COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Control of Pathogenic and Spoilage Microorganisms in Fresh-cut Fruits and Fruit Juices by Traditional and Alternative Natural Antimicrobials

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ABSTRACT: Traditional antimicrobials have been extensively used for many years. However, consumers are currently demanding wholesome, fresh-like, and safe foods without addition of chemically synthesized preservatives. The application of novel natural antimicrobials to assure safety of fresh-cut fruits and unpasteurized juices while preventing quality loss is a promising alternative. The effectiveness of these natural substances added to fruit derivatives has been studied by different researchers. Antimicrobials of animal (lactoperoxidase, lysozyme, and chitosan), plant (essential oils, aldehydes, esters, herbs, and spices), and microbial origin (nisin) can be used to effectively reduce pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices. Nevertheless, the use of these compounds at a commercial level is still limited due to several factors such as impact on sensory attributes or, in some cases, regulatory issues concerning their use. Therefore, extensive research on the effects of each antimicrobial on food sensory characteristics is still needed so that antimicrobial substances of natural origin can be regarded as feasible alternatives to synthetic ones.

Introduction

Consumption of ready-to-eat fresh-cut fruits and fruit juices has substantially risen over the last few years, mostly due to the increasing demand for low-caloric food products with fresh-like characteristics. In addition, there is scientific evidence that consumption of fruits and vegetables helps prevent many degenerative diseases such as cardiovascular problems and several cancers (Rico and others 2007). However, as a consequence of inappropriate manipulation and storage conditions, both pathogenic and/or deteriorative microorganisms may contaminate a product, thus increasing the risk of microbial diseases and spoilage (Beuchat 1996; Díaz-Cinco and others 2005). In fact, the number of outbreaks and cases of illness caused by consumption of fresh-cut fruits and unpasteurized juices has increased in the last years (Harris and others 2003).

Quality losses in fresh-cut fruits and unpasteurized juices may occur as a consequence of microbiological, enzymatic, chemical, or physical changes. Safety and quality losses by microbiological causes are very important due to 2 reasons: first, because they constitute a hazard for consumers by the possible presence of microbial toxins or pathogenic microorganisms in the product, and second, by economic losses as a result of microbial spoilage. Many food preservation strategies such as chilling, freezing, water activity reduction, nutrient restriction, acidification, modified atmosphere packaging, fermentation, nonthermal physical treatments or the use of antimicrobials have been traditionally applied to control microbial growth (Davidson 2001). However, interest in the use of natural substances to prevent fresh-cut fruits

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and unpasteurized juices from microbiological spoilage while assuring safety and maintaining quality characteristics has significantly increased in the last years, due to the high demand of healthy, fresh-like, and safe foods that contain as low amounts of preservatives as possible (Soliva-Fortuny and Martín-Belloso 2003).

Antimicrobial agents are considered as food additives. Therefore, their use in foods is ruled by both international and national regulations. Hence, different countries have their own regulations with lists of approved additives (European Parliament and Council Directive N° 95/2/EC 1995; USFDA 2006, 2007; USCFR 2009). The U.S. Food and Drug Act, the European Union standards, and the Codex Alimentarius, which constitutes the FAO/WHO joint regulatory document, are the foremost governmental regulations concerning food additives (Raju and Bawa 2006). According to these regulations, the majority of natural antimicrobials are generally recognized as safe (GRAS); however, this will depend on their origin in an edible or inedible commodity and demonstrated absence of toxicity in concentrated form. Therefore, some limits based on these conditions, effects on sensory attributes, and the allowed acceptable daily intake (ADI) can be established in each case.

This review presents a compilation of the different studies on the use of natural antimicrobials in fresh-cut fruits and juices to maintain their safety and quality.

Microorganisms Associated with Fresh-Cut Fruits and Fruit Juices

Foods of plant origin such as fruits and vegetables have heterogeneous characteristics with regard to their compositions. Consequently, the microbiota in these products may substantially differ depending on medium pH, nutrient availability, and water activity, among other factors (Kalia and Gupta 2006). Fruits may become contaminated with pathogenic and spoilage microorganisms either during their growing in fields, orchards, vineyards, or greenhouses, or during harvesting, postharvest handling, and distribution (Beuchat 2002). Fresh fruits have a natural protective barrier (skin) that acts effectively against most plant spoilage and pathogenic microorganisms; however, this protection may be eliminated during the processing, thus exposing the fruit flesh to unfavorable environmental conditions as well as to a possible contamination with pathogenic microorganisms including bacteria, viruses, and parasites during the handling, cutting, shredding, and maintenance of the fresh-cut fruit at ambient temperature (Brackett 1994; Nguyen-The and Carlin 1994; Balla and Farkas 2006). Hence, the number of documented outbreaks of human infections associated with consumption of fresh-cut fruits (ranged from 1 to 6 per year) and unpasteurized fruit juices (ranged from 1 to 5 per year) has increased in the last 2 decades in comparison with previous decades (ranged from 0 to 1 per year) (Table 1 and 2).

Very few surveys analyzing the incidence of pathogens in fresh-cut fruits and fruit juices have been carried out and reported in the literature. Harris and others (2003) reported absence of *Salmonella* in a total of 336 samples of freshly peeled oranges or tangerines. Likewise, Martínez and others (2000) reported absence of *L. monocytogenes* in minimally processed fruit salads including papaya, apple, watermelon, grape, guava, and pineapple. On the other hand, Sado and others (1998) found that 2 samples of a total of 50 analyzed, corresponding to an apple juice and an apple/raspberry juice, were positive for *L. monocytogenes*. In contrast, survival and growth of pathogenic microorganisms in fresh-cut fruits and fruit juices have been more extensively studied. For example, challenge studies have been performed to evaluate the behavior of *Campylobacter jejuni*, *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, *Staphylococcus aureus*, and *Shigella* in several products (Table 3 and 4).

The causal agents of microbiological spoilage in fruits and derivatives can be bacteria, as well as yeasts and molds. The latter are considered the main spoilage agents due to the low pH of most fruits. Nevertheless, some bacteria such as *Erwinia* spp., *Enterobacter* spp., *Alicyclobacillus* spp., *Propionibacterium cyclohexanicum*, *Pseudomonas* spp., and lactic acid bacteria have been reported as deteriorative in cut fruit and juices (Pao and Petracek 1997; Brackett 2001; Chang and Kang 2004; Walker and Phillips 2008). Certain common molds such as *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Alternaria* spp., *Cladosporium* spp., *Paecilomyces* spp., and *Botrytis* spp. have been shown to be involved in the spoilage of fresh fruits and some processed fruit derivatives including the thermally processed (Splittstoesser 1991; Beuchat and Pitt 1992; Lund and Snowdon 2000). On the other hand, Jay and others (2005) reported the occurrence of yeasts such as *Saccharomyces* spp. *Cryptococcus* spp., and *Rhodotorula* spp. in fresh fruits, and *Zygosaccharomyces* spp., *Hanseniaspora* spp., *Candida* spp., *Debaryomyces* spp., and *Pichia* spp. in dried fruits. Although both molds and yeasts are able to grow in fruit tissue, the latter are more often associated with spoilage of cut fruits due to their ability to grow faster than molds.

Four types of factors determine the colonization of fresh-cut fruits and derivatives by microorganisms: 1) intrinsic factors, which are dependent on food composition, such as water activity, pH, redox potential, nutrients, structures, and antimicrobial agents; 2) technological treatments, which can modify the initial microbiota; 3) extrinsic factors or environmental conditions of the medium such as temperature, relative humidity, and atmosphere; 4) implicit factors, which depend on the developing microbiota and the handling of both the raw material and the product during processing and storage (Montville and Matthews 2001).

Antimicrobials for the Preservation of Fresh-Cut Fruits and Fruit Juices

Whole fresh fruits before processing are washed with water containing chemical sanitizer agents such as chlorine, chlorine dioxide, trisodium phosphate, hydrogen peroxide, organic acids, and ozone to decontaminate the surface of the fruit; with chlorine being among the more effective chemical additives in reducingpathogenic or naturally occurring microorganisms (by the order of 10- to 100-fold) (Balla and Farkas 2006). So, if the initial microbial load of the fruit surface is high (>100,000 cells/cm^2), then it would be ineffective. Several nonthermal physical treatments, however, such as ionizing irradiation, high hydrostatic pressure, pulsed electric field (for liquid foods), ultraviolet light, pulsed light, and ultrasound are emerging to improve the microbiological safety and quality of minimally processed foods including fruit products (Ross and others 2003). Nevertheless, the high treatment intensities required for microbial inactivation by some of these physical treatments during processing can cause adverse changes in the sensory or nutritional properties of the food (Ross and others 2003). Moreover, some emerging nonthermal technologies have been considered too energy expensive or costly to be practical for use in food processing (Raso and others 1998). On the other hand, the resilience of bacterial spores and the existence of highly resistant microbial subpopulations could also currently limit the efficacies of emerging nonthermal technologies (Ross and others 2003). Therefore in this review, traditional and alternative natural antimicrobial

Table 1—Outbreaks of foodborne illness caused by pathogenic bacteria associated with fresh fruits.

Causal agent	Year	Fruits	Cases (death)	Place
E. coli O157:H7	2005	Fruit salad	18	Home
Salmonella ser. Braenderup	2005	Roma tomatoes	84	Restaurant or deli
Salmonella ser. Braenderup	2004	Roma tomatoes	137	Restaurant or delicatessen, home
Salmonella multiserotypes	2004	Roma tomatoes	429	–
Salmonella spp.	2003	Strawberry	13	–
Salmonella ser. Muenchen	2003	Cantaloupe, Honeydew melons	58	–
Salmonella ser. Newport	2003	Honeydew melons	68	–
Salmonella ser. Berta	2002	Watermelon	29	–
Salmonella ser. Poona	2002	Cantaloupe melon	26	–
Salmonella ser. Newport	2002	Tomatoes	510	–
Salmonella ser. Newport	2002	Fruit salad	51	–
Salmonella ser. Poona	2001	Honeydew melons, watermelon	23	Restaurant
Salmonella ser. Saintpaul	2001	Mango	26	Private home
Salmonella ser. Poona	2001	Watermelon	23	–
Salmonella ser. Poona	2001	Cantaloupe, Honeydew melons	50	Private home
Salmonella ser. Senftenberg	2001	Green grapes	40	Private home
E. coli O157:H7	2001	Pear	14	School
E. coli O157:H7	2000	Watermelon	736	Restaurant
E. coli O157:H7	2000	Red grape	14	Grocery store
Salmonella ser. Poona	2000	Cantaloupe melon	46	–
Salmonella ser. Thompson	2000	Tomato	43	Private home
Salmonella ser. Enteritidis	1999	Honey dew melons/watermelon	82	School
Salmonella ser. Newport	1999	Mango	79	Multiple
Salmonella ser. Baildon	1998	Tomatoes	>85(3)	Multiple
Salmonella ser. Oranienburg	1998	Mango	9	Private home
Salmonella ser. Oranienburg	1998	Cantaloupe	22	Various
E. coli O157:H7	1997	Melon	9	Private home
Salmonella *ser. Saphra*	1997	Cantaloupe melon	24	Restaurant, home, grocery store
E. coli O157:H7	1993	Cantaloupe	27	Restaurant
Salmonella ser. Montevideo	1993	Tomatoes	100	Restaurant
Salmonella *ser. Poona*	1991	Cantaloupe	>400	Multiple
Salmonella ser. Javiana	1990	Fresh tomatoes	176	Day care center, restaurant
Salmonella ser. Chester	1990	Cantaloupe	25000(2)	–
Salmonella ser. Miami	1954	Watermelon	17(1)	Supermarket

Adapted from Harris and others (2003) and CDC (2007).

Table 2—Outbreaks of foodborne illness caused by pathogenic bacteria associated with fruit juices.

Causal agent	Year	Fruit juice	Cases (death)	Place
Salmonella ser. Typhimurium and Saintpaul	2005	Orange juice unpasteurized	157	Restaurant, deli, private home
Salmonella ser. Enteritidis	2000	Orange, grapefruit, and lemonade juice	74	Multiple places
Salmonella ser. Muenchen	1999	Orange juice unpasteurized	398 (1)	–
Salmonella ser. Typhimurium	1999	Orange juice	427	Retail
Salmonella ser. Anatum	1999	Orange juice unpasteurized	10	Other
Salmonella ser. Typhimurium	1999	Mamey juice unpasteurized	13	–
E. coli O157:H7	1999	Apple cider unpasteurized	5	Private home
E. coli O157:H7	1998	Apple juice	14	Farm, home
E. coli O157:H7	1997	Apple cider unpasteurized	6	Farm
E. coli O157:H7	1996	Apple cider unpasteurized	56	Multiple
E. coli O157:H7	1996	Apple juice unpasteurized	71(1); 14HUS	Community
E. coli O157:H7	1996	Apple cider unpasteurized	14 (3)	Small cider mill
E. coli O157:H7	1996	Apple cider unpasteurized	6	Small cider mill
Salmonella ser. Hartford, Gaminara and Rubislaw	1995	Orange juice	62	Theme park
E. coli O157:H7	1992	Orange juice	6	Roadside vendor
E. coli O157:H7	1991	Apple cider	23; 4HUS	Community
Salmonella ser. Javiana	1991	Watermelon juice	39	Indoor picnic, school party
Salmonella ser. Enteritidis	1991	Orange juice	600	–
E. coli O157:H7	1980	Apple juice unpasteurized	14 (1); 14HUS	Local market
Salmonella ser. Typhimurium	1974	Apple cider	296	Farm and small retail outlets

HUS = people with hemolytic uremic syndrome.
Adapted from Powell and Luedtke (2000), Harris and others (2003), and CDC (2007).

Table 3—Survival and growth of pathogenic bacteria in fresh-cut fruits.

