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Application of Natural Antimicrobials for Food Preservation

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In this review, antimicrobials from a range of plant, animal, and microbial sources are reviewed along with their potential applications in food systems. Chemical and biochemical antimicrobial compounds derived from these natural sources and their activity against a range of pathogenic and spoilage microorganisms pertinent to food, together with their effects on food organoleptic properties, are outlined. Factors influencing the antimicrobial activity of such agents are discussed including extraction methods, molecular weight, and agent origin. These issues are considered in conjunction with the latest developments in the quantification of the minimum inhibitory (and noninhibitory) concentration of antimicrobials and/or their components. Natural antimicrobials can be used alone or in combination with other novel preservation technologies to facilitate the replacement of traditional approaches. Research priorities and future trends focusing on the impact of product formulation, intrinsic product parameters, and extrinsic storage parameters on the design of efficient food preservation systems are also presented.

KEYWORDS: Antimicrobial activity; chemical compounds; plant/animal/microbial antimicrobials mechanism; minimum inhibitory concentration

INTRODUCTION

A number of nontraditional preservation techniques are being developed to satisfy consumer demand with regard to nutritional and sensory aspects of foods. Generally, foods are thermally processed by subjecting them to temperatures varying from 60 to 100 °C for the duration of a few seconds to a minute in order to destroy vegetative microorganisms. During this period of treatment, a large amount of energy is transferred to the food. However, this energy can trigger unwanted reactions, leading to undesirable organoleptic and nutritional effects (1). Ensuring food safety and at the same time meeting such demands for retention of nutrition and quality attributes has resulted in increased interest in alternative preservation techniques for inactivating microorganisms and enzymes in foods. Quality attributes of importance include flavor, odor, color, texture, and nutritional value. This increasing demand has opened new dimensions for the use of natural preservatives derived from plants, animals, or microflora. In biopreservation, storage life is extended, and/or safety of food products is enhanced by using natural or controlled microflora, mainly lactic acid bacteria (LAB) and/or their antibacterial products such as lactic acid, bacteriocins, and others (2). Typical examples of investigated compounds are lactoperoxidase (milk), lysozyme (egg white, figs), saponins and flavonoids (herbs and spices), bacteriocins (LAB), and chitosan (shrimp shells) (3). Antimicrobial compounds present in foods can

extend the shelf life of unprocessed or processed foods by reducing the microbial growth rate or viability (4). Originally, spices and herbs were added to change or to improve taste. Some of these substances are also known to contribute to the self-defense of plants against infectious organisms (5, 6).

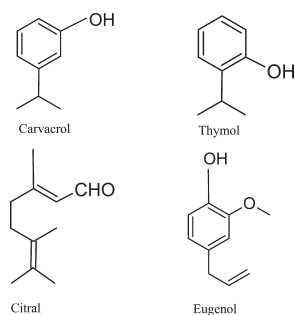
Extensive research has investigated the potential application of natural antimicrobial agents in food preservation. In this review, antimicrobials and their chemical and biochemical components from a range of natural sources and their applications in food systems are reviewed. Natural antimicrobials in food preservation can be used alone or in combination with other nonthermal technologies. Naturally derived antimicrobial systems from plant, animal, and microbial origin are detailed, and the latest developments in the quantification of the minimum (and noninhibitory) concentration of antimicrobials and/or their components are presented.

PLANT ORIGIN ANTIMICROBIAL AGENTS

Edible, medicinal, and herbal plants and their derived essential oils (EO) (and their hydrosols, i.e., byproducts of an essential oil purification procedure) and isolated compounds contain a large number of secondary metabolites that are known to retard or inhibit the growth of bacteria, yeast, and molds (7, 8). Many of these compounds are under investigation and are not yet exploited commercially. The antimicrobial compounds in plant materials are commonly found in the essential oil fraction of leaves (rosemary, sage, basil, oregano, thyme, and marjoram), flowers or buds (clove), bulbs (garlic and onion), seeds (caraway,

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Scheme 1. Plant Origin Antimicrobial Agents



fennel, nutmeg, and parsley), rhizomes (asafetida), fruits (pepper and cardamom), or other parts of plants (9, 10). Plant EOs and their constituents have been widely used as flavoring agents in foods since the earliest recorded history, and it is well established that many have a wide spectra of antimicrobial action (11–15). These compounds may be lethal to microbial cells or they might inhibit the production of secondary metabolites (e.g., mycotoxins) (16). Plant essential oils are generally more inhibitory against Gram-positive than Gram-negative bacteria (10, 17, 18). While this is true for many EOs, there are some agents that are effective against both groups, such as oregano, clove, cinnamon, and citral (19–21). The major EO components with antimicrobial effects found in plants, herbs, and spices are phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids, and iso-flavonoids (8, 22–27). Chemical analysis of a range of EOs revealed that the principal constituents of many include carvacrol, thymol, citral, eugenol (see Scheme 1 for their chemical structure), and their precursors (8, 28–30). It has been reported that some nonphenolic constituents of EOs are more effective or quite effective against Gram-negative bacteria, e.g., allyl isothiocyanate (AIT) (31) and garlic oil (32), respectively. In addition, AIT is also effective against many fungi (33). Generally, the antimicrobial efficacy of EOs is dependent on the chemical structure of their components as well as the concentration. Many of the antimicrobial compounds present in plants can be part of their pre- or postinfectious defense mechanisms for combating infectious or parasitic agents (34). Consequently, plants that manifest relatively high levels of antimicrobial action may be sources of compounds that inhibit the growth of foodborne pathogens (35). Compounds are also generated in response to stress from inactive precursors (36), which may be activated by enzymes, hydrolases or oxidases, usually present in plant tissues (37). In mustard and horse radish, precursor glucosinolates are converted by enzyme myrosinase to yield a variety of isothiocyanates including the allyl form, which is a strong antimicrobial agent (38).

