. 1998. Effects of fatty acid type on dispersed phase particle size distributions in emulsion edible films. *J. Agric. Food Chem.* 46:4534–4538.
165. Siragusa, G. R., and J. S. Dickson. 1993. Inhibition of *Listeria monocytogenes, Salmonella* Typhimurium and *Escherichia coli* O157:H7 on beef muscle tissue by lactic or acetic acid contained in calcium alginate gels. *J. Food Saf.* 13:147–158.
166. Smith, D. M., and J. D. Culbertson. 2000. Proteins: functional properties, p. 131. *In* G. L. Christen and J. S. Smith (ed.), Food chemistry: principles and applications. Science Technology System, West Sacramento, Calif.
167. Smolelis, A. N., and S. E. Hartsell. 1952. Factors affecting the lytic activity of lysozyme. *J. Bacteriol.* 63:665–674.
168. Smulders, F. J. M. 1986. Prospectives for microbial decontamination of meat and poultry by organic acids with special reference to lactic acid. *In* F. J. M. Smulders (ed.), Proceedings of an international symposium: Prevention of Contamination in the Meat Industry, Zeist, The Netherlands.
169. Sofos, J. N. 1989. Chemistry, p. 13. *In* N. Sofos (ed.), Sorbate food preservative. CRC Press, Boca Raton, Fla.
170. Sofos, J. N., and F. F. Busta. 1981. Antimicrobial activity of sorbate. *J. Food Prot.* 44:614–618.
171. Soomers, C. H., and D. W. Thayer. 2000. Survival of surface-inoculated *Listeria monocytogenes* on commercially available frankfurters following gamma irradiation. *J. Food Saf.* 20:127–137.
172. Sperber, W. H. 1983. Influence of water activity on foodborne bacteria—a review. *J. Food Prot.* 46:142–150.
173. Sprong, R. C., M. F. Hulstein, and R. Meer. 1999. High intake of milk fat inhibits intestinal colonization of *Listeria* but not of *Salmonella* in rats. *J. Nutr.* 129:1382–1389.
174. Stuchell, Y. M., and J. M. Krochta. 1994. Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.* 59:1332–1337.
175. Stuchell, Y. M., and J. M. Krochta. 1995. Edible coatings on frozen King salmon: effect of whey protein isolate and acetylate monoglycerides on moisture loss and lipid oxidation. *J. Food Sci.* 60:28–32.
176. Swenson, H. A., J. C. Miers, T. H. Schultz, and H. S. Owens. 1953. Pectinate and pactate coatings. II. Application to nut and fruit products. *Food Technol.* 7:232–236.
177. Szyperski, R. T., and J. P. Gibbons. 1963. Zein systems developed for heat cured coatings. *Paint Varnish Prod.* 53:65–73.
178. Tabanca, N., N. Kirimer, B. Demirci, F. Demirci, and H. C. Baser. 2001. Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phyrygia* and the enantiomeric distribution of borneol. *J. Agric. Food Chem.* 49:4300–4303.
179. Tassou, C., and G. J. E. Nychas. 1994. The inhibitory effect of the essential oils from basil and sage in broth and in model food system. *Food Flavors* 37:1925–2935.
180. Teerakarn, A., D. E. Hirt, J. C. Acton, J. R. Rieck, and P. L. Dawson. 2002. Nisin diffusion in protein films: effects of films type and temperature. *J. Food Sci.* 67:3019–3025.
181. Teeraporn, K. 1995. Antimicrobial activity of garlic and cloves on *Salmonella* Typhimurium and *S. aureus* in chilled chicken. *Bangkok* 4:117–125.
182. Thomas, L. V., J. W. T. Wimpenny, and J. G. Davis. 1993. Effect of three preservatives on the growth of *Bacillus cereus,* Vero cytotoxigenic *Escherichia coli* and *Staphyloccoccus aureus* on plates with gradients of pH and sodium chloride concentration. *Int. J. Food Sci.* 17:289–301.
183. Thompson, D. P. 1991. Effect of butylated hydroxyanisole on conidial germination of toxigenic species of *Aspergillus* species of *Aspergillus flavus* and *Aspergillus parasiticus*. *J. Food Prot.* 54:375–379.
184. Tomita, M., W. Bellamy, M. Takase, K. Yamauchi, H. Wakabayashi, and K. Kawase. 1992. Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. *J. Dairy Sci.* 74:4137–4142.
185. Torres, J. A., J. O. Bouzas, and M. Karel. 1985. Microbial stabilization of intermediate moisture food surfaces. II. Control of surface pH. *J. Food Process. Preserv.* 9:93–106.
186. Torres, J. A., and M. Karel. 1985. Microbial stabilization of intermediate food surfaces. III. Effects of surface preservative concentration and surface pH control on microbial stability of an intermediate moisture cheese analog. *J. Food Process. Preserv.* 9:107–111.
187. Tsai, S. H., and C. C. Chou, 1996. Injury, inhibition and inactivation of *Escherichia coli* O157:H7 by potassium sorbate and sodium

nitrite as affected by pH and temperature. *J. Sci. Food Agric.* 71: 10–12.