Pathogen	Fresh-cut fruit	Fruit pH	Temperature (°C)	Storage time (h)	Initial/final counts (log CFU/g)	Reference
Campylobacter jejuni	Watermelon	3.0	25 to 29	6	2.9/2.0	Castillo and Escartín (1994)
Campylobacter jejuni	Watermelon	5.5	25 to 29	6	2.7/2.1	Castillo and Escartín (1994)
Campylobacter jejuni	Papaya	3.0	25 to 29	6	3.3/Nd	Castillo and Escartín (1994)
Campylobacter jejuni	Papaya	5.0	25 to 29	6	2.8/1.7	Castillo and Escartín (1994)
Escherichia coli O157:H7	Melon (Cantaloupe)	7.01	25 and 5	34 and 34	3.0/7.0 and 3.1/3.1	Del Rosario and Beuchat (1995)
Escherichia coli O157:H7	Watermelon	5.56	25 and 5	34 and 34	3.0/8.7 and 3.0/3.0	Del Rosario and Beuchat (1995)
Escherichia coli O157:H7	Apples (Red delicious)	4.10	4, 10, and 25	432, 288, and 120	7.5/6.8, 7.5/5.8, and 7.5/8.5	Fisher and Golden (1998a)
Escherichia coli O157:H7	Apples (Golden delicious)	3.84	4, 10, and 25	432, 288, and 120	7.5/7.2, 7.5/6.8, and 7.5/8.2	Fisher and Golden (1998a)
Escherichia coli O157:H7	Apples (Rome)	3.70	4, 10, and 25	432, 288, and 120	7.5/6.8, 7.5/7.0, and 7.5/7.5	Fisher and Golden (1998a)
Escherichia coli O157:H7	Apples (Winesap)	3.47	4, 10, and 25	432, 288, and 120	7.5/7.0, 7.5/7.0, and 7.5/7.0	Fisher and Golden (1998a)
Escherichia coli O157:H7	Oranges (Hamlin)	6.25	4, 8, and 24	336, 336, and 24	3.5/3.2, 3.5/2.5, and 3.5/7.5	Pao and others (1998)
Escherichia coli O157:H7	Apples (Golden delicious)	—	24	48	2.0/5.5	Janisiewicz and others (1999)
Escherichia coli O157:H7	Pear (Cactus)	5.9	4, 8, 12, and 20	336	3.0/4.8, 3.0/5.1, 3.0/3.8, and 3.0/Nd	Corbo and others (2005)
Salmonella multiserotypes	Melon (Cantaloupe)	6.67	23 and 5	24 and 24	2.0/7.2, 2.0/1.6	Golden and others (1993)
Salmonella multiserotypes	Melon (Honeydew)	5.95	23 and 5	24 and 24	2.0/8.0 and 2.0/1.8	Golden and others (1993)
Salmonella multiserotypes	Watermelon	5.90	23 and 5	24 and 24	2.0/8.6 and 2.0/1.9	Golden and others (1993)
Salmonella ser. Typhi	Papaya	5.69	25 to 27	6	2.9/4.3	Fernandez-Escartin and others (1989)
Salmonella ser. Typhi	Papaya	3.59	25 to 27	6	3.0/3.8	Fernandez-Escartin and others (1989)
Salmonella spp.	Oranges (Hamlin)	6.25	4, 8, and 24	336, 336, and 24	4.4/3.5, 4.4/2.5, and 4.4/7.6	Pao and others (1998)
Salmonella ser. Chester	Apples (Golden delicious)	4.1	8	66	5.5/5.3	Liao and Sapers (2000)
Salmonella ser. Chester	Apples (Golden delicious)	4.1	20	66	5.5/10.4	Liao and Sapers (2000)
Salmonella ser. Enteritidis	Apple (Granny Smith)	4.1	20	150	4.5/7.7	Lanciotti and others (2003)
Listeria monocytogenes	Oranges (Hamlin)	6.25	4, 8, and 24	336, 336, and 24	3.9/4.0, 3.9/3.3, and 3.9/5.5	Pao and others (1998)
Listeria monocytogenes	Fruits salad	4.22	5	192	3.5/3.0	Mejía and Díaz (1998)
Listeria monocytogenes	Apple (Granny Smith)	4.1	20	96	4.5/approximately 4.5	Lanciotti and others (2003)
Listeria monocytogenes	Watermelon	5.50	10, 20, and 30	168, 48, and 24	2.5/6.0, 2.5/7.2, and 2.5/9.0	Penteado and Leitao (2004)
Listeria monocytogenes	Papaya	4.87	10, 20, and 30	168, 48 and 24	2.5/4.7, 2.5/4.3, and 2.5/7.3	Penteado and Leitao (2004)
Listeria monocytogenes	Melon	5.87	10, 20, and 30	168, 48, and 24	2.5/9.0, 2.5/9.0, and 2.5/9.0	Penteado and Leitao (2004)
Listeria monocytogenes	Pear (Cactus)	5.9	4, 8, 12, and 20	336	4.6/5.7, 4.6/7.6, 4.6/7.6, and 4.6/7.6	Corbo and others (2005)
Staphylococcus aureus	Oranges (Hamlin)	6.25	4, 8, and 24	336, 336, and 24	2.8/2.0, 2.8/2.0, and 2.8/3.5	Pao and others (1998)
Shigella flexneri	Watermelon	—	22 to 26	6	2.8/4.5	Fernandez Escartin and others (1989)
Shigella spp.	Papaya	5.69	25 to 27	6	2.0 to 2.4/3.8 to 4.2	Fernandez Escartin and others (1989)

Nd = not determined.

Table 4—Survival and growth of pathogenic bacteria in fruit juices.

Pathogen	Juice type	pH	Temperature (°C)	Storage time (h)	Initial/final counts (log CFU/mL)	Reference
Escherichia coli O157:H7	Apple cider	3.6 to 4.0	25	72 to 144	5.0/<1.0	Zhao and others (1993)
Escherichia coli O157:H7	Apple cider	3.6 to 4.0	8	360 to 816	5.0/<1.0	Zhao and others (1993)
Escherichia coli O157:H7	Apple cider	3.6 to 4.0	8	264 to 360	2.0/<1.0	Zhao and others (1993)
Escherichia coli O157:H7	Apple cider	3.7 to 3.9	4	240	4.5/9.4	Miller and Kaspar (1994)
Escherichia coli O157:H7	Apple cider	3.5	21 and 4	168	5.3/<1.5 and 5.3/2.2	Uljas and Ingham (1999)
Escherichia coli O157:H7	Apple cider	3.7	26	144	6.0/4.5	Janisiewicz and others (1999)
Escherichia coli O157:H7	Apple cider	3.6 to 4.2	20 to 25	168	4.3/2.5 to 4.1	Dingman (2000)
Escherichia coli O157:H7	Apple juice	—	8 and 25	336 and 72	5.1/5.0 and 5.3/5.1	Ceylan and others (2004)
Escherichia coli O157:H7	Apple cider	3.5 to 3.6	5	72	8.0/7.9	Ingham and others (2006)
Listeria monocytogenes	Apple juice	3.7	5 and 20	72 and 24	4.5/Nd and 4.5/Nd	Yuste and Fung (2002)
Listeria monocytogenes	Apple cider	3.5 to 3.6	5	72	5.8/1.5	Ingham and others (2006)
Salmonella spp.	Apple cider	3.5 to 3.6	5	72	7.9/6.4	Ingham and others (2006)
Salmonella ser. Enteritidis	Apple juice	4.2	35	24	5.0/3.01	Raybaudi-Massilia and others (2006)
Salmonella ser. Enteritidis	Pear juice	4.0	35	24	5.0/4.1	Raybaudi-Massilia and others (2006)
Salmonella ser. Enteritidis	Melon juice	5.9	35	24	5.0/7.9	Raybaudi-Massilia and others (2006)

substances, with a relatively lower cost than physical treatments and simple use, in addition to their potentials to suppress outgrowth of surviving populations during subsequent storage of the fresh-cut fruit and fresh fruit juices are discussed in detail in this section.

Food antimicrobials are chemical compounds or substances that may delay microbial growth or cause microbial death when finding their way into a food matrix (Davidson and Zivanovic 2003). The major targets for antimicrobials are food poisoning microorganisms (infective agents and toxin producers) and spoilage microorganisms whose metabolic end products or enzymes cause off-odors, off-flavors, texture problems, and discoloration (Davidson 2001).

The classification of antimicrobials is extremely difficult. They can be divided into traditional and novel substances (called "naturals") depending on their origin. Nevertheless, this classification does not imply that the synthetic or traditional preservatives are less effective from a microbiological point of view than one of natural origin. Antimicrobials are called traditional when: 1) they have been used for many years, 2) they have been approved by many countries for inclusion as antimicrobials in foods, or 3) they have been produced by chemical synthesis. Ironically, many synthetic traditional antimicrobials are found in nature. This is the case of benzoic acid (in cranberries), sorbic acid (in rowanberries), citric acid (in lemons), malic acid (in apples), or tartaric acid (in grapes).

Most food antimicrobial agents are only biostatic and are not biocides. Therefore, their actions on foods are rather limited and the shelf life of the product will depend on the storage conditions. On the other hand, the use of combinations of antimicrobials is usually more effective than adding just one antimicrobial because some microorganisms are not inhibited or killed by the doses that are legally approved or accepted flavorwise (Beuchat 2001). The combined use of 2 or more antimicrobial compounds can result in synergistic, additive, or antagonistic effects. Similar results can be expected by combining them with other preservation methods such as heat, pulsed electric fields, ultraviolet light, ultrasound, and high hydrostatic pressure. However, these combinations of techniques must be tested for each specific food product before application to find desirable synergies and to avoid antagonistic effects (Wiley 1994).

Generally, antimicrobials have different concentration thresholds for inhibition or inactivation. These thresholds depend on the specific targets of the antimicrobial substance, including cell wall, cell membrane, metabolic enzymes, protein synthesis, and genetic systems. The exact mechanism(s) or target(s) for food antimicrobials are often not known or well defined. It is difficult to identify a specific action site where many interacting reactions take place simultaneously. For example, membrane-disrupting compounds could cause leakage of cellular content, interference with active transport or metabolic enzymes, or dissipation of cellular energy in ATP form (Davidson 2001).

The efficiency of a certain antimicrobial will also depend on the type, genus, species, and strain of the target microorganism. Likewise, it will also depend on environmental factors such as pH, water activity (a_w), temperature, atmosphere composition, initial microbial load, and acidity of the food substrate (Gould 1989). Many of these environmental factors can be considered individually as preservation methods; whereas the combined use of some of these treatments has been the basis of the hurdle concept which consists in the use of more than one treatment in a logical sequence to enhance microbiological safety as well as to provide fresh-like quality food products (Wiley 1994; Leistner 1995).

The antimicrobial nature of any compound is mostly determined by its chemical properties, notably the pKa value, hydrophobicity/lipophilicity ratio as measured by the partition coefficient log P_{oct}, solubility, and volatility, particularly in opened systems (Stratford and Eklund 2003). The pH and polarity are perhaps the most prominent factors influencing the effectiveness of a food antimicrobial. Polarity is related to both the ionization of the molecule and the contribution of any alkyl side groups or hydrophobic parent molecules (Davidson 2001). Therefore, it is very important to know the specific characteristics of the food system that needs to be preserved since a high proportion of lipids could limit the effectiveness of some antimicrobial agents, especially of those with hydrophobic properties. On the other hand, hydrophobic or partially hydrophobic characteristics of some antimicrobial substances makes difficult their dissolution in water, and therefore, they can not be used to prepare dipping solutions, which is a common technique in fresh-cut fruit processing.

Traditional antimicrobials

Organic acids. Organic acids have been traditionally used in the food industry as preservative agents, since pH, as affected by the concentration of hydrogen ions, has a great impact on the survival and growth of microorganisms in foods. In general, bacteria prefer a pH close to neutrality (pH 6.5 to 7.5), but tolerate a pH range of 4 to 9. Yeasts are more tolerant than bacteria to low pH values, whereas molds can grow in the widest range of pH conditions. Therefore, one effective way of limiting microbial growth is to increase the acidity of a food by either adding an acidifier or enhancing natural fermentation to develop a change in acidity (Doores 1993).

Given the metabolic complexity of the microbial cell, either prokaryote (bacteria) or eukaryote (yeasts and molds), it is very unlikely for a chemical compound to affect a single site of action only. Thus, organic acids are likely to affect a number of systems in the target organism. The effect on each point of action will depend, in turn, on variables such as acid type and concentration, conditions of use, pH, temperature, and nature of the target microorganism. Possible mechanisms of action of organic acids (Figure 1) include: direct pH reduction of the substrate or growth medium due to an increase in proton concentration, depression of the internal cellular pH by ionization of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability. In addition to inhibiting substrate transport, organic acids may also inhibit NADH oxidation, thus eliminating supplies of reducing agents to electron transport systems (Davidson 2001). Because the undissociated portion of an acid molecule is primarily responsible for the antimicrobial activity, effectiveness at a given pH depends largely on the dissociation constant (pKa) of the acid (Beuchat 2000). Fully dissociated "strong" acids such as hydrochloric or sulfuric acids affect microbes only through alteration of pH (proton concentration), since chloride or sulfate concentrations appear to have little effect. However, when media are acidified with "weak" acids, such as citric, acetic, or lactic acids, the antimicrobial effects are more pronounced, indicating that "weak" acids inhibit microbes by other mechanisms in addition to that of merely lowering pH (Stratford and Eklund 2003). Undissociated forms of organic "weak" acids can penetrate the cell membrane lipid bilayer more easily. Once inside the cell, the acid is forced to dissociate into charged anions and protons because the cell interior has a higher pH than the exterior. Protons generated from intracellular dissociation cause a progressive decline in intracellular pH, which, in turn, may inhibit glycolysis, affect cell signaling, and inhibit active transport (Stratford and Eklund 2003). Bacteria have to exclude the protons generated outside the cell to prevent conformational changes in the cell structural proteins, enzymes, nucleic acids, and phospholipids. According to the chemiosmotic theory, the cytoplasmic membrane is impermeable to protons, which must be transported to the exterior implying an energetic cost in the ATP form, which will eventually deplete the cellular energy (Davidson 2001). Organic acids also interfere with membrane permeability. Thus, Sheu and Freese (1972) suggested that short-chain organic acids interfere with energy metabolism by alteration of the structure of the cytoplasmic membrane due to an interaction with membrane proteins.