The application of plant EOs for controlling the growth of foodborne pathogens and food spoilage bacteria requires evaluation of the range of activity against the organisms of concern to a particular product, as well as effects on a food's organoleptic properties. Plant EOs are usually mixtures of several components. Oils with high levels of eugenol (allspice, clove bud and leaf, bay, and cinnamon leaf), cinnamamic aldehyde (cinnamon bark and cassia oil), and citral (lemon myrtle, *Litsea cubeba*, and lime) are usually strong antimicrobials (39, 40). The EOs from *Thymus* spp. possess significant quantities of phenolic monoterpenes and have reported antiviral (41), antibacterial (42, 43), and antifungal (44, 45) properties. The volatile terpenes carvacrol, *p*-cymene, γ -terpinene, and thymol contribute to the antimicrobial activity of oregano, thyme, and savory (18). The antimicrobial activity of sage and rosemary can be attributed to borneol and other phenolic compounds in the terpene fraction. Davidson and Naidu (40) reported that the terpene thejone was responsible

for the antimicrobial activity of sage, whereas in rosemary, a group of terpenes (borneol, camphor, 1,8 cineole, *a*-pinene, camphone, verbenonone, and bornyl acetate) was responsible. Plant EOs such as cumin, caraway, and coriander have inhibitory effects on organisms such as *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* (46, 47), marjoram and basil have high activity against *B. cereus*, *Enterobacter aerogenes*, *Escherichia coli*, and *Salmonella*, and lemon balm and sage EOs appear to have adequate activity against *L. monocytogenes* and *S. aureus* (10). Gutierrez et al. (10) showed that oregano and thyme EOs had comparatively high activity against enterobacteria (minimum inhibitory concentration (MIC) of oregano and thyme at a range of 190 ppm and 440 ppm, respectively, for *E. cloacae*), lactic acid bacteria (MIC of oregano and thyme at a range of 55 ppm and 440 ppm, respectively, for *Lactobacillus brevis*), *B. cereus* (MIC of oregano and thyme at a range of 425 ppm and 745 ppm, respectively, for *Lactobacillus brevis*), and *Pseudomonas* spp (MIC of oregano and thyme at a range of 1500 ppm for *P. putida*), although in general *Pseudomonas* species are consistently highly resistant to plant antimicrobials (10, 48). One of the attributed factors can be the production of exopolysaccharide layers forming biofilms of the microorganism that can delay penetration of the antimicrobial agent (49).

Lee et al. (50) investigated the antibacterial activity of vegetables and juices and concluded that green tea and garlic extracts have broad applications as antibacterial agents against a wide range of pathogens. Arrowroot tea extract has reported antimicrobial activity against *E. coli* O157:H7 (19). Ibrahim et al. (35) reported the potential of caffeine at a concentration of 0.5% or higher as an effective antimicrobial agent for the inactivation of *E. coli* O157:H7 in a liquid system (i.e., brain heart infusion (BHI)).

Mechanisms of Antimicrobial Action. The possible modes of action for phenolic compounds (EO fractions) as antimicrobial agents have been previously reviewed (16, 24, 27, 36, 51–53). However, the exact mechanism of action is not clear. The effect of phenolic compounds can be concentration dependent (54). At low concentration, phenols affect enzyme activity, particularly those associated with energy production, while at high concentrations, they cause protein denaturation. The antimicrobial effect of phenolic compounds may be due to their ability to alter microbial cell permeability, thereby permitting the loss of macromolecules from the interior (for example ribose and Na glutamate) (55). They could also interfere with membrane function (electron transport, nutrient uptake, protein, nucleic acid synthesis, and enzyme activity) (55) and interact with membrane proteins, causing deformation in structure and functionality (56–58). The high antibacterial activity of phenolic components can be further explained in terms of alkyl substitution into the phenol nucleus (25). The formation of phenoxyl radicals that interact with alkyl substituents does not occur with more stable molecules such as the ethers myristicin or anethole, which was related to the relative lack of antimicrobial activity of fennel, nutmeg, or parsley EOs (10).

Delaquis and Mazza (38) reported that the antimicrobial activity of isothiocyanates derived from onion and garlic is related to the inactivation of extracellular enzymes through oxidative cleavage of disulfide bonds and that the formation of the reactive thiocyanate radical was proposed to mediate the antimicrobial effect. Carvacrol, (+)-carvone, thymol, and *trans*-cinnamaldehyde are reported to decrease the intracellular ATP (adenosine triphosphate) content of *E. coli* O157:H7 cells while simultaneously increasing extracellular ATP, indicating the disruptive action of these compounds on the plasma membrane (59). Inactivation of yeasts can be attributed to the disturbance of several enzymatic systems, such as energy production and structural component synthesis (60).

193 **Factors Affecting Antimicrobial Activity.** Antimicrobial activity
194 of EOs is influenced by a number of factors including botanical
195 source, time of harvesting, stage of development, and method of
196 extraction (61). For example, Chorianopoulos et al. (62) reported
197 that *Satureja* EOs obtained during the flowering period were the
198 most potent with bactericidal properties. The composition, struc-
199 ture as well as functional groups of the oils play an important role
200 in determining their antimicrobial activity. Usually compounds
201 with phenolic groups are the most effective (5, 25). Most studies
202 related to the antimicrobial efficacy of EOs have been conducted
203 in vitro using microbiological media (63–71). Consequently,
204 there is less understanding related to their efficacy when applied
205 to complex food systems. Key areas requiring further knowledge
206 for optimized application of natural antimicrobials in food
207 include targeting the microorganism of concern, the intelligent
208 use of combinations to provide a synergy of activity, matching the
209 activity of the compounds to the composition, and processing and
210 storage conditions of the food (9, 72).