188. Tsuzuki, T., and E. R. Liberman. 1972. Process for making edible collagen casings. U.S. patent 3,681,093.
189. Tuncan, E. U., and S. E. Martin. 1985. Effect of pH, temperature and potassium sorbate on amino acid uptake in *Salmonella* Typhimurium 7136. *Appl. Environ. Microbiol.* 49:505–508.
190. Ultee, A., E. P. W. Kets, M. Alberta, F. A. Hoekstra, and E. J. Smid. 2000. Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. *Arch. Microbiol.* 174:233–238.
191. U.S. Department of Agriculture Food Safety and Inspection Service. 1999–2001. Recall information center. Available at: http://www.fsis.usda.gov/OA/recalls/rec_intr.htm. Accessed 15 September 2001.
192. Vojdani, F., and J. A. Torres. 1989. Potassium sorbate permeability of polysaccharide films: chitosan, methylcellulose and hydroxypropyl methylcellulose. *J. Food. Proc. Eng*. 12:33–37.
193. Vojdani, F., and J. A. Torres. 1989. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose multi-layer films. *J. Food Process. Preserv.* 13:417–430.
194. Vojdani, F., and J. A. Torres. 1990. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings effect of fatty acids. *J. Food Sci.* 55:841–846.
195. Wakabayashi, H., W. Bellamy, M. Takase, and M. Tomita. 1992. Inactivation of *Listeria monocytogenes* by lactoferrin, a potent antimicrobial peptide derived from cow's milk. *J. Food Prot.* 55:238–242.
196. Wang, L., and E. A. Johnson. 1992. Inhibition of *Listeria monocytogenes* by fatty acids and monoglycerides. *Appl. Environ. Microbiol.* 58:624–629.
197. Wanstedt, K. G., S. C. Seideman, L. S. Donnelly, and N. M. Ouenzer. 1981. Sensory attributes of precooked, calcium alginate-coated pork patties. *J. Food Prot.* 44:732–735.
198. Wendorff, W. L. 1989. Effect of smoke flavorings on *Listeria monocytogenes* in skinless franks. Presented at the Department of Food Science, University of Wisconsin–Madison, 13 January 1989.
199. Were, L., N. S. Hettiarachchy, and M. Coleman. 1999. Properties of cysteine-added soy protein–wheat gluten films. *J. Food Sci.* 64: 514–518.
200. Wong, D. W. S., F. A. Gastineau, K. S. Gregorski, S. J. Tillin, and A. E. Pavlath. 1992. Chitosan-lipid films: microstructure and surface energy. *J. Agric. Food Chem.* 40:540–544.
201. Woods, L. F., J. M. Wood, and P. A. Gibbs. 1981. The involvement of nitric oxide in the inhibition of the phosphoroclastic system in *Clostridium sporogenes* by sodium nitrite. *J. Gen. Microbiol.* 125: 399–403.
202. Wu, Y., C. L. Weller, F. Hamouz, S. Cuppett, and M. Schnepf. 2001. Moisture loss and lipid oxidation for precooked ground-beef patties packaged in edible starch–alginate-based composite films. *J. Food Sci.* 66:486–493.
203. Xie, L., N. S. Hettiarachchy, Z. Y. Ju, J. Meullenet, H. Wang, M. F. Slavik, and M. E. Janes. 2002. Edible film coating to minimize eggshell breakage post-wash bacterial contamination measured by penetration in eggs. *J. Food Sci.* 67:280–284.
204. Yang, T. 1985. Mechanism of nitrite inhibition of cellulose respiration in *Pseudomonas aeruginosa*. *Curr. Microbiol.* 12:35–40.
205. Yashitake, S., and A. Shininichiro. 1977. Use of egg-white lysozyme in the food industry. *New Food Ind.* 19:17–23.
206. Young, D. H., H. Kohle, and H. Kauss. 1982. Effect of chitosan on membrane permeability of suspension-cultured *Glycine max* and *Phasaolus vulgaris* cells (soybeans, kidney beans). *Plant Physiol.* 70:1449–1454.
207. Yousef, A. E., J. B. Luchansky, A. J. Degnan, and M. P. Doyle. 1991. Behavior of *Listeria monocytogenes* in wiener exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25°C. *Appl. Environ. Microbiol.* 57:1461–1467.
208. Yuste, J. R. P., M. Capallas, E. Ponce, and M. Mor-Mur. 2000. High-pressure processing applied to cooked sausages: bacterial populations during chilled storage. *J. Food Prot.* 63:1093–1099.
209. Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Appl. Environ. Microbiol.* 61: 2127–2131.
210. Zhuang, R. Y., L. R. Beuchat, M. S. Chinnan, R. L. Shewfelt, and Y. W. Huang. 1996. Inactivation of *Salmonella* Montevideo on tomatoes by applying cellulose-based edible films. *J. Food Prot.* 59: 808–812.