Different studies have demonstrated the effectiveness of organic acids added to fresh-cut fruits and fruit juices to inhibit or reduce populations of spoilage and pathogenic microorganisms (Table 5 and 6). Nonetheless, it has been shown that the use of organic acids in combination with other preservation methods such as mild heat, high-intensity pulsed electric fields, dehydration, freezing-thawing, and low temperatures has an enhanced antimicrobial effect in fresh-cut fruits and/or fruit juices (Uljas and Ingham 1999; Comes and Beelman 2002; Chikthimmah and

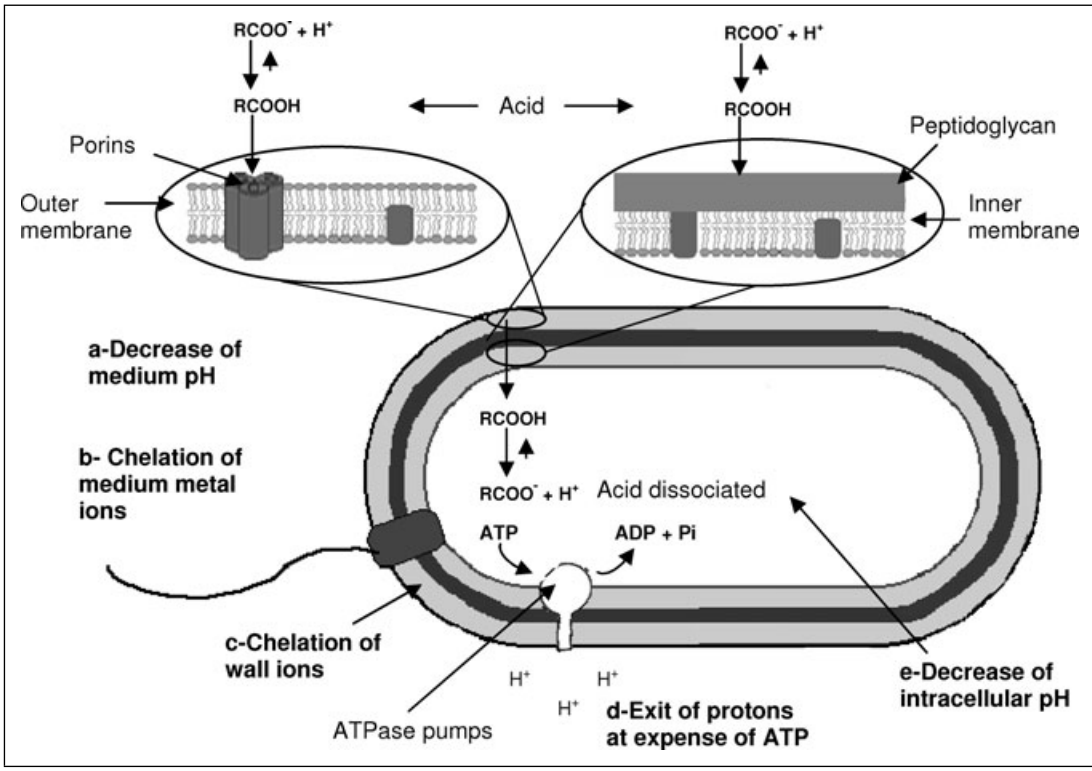

Figure 1—Mechanisms of action of organic acids in a bacterial cell (a to e). The left amplification illustrates how the organic acids can pass through the outer membrane in Gram-negative bacteria, whereas the right amplification shows how they can pass through the inner membrane in Gram-positive (adapted from Davidson 2001).

Table 5—Traditional antimicrobials used in fresh-cut fruits to control pathogenic and spoilage microorganisms.

Antimicrobial	Amount applied (%)	Time of exposition (min)	Mode of application	Fresh-cut fruit	Product pH	Target microorganism	Storage conditions		Effect	Reference
							Temp. (°C)	Time		
Ascorbic acid	3.4	10	Direct by immersion	Apple var. Gala	3.77	*Salmonella* ser. Typhimurium, *agona*, and *michigan*	25	0 h	Reduced up to 0.7 log CFU/g	DiPersio and others (2003)
Ascorbic acid	2.8	10	Direct by immersion	Apple var. Gala	4.05	*E. coli* O157:H7	25	0 h	Reduced up to 1.3 log CFU/g	Derrickson-Tharrington and others (2005)
Citric acid	0.21	10	Direct by immersion	Apple var. Gala	4.14	*Salmonella* ser. Typhimurium, *agona*, and *michigan*	25	0 h	Reduced up to 0.9 log CFU/g	DiPersio and others (2003)
Citric acid	1.7	10	Direct by immersion	Apple var. Gala	3.55	*E. coli* O157:H7	25	0 h	Reduced up to 1.3 log CFU/g	Derrickson-Tharrington and others (2005)
Citric acid	0.5 1.0	2	Direct by immersion	Orange var. Valencia or Hamlin	2.75 2.25	Total aerobic microorganisms	4 8 or 21	21 d	Delayed the microbial growth and extended the shelf-life of peeled cut-orange by >21 d	Pao and Petracek (1997)
Malic acid + N-acetyl-L-cysteine + Glutathione + Calcium lactate	2.5 1.0 1.0 1.0	1	Direct by immersion	Apple var. Fuji	3.9	a) *Listeria monocytogenes* b) *Salmonella* Enteritidis c) *E. coli* O157:H7	5	30 d	a) >5 log CFU/g at 0 d b) >5 log CFU/g at 0 d c) >5 log CFU/g after 3 d	Raybaudi-Massilia and others (2009a)
Malic acid + N-acetyl-L-cysteine + Glutathione + Calcium lactate	2.5 1.0 1.0 1.0	1	Direct by immersion	Apple var. Fuji	3.9	Mesophilic bacteria, psychrophilic bacteria, mold and yeast populations	5	30 d	Delayed the microbial growth and extended the shelf-life of apple slices by >13 d in comparison with control	Raybaudi-Massilia and others (2007)
Malic acid + N-acetyl-L-cysteine + Glutathione + Calcium lactate	2.5 1.0 1.0 1.0	2	Direct by immersion	Pear var. Flor de Invierno	4.2	a) *Listeria monocytogenes* b) *Salmonella* Enteritidis c) *E. coli* O157:H7 d) Mesophilic, psychrophilic, mold and yeast populations	5	30 d	a) y b) >5 log CFU/g at 0 d c) >5 log CFU/g after 14 d d) Delayed the microbial growth and extended the shelf-life of pear slices by >21 d with respect to control	Raybaudi-Massilia and others (2009b)

Continued

Table 5—Continued.

Antimicrobial	Amount applied (%)	Time of exposition (min)	Mode of application	Fresh-cut fruit	Product pH	Target microorganism	Storage conditions		Effect	Reference
							Temp. (°C)	Time		
Malic acid + N-acetyl-L-cysteine + Glutathione + Calcium lactate	2.5 1.0 1.0 2.0	2	Incorproted into an edible coating of alginate	Apple var. Fuji	–	a) *E. coli* O157:H7 b) Mesophilic, psychrophilic, mold and yeast populations	5	30 d	a) Reduced up to 4.1 log CFU/g after 30 d b) Delayed the microbial growth and extended the shelf-life of apple slices by >13 d with respect to control	Raybaudi-Massilia and others (2008c)
Malic acid + Calcium lactate	2.5 2.0	2	Incorproted into an edible coating of alginate	Melon var. Piel de sapo	–	a) *Salmonella* Enteritidis b) Mesophilic, psychrophilic, mold and yeast populations	5	21 d	a) Reduced up to 3.1 log CFU/g after 21 d b) Delayed the microbial growth and extended the shelf-life of apple slice by >6 d in comparison with control	Raybaudi-Massilia and others (2008b)
Sodium metabisulfite	4.18	10	Direct by immersion	Apple var. Gala	3.78	*Salmonella* ser. Typhimurium, Agona, and Michigan	25	0 h	Reduced up to 0.4 log CFU/g	DiPersio and others (2003)

others 2003; DiPersio and others 2003; Derrickson-Tharrington and others 2005; Ingham and others 2006; Mosqueda-Melgar and others 2008a, 2008b, 2008c).

Sulfites. While sulfites now have multiple uses as food additives, they were originally used for antimicrobial purposes. As antimicrobials, sulfites are used primarily in fruit and vegetable products to control 3 groups of microorganisms: 1) spoilage and fermentative yeasts and molds on fruits and fruit products, 2) acetic acid bacteria, and 3) malolactic bacteria. The antimicrobial activity of sulfites is substantially enhanced at a pH below 4. In addition, sulfites inhibit enzymatic and nonenzymatic browning (Davidson 2001).

Because of their extreme reactivity, it is difficult to pinpoint the exact antimicrobial mechanism for sulfites. This reactivity is due to the ability of sulfites to act as reducing agents or to take part in nucleophilic attacks. Sulfites react with disulfide bonds of proteins and with glutathione-forming thiosulfonates. The most likely targets for inhibition by sulfites include: cytoplasmic membrane, DNA replication, protein synthesis, cytoplasmic enzymes, or individual components in metabolic pathways (Davidson 2001).

Traditionally, sulfites have been used to prevent enzymatic browning of fresh-cut products, and to inhibit the growth of microorganisms in fermented foods such as wines, ciders, and juices (Table 5 and 6). However, their use as food additive has been restricted by the U.S. Food and Drug Administration (FDA) since 1990 because they can cause dangerous side effects for people with asthma. For this reason, there is increasing interest in substitutes for sulfites (USFDA 1994; Ahvenainen 1996).

Alternative natural antimicrobials of animal origin

Enzymes. Lactoperoxidase is a hemoprotein present in milk and other secretions, which catalyzes the oxidation of thiocyanate (SCN^-) and iodide ions to generate highly reactive oxidizing agents. These products have a broad spectrum of antimicrobial effects against bacteria, fungi, and viruses (Naidu 2000). Lactoperoxidase is primarily active against H_2O_2-producing bacteria such as *Lactobacillus* and *Streptococcus* spp., although certain catalase-positive Gram-negative microorganisms may also be inhibited. The lactoperoxidase system exerts its antimicrobial action through short-life oxidation products, mainly hypothiocyanate ($OSCN^-$) and hypothiocyanous acid (HOSCN), which produce microbiocidal or microbiostatic effects by the oxidation of thiol groups (-SH) of cytoplasmic enzymes and damage to the outer membrane, cell wall or cytoplasmic membrane, transport systems, glycolytic enzymes, and nucleic acids (Beuchat and Golden 1989; Touch and others 2004).

The Food Standards Australia New Zealand (FSANZ), in 2002, in its regulatory status pointed out that lactoperoxidase is not a known allergen and the presence of known allergens in commercial lactoperoxidase seems insufficient to elicit allergic reaction in the vast majority of milk-allergic individuals, since there is little evidence to suggest that lactoperoxidase may be capable of sensitizing susceptible individuals. However, they recommended that consumers be informed by appropriate labeling of food products for the presence of this milk protein. This regulatory organization has permitted the use of the lactoperoxidase system for the treatment of meat (including poultry), fish, and milk products as an antimicrobial at maximum levels of 20 mg/kg meat or 30 mg/L milk. In addition, the USFDA, in 2006, informed that lactoperoxidase is generally recognized as safe (GRAS) (Table 7), through scientific procedure, for use as an ingredient of foods including dairy products (up to 1000 mg/kg L), fruit and vegetable juices (up to 167 mg/L).

Studies reporting the effect of enzymes on pathogenic or spoilage microorganisms naturally present or intentionally

Table 6—Traditional antimicrobials used in fruit juices to control pathogenic and spoilage microorganisms.