211 Plant EOs of thyme, clove, and pimento were tested against
212 *Listeria monocytogenes* and were found to be highly effective in
213 peptone water. However, when the EOs were applied in a food
214 system, Singh et al. (73) concluded that efficacy of EOs was reduced
215 due to interaction with food components. In general, higher
216 concentrations of EOs are required in foods than in laboratory
217 media. Combinations of EOs could minimize the application
218 concentrations required, thereby reducing any adverse organolep-
219 tical impact; however, their application for microbial control may
220 also be affected by food composition (74). The antimicrobial
221 efficacy of EOs was found to be a function of ingredient manipula-
222 tion, for example, the antimicrobial activity of thyme is increased in
223 high protein concentrations, concentrations of sugars above 5% on
224 the microbial growth medium did not reduce EO efficacy, and high
225 potato starch concentrations decreased the EO antimicrobial
226 activity of oregano and thyme on *L. monocytogenes* in food model
227 systems (74, 75). Finally, low pH values (of the range of 5) seemed to
228 have the highest impact on the increase of the antimicrobial effect of
229 EOs on *L. monocytogenes* (74). Low pH values appear to increase
230 the hydrophobicity of EOs, consequently enabling easier dissolu-
231 tion in the lipids of the cell membrane of target bacteria (54).

232 Accordingly, the challenge for practical application of EOs is
233 to develop optimized low dose combinations to maintain product
234 safety and shelf life, thereby minimizing the undesirable flavor
235 and sensory changes associated with the addition of high con-
236 centrations of EOs.

237 ANIMAL ORIGIN ANTIMICROBIAL AGENTS

238 There are numerous antimicrobial systems of animal origin,
239 where they have often evolved as host defense mechanisms.
240 Lysozyme is a bacteriolytic enzyme, commercially sourced from
241 hen's egg white which is reported to inhibit the outgrowth of
242 *Clostridium tyrobutyricum* spores in semihard cheeses (76). Lyso-
243 zyme has found commercial applications; inovapure is said to be
244 effective against a wide range of food spoilage organisms and can
245 be successfully used to extend the shelf life of various food
246 products, including raw and processed meats, cheese, and other
247 dairy products. The lactoperoxidase system, which is naturally
248 active in milk, has strong antimicrobial effects against both
249 bacteria and fungi. A wide range of both Gram-negative (77)
250 and Gram-positive bacteria (78) are inhibited by the lactoperox-
251 idase system. However, studies have shown that Gram-negative
252 bacteria were generally found to be more sensitive to lactoperox-
253 idase mediated food preservation than Gram-positive species
254 (79, 80). Many of the antimicrobial agents inherent to animals
255 are in the form of antimicrobial peptides (polypeptides).

Antimicrobial peptides were first isolated from natural sources
in the 1950s when nisin was isolated from lactic acid bacteria for
potential application as a food preservative (81). Subsequently,
antimicrobial peptides were isolated from other natural sources,
such as plants, insects, amphibians, crustaceans, and marine
organisms (82–84). Antimicrobial peptides (AMPs) are widely
distributed in nature and are used by many if not all life forms as
essential components of nonspecific host defense systems. The list
of discovered AMPs has been constantly increasing, with much
discovery in the last two decades. The list of AMPs produced by
animal cells includes magainin (85), MSI-78 (86), PR-39 (87),
spheniscin (88), pleurocidin (89), dermaseptin S4 (90), K4S4-
(1-14) (91), cecropin P1 (92), melittin (93), LL-37 (94), clavanin
A (92), and curvacin A (95). Antimicrobial peptides present a
promising solution to the problem of antibiotic resistance because,
unlike traditional antimicrobial agents, specific molecular sites are
not targeted, and their characteristic rapid destruction of mem-
branes does not allow sufficient time for even fast-growing
bacteria to mutate. Some of the potential antimicrobials of animal
origin which could be used as food additives are discussed below.

276 **Pleurocidin.** Pleurocidin, a 25 amino acid peptide isolated from
277 the skin mucus membrane of the winter flounder (*Pleuronectes*
278 *americanus*) is active against Gram-positive and Gram-negative
279 bacteria. It is heat-stable, salt-tolerant, and insensitive to physio-
280 logical concentrations of magnesium and calcium (96). Pleuroci-
281 din has potential for use in food applications and was found to be
282 effective against foodborne organisms including *Vibrio paraha-*
283 *molyticus*, *L. monocytogenes*, *E. coli* O157:H7, *Saccharomyces*
284 *cerevisiae*, and *Penicillium expansum* (97). The antimicrobial
285 activity of pleurocidin against foodborne microorganisms was
286 reported at levels well below the legal limit for nisin (10,000 IU/g)
287 without significant effect on human red blood cells (97), thereby
288 indicating its potential as a food preservative and a natural
289 alternative to conventional chemicals. However, pleurocidin
290 was inhibited by magnesium and calcium (96), which may limit
291 the use of this AMP in environments rich in these cations.

292 **Defensins.** Defensins are another group of antimicrobial pep-
293 tides widely found in nature including mammalian epithelial cells
294 of chickens, turkeys, etc. They are abundant in cells and tissues
295 active in host defense against microorganisms (98, 99). They are
296 reported to have a broad spectrum of antimicrobial activity (100),
297 including Gram-positive, Gram-negative bacteria, fungi, and
298 enveloped viruses (101, 102).

299 **Lactoferrin.** Bovine and activated lactoferrin (ALF), an iron-
300 binding glycoprotein present in milk, has antimicrobial activity
301 against a wide range of Gram-positive and negative bacteria (102)
302 fungi, and parasites (103). Lactoferrin has been applied in meat
303 products (104–106) as it has recently received approval for
304 application on beef in the USA (USDA-FSIS 2008. FSIS Direc-
305 tive 7120.1 Amendment 15).

306 **Other AMPs.** Protamine, like salmine and clupeine, has been
307 reported to be isolated from fish and is found to be effective against
308 Gram-negative and Gram-positive bacteria, yeasts, and molds
309 (108–111). Magainin peptides isolated from frogs (112) have been
310 found effective against a range of food-related pathogens (113),
311 implying a possible application as food preservatives (91, 114, 115).