Antimicrobial	Amount applied (%)	Fruit juice	Product pH	Target microorganism	Temp. (°C)	Storage time	Effect	Reference
Benzoic acid	0.1	Apple cider	3.6 to 4.0	*E. coli* O157:H7	8	7 d	Reduced 3 to 5 log CFU/mL	Zhao and others (1993)
Benzoic acid	0.01	Grape juice	—	Yeast populations	1	70 to 78 d	Inhibited the growth	Pederson and others (1961)
Capric & Caprylic acid	0.01	Grape juice	—	Yeast populations	1	70 to 78 d	Inhibited the growth	Pederson and others (1961)
Citric acid	0.1	Apple cider	3.3	*E. coli* O157:H7	4	48 h	Do not reductions were found	Comes and Beelman (2002)
Citric acid	0.5 to 2.0	Tomato juice	3.7 to 3.0	*Salmonella* Enteritidis	22	60 min	Reduced < 0.1 log CFU/mL	Mosqueda-Melgar and others (2008a)
Citric acid	0.5 to 2.0 0.5 to 2.0 0.5 to 2.0 0.5 to 2.0	Apple juice Pear juice Orange juice Strawberry juice	3.7 to 2.9 3.4 to 2.7 3.1 to 2.7 2.9 to 2.5	*E. coli* O157:H7 *Salmonella* Enteritidis	22	60 min	Reduced < 1.2 log CFU/mL of both pathogenic bacteria	Mosqueda-Melgar and others (2008b)
Citric acid	0.5 to 2.0	Melon juice	4.2 to 3.1	*E. coli* O157:H7 *Salmonella* Enteritidis *Listeria monocytogenes*	22	60 min	Reduced < 0.5 log CFU/mL of pathogenic microorganisms	Mosqueda-Melgar and others (2008c)
Citric acid	0.5 to 2.0	Watermelon juice	3.8 to 3.0	*E. coli* O157:H7 *Salmonella* Enteritidis *Listeria monocytogenes*	22	60 min	Reduced less than 1.5 log CFU/mL of pathogenic microorganisms	Mosqueda-Melgar and others (2008c)
Fumaric acid	0.10 0.15 0.20	Apple cider	3.3	*E. coli* O157:H7	4	48 h	Reduced up to 2.0 log CFU/mL Reduced up to 2.9 log CFU/mL Reduced up to 3.4 log CFU/mL	Comes and Beelman (2002)
Lactic acid	0.1	Apple cider	3.7 4.1 4.1 4.1	a) *E. coli* O157:H7 b) *E. coli* O157:H7 c) *Salmonella* Typhimurium d) Molds and yeasts	35 35 4 or 25 35	4 h 6 h 12 h 6 h	a) Reduced > 5 log CFU/mL b) Reduced up to 2.0 log CFU/mL c) Had no significant lethal effect d) Were inhibited	Uljas and Ingham (1999)
Malic acid	0.1	Apple cider	3.3	*E. coli* O157:H7	4	48 h	Do not reductions were found	Comes and Beelman (2002)
Malic acid	0.2/0.4/0.6 0.8/0.8/1.0 0.8/0.8/2.0	Apple juice	3.6/3.3/3.1 3.1/3.1/3.0 3.1/3.1/2.7	*Listeria monocytogenes* *Salmonella* Enteritidis *E. coli* O157:H7	35/20/5	24 h	Reduced > 5 log CFU/mL	Raybaudi-Massilia and others (2008a)
Malic acid	0.6/0.6/0.8 0.8/0.8/1.5 1.5/1.5/2.0	Pear juice	3.3/3.3/3.2 3.2/3.2/2.8 2.8/2.8/2.6	*Listeria monocytogenes* *Salmonella* Enteritidis *E. coli* O157:H7	35/20/5	24 h	Reduced > 5 log CFU/mL	Raybaudi-Massilia and others (2008a)
Malic acid	0.6/0.8/1.0 0.6/0.8/1.5 0.8/2.0/2.5	Melon juice	3.6/3.5/3.3 3.6/3.5/3.1 3.5/3.0/2.9	*Listeria monocytogenes* *Salmonella* Enteritidis *E. coli* O157:H7	35/20/5	24 h	Reduced > 5 log CFU/mL	Raybaudi-Massilia and others (2008a)
Potassium sorbate	0.5 to 1.0	Apple juice	—	*Alicyclobacillus acidoterrestris*	30	29 d	Reduced up to 2 and 1.5 log CFU/mL of vegetative cells and spores, respectively, after 29 d	Walker and Phillips (2008)
Potassium sorbate	a) 0.5 b) 1.0	Orange juice	—	*Propionibacterium cyclohexanicum*	30	29 d	a) Had no significant lethal effect b) > 5 log CFU/mL after 15 d	Walker and Phillips (2008)

Continued

Table 6—Continued.

Antimicrobial	Amount applied (%)	Fruit juice	Product pH	Target microorganism	Temp. (°C)	Storage time	Effect	Reference
Potassium sorbate	0.1	Apple juice	3.75	*E. coli* O157:H7	a) 8 b) 25	a) 14 d b) 3 d	a) Reduced up to 3.8 log CFU/mL b) Reduced up to 4.0 log CFU/mL	Ceylan and others (2007)
Potassium sorbate	0.05 to 0.1	Apple juice	3.3	*Listeria innocua*	30	4 – 8 h	Reduced up to 5 log CFU/mL	Ferrante and others (2004)
Potassium sorbate	0.015	Apple juice	3.5	*Byssochlamys nivea*	21 to 37	25 d	Retarded the microbial growth	Roland and Beuchat (1984)
Propionic acid	0.1	Apple cider	4.1	*E. coli* O157:H7	35	6 h	Reduced < 1 log CFU/mL	Uljas and Ingham (1999)
Sodium benzoate	0.045	Apple cider	3.5	*E. coli* O157:H7	a) 4 b) 10 c) 25	18 d	a) >5 log CFU/mL after 12 d b) >5 log CFU/mL after 15 d c) >5 log CFU/mL after 2 d	Fisher and Golden (1998b)
Sodium benzoate	0.5 to 1.0	Apple juice	–	*Alicyclobacillus acidoterrestris*	30	29 d	Vegetative cells and spores were inhibited for 29 d	Walker and Phillips (2008)
Sodium benzoate	0.5 to 1.0	Orange juice	–	*Propionibacterium cyclohexanicum*	30	29 d	Reduced 5 log CFU/mL after 8 d	Walker and Phillips (2008)
Sodium benzoate	0.1	Apple juice	3.75	*E. coli* O157:H7	a) 8 b) 25	a) 14 d b) 3 d	a) Reduced up to 4.9 log CFU/mL b) Reduced up to 4.8 log CFU/mL	Ceylan and others (2004)
Sodium benzoate	0.05	Apple juice	3.5	*Byssochlamys nivea*	21 to 37	25 d	Retarded the microbial growth	Roland and Beuchat (1984)
Sodium bisulfite	0.0046	Apple cider	3.4	*E. coli* O157:H7	a) 4 b) 10 c) 25	18 d	a) >5 log CFU/mL after 18 d b) >5 log CFU/mL after 18 d c) >5 log CFU/mL after 3 d	Fisher and Golden (1998b)
Sorbic acid	0.1	Apple cider	3.7	*E. coli* O157:H7	35 25	4 h 12 h	Reduced > 5 log CFU/mL	Uljas and Ingham (1999)
Sorbic acid	a) 0.010 b) 0.015	a) Grape juice b) Apple and strawberry	–	Yeasts populations	1	27 d	Inhibited the growth	Pederson and others (1961)
Sorbic acid	0.1	Apple cider	4.1	a) *E. coli* O157:H7 and *Salmonella* Typhimurium b) Moulds and yeasts	35	6 h	a) Reduced > 5 log CFU/mL b) Yeasts were inhibited and moulds were reduced by 1.4 log CFU/mL	Uljas and Ingham (1999)
Sulfur dioxide (SO_2)	0.0075	Apple juice	3.5	*Byssochlamys nivea*	21 to 37	25 d	Retarded the microbial growth	Roland and Beuchat (1984)
Fumaric acid + Sodium benzoate + Potassium sorbate	0.15 0.05 0.05	Apple cider	3.5	*E. coli* O157:H7	25	6 h	Reduced > 5 log CFU/mL	Comes and Beelman (2002)
Fumaric acid + Sodium benzoate	0.15 0.05	Apple cider	3.4	*E. coli* O157:H7	25	6 h	Reduced > 5 log CFU/mL	Comes and Beelman (2002)
Fumaric acid + Sodium benzoate	0.15 0.05	Apple cider	3.2	*E. coli* O157:H7	5 15 25	55 h 16 h 4 h	Reduced > 5 log CFU/mL	Chikthimmah and others (2003)
Sodium benzoate + Sodium bisulfite	0.045 0.0046	Apple cider	3.5	*E. coli* O157:H7	a) 4 b) 10 c) 25	18 d	a) >5 log CFU/mL after 15 d b) >5 log CFU/mL after 12 d c) >5 log CFU/mL after 2 d	Fisher and Golden (1998b)

Table 7—Natural antimicrobials applied on fresh-cut fruits and fruit juices to control pathogenic and spoilage microorganisms.

Antimicrobial	Application	Antimicrobial effectiveness	Effect on the product flavor	Regulatory status	Reference
Chitosan	• Fresh-cut cantaloupe, pineapple, litchi, papayas, and mangoes applied as edible coatings. • Apple and apple-elderflower juices.	• Reduces native mesophilic bacteria and populations of inoculated *E. coli* and *S. cerevisiae*; and inhibits the growth of naturally occurring microorganisms on coated fresh-cut fruits. • Enhances the survival of *E. coli* O157:H7 and *S.* Typhimurium; reduces populations of native yeast and molds; and inhibits the growth of inoculated *S. cerevisiae* and *Pichia* spp. in fruit juices.	• Concentrations up to 3% of chitosan applied as an edible coating did not change the sensory attributes. A higher acceptability of the sensory attributes was obtained.	GRAS; GRN 73; GRN 170	Roller and Covill (1999), Rhoades and Roller (2000), Dong and others (2004), González-Aguilar and others (2005), Kiskó and others (2005), Chien and others (2007), USFDA (2007), Sangsuwan and others (2008)
Cinnamon powder	Apple juice.	• Reduces populations of *L. monocytogenes* and *E. coli* O157:H7.	• Not reported.	GRAS; 21 CFR 182.10 to 20;	Iu and others (2001), Yuste and Fung (2002), USFDA (2002), Ceylan and others (2004)
Essential oils of plants and active compounds (carvacrol, cinnamon, cinnamaldehyde, citral, cinnamic acid, citrus, clove, eugenol, garlic, geraniol, lemon, lime, lemongrass, mandarin, oregano, palmarose)	• Fresh-cut apple, pear, grape, peach, honeydew, tomato, and kiwifruits. • Fresh-cut apple and melons applied as edible coatings. • Apple, pear, melon, orange, strawberry, tomato, and watermelon juices.	• Reduces naturally occurring microbiota and populations of inoculated *E. coli* and *S. crevisiae* in fresh-cut fruits. • Inhibits naturally occurring microbiota; reduces populations of inoculated *E. coli* O157:H7, *S.* Enteritidis, and *L. innocua* in coated fresh-cut fruits. • Reduces populations of inoculated *S.* Hadar, *E. coli*, *E. coli* O157:H7, *L. innocua*, and *L. monocytogenes*, in fruit juices.	• Concentrations $\leq$ 0.015% of carvacrol and cinnamic acid; $\leq$0.020% of citrus, mandarin, lemon, and lime oils, did not affect the sensory attributes. • Concentrations $\geq$ 0.7% of cinnamon, lemongrass, palmarosa, and clove oils affected the sensory attributes.	GRAS; 21 CFR 182.10; 21 CFR 182.20; 21 CFR 182.1317; 21 CFR 182.60; 21 CFR 184.1257	Roller and Seedhar (2002), USFDA (2002), Friedman and others (2004), Lanciotti and others (2004), Liang and others (2006), Raybaudi-Massilia and others (2006, 2008b, 2008c), Ayala-Zavala and others (2007), Ferrante and others (2007), Rojas-Graü and others (2007a), Mosqueda-Melgar and others (2008a, 2008b, 2008c)
Hexanal, (E)-2-Hexenal, Trans-2-Hexenal, Hexyl acetate	• Fresh-cut apples.	• Prolongs the lag phase of native yeasts and mesophilic bacteria, and inoculated *E. coli*, *S.* Enteritidis, and *Pichia subpelliculosa*. • Reduces populations of *L. monocytogenes.*	• Not reported.	GRAS; 21 CFR 172.515	Lanciotti and others (1999), Corbo and others (2000), Lanciotti and others (2003)

Continued

Table 7—Continued.

Antimicrobial	Application	Antimicrobial effectiveness	Effect on the product flavor	Regulatory status	Reference
Lactoperoxidase	• Apple, orange, tomato, and pink grape juices.	• Reduces populations of *E. coli* O157:H7, *S.* Enteritidis and *Shigella* spp.	• Not reported.	GRAS; GRN 196; A404	FSANZ (2002), Touch and others (2004), Van Opstal and others (2006), USFDA (2007)
Lysozyme	• Orange juice.	• Reduces slightly populations of *S.* Typhimurium.	• Not reported.	GRAS, GRN 64; E-1105	Liang and others (2002), USFDA (2007)
Mint	• Tomato juice.	• Reduces occurring naturally microorganisms.	• Not reported.	GRAS; 21 CFR 182.20	USFDA (2002), Nguyen and Mittal (2007)
Methyl jasmonate	• Fresh-cut kiwifruit, pineapple, and tomato.	• Inhibits the growth of molds and native microbiota.	• Not reported.	GRAS; PAFA 980	Wang and Buta (2003), Martín-Ferrer and Harper (2005), Ayala-Zavala and others (2007), USFDA (2008)
Nisin	• Orange juice and apple cider.	• Reduces populations of *S.* Typhimurium and *E. coli* O157:H7.	• Not reported.	GRAS; GRN 65; 21 CFR 184.1538; E-234; A565; Codex Standard A-8	Iu and others (2001), Liang and others (2002), FSANZ (2007), USFDA (2007)
Vanillin	• Fresh-cut apples applied as edible coatings. • Fresh-cut mangoes. • Orange and apple juices. • Strawberry, apple, and banana purees.	• Reduces populations of *L. innocua*, *L. monocytogenes*; inhibits the growth of psychrophilic and mesophilic bacteria, yeast, and molds.	• Concentrations of 0.10 and 0.15% affected the sensory attributes, but pleasant flavor was observed. • Concentrations $\geq$ 0.2% affected the sensory attributes.	GRAS; 21 CFR 182.60	Cerruti and Alzamora (1996), Cerruti and others (1997), USFDA (2002), Corte and others (2004), Fitzgerald and others (2004b), Ngarmsak and others (2006), Vasantha-Rupasinghe and others (2006), Ferrante and others (2007), Rojas-Graü and others (2007a)

GRAS: generally recognized as safety; GRN = GRAS notice according to USFDA; A = application according to FSANZ; 21 CFR = Title 21 of the U.S. Code of Federal Regulations; E = number code of food additive of the European Union; PAFA = priority-based assessment for food additive.

inoculated in fresh-cut fruits are not available in the literature. However, their effects on pathogenic bacteria in fruit juices have been investigated. Van Opstal and others (2006) inactivated *E. coli* O157:H7 and *Shigella* spp. in freshly squeezed and pasteurized apple, orange, tomato, and pink grape juices with peroxidase systems such as lactoperoxidase (LPER)-thiocyanate and soybean peroxidase (SBP)-thiocyanate. Since, in the absence of peroxidase systems, these pathogenic microorganisms might survive for at least 24 h at 6 and 20 °C. These researchers concluded that LPER systems have more interesting properties as biopreservatives in acid juices than SPB systems; because the latter produced significant browning on some juices and caused little or no inactivation of *E. coli* O157:H7 and *Shigella* spp. in the respective juices. Reductions of more than 5 log CFU/mL of *E. coli* O157:H7 and *Shigella* spp. in freshly extracted and pasteurized apple juice stored at 6 and 20 °C for 24 h were found using 30 μg/mL of LPER. Addition of the same concentration to pasteurized commercial orange juice resulted in reductions of 2 and 5 log CFU/mL in *E. coli* O157:H7 and *Shigella* spp. counts, respectively. Nevertheless, no significant activity against inoculated pathogens was reported in freshly extracted orange juice regardless of storage temperature. This fact could be attributed to the additives added into pasteurized commercial orange juice, which may give an additional antimicrobial effect on microorganisms and/or pulp present in fresh juice, which could exert a protective effect on microorganisms. On the other hand, a slight effect, leading to $\leq$1 log CFU/mL reductions of *Shigella* spp., was observed in pasteurized tomato juice stored for 24 h at 20 °C. However, Touch and others (2004) reduced more than 5 log CFU/mL of *S.* Enteritidis in tomato juice treated with 14.8 μg/mL of a lactoperoxidase system after 3 (acid-adapted cells) and 4 h (nonadapted cells) of storage at 30 °C, in comparison with nontreated tomato juice, where any microbial reduction was observed. The differences found between both studies could be attributed to the sensitivity of each microorganism to the antimicrobial and storage temperature used, being the latter factor the most important in regulating the effectiveness.