312 **Chitosan.** Chitosan, a natural biopolymer obtained from the
313 exoskeletons of crustaceans and arthropods, is known for its
314 unique polycationic nature and has been used as active material
315 for its antifungal activity (72, 116) and antibacterial activity (117–
316 120). Liu et al. (121) studied the efficacy of chitosan against *E. coli*
317 and concluded that low molecular weight chitosan is effective for
318 controlling growth. The strong antibacterial activity of chitosan
319 was also observed against *S. aureus*, while its molecular weight
320 appeared to be a significant parameter defining its activity (122).

Lipids. Like lipids of plant origin, lipids of animal origin have antimicrobial activity against a wide range of microorganisms. Free fatty acids at mucosal surfaces have been shown to inactivate *S. aureus* (123). Milk lipids have recorded activity for inactivation of Gram-positive bacteria including *S. aureus*, *Cl. botulinum*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, Gram-negative bacteria such as *P. aeruginosa*, *E. coli*, and *Salmonella enteritidis* (124–126), and also against various fungi such as *Aspergillus niger*, *Saccharomyces cerevisiae*, and *C. albicans* (36, 124). Lipids may serve to inhibit the proliferation as well as the prevention of the establishment of pathogenic or spoilage microorganisms in food matrices.

Shin et al. (127) studied eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are formed in animal (including fish and shellfish) tissues but not plant tissues (18:3 ω -3). DHA is a component of membrane structural lipids that are enriched in certain phospholipid components of the retina and nonmyelin membranes of the nervous system in animals. Bioconverted EPA and DHA exhibited antibacterial activities against four Gram-positive bacteria, *B. subtilis*, *L. monocytogenes*, *Staphylococcus aureus* ATCC 6538, *S. aureus* KCTC 1916, and seven Gram-negative bacteria, *E. aerogenes*, *E. coli*, *E. coli* O157:H7, *E. coli* O157:H7 (human), *P. aeruginosa*, *Salmonella enteritidis*, and *S. typhimurium* (127). The growth inhibition by both EPA and DHA was similar against Gram-positive bacteria, while the bioconverted extract of DHA was more effective than EPA against Gram-negative bacteria.

Mechanism of Antimicrobial Action. The mechanism of action of AMPs seems to involve multiple targets. The plasma membrane is the most cited target; however, recent studies suggest intracellular targets at least for some peptides (128, 129). Although most AMPs act by nonspecific mechanisms, they often display some selectivity between different microorganisms, for example, Gram-negative compared with Gram-positive bacteria (130, 131) and susceptibility of fungal cells compared with other eukaryotic cells (132). Antimicrobial peptides can assume amphipathic structures, which are able to interact directly with the microbial cell membrane, rapidly disrupting the membrane in several locations, resulting in leaching out of vital cell components (96, 133). Previous studies conducted on the mechanism of action of pleurocidin revealed that it exhibits strong membrane translocation and pore-formation ability, reacting with both neutral and acidic anionic phospholipid membranes (134). Lipids inactivate microorganisms mainly by disruption of bacterial cell wall or membrane, inhibition of intracellular replication, or inhibition of an intracellular target (135). Monoacylglycerols lower the heat resistance of certain bacteria and fungi; therefore, they may find application in reducing the required heat treatment for certain foods (36). Lysozyme hydrolyses the β -1,4-glycosidic linkage in sugar polymers such as *N*-acetylmuramic acid and *N*-acetylglucosamine linkages found in bacterial peptidoglycan (136).

MICROBIAL ORIGIN ANTIMICROBIAL AGENTS

Bacteria produce many compounds that are active against other bacteria, which can be harnessed to inhibit the growth of potential spoilage or pathogenic microorganisms. These include fermentation end products such as organic acids, hydrogen peroxide, and diacetyl, in addition to bacteriocins and other antagonistic compounds such as reuterin (137). Both Gram-negative and Gram-positive bacteria produce bacteriocins. Bacteriocins are proteinaceous antibacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides (138). Bacteriocin production can be exploited by food processors to provide an additional barrier to undesirable bacterial growth in foods (Table 1).

Bacteriocins are cationic peptides that display hydrophobic or amphiphilic properties, and in most cases, the target for their activity is the bacterial membrane. Depending on the producer organism and classification criteria, bacteriocins can be categorized into several groups (139–142) with as many as five classes of bacteriocins proposed (143–145). The majority fall into classes I and II, which are the most intensively researched to date. The class I group, termed lantibiotics, are small peptides that are characterized by their content of several unusual amino acids (146). The class II bacteriocins are small, nonmodified, heat stable peptides (147). Another classification is with respect to the producing microorganism and is specifically named after the genus, species, or the group of microorganisms, e.g., lantibiotics for bacteriocins of lactic acid bacteria, colicins of *E. coli*, klebsins of *Klebsiella pneumoniae* (148). A large number of bacteriocins have been isolated and characterized from lactic acid bacteria, and some have acquired a status as potential food preservatives because of their antagonistic effect on important pathogens. Many bacteriocins are active against food borne pathogens and spoilage bacteria (149–152). The important ones include nisin, diplococcin, acidophilin, bulgarican, helveticin, lactacin, and plantaricin (153). Nisin is produced by various *Lactococcus lactis* strains, is the most thoroughly studied bacteriocin to date, and is applied as an additive in food worldwide (154). While the antimicrobial polypeptide nisin and related compounds such as pediocin are the only bacteriocins widely used for food preservation (155, 156), many other bacteriocins have been reported and have shown potential for food preservation and safety applications.

Reuterin. Reuterin (β -hydroxypropionaldehyde) is a water-soluble nonproteinaceous metabolite of glycerol (157). It is a broad spectrum antimicrobial compound produced by some strains of *Lactobacillus reuteri*, with recorded activity against Gram-negative and Gram-positive bacteria, yeasts, and filamentous fungi (158). Reuterin was isolated, purified, and identified by Talarico and Dobrogosz (159) and is active over a wide range of pH values and resistant to the action of proteolytic and lipolytic enzymes (160). Reuterin is reported to exhibit bacteriostatic activity against *Listeria monocytogenes* but was only slightly bactericidal against *Staphylococcus aureus* at 37 °C. However, higher bactericidal activity was reported against *E. coli* O157:H7, *S. choleraesuis* subsp. *Choleraesuis*, *Y. enterocolitica*, *A. hydrophila* subsp. *Hydrophila*, and *C. jejuni* (161).

Pediocin. Pediocin is produced by strains of *Pediococcus acidilactici* and *P. pentosaceus* and is designated generally recognized as a safe (GRAS). The organism is commonly isolated from and used in fermented sausage production. The bacteriocins produced by *P. acidilactici* are AcH, PA-1, JD, and 5, and those produced from *P. pentosaceus* are A, N5p, ST18, and PD1 (162). Most pediocins are thermostable proteins and function over a wide range of pH values. Pediocin AcH has proven efficacy against both spoilage and pathogenic organisms, including *L. monocytogenes*, *Enterococcus faecalis*, *S. aureus*, and *Cl. Perfringens* (163). Natamycin is an antifungal produced by *Streptomyces natalensis* that is effective against nearly all molds and yeasts but has little or no effect on bacteria.

Nisin. Nisin is the most widely used bacteriocin. To date, nisin is the only natural antimicrobial peptide (see Scheme 2 for its structure) approved by the FDA for use as a food preservative; however, it has a limited spectrum of activity, does not inhibit Gram-negative bacteria or fungi, and is only effective at low pH (164, 165). Nisin is produced by fermentation of a modified milk medium by certain strains of lactic acid bacterium, *Lactococcus lactis*. Nisin functions by interacting with the phospholipids

Table 1. Effect of Natural Antimicrobial Agents on Food Preservation and Quality^a

food product	antimicrobial agent (concentrations)	microbial dynamics	quality attributes	reference
fruit yoghurt	vanillin (2000 ppm)	yeast, bacterial (delays growth)	shelf life (†)	(232)
tomato juice	clove oil (0.1%)	total plate count (3.9LR)	shelf life (†), vitamin C (~)	(208)
	mint extract (1.0%)	total plate count (8.34LR)		
	nisin (0.004%)	total plate count (↓)		
ready-to-eat fruit salad	citral (25–125 ppm)	yeasts and lactic acid bacteria (LAB) (delays growth)	shelf life (†)	(233)
	citron (300–900 ppm)			
	citron (600 ppm)	<i>Salmonella enteritidis</i> E4 (2 LR), <i>Escherichia coli</i> 555 (<4.5 LR) <i>Listeria monocytogenes</i> Scott A (4 LR)	sensory characteristics (~)	
raspberries	methyl jasmonate (MJ), allyl isothiocyanate (AITC) EO of <i>Melaleuca alternifolia</i> (tea tree oil)		AC (†) AC (↓) AC (†)	(234)
fresh cut water melon	nisin (25 µg/mL)	<i>L. monocytogenes</i> (0.8 LR)	quality (†)	(235)
lettuce	thyme oil (1 mL/l)	<i>E. coli</i> (6.32LR)		(236)
baby carrot		<i>E. coli</i> (5.57LR)		
minimally processed carrots	oregano oil (250 ppm)	background spoilage microflora total viable count (TVC) (>1 LR) lactic acid bacteria (LAB) (>1 LR) <i>Pseudomonas</i> (<1 LR)	sensory characteristics (~)	(205)
minimally processed vegetables	thyme oil (1%)	<i>Aeromonas spp</i> (2 LR)	sensory properties (↓), shelf life (†)	(237)
		psychrotrophic aerobic plate count (4.19 LR) plate count agar (5.44 LR)		
wine	nisin	LAB (minimum inhibitory concentration, MIC = 0.39 mg/mL) <i>Oenococcus oeni</i> (MIC 0.01 mg/mL) acetic acid bacteria (MIC 1.5 mg/mL)		(238)
milk	reuterin (8 AU/ml)	<i>L. monocytogenes</i> (4.59 LR)		(161)
	nisin (100 IU/ml)	<i>S. aureus</i> counts (5.45 LR)		
skimmed milk powder	nisin (100 IU/ml)	<i>L. innocua</i> (3.8 LR)		(240)
chicken meat	nisin	<i>E. coli</i> (<1 LR)	proximate composition (~), shelf life (†)	(209)
		<i>Brochothrix thermosphacta</i> (~) <i>Lactobacillus alimentarius</i> (~) <i>Brochothrix thermosphacta</i> (~) <i>Lactobacillus alimentarius</i> (delays growth)		
fish	EOs (0.5% carvacrol + 0.5% thymol)	TVC (2.5LR)	shelf life (†), lipid oxidation (↓) sensory characteristics (~)	(241)
red meat	tea catechins (300 mg/kg)		shelf life (†), lipid oxidation (↓)	(242)
beef hot dog	clove oil (5 mL/l)	<i>L. monocytogenes</i> (1.15–1.71LR)		(73)
	thyme oil (1 mL/l)	<i>L. monocytogenes</i> (0.67–1.05 LR)		
pork bologna	nisin (125 µg/mL)	<i>L. monocytogenes</i> (1.5LR)		(169)
minced beef	<i>Capsicum annum</i> extract	<i>Salmonella typhimurium</i> (Minimum lethal concentration, MLC 15 g/kg) <i>Pseudomonas aeruginosa</i> (MLC 30 g/kg)		(199)
chicken frankfurter	clove oil (1% v/w)	<i>L. monocytogenes</i> (4.5 LR)		(197)
cooked beef	grape seed extract (1%)	<i>Escherichia coli</i> (1.7 LR) <i>S. Typhimurium</i> (2.0 LR) <i>L. monocytogenes</i> (0.8 LR) <i>Aeromonas hydrophila</i> (0.4 LR)	color (~), lipid oxidation (↓)	(200)