Results reported here point out that lactoperoxidase system could be a good alternative thermal treatment for fruit juices, because more than 5 log reductions of pathogenic microorganisms might be reached; however, more studies are necessary to determine whether effective concentrations can alter their sensory attributes.

The use of other enzymes such as lysozyme to inactivate pathogenic and spoilage microorganisms has also been reported for fruit juices. Lysozyme is a protein present in milk and eggs that catalyzes the hydrolysis of the β-1,4 linkages between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of the bacterial cell wall. The FAO/WHO joint and several countries including Austria, Australia, Belgium, Denmark, Finland, France, Germany, Italy, Japan, Spain, and United Kingdom have approved its use in some foods when used in accordance with good manufacturing practices (GMP) (Losso and others 2000). Likewise, in 2000, the USFDA considered to lysozyme as GRAS (Table 7), through scientific procedures, for use as antimicrobial agent in casings for frankfurters (up to 5.5 mg/kg), on cooked meat and poultry products (up to 4.4 mg/kg), and cheese production (according to GMP) (in preventing late blowing caused by the bacterium *Clostridium tyrobutyricum*). However, hen eggs white proteins including ovomucoid (Gal d 1), ovalbumin (Gal d 2), conalbumin (Gal d 3), and lysozyme (Gal d 4), which reside in the egg white fraction, have traditionally been implicated in the development of food allergy. Although clinical reactions to lysozyme have rarely been reported, an immunoglobulin E (IgE)-mediated hypersensitivity reaction could occur in patients allergic to this enzyme (Pérez-Calderón and others 2007). Therefore, a condition for the safe use of lysozyme would be labeling to alert the sensitive population to the possible presence of this enzyme in foods (USFDA 2007).

Lysozyme is generally active against most Gram-positive bacteria, particularly thermophilic spore formers (Beuchat and Golden 1989). Hughey and Johnson (1987) reported that lysozyme is inhibitory to several food spoilage organisms as well as to some pathogens, including *L. monocytogenes*, *C. jejuni*, *B. cereus*, and *C. botulinum*. Gram-positive bacteria are more susceptible to lysozyme than Gram-negative bacteria due to the different contents of peptidoglycan in their cell walls. The former contain about 90% peptidoglycan and the latter one, 5% to 10%.

Very few studies have reported the use of lysozyme in fruit juices. Liang and others (2002) did not find significant reductions of *S.* Typhimurium in freshly squeezed nonpulpy (0.05 log CFU/mL) and pulpy (0.08 log CFU/mL) orange juice with added lysozyme (0.1 μg/mL) in comparison with the control juice (0.06 log CFU/mL); whereas in pasteurized juice, treated with the same concentration of lysozyme, a slight reduction was observed (1.3 log CFU/mL). The lower pH value of the pasteurized juice (pH 3.8) with respect to fresh juices (pH 4.06) might have contributed to the more extensive microbial inactivation. This fact suggests that antimicrobial action of lysozyme could be favored by low pH. In addition, Liang and others (2002) indicated that lysozyme combined with pulsed electric fields (PEF) and/or nisin had a greater bactericidal effect than either of them alone. Other studies have also reported the antimicrobial effect of lysozyme combined with nisin, PEF, high hydrostatic pressure (HHP), or heat in red grape and banana juice, and apple cider against *S.* Typhimurium, *Shigella flexneri*, *E. coli* O157:H7, *Yersinia enterocolitica*, and spoilage microorganisms (Wu and others 2005; Liang and others 2006; Nakimbugwe and others 2006).

The results indicate that lysozyme added alone to that concentration into fruit juices is ineffective against Gram-negatives. Therefore, other preservation methods such as thermal or nonthermal processing are needed to increase its antimicrobial effectiveness.

Polysaccharides. Chitosan is a heteropolysaccharide composed of $\beta-1$, 4-linked 2-amino-2-deoxy-β-D-glucose obtained commercially by deacetylation of chitin, which is an abundant constituent of crustacean shells and fungi (Rhoades and Roller 2000; Sebti and others 2005). Chitosan is considered a biocompatible, nonantigenic, nontoxic, and biofunctional food additive (Novack and others 2003; No and others 2007). In addition, shrimp-derived chitosan was admitted as GRAS (Table 7) in 2005 by the USFDA (2007), based on scientific procedures for use in foods in general in accordance with GMP. It is marketed as food additive or supplement in Japan, Korea, England, Italy, Portugal, and today in the United States (Novack and others 2003; No and others 2007). However, Barney (1998) indicated that anyone with shellfish allergy, pregnant, or nursing should avoid the use of chitosan products. Consequently, its use as an antimicrobial may be limited, and adequate labeling for alerting the population of its presence would be necessary.

The general properties and applications of chitin, chitosan, and their derivatives in foods have extensively been studied (Shahidi and others 1999; No and others 2007). However, their antimicrobial properties have been scarcely evaluated. Although it is more active against spoilage yeasts and molds (Rhoades and Roller 2000), chitosan has also been shown to inhibit some Gram-negative bacteria including *E. coli*, *Pseudomonas aeruginosa*, and *S.* Typhimurium (Helander and others 2001). The antimicrobial activity of chitosan towards Gram-negative bacteria may be attributed to its chemical and structural properties. Because of its macromolecular polymeric structure, chitosan is unable to pass

the outer membrane of Gram-negative bacteria (Nikaido 1996). Therefore, chitosan penetration into microbial cells is unlikely to occur. A key feature of chitosan is its positive charge of the amino group at C-2 below its p*Ka* (pH 6.3), which can create a polycationic structure and interact with anionic components such as lipopolysaccharides and proteins of the membrane cell surface (Begin and Van Calsteren 1999; Helander and others 2001). Binding of polycationic molecules has been shown to disrupt the integrity of the outer membrane resulting in loss of the barrier function but lacking direct bactericidal activity (Helander and others 2001).

The antimicrobial properties of chitosan-based coatings applied to fresh-cut fruits have been evaluated by several researches. Sangsuwan and others (2008) reduced populations of *E. coli* inoculated on fresh-cut cantaloupe by more than 5 log CFU/piece in 8 d at 10 °C using a chitosan (1.5% w/v)/methyl cellulose (0.5% w/v) film. Nevertheless, populations of *E. coli* on noncoated fresh-cut cantaloupe were also reduced by 4 log CFU/piece in 8 d at 10 °C. That latter fact could be due to storage temperature, whereas differences in reductions between coated and noncoated fresh-cut cantaloupe might be due to antimicrobial effect of chitosan. On the other hand, these same reserachers indicated that populations of *S. cerevisiae* on fresh-cut cantaloupe melon and pineapple coated with that antimicrobial film were reduced about 3 log CFU/piece in 4 d of storage at 10 °C. Similarly, González-Aguilar and others (2005) reported that the incorporation of chitosan of low and medium molecular weight at concentrations of 1% and 2% (w/v) into edible coatings affected the growth of mesophilic bacteria and fungi in coated fresh-cut papayas stored at 5 °C for 15 d. These researchers reported a 3 log CFU/g reduction in mesophilic bacteria counts during the storage time when chitosan coatings of low (2% w/v) and medium (1% and 2% w/v) molecular weight were used, in comparison with control sample, where microbial growth was observed during storage. In addition, the growth of yeasts and molds was completely inhibited throughout storage. Likewise, Chien and others (2007) found a substantial antimicrobial effect of an edible chitosan coating applied to sliced mango at concentrations of 0.5%, 1%, and 2% (w/v) and stored at 6 °C. A delay in the growth of naturally occurring microorganisms (from 3.82 to 5.53 log CFU/g) in comparison with the control (from 3.82 to 6.41 log CFU/g) was observed during storage. However, increasing the concentration of chitosan from 0.5 to 2% did not further delay microbial growth.

The effect of chitosan on pathogenic and spoilage microorganisms in fruit juices has also been reported. Kiskó and others (2005) observed that the addition of chitosan (0.05% or 0.1% w/v) to apple juice (pH 3.2) enhanced the survival of *E. coli* O157:H7 and *S.* Typhimurium from 1 to 2 d at 25 °C, and from 3 to 5 d at 4 °C only for *E. coli* O157:H7. However, these researchers indicated that yeasts such as *Metschnikowia pulcherrima* and *Kloeckera apiculata* were inactivated in apple juice supplemented with 0.05% chitosan and stored for 12 d at 25 °C, whereas the growth of *S. cerevisiae* and *Pichia* spp. was delayed for the duration of the experiment (12 d) in comparison with the control, where yeast growth reached levels of 7 to 8 log CFU/mL within 4 d. Roller and Covill (1999) demonstrated that chitosan at concentrations from 0.01% to 0.5% (w/v) was effective to inhibit the growth of yeasts and molds in apple juice (pH 3.4) stored at 25 °C for 10 d, since high levels (7 to 8 log CFU/mL) of fungi in control sample was observed at 1st day of storage. Likewise, Rhoades and Roller (2000) reported that the addition of 0.03% (w/v) chitosan to apple-elderflower juice (pH 3.3) completely inactivated yeasts during 13 d of storage at 7 °C, and the total microbial and lactic acid bacteria counts increased at lower rates than those observed in nontreated juices.

In general, chitosan has demonstrated to be effective in low concentrations (<1%) against mesophilic, yeast and mold populations in both fresh-cut fruits and fruit juices. Nevertheless, it has also shown to enhance the survival of pathogenic microorganisms in fruit juices, in contrast with fresh-cut fruit coated with an edible film containing chitosan, where a microbial reduction was noted. Therefore, new investigations are suggested to clear the effect of chitosan on pathogenic microorganisms.

Alternative natural antimicrobials from plant origin

Plant extracts. *Essential oils*. Essential oils (EOs), also called volatile or ethereal oils, are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twig bark, herbs, wood, fruits, and roots), which can be obtained by fermentation, extraction, or distillation, with distillation being the most commonly used method for the commercial production of these oils (Burt 2004). Essential oils are constituted of a complex mix of compounds including terpenes, alcohols, cetones, phenols, acids, aldehydes, and esters (Burt 2004; Ayala-Zavala and others 2005). EOs are mainly used as food flavorings, in perfumes (fragrances and aftershaves), and as functional components in pharmaceuticals (Nychas and others 2003). Individual components of EOs are also used as food flavorings; they are either extracted from plant material or are synthetically manufactured (Burt 2004). Although the majority of the EOs are classified as GRAS substances (Table 7) (USFDA 2006), their use in food as preservatives is often limited due to flavor considerations (Lambert and others 2001). Many herbs and plant extracts possess antimicrobial activities against a wide range of bacteria, yeasts, and molds (Beuchat 2001; Friedman and others 2002, 2004; Burt 2004; Raybaudi-Massilia and others 2006, 2008b, 2008c; Rojas-Graü and others 2006, 2007a, 2007b; Mosqueda-Melgar and others 2008a, 2008b, 2008c).

Although the antimicrobial properties of EOs and their components have been reviewed in the past, their mechanisms of action have not been studied in great detail. Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but to action over several specific targets in the cell (Burt 2004). Nychas and others (2003) and Burt (2004) have reported the location and mechanisms of action in the bacterial cell of EOs, for instance: degradation of the cell wall, damage to cytoplasmic membrane and membrane proteins, leakage contents out of the cell, coagulation of cytoplasm, and depletion of the proton motive force (Figure 2). Nychas and others (2003) indicated that the mode of action of EOs is concentration-dependent, indicating that low concentrations inhibit enzymes associated with energy production, while higher amounts may precipitate proteins.

EOs of oregano, savory, lemongrass, and active compounds such as thymol, eugenol, and carvacrol have been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of several microorganisms such as *E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *Lactobacillus sakei*, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis, and *S. aureus* (Lambert and others 2001; Gill and Holley 2006; Oussalah and others 2006; Raybaudi-Massilia and others 2006). However, Oussalah and others (2006) and Gill and Holley (2004, 2006) indicated that cinnamon oil and cinnamaldehyde produced a decrease in the intracellular ATP by ATPase activity without apparent changes on the cell membrane of *E. coli*, *E. coli* O157:H7, and *L. monocytogenes*. This fact could be attributed to interaction of cinnamaldehyde with the cell membrane, which may cause enough disruption to disperse the proton motive force by leakage of small ions but without leakage of larger cell molecules such as ATP. Wendakoon and

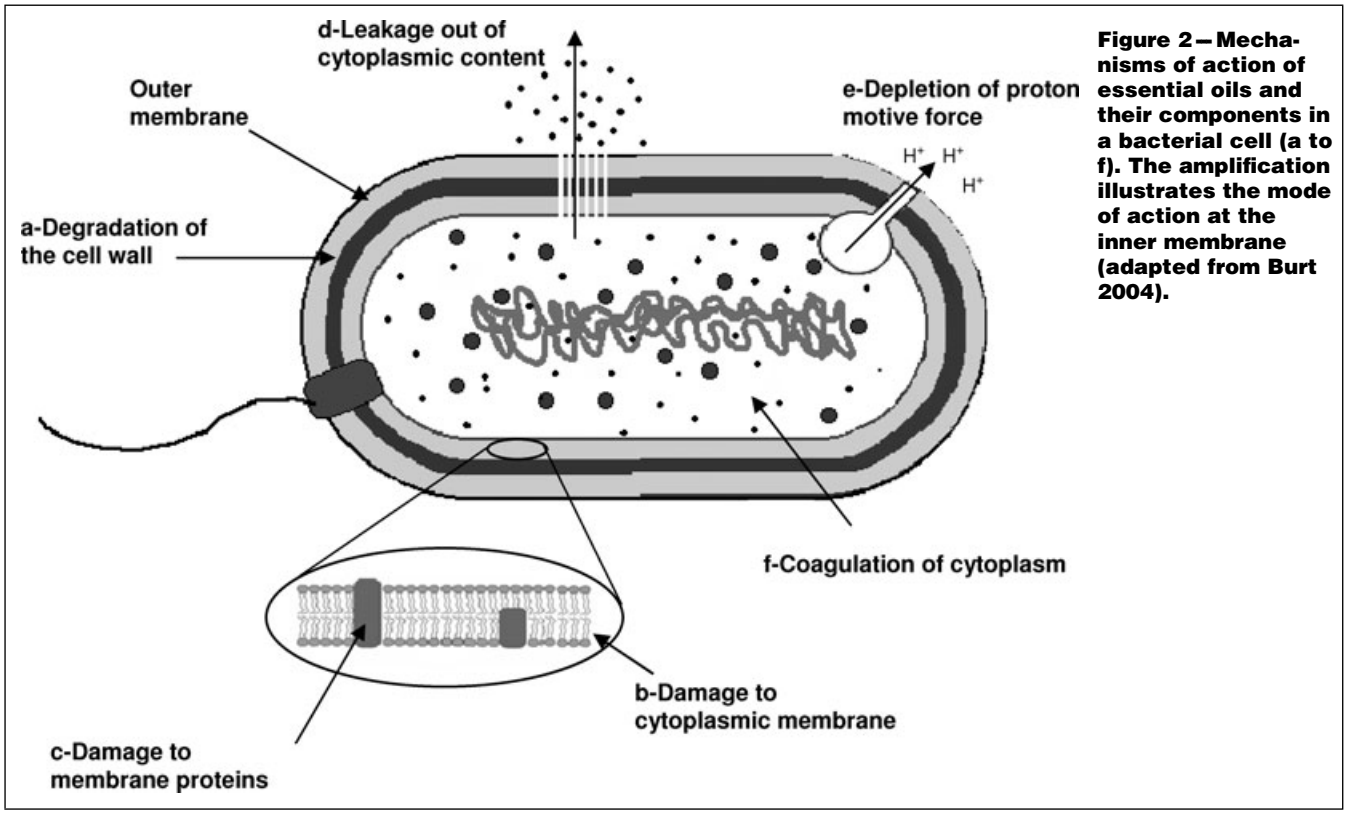

Figure 2—Mechanisms of action of essential oils and their components in a bacterial cell (a to f). The amplification illustrates the mode of action at the inner membrane (adapted from Burt 2004).

Sakaguchi (1995) reported a possible action of cinnamaldehyde on the embedded proteins in the cytoplasmic membrane of *Enterobacter aerogenes* by inhibition of amino acid decarboxylase enzymes, which are necessary for amino acid biosynthesis and biodegradation.

Different studies have demonstrated the effectiveness of EOs and their active compounds to control or inhibit the growth of pathogenic and spoilage microorganisms in both fresh-cut fruit and fruit juices (Table 8 and 9). That effectiveness depended on the pH of the fruit product, kind and concentration of used EOs or active compound, and microorganism type. In this way, Raybaudi-Massilia and others (2008c) using EOs (cinnamon and lemongrass) or their active compounds (eugenol, and citral) incorporated into an alginate-based edible coating and applied on fresh-cut apples found a higher effectiveness of those substances for reducing populations of inoculated *E. coli* O157:H7 during storage time than for populations of *S.* Enteritidis inoculated onto fresh-cut melons coated with the same edible coating (Raybaudi-Massilia and others 2008b) (Table 8). Differences between data could be attributed to the kind of fruit used, because fresh-cut apple had a lower pH value than fresh-cut melon (Table 8). Similar results with regard to pH differences were obtained by Mosqueda-Melgar and others (2008a), who reported higher reductions of *S.* Enteritidis and *E. coli* O157:H7 in strawberry and orange juices containing 0.1% (v/v) of cinnamon bark oil than in apple and pear juices under same conditions. Likewise, Mosqueda-Melgar and others (2008c) and Raybaudi-Massilia and others (2006) reported this same microbial behavior between melon and watermelon juices with added cinnamon bark oil; and among apple and pear juices in comparison with melon juice containing cinnamon oil, lemongrass oil, or geraniol. Burt (2004) stated that the bacterial susceptibility to EOs increases with a reduction in pH of the food, since at low pH the hydrophobicity of the oil increases, enabling it to more easily dissolve in the lipids of cell membrane of the target bacteria.

On the other hand, Raybaudi-Massilia and others 2008c indicated that lemongrass oil and its main active compound (citral) acted faster against *E. coli* O157:H7 at 0 d than cinnamon and clove oils or their active compounds (cinnamaldehyde and eugenol). This fact suggests that partition coefficients of the substances might influence its diffusion rate through the cell membrane, because a higher value of partition coefficient of the formers was reported (Raybaudi-Massilia and others 2008c). In addition, when higher concentrations of EOs were added onto fresh-cut fruit and fruit juices, a greater antimicrobial effectiveness of them on microorganisms was observed, but sensory attributes were seriously affected (Raybaudi-Massilia and others 2006, 2008b, 2008c).

Mosqueda-Melgar and others (2008c) indicated that *L. monocytogenes* was more sensitive to cinnamon bark oil than *E. coli* O157:H7 and *S.* Enteritidis inoculated into melon and watermelon juices (Table 9). This fact could be attributed to the outer membrane and lipopolysaccharide layer that possess the Gram-negative microorganisms (absent in Gram-positives), which can, in part, restrict diffusion of hydrophobic compounds toward the inside of the cell (Brul and Coote 1999; Burt 2004; Mosqueda-Melgar and others 2008c). However, Burt (2004) reported that not all studies on EOs have concluded that Gram-positive microorganisms are more susceptible than Gram-negative microorganisms. Therefore, more studies on this phenomenon in real food systems should be carried out in the future. Storage temperature is another important factor that may influence the antimicrobial effectiveness of EOs in fruit products. In such sense, Friedman and others (2004) observed that the bactericidal activity of different EOs or their active components against *E. coli* O157:H7 and *S.* Hadar in apple juice was higher at 37 °C than at 4 and 21 °C.

Table 8—Use of essential oils and their active compounds in fresh-cut fruits to control spoilage and pathogenic microorganisms.

Essential oil or active compound	Amount applied (%)	Mode of application	Fresh-cut fruit	pH	Target microorganism	Storage conditions		Effect	Reference
						Temp. (°C)	Time		
Cinnamon oil	a) 0.7	a) Incorporated into an alginate-based edible coating.	a) Apples.	a) 4.33	a) *E. coli* O157:H7 and native microbiota	a) 5	a) 30 d	a) Reduced > 4 log CFU/g of *E. coli* O157:H7 in 3 d. Inhibited native microbiota by 30 d.	a) Raybaudi-Massilia and others (2008c)
	b) 0.7	b) Incorporated into an alginate-based edible coating.	b) Melons.	b) 6.06	b) *S.* Enteritidis and native microbiota	b) 5	b) 21 d	b) Reduced > 4 log CFU/g of *S.* Enteritidis in 21 d. Inhibited native microbiota by 30 d.	b) Raybaudi-Massilia and others (2008b)
Citrus oil	0.02	Direct.	A mix of apple, pear, grape, peach, and kiwifruits.	–	*E. coli*, *S. cerevisiae*, and native microbiota	13	17 d	Inhibited native microbiota and inoculated *S. cerevisiae* by 17 d. Reduced the growth rate of *E. coli.*	Lanciotti and others (2004)
Clove oil	0.7	Incorporated into an alginate-based edible coating.	Apples.	4.33	*E. coli* O157:H7	5	30 d	Reduced > 4 log CFU/g in 7 d.	Raybaudi-Massilia and others (2008c)
Garlic oil	0.0333	As vapor.	Tomato.	4.34	Native microbiota	5	15 d	Inhibited native microbiota by 15 d.	Ayala-Zavala and others (2007)
Lemon oil	0.02	Direct.	A mix of apple, pear, grape, peach, and kiwifruits.	–	*E. coli*, *S. cerevisiae*, and native microbiota	13	17 d	Inhibited native microbiota and inoculated *S. cerevisiae* by 17 d. Reduced the growth rate of *E. coli.*	Lanciotti and others (2004)
Lemongrass oil	a) 1.0 to 1.5	a) Incorporated into an apple puree-alginate edible coating.	a) Apples.	a) 3.5 to 4.7	a) *L. innocua* and native microbiota	a) 5	a) 21 d	a) Reduced > 4 log CFU/g of *L. innocua* in 7 d. Inhibited native microbiota by 21 d.	a) Rojas-Graü and others (2007a)
	b) 0.7	b) Incorporated into an alginate-based edible coating.	b) Apples.	b) 4.33	b) *E. coli* O157:H7	b) 5	b) 30 d	b) Reduced > 4 log CFU/g of *E. coli* O157:H7 in 0 d. Inhibited native microbiota by 30 d.	b) Raybaudi-Massilia and others (2008c)
	c) 0.7	c) Incorporated into an alginate-based edible coating.	c) Melons.	c) 6.06	c) *S.* Enteritidis and native microbiota	c) 5	c) 21 d	c) Reduced > 4 log CFU/g of *S.* Enteritidis in 21 d. Inhibited native microbiota by 30 d.	c) Raybaudi-Massilia and others (2008b)

Continued

Table 8—Continued.

Essential oil or active compound	Amount applied (%)	Mode of application	Fresh-cut fruit	pH	Target microorganism	Storage conditions		Effect	Reference
						Temp. (°C)	Time		
Lime oil	0.02	Direct.	A mix of apple, pear, grape, peach, and kiwifruits.	–	*E. coli*, *S. cerevisiae*, and native microbiota	13	17 d	Inhibited native microbiota and inoculated *S. cerevisiae* by 17 d. Reduced the growth rate of *E. coli*.	Lanciotti and others (2004)
Mandarin oil	0.02	Direct.	A mix of apple, pear, grape, peach, and kiwifruits.	–	*E. coli*, *S. cerevisiae*, and native microbiota	13	17 d	Inhibited native microbiota and inoculated *S. cerevisiae* by 17 d. Reduced the growth rate of *E. coli*.	Lanciotti and others (2004)
Oregano oil	0.1 to 0.5	Incorporated into an apple puree-alginate edible coating.	Apples.	3.5 to 4.7	*L. innocua* and native microbiota	5	21 d	Reduced > 4 log CFU/g of *L. innocua* in 4 d. Inhibited native microbiota by 21 d.	Rojas-Graü and others (2007a)
Palmarose oil	0.7	Incorporated into an alginate-based edible coating.	Melons.	6.06	*S.* Enteritidis and native microbiota	5	21 d	Reduced > 4 log CFU/g of *S.* Enteritidis in 21 d. Inhibited native microbiota by 30 d.	Raybaudi-Massilia and others (2008b)
Cinnamic acid	0.015	Direct.	a) Kiwifruits and b) Honeydew melons.		Native microbiota	4 or 8	a) 5 d b) 10 d	a) Inhibited native microbiota by 5 d. b) Inhibited native microbiota by 8 d.	Roller and Seedhar (2002)
Citral	a) 0.5	Incorporated into an alginate-based edible coating.	a) Apples.	a) 4.33	a) *E. coli* O157:H7	5	a) 30 d	a) Reduced > 4 log CFU/g of *E. coli* O157:H7 in 14 d. Inhibited native microbiota by 30 d.	a) Raybaudi-Massilia and others (2008c)
	b) 0.5		b) Melons.	b) 6.06	b) *S.* Enteritidis and native microbiota		b) 21d	b) Reduced < 3.5 log CFU/g of *S.* Enteritidis in 21 d. Inhibited native microbiota by 30 d.	b) Raybaudi-Massilia and others (2008b)
Cinnamaldehyde	0.5	Incorporated into an alginate-based edible coating.	Apples.	4.33	a) *E. coli* O157:H7	5	30 d	Reduced > 4 log CFU/g of *E. coli* O157:H7 in 3 d. Inhibited native microbiota by 30 d.	Raybaudi-Massilia and others (2008c)
Eugenol	a) 0.5	Incorporated into an alginate-based edible coating.	a) Apples.	a) 4.33	a) *E. coli* O157:H7	5	a) 30 d	a) Reduced > 4 log CFU/g of *E. coli* O157:H7 in 14 d. Inhibited native microbiota by 30 d.	a) Raybaudi-Massilia and others (2008c)
	b) 0.5		b) Melons.	b) 6.06	b) *S.* Enteritidis and native microbiota		b) 21d	b) Reduced < 3.5 log CFU/g of *S.* Enteritidis in 21 d. Inhibited native microbiota by 30 d.	b) Raybaudi-Massilia and others (2008b)
Geraniol	0.5	Incorporated into an alginate-based edible coating.	Melons	6.06	*S.* Enteritidis and native microbiota	5	21 d	Reduced < 3.5 log CFU/g of *S.* Enteritidis in 21 d. Inhibited native microbiota by 30 d.	Raybaudi-Massilia and others (2008b)

Table 9—Use of essential oils and their active compounds in fruit juices to control pathogenic and spoilage microorganisms.