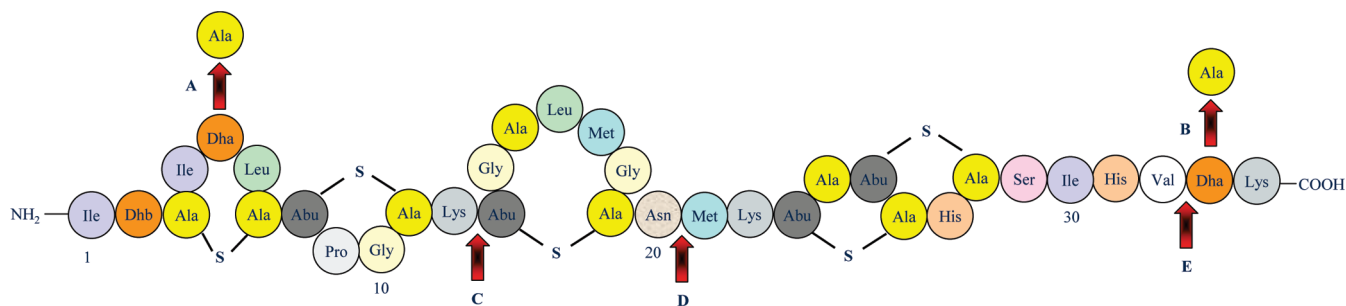
^a AU: arbitrary units were defined as the reciprocal of the highest two-fold dilution that did not allow the growth of the indicator strain. AC: anthocyanin content. † and ↓ indicate increase and decrease, respectively, while ~ shows no significant difference. LR: microbial log reduction.

449 in the cytoplasmic membrane of bacteria, thus disrupting
450 membrane function and preventing outgrowth of spores by
451 inhibiting the swelling process of germination. It is highly
452 active against many of the Gram-positive bacteria and speci-
453 fically used by the cheese industry to control the growth of
454 *Clostridium spp.* (166). Substantial research has evaluated the
455 efficacy of nisin against various pathogens and its use for
456 different food products (167–174). Nisin has been used to
457 inhibit microbial growth in beef (173), sausages (2), liquid
458 whole egg (174), ground beef (175), and poultry (176). It has
459 also been reported to reduce initial levels of *Listeria mono-*
460 *cytogenes* and suppress subsequent growth in ready-to-eat
461 (RTE) meat products (177, 178). Komitopoulou et al. (179)
462 reported that nisin could be used for the effective control of

Alicyclobacillus acidoterrestris in fruit juices. A nisin level of 463
464 6.25 µg/g could inhibit lactic acid bacteria (LAB) growth for
465 over 28 days and for 35 days with 25 µg/g (180). The effects of
466 three types of phosphate (used as emulsifiers) on nisin activity
467 in sausage were compared, and LAB growth rate was fastest in
468 samples containing orthophosphate and slowest in sausages
469 containing diphosphate.

Mechanism of Antimicrobial Action. The antimicrobial action 470
471 of bacteriocins is based on pore formation in the cytoplasmic
472 membrane of the target microorganism. This leads to a loss of
473 small intracellular molecules and ions and a collapse of the proton
474 motive force (181). Nisin is less effective on Gram-negative
475 bacteria, as the outer membrane disables the entry of this
476 molecule to the site of action (50, 119, 182, 183). The first step

Scheme 2. Structure of Nisin



477 in the mode of action of nisin is to pass through the cell wall of
 478 Gram-positive bacteria. Generally, it is assumed that nisin
 479 passes the cell wall by diffusion. However, the Gram-positive
 480 cell wall can act as a molecular sieve against nisin depending
 481 on its composition, thickness, or hydrophobicity (184). The
 482 removal of the cell wall from nisin-resistant *Listeria* resulted in
 483 the removal of nisin resistance, suggesting that the cell wall plays
 484 a role in the differences in susceptibility toward nisin (185). The
 485 next step of the antimicrobial process of nisin is to associate with
 486 the cytoplasmic membrane of the target microorganism. It has
 487 been suggested that nisin interacts electrostatically with the
 488 negatively charged phosphate groups of surface membrane
 489 phospholipids (173).

490 **Factors Affecting Antimicrobial Activity.** Various factors can
 491 impact the antimicrobial efficacy of bacteriocins. These include
 492 the emergence of bacteriocin-resistant bacteria, conditions that
 493 destabilize the biological activity of proteins such as proteases or
 494 oxidation processes, binding to food components such as fat
 495 particles or protein surfaces, inactivation by other additives, poor
 496 solubility, and uneven distribution in the food matrix and/or pH
 497 effects on bacteriocin stability and activity (137). The application
 498 of bacteriocins in combination with other preservation hurdles
 499 has been proposed to reduce the selection for resistance to
 500 bacteriocins in target strains and/or to extend its inhibitory
 501 activity to Gram-negative species (182). Interactions between
 502 bacteriocin and the food matrix may result in a decrease in the
 503 efficacy of the bacteriocin. The combination of bacteriocins with
 504 other minimal or nonthermal preservation technologies may
 505 prove useful for practical applications. This approach is of value
 506 for the control of Gram-negative bacteria as their outer mem-
 507 brane acts as an efficient barrier against hydrophobic solutes and
 508 macromolecules, such as bacteriocins (119).

509 QUANTIFICATION OF THE MINIMUM AND NONINHIBITORY 510 CONCENTRATION

511 The use of antimicrobials as preservatives in food systems
 512 can be constrained when effective antimicrobial doses exceed
 513 organoleptic acceptable levels (especially for essential oils) or
 514 when they are added to complex food systems. Two specific
 515 concentrations appear to be of interest, i.e., the noninhibitory
 516 concentration, NIC, the concentration above which the inhibi-
 517 tor begins to have a negative effect on growth, and the
 518 minimum inhibitory concentration, MIC, which marks the
 519 concentration above which no growth is observed by compar-
 520 ison with the control (186). Therefore, these concentrations are
 521 quantified with the aim of defining the boundaries of sensory
 522 acceptability and antimicrobial efficacy of antimicrobials (26).
 523 Most of the studies on the calculation of MIC and NIC are
 524 semiquantitative, while quantitative approaches have been
 525 mainly applied on studies concerning the antimicrobial activity
 526 of plant origin antimicrobial agents, i.e., essential oils and their
 527 components.