Essential oil or active compound	Concentration (%)	Fruit juice (pH)	Target microorganism	Storage conditions		Effect	Reference
				Temp. (°C)	Time		
Cinnamon oil	a) 0.2 b) 0.8 c) 0.1 to 0.3 d) 0.3 e) 0.01 to 0.06	a) Apple (4.20) and pear (3.97) b) Melon (5.91) c) Strawberry (3.16), orange (3.44), apple (4.46), pear (4.40), and tomato (4.30) d) Melon (6.11) and watermelon (5.73) e) Apple (3.7)	a and b) *L. innocua*, *S.* Enteritidis, *E. coli* c) *S.* Enteritidis *E. coli* O157:H7 d) *S.* Enteritidis *E. coli* O157:H7, *L. monocytogenes* e) *S.* Hadar, *E. coli* O157:H7	a and b) 35 c) 22 d) 22 e) 37	a and b) 24 h c) 1 h d) 1 h e) 1 h	a to c) Reduced > 5 log CFU/mL d) Reduced 3.1 to 3.9, 1.4 to 1.9, and 3.4 to 4.4 log CFU/mL of *S.* Enteritidis, *E. coli* O157:H7, and *L. monocytogenes*, respectively e) Reduced 50% of bacterial population	a and b) Raybaudi-Massilia and others (2006) c) Mosqueda-Melgar and others (2008a,b) d) Mosqueda-Melgar and others (2008c) e) Friedman and others (2004)
Clove oil	a) 3 to 5 b) 0.1 c) 0.019 to 0.075	a) Freshly squeezed apple cider (—) b) Tomato (4.2) c) Apple (3.7)	a) Native microbiota b) Native microbiota c) *S.* Hadar, *E. coli* O157:H7	a) 50 b) 50 c) 37	a) — b) 0.5 h c) 1 h	a) Reduced 0.06 to 0.07 log CFU/mL b) Reduced 3.9 log CFU/mL c) Reduced 50% of bacterial population	a) Liang and others (2006) b) Nguyen and Mittal (2007) c) Friedman and others (2004)
Lemon oil	0.008 to 0.093	Apple (3.7)	*S.* Hadar *E. coli* O157:H7	37	1 h	Reduced 50% of bacterial population	Friedman and others (2004)
Lemongrass oil	a) 0.2 b) 0.5 c) 0.097 to 0.079	a) Apple (4.20) and pear (3.97) b) Melon (5.91) c) Apple (3.7)	a and b) *L. innocua*, *S.* Enteritidis, *E. coli* c) *S.* Hadar, *E. coli* O157:H7	a and b) 35 c) 37	a and b) 24 h c) 1 h	a and b) Reduced > 5 log CFU/mL c) Reduced 50% of bacterial population	a and b) Raybaudi-Massilia and others (2006) c) Friedman and others (2004)
Lime oil	0.038 to 0.23	Apple (3.7)	*S.* Hadar *E. coli* O157:H7	37	1 h	Reduced 50% of bacterial population	Friedman and others (2004)
Oregano oil	0.006 to 0.023	Apple (3.7)	*S.* Hadar *E. coli* O157:H7	37	1 h	Reduced 50% of bacterial population	Friedman and others (2004)
Carvacrol	0.004 to 0.018	Apple (3.7)	*S.* Hadar *E. coli* O157:H7	37	1 h	Reduced 50% of bacterial population	Friedman and others (2004)
Cinnamaldehyde	0.018 to 0.094	Apple (3.7)	*S.* Hadar *E. coli* O157:H7	37	1 h	Reduced 50% of bacterial population	Friedman and others (2004)
Citral	a) 0.01 b) 0.008 to 0.07	a) Orange (3.5) b) Apple (3.7)	a) *L. monocytogenes* b) *S.* Hadar *E. coli* O157:H7	a) 45 b) 37	a) 0.5 h b) 1 h	a) Reduced 1.1 to 1.3 log CFU/mL b) Reduced 50% of bacterial population	a) Ferrante and others (2007) b) Friedman and others (2004)
Eugenol	0.02 to 0.05	Apple (3.7)	*S.* Hadar *E. coli* O157:H7	37	1 h	Reduced 50% of bacterial population	Friedman and others (2004)
Geraniol	a) 0.2 b) 0.6 c) 0.0069 to 0.025	a) Apple (4.20) and pear (3.97) b) Melon (5.91) c) Apple (3.7)	a and b) *L. innocua*, *S.* Enteritidis, *E. coli* c) *S.* Hadar, *E. coli* O157:H7	a and b) 35 c) 37	a and b) 24 h c) 1 h	a and b) Reduced > 5 log CFU/mL c) Reduced 50% of bacterial population	a and b) Raybaudi-Massilia and others (2006) c) Friedman and others (2004)

A greater fluidity of the microbial cell membrane at high temperatures could explain an increased cellular diffusion of antimicrobial substances. Aronsson and Rönner (2001) indicated that the temperature of the medium in which cells are suspended has a significant influence on determining membrane fluidity properties. At low temperatures, the phospholipids are closely packed into a rigid gel structure while at high temperatures, they are less ordered and the membrane has a liquid-crystalline structure.

In general, EOs have shown to possess strong antimicrobial activity against both pathogenic and spoilage microorganisms being greater in fruit products of low pH and stored above refrigeration temperatures. On the other hand, a higher value of partition coefficient of the main active compound of the EO would favor its diffusion through the cell membrane, and consequently, its antimicrobial action. In addition, the use of high concentrations of EOs or their active compounds as antimicrobial agents in fresh-cut fruit and juices would not be recommended because of their adverse effects on the sensory properties. Therefore, combinations with other preservation methods are required to decrease their impact on food flavor.

Aldehydes. Aldehydes are dominant compounds released by plant tissue through the lipoxygenase pathway after some damage (Lanciotti and others 1999). The precise action mode is not yet clear but passive diffusion across the plasma membrane is likely to occur. Once inside cells, aldehydes would react with nucleophilic groups playing a key role in living cells, namely sulfydryl groups present in proteins and lower-molecular-weight compounds such as glutathione. Although the precise targets in microbial cells remain unclear, the toxicity of these molecules seems to be dependent on affinity to membrane phospholipidic bilayer (Lanciotti and others 2003; Patrignani and others 2008).

On the other hand, Lanciotti and others (2003) pointed out that the effectiveness at low levels, the natural occurrence in several fruits, and the nonregulated doses, in addition to its GRAS status (Table 7) as flavoring agents makes these volatile compounds good candidates as antimicrobial agents to improve the safety and quality of minimally processed fruits. Nevertheless, high concentrations of these compounds may produce an undesirable hay-like flavor due to the oxidative rancidity of fatty acids through lipoxygenase pathway, thus limiting its use as antimicrobial in some foods (Fritsch and Gale 1977; Su and Wiley 1998; Lei and Boatright 2008).

The antimicrobial activity against pathogenic and spoilage species of some aldehydes such as hexanal, (E)-2-hexenal, trans-2-hexenal, and hexyl acetate, which are components of the aroma of many fruits and vegetables, has been demonstrated (Lanciotti and others 1999, 2003; Corbo and others 2000). Lanciotti and others (2003) reported significant extensions of the lag phase of *E. coli* (from 5 to approximately 35 h) and *S. Enteritidis* (from 10 to approximately 44 h) inoculated onto apple slices treated with hexanal (150 μL/L), hexyl acetate (150 μL/L), and (E)-2-hexenal (20 μL/L) stored at 20 °C. Whereas for *L. monocytogenes*, a bactericidal effect (4 to 5 log CFU/g) after 4 d, using volatile compounds, was found under the same experimental conditions, in comparison with control sample, where survival but not growth of *L. monocytogenes* in apple slices was detected. These authors indicated that higher resistance of Gram-negative bacteria to volatile compounds than Gram-positive bacteria is mainly attributed to the outer membrane, which acts as an efficient permeability barrier against macromolecules and hydrophobic substances, as well as to the high content in cyclopropane fatty acids of the inner membrane.

Lanciotti and others (1999) reported that the use of 0.225 μL/L hexanal prolonged the lag phase of native yeasts for 8 d, in comparison with control sample, on sliced apple stored at 15 °C under modified atmosphere (80% N_2 and 20% CO_2); whereas mesophilic bacteria growth was retarded by more than 20 d under the same conditions. Likewise, Corbo and others (2000) attained an extension of 13 (at 25 °C) and 10 (at 5 °C) d with regard to control in the lag phase of an inoculated spoilage yeast (*Pichia subpelliculosa*) on sliced apple treated with 0.3 μL/L of hexanal in vapor form (soaked filter paper disks introduced inside package before sealing) and packed under modified atmosphere (70% N_2 and 30% CO_2). On the other hand, concentrations of 0.06 μL/L of trans-2-hexenal applied in the same form than hexanal extended the lag phase up to 2 (at 25 °C) and 8 (at 5 °C) d with regard to control samples. Therefore, the antimicrobial activity of hexanal and trans-2-hexenal was influenced by the vapor pressure of the used compounds, which is temperature-dependent.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a phenolic aldehyde present in vanilla beans. Based on the studies conducted with *E. coli*, *Lactobacillus plantarum*, and *L. innocua* the inhibitory activity of vanillin resides primarily in its ability to negatively affect the integrity of the cytoplasmic membrane, with loss of ion gradients, pH homeostasis, and inhibition of respiratory activity, but keeping energy generation largely unaffected (Fitzgerald and others 2004a).

Vanillin is a GRAS flavoring compound (Table 7) widely used in ice cream, beverages, biscuits, chocolate, confectionary, desserts, and more (Walton and others 2003). However, its use as an antimicrobial in fruit juices and fruit juice-containing drinks may be limited, due to the formation of guaiacol (an "off-flavor" metabolic compound) catabolized from vanillin by several microorganisms including *Alicyclobacillus acidoterrestris*, *Bacillus megaterium*, *Streptomyces* spp., and *Rhodotorula rubra* (Lee and others 2002; Álvarez-Rodríguez and others 2003; Bahçeci and others 2005).

The bactericidal activity of vanillin on *L. innocua* (as microbial surrogate for *L. monocytogenes*) inoculated onto fresh-cut "Fuji" apples coated with an apple puree-alginate edible layer was studied (Rojas-Gräu and others 2007a). Significant reductions (3 log CFU/g) of *L. innocua* populations were reported on coated apple pieces compared to control samples by incorporating vanillin (0.3 and 0.6% w/v) into the edible coating formulations. The incorporation of vanillin was also effective in inhibiting the growth of psychrophilic bacteria and fungi on apple pieces, with maximal populations down to 10^3 CFU/g after 21 d of refrigerated storage, whereas in control sample that microbial level was found before 10 d. In the same way, Vasantha-Rupasinghe and others (2006) demonstrated that the incorporation of vanillin (0.18% w/v) into dipping treatments inhibited microbial growth on "Empire" and "Crispin" apple slices during 19 d of storage at 4 °C with regard to control. Likewise, Ngarmsak and others (2006) delayed the development of total aerobic bacteria and yeast and mold populations for up to 14 and 7 d in fresh-cut mangoes stored at 5 and 10 °C, respectively, using vanillin at 0.12% (w/v).

On the other hand, vanillin has also been used in fruit juice and purees to control pathogenic and spoilage microorganisms. Ferrante and others (2007) reduced *L. monocytogenes* populations in orange juice (pH 3.6) by 2 to 3 log cycles after a 30-min exposure to 0.10% or 0.15% (w/v) vanillin at 45 °C, but when 0.20% vanillin was applied 4 log CFU/mL reductions in less than 15 min were achieved. However, the latter concentration of vanillin imparted a strong flavor to the orange juice when panelists tested it. Similar reductions (4 to 5 log cycles) but in *L. innocua* counts were found by Corte and others (2004) adding to apple juice (pH 3.3) a higher concentration of vanillin (0.30% w/v), applying a higher exposure time (4 to 8 h), and a lower temperature (30 °C). Likewise, Moon and others (2006) reduced more than 5 log CFU/mL of *L. monocytogenes* and *E. coli* O157:H7 in apple juice (pH 3.42) supplemented with 0.6% (w/v) after 24 h of storage at 4 or 15 °C. In addition,

these researchers indicated that the antimicrobial effect of vanillin was enhanced when lower pH and higher temperature were applied. Cerruti and others (1997) evaluated the use of vanillin as a natural antimicrobial for producing shelf-stable strawberry puree (pH 3.1). They prevented the growth of both native (aerobic, anaerobic mesophilic, and yeasts and molds) and inoculated flora (*S. cerevisiae*, *Zygosaccharomyces rouxii*, *Z. bailii*, *Schizosaccharomyces pombe*, *Pichia membranaefaciens*, *Botrytis* spp., *Byssochlamys fulva*, *Bacillus coagulans*, and *Lactobacillus delbrueckii*) for more than 60 d of storage at room temperature in pasteurized strawberry puree containing 0.3% (w/v) vanillin. In the same way, Cerruti and Alzamora (1996) inhibited the growth of *S. cerevisiae*, *Z. rouxii*, *Debaryomyces hansenii*, and *Z. bailii* in apple purée (pH 3.5) containing 0.2% (w/v) of vanillin for 40 d stored at 27 °C. But the addition of vanillin to the banana puree (pH 4) at the same concentration was not effective to inhibit the growth of *Z. rouxii* and *S. cerevisiae*. These researchers attributed the lack of antimicrobial activity to the high lipid/protein levels found in bananas, since interactions could reduce the quantity of vanillin available to act as an antimicrobial. On the other hand, Fitzgerald and others (2004b) reported that concentrations of 0.30% and 0.15% (v/v) vanillin added to pasteurized apple juice (pH 3.5) and a peach-flavored soft drink (pH 3.1), respectively, were required to inactivate (about 10^4 CFU/mL) and inhibit both inoculated *S. cerevisiae* and *Candida parapsilosis* over 56 d of storage at 25 °C. Nonetheless, when storage temperature was reduced to 8 °C the effective levels of vanillin were 0.075% and 0.015% (v/v).