528 The MIC and NIC are dependent on experimental conditions.
 529 The influencing conditions include the incubation temperature,
 530 organism, and inoculum size, and therefore, they should be
 531 reported in studies where MIC and NIC are evaluated (187,
 532 188). In vitro studies for identifying the MIC can be divided into
 533 groups such as diffusion, dilutions, impedance, and optical
 534 density (or absorbance) methods (see for e.g., refs (189–191)).
 535 Most of these evaluations are based on an end-point approach for
 536 evaluating the MIC, i.e., end result in which no growth is obtained
 537 for a test level of preservative, into which an inoculum of
 538 microbes is added. This kind of approach is considered semi-
 539 quantitative (188).

540 Lambert and Pearson (188) examined the inhibitory activity of
 541 single compounds of EOs and developed a fully quantitative
 542 approach. This is given by the Lambert–Pearson model (LPM)
 543 inspired by a modified Gompertz equation (eq 1) to evaluate the
 544 dose–responses of microorganisms against several inhibitors.
 545 This modeling approach has already been examined for optical
 546 density, O.D. (187, 188), and impedance microbial measure-
 547 ments (62).

$$548 \quad fa = \exp \left[- \left(\frac{x}{P_1} \right)^{P_2} \right] \quad (1)$$

549 In eq 1, *fa* is the fractional area which is defined as the ratio of
 550 inhibited growth to uninhibited growth as measured by the
 551 applied method (impedance, optical density, etc.), *x* is the
 552 inhibitor concentration (mg/L), *P*₁ is the concentration at max-
 553 imum slope (of a log *x* vs *fa* plot; see Figure 1 for a graphical
 554 example of this equation), and *P*₂ is a slope parameter. Observe
 555 that *fa* can be measured by using the trapezoidal rule under the O.
 556 D. (or other microbial measurements)/time curves and then
 557 taking the ratio of the test area to that of the control (187).
 558 Therefore, the range of *fa* will be between 0 and 1 (Figure 1). The
 559 routine, trapz, provided by Matlab is an example of a software
 560 package that can be used for performing a trapezoidal numerical
 561 integration.

562 The MIC (eq 2) and the NIC (eq 3) can then be calculated as the
 563 intercept of the concentration axis to the tangent at the maximum
 564 gradient of the *fa*/log concentration curve and the intercept of the
 565 tangent at the maximum gradient of the *fa*/log concentration
 566 curve to the *fa* = 1 contour.

$$567 \quad MIC = P_1 \cdot \exp \left(\frac{1}{P_2} \right) \quad (2)$$

$$568 \quad NIC = P_1 \cdot \exp \left(\frac{1-e}{P_2} \right) \quad (3)$$

569 Guillier et al. (192) developed another approach for evaluating
 570 the MIC based on the use of growth rate models. After estimation
 571 of the maximum specific growth rates (μ_{max}) from optical density
 572 measurements

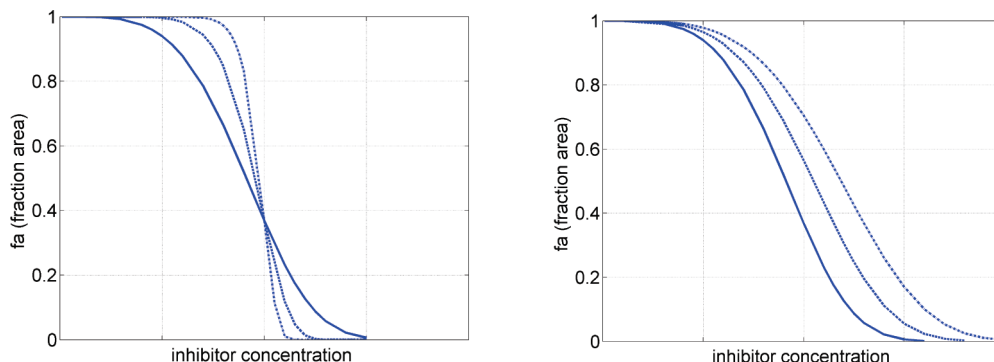


Figure 1. Hypothetical inhibition profile as can be described by eq 1 for increasing values of P_2 and constant P_1 (left panel) and increasing values of P_1 and constant P_2 (right panel). Inhibitor concentration is expressed on a logarithmic scale.

570 growth kinetics by a modified Gompertz model, they assessed the
571 antimicrobial concentration dependence on μ_{\max} (eq 4).

$$\sqrt{\mu_{\max}} = \sqrt{\mu_{\max}(c=0) \cdot f(c)} \quad (4)$$

572 $f(c)$ can be described either as eq 5, i.e., the SR_{μ} model, or as eq 6,
573 i.e., the LP_{μ} model.

$$f(c) = \left(1 - \frac{c}{MIC}\right)^{\beta}, c < MIC \text{ or } 0, c \geq MIC \quad (5)$$

$$f(c) = \exp \left[- \left(\frac{c}{MIC / \exp \left(\frac{\ln(NIC/MIC)}{-e} \right)} \right)^{-e / (\ln(NIC/MIC))} \right] \quad (6)$$

574 $\mu_{\max}(c=0)$ is the growth rate in the absence of the antimicrobial
575 ($c=0$) and β a shape parameter representing the sensitivity of the
576 microorganism to an antimicrobial in eq 5. These two approaches
577 appeared to give equivalent results. Observe that for estimating
578 the parameters of MIC, NIC, and $\mu_{\max}(c=0)$ of eq 6, a regression
579 is performed for the data that relate the maximum specific growth
580 rates (μ_{\max}) with the concentration of the inhibitor.

581 Lambert et al. (26) argued that the majority of antimicrobial
582 activity could be attributed to two components acting independ-
583 dently. Therefore, they also suggested another expression for a
584 mixture of two inhibitors that could be extended in case there are
585 more inhibitors as presented in eq 7:

$$f_{a_{x_1, \dots, x_k}} = \exp \left\{ - \left[\left(\frac{x_1}{C_{i,1}} \right)^{C_{i,2}} + \dots + \left(\frac{x_k}{C_{k,1}} \right)^{C_{k,2}} \right]^{C_Q} \right\} \quad (7)$$

586 where parameters $C_{i,1}$ are the concentrations of the x_i inhibitors at
587 the maximum slope. The main difference is that the current
588 expression takes into account interactions between the antimi-
589 crobials, which means that it could be considered for any additive,
590 antagonistic, and synergistic activity between the studied inhibi-
591 tors. For an example in which a mixture of two antimicrobials is
592 studied reference is made to Lambert and Lambert (187). In that
593 case, the MIC of any of the x_i antimicrobials is then given by eq 8.

$$MIC = C_{i,1} \cdot \exp \left(\frac{1}{C_{i,2} + C_Q} \right) \quad (8)$$

595 Another interesting quantitative approach for evaluating the
596 bactericidal effect of different agents has been suggested by Lui
597 et al. (193). This is based on a concentration killing curve
598 approach and the estimation of the so-called median bactericidal
599 concentration and bactericidal intensity. The developed method

is based on the correlation (by the use of a sigmoidal curve with an
inflection point) of the population size (number CFU per plate)
with respect to the concentration of the agent. This approach has
been applied for quantifying the bactericidal potency of anti-
biotics against *E. coli* and might have to be further investigated
for different antimicrobials. Similar to the discussed approaches,
novel modeling methods for quantitatively expressing the effect
of antimicrobials through MIC and NIC values can be developed
by knowledge coming from predictive microbiology. An overview
of representative cases for different modeling expressions tackling
the effect of both chemical and natural inhibitory compounds can
be found in Devlieghere et al. (194).

Accurate quantitative evaluations of MIC and NIC are
important for designing effective preservation methods that
are based on the use of the discussed antimicrobials. These
quantitative methods can be exploited to give insight to optimal
concentrations or combinations for real food systems by direct
comparison of the antimicrobial efficacy of different antimi-
crobials, their individual or combined components, or their mix-
tures, and for efficient design of preservation for food products
based on the principles of hurdle technology. These approaches
have not received much attention for evaluating the MIC or the
minimum bactericidal concentration of the antimicrobials of
animal and microbial origin, but their potential is evident.

APPLICATIONS OF NATURAL ANTIMICROBIALS IN FOOD

The extrapolation of results obtained from in vitro experiments
with laboratory media to food products is not straightforward as
foods are complex, multicomponent systems consisting of differ-
ent interconnecting microenvironments. Though there is vast
potential for natural antimicrobial agents in food preservation,
most of the literature presents inactivation data from model foods
or laboratory media. **Table 1** reports inactivation studies in real
food systems. The level of natural preservatives required for
sufficient efficacy in food products in comparison with laboratory
media may be considerably higher, which may negatively impact
the organoleptic properties of food.

Monoacylglycerols have increased the shelf life of various
foods including soy sauce, miso, sausages, cakes, and noodles (36).
The lauric acid ester of monoacylglycerol has reported antimi-
crobial potential in seafood salads and various flesh foods
including deboned chicken meat, minced fish, refrigerated beef
roasts, and frankfurter slurries (126, 195). Hao et al. (196) studied
the efficacy of a range of plant extracts for inhibition of
A. hydrophila and *L. monocytogenes* in refrigerated cooked
poultry and found that eugenol reduced pathogen counts by
4 log₁₀ cfu/g over a 14 day storage trial. Similarly, 1–2% w/w clove
oil inhibited the growth of a range of *Listeria* spp. in chicken
frankfurters over 2 weeks at 5 °C (197). Conversely, Shekarforoush

648 et al. (198) found that EOs of oregano and nutmeg were effective
649 against *E. coli* O157:H7 in a broth system but had no effect in
650 ready-to-cook chicken. Careaga et al. (199) recorded that 1.5 mL/
651 100 g of capsicum extract was sufficient to prevent the growth of
652 *S. typhimurium* in raw beef but that 3 mL/100 g was required for a
653 bactericidal effect against *P. aeruginosa*. Ahn et al. (200) also found
654 a range of plant extracts to be useful for reduction of pathogens
655 associated with cooked beef and quality maintenance; however,
656 Uhart et al. (201) concluded that when in direct contact, spices
657 inactivated *S. typhimurium* DT104 but that the activity decreased
658 considerably when added to a complex food system such as ground
659 beef. Gutierrez et al. (74, 75) concluded that plant essential oils are
660 more effective against food-borne pathogens and spoilage bacteria
661 when applied to ready-to-use foods containing a high protein level
662 at acidic pH as well as lower levels of fats or carbohydrates and
663 moderate levels of simple sugars. The success of plant derived
664 antimicrobials when applied to fruit and vegetable products is also
665 documented in the literature. Karapinar et al. (202) recommended
666 unripe grape juice as an alternative antimicrobial agent for
667 enhancing the safety of salad vegetables, and Martinez-Romero
668 et al. (203) suggested that carvacrol could be applied as a novel tool
669 for the control of fungal decay on grapes. Although Valero and
670 Frances (204) found that low concentrations of carvacrol, cinnamaldehyde, or thymol had a clear antibacterial effect against
671 *B. cereus* in carrot broth, cinnamaldehyde retained a significant
672 activity at storage temperatures of 12 °C. Gutierrez et al. (205)
673 found that the efficacy of oregano EO was comparable with
674 chlorine as a decontamination treatment for ready-to-eat carrots.
675 Use of this essential oil contributed to the acceptability of sensory
676 quality and appreciation. A novel application of plant extracts is
677 for the production of chocolate; Kotzekidou et al. (206) reported
678 enhanced inhibitory effects of plant extracts against an *E. coli*
679 cocktail at 20 °C.

681 Antimicrobials from microbial sources, especially nisin, find
682 application in a number of foods such as milk, orange juice (207),
683 and tomato juice (208), and for increasing the shelf life of chicken
684 meat without altering sensory properties of the product (209). The
685 efficacy of enterocin AS-48 for inhibition of *B. cereus* in rice and
686 *S. aureus* in vegetable sauces