Vanillin has been demonstrated to be effective against both pathogenic and spoilage microorganisms in fresh-cut fruit and fruit juices; however, effective concentrations (>0.2%) may be a hurdle, because a strong flavor in the fruit products can be imparted. Therefore, combinations of preservation methods are required for decreasing its impact on the flavor.

Esters. Methyl jasmonate is a natural compound derivate of jazmine (Ayala-Zavala and others 2005) with GRAS status (Table 7) in accordance with USFDA (2008), which is found as a lipid of plant cell membranes, synthesized via the lipoxygenase pathway (Ippolito and Nigro 2003). Some of its properties are: to regulate plant growth, to promote the closing of stomas, to act as second messenger, and to decrease the pathogen's attack (Ayala-Zavala and others 2005).

No references reporting the use of methyl jasmonate in fresh-cut fruits to control pathogenic microorganisms are available, although different studies have been published about the effectiveness of methyl jasmonate to reduce the spoilage of whole products of plant origin (Ayala-Zavala and others 2005). Wang and Buta (2003) reported that methyl jasmonate in concentrations of 11.2 and 22.4 μL/L applied as vapor (spotting the volatile compound onto filter paper strip hanging inside the container before the lids were covered) was effective for preventing mold growth in fresh-cut kiwifruit during 3 wk of storage at 10 °C. Likewise, Martínez-Ferrer and Harper (2005) reached 3 log CFU/g reductions of the native microbiota on fresh-cut pineapple after 12 d of storage at 7 °C when treating with an emulsion of methyl jasmonate in a concentration of 15 μL/L. In addition, those authors indicated that methyl jasmonate in the same concentration, but applied as a vapor (on cotton soaked with the volatile compound into container), was less effective in reducing the microbial population. Consistently, Ayala-Zavala and others (2007) demonstrated that methyl jasmonate in a concentration of 22.4 μL/L applied as vapor (spotting the volatile compound onto filter paper strip hanging inside the container before the lids were covered) suppressed microbial proliferation in fresh-cut tomato stored at 5 °C for 15 d. However, a combination of methyl jasmonate (22.4 μL/L) and ethanol (300 μL/L) applied as vapor was more effective in inhibiting the microbial growth through the storage period than the individual treatments with each volatile compound.

Methyl jasmonate in fruit products has only been proved on naturally occurring microorganisms with demonstrated effectiveness on them. Nonetheless, more inhibition than reduction was observed when applied as vapor. Thus, further studies on effective concentrations and behavior of pathogenic microorganisms in foods treated with this volatile compound are still necessary.

Herbs and spices. Mint belonging to the genus *Mentha* in the family Lamiaceae, consisting of about 25 to 30 species, including peppermint (*Mentha piperita* L.) and spearmint (*Mentha spicata*) as the most common species. This family is a rich source of polyphenolic compounds, flavonoids, terpenoids, and other volatile compounds, hence could possess strong antimicrobial and antioxidant properties (Gulluce and others 2007). The antimicrobial properties of mint against pathogenic microorganisms have already been demonstrated (Tassou and others 1995, 2000). However, the use of mint extract to control pathogenic and deteriorative microorganisms in fresh-cut fruits has not yet been reported in the literature. Indeed, scarce information is available on fruit juice applications. Nguyen and Mittal (2007) reported 4.77 and 8.34 log CFU/mL reductions in the native flora of pasteurized tomato juice intentionally spoiled when mint crystals at 0.1% and 1.2% (w/v) were used, respectively, with heat (50 °C). On the other hand, these researchers reduced 0.74 CFU/mL of native microorganisms when without mint tomato juice was only heated at 50 °C. Therefore, mint has demonstrated to be an alternative agent; however, studies about its sensory impact are still needed.

Cinnamon powder obtained from bark is widely used as a spice with antioxidant and antimicrobial activities. It contains cinnamaldehyde and eugenol as the major compounds with antimicrobial effects. The use of this spice to control pathogenic and spoilage microorganisms in fresh-cut fruits has not been reported in the literature. However, its use to inactivate pathogenic microorganisms such as *L. monocytogenes* and *E. coli* O157:H7 in fruit juices has been studied. Yuste and Fung (2002) reached 4 to 6 log CFU/mL reductions of *L. monocytogenes* inoculated in pasteurized apple juice with 0.1%, 0.2%, and 0.3% (w/v) of ground cinnamon after 1 h of incubation at 5 and 20 °C. In addition, no growth of the microorganism occurred during 7 d of storage. On the other hand, Ceylan and others (2004) demonstrated that the addition of 0.3% (w/v) cinnamon powder into pasteurized apple juice gradually decreased the counts of *E. coli* O157:H7 in 1.6 (8 °C) and 2 (25 °C) log CFU/mL after 14 and 3 d, respectively. In contrast, Iu and others (2001) reported an immediate 2 log CFU/mL reduction of *E. coli* O157:H7 in unpasteurized apple cider maintained at 42 °C by adding 2% (w/v) cinnamon powder.

The results obtained show that cinnamon powder was more effective against *L. monocytogens* than *E. coli* O157:H7 under similar experimental conditions. Moreover, storage temperature plays an important role in the antimicrobial effectiveness of it, being more effective at higher temperatures.

Natural antimicrobials of microbial origin

Bacteriocins. Nisin is a small, heat-stable antimicrobial peptide of 34 amino acids produced by *Lactococcus lactis* subsp. *lactis* (Davidson and Zivanovic 2003), which has been described as a class 1 bacteriocin, a group that comprises lantibiotics, which are a family of membrane-active peptides containing the unusual thioether amino acids lanthionine and β-methyl lanthionine, as well as other modified amino acids such as dehydrated serine and threonine. Nisin has shown a narrow antimicrobial spectrum, inhibiting only Gram-positive bacteria, including *Alicyclobacillus*, *Bacillus cereus*, *Brochothrix thermosphacta*, *C. botulinum*,

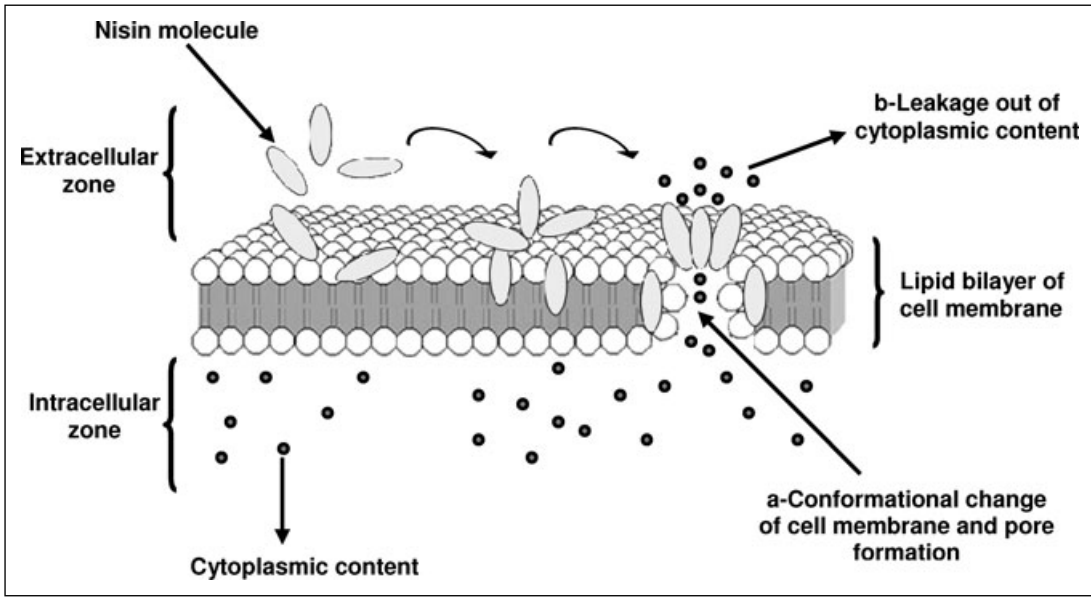

Figure 3—Mechanism of action of nisin in a bacterial cell (a and b).

C. sporogenes, Desulfotomaculum, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *L. monocytogenes*, *Micrococcus*, *Pediococcus*, *Sporolactobacillus*, and *Staphylococcus*. Against bacterial spores, nisin is sporostatic rather than sporicidal. On the other hand, nisin does not generally inhibit Gram-negative bacteria, yeasts, or molds. The activity spectrum includes Gram-negative bacteria when used in combination with chelating agents (such as EDTA). Nisin activity generally increases at low pH and low initial microbial loads (Davidson and Zivanovic 2003; Ross and others 2003).

In vegetative cells the primary site of action for nisin is the cytoplasmic membrane where it forms pores, thus destroying membrane integrity (Figure 3), and acts as a voltage-dependent polarizer (Abee and others 1994; Ross and others 2003). Pore formation results in depletion of proton motive force and loss of cellular ions, amino acids, and ATP (Crandall and Montville 1998; Davidson and Zivanovic 2003). Other action mechanisms against vegetative cells have been proposed for nisin, including interference with cell wall biosynthesis, although some researchers have indicated that this may simply be a consequence of energy loss and membrane depolarization resulting from pore formation and induction of autolysis (Thomas and others 2000).

The FSANZ (2007) and Codex Standards have permitted the use of nisin in foods including meat (up to 12.5 mg/kg), poultry (up to 12.5 mg/kg), and dairy (according to GMP) products, fruit and vegetables juices (according to GMP), and egg products (according to GMP). Likewise, the USFDA (2007) approved nisin as GRAS, through scientific procedures, for use on casing for frankfurters (up to 6.9 mg/kg), meat and poultry products (up to 5.5 mg/kg).

The effectiveness of nisin used alone against pathogenic microorganisms in fresh-cut fruits has not been found in the literature. However, Ukuku and Fett (2004) reported reductions of 1 and 1.4 log CFU/g *Salmonella* in fresh-cut cantaloupe melon using combinations of nisin (50 μg/mL), EDTA (0.02 M), sodium lactate (2% v/v), and potassium sorbate (0.02% v/v). On the other hand, Ukuku and Fett (2002) reached 2 log CFU/g reductions in the mesophilic aerobic and lactic acid bacteria populations on fresh-cut cantaloupe melon after washing with a solution containing 10 μg/mL nisin and 0.02 M EDTA. Nevertheless, those researchers indicated that the growth of Gram-negative bacteria such as Pseudomonas and yeasts and molds during 15 d of storage at 5 °C was not inhibited with that dipping treatment.

The effect of nisin alone or in combination with other preserving technologies over pathogenic microorganisms in fruit juices has been evaluated by several researchers. Liang and others (2002) did not find significant reductions of *S.* Typhimurium in nonpulpy (0.1 log CFU/mL), pulpy (0.1 log CFU/mL), and pasteurized freshly squeezed (1.5 log CFU/mL) orange juice by adding 0.1 μg/mL nisin in comparison with control samples. However, they found that the use of a combination of a PEF treatment (30 pulses of 90kV/cm) with nisin slightly reduced *S.* Typhimurium counts in nonpulpy (0.25 log CFU/mL), pulpy (0.20 log CFU/mL), and pasteurized freshly squeezed (2.95 log CFU/mL) orange juice. Likewise, Iu and others (2001) achieved a 4.63 log CFU/mL reduction of *E. coli* O157:H7 in unpasteurized apple cider using 20 μg/mL nisin; whereas a higher reduction (8.78 log CFU/mL) was achieved when nisin was combined with a PEF treatment (10 pulses of 80 kV/cm). Greater reductions found in this latter study were due probably to the higher concentrations of nisin used in comparison with the former one.

On the other hand, the use of nisin in combination with other preservation methods has been shown to effectively control naturally occurring microbes in fruit juices. Thus, Wu and others (2005) reported a 6.2 log CFU/mL reduction of the naturally occurring biota of intentionally spoiled pasteurized red grape juice when applying a combination of nisin (4 μg/mL), heat at 51 °C and PEF (20 pulses of 80 kV/cm). On the other hand, Nguyen and Mittal (2007) achieved a 0.85 log CFU/mL reduction in the naturally occurring microbiota of intentionally spoiled pasteurized tomato juice by applying a combination of nisin (4 μg/mL) with a thermal treatment at 50 °C, whereas heat treatment of juice caused only a reduction of 0.74 log CFU/mL. Nonetheless, those researchers found a higher reduction (4.4 log CFU/mL) of the natural microbiota when a combination of nisin, heat at 50 °C, and PEF (20 pulses of 80 kV/cm) was used.

The application of nisin alone in low concentrations as an antimicrobial agent in fruit products showed scarce effectiveness against Gran-negative bacteria; therefore, combinations of preservation methods to achieve a higher effectiveness are necessary.

Conclusions

The information compiled in this review demonstrates that different natural antimicrobials of animal, plant, and microbial origin, directly or indirectly added to fresh-cut fruits and fruit juices, can effectively reduce or inhibit pathogenic and spoilage microorganisms, thus representing a good alternative to the use of traditional antimicrobials. However, the extraction and purification of some natural antimicrobials can be difficult and expensive. Isolation and purification procedures that may avoid denaturalization, breakdown, volatilization, and/or loss of functional properties of active compounds, as well as safety and toxicology evaluations could be implicated.

On the other hand, the addition of antimicrobials to these products without adversely affecting the sensory characteristics is still a challenge for researchers, since the concentrations that are necessary to ensure safety (up to 5 log CFU/g reductions in the most resistant pathogenic microorganism, based on USFDA (2002) regulation) of fresh-cut fruits and fruit juices are several times higher than those accepted by consumers from sensory point of view. Therefore, new studies combining the use of antimicrobials with other methodologies of food preservation are necessary to reduce the impact of these compounds on sensory properties.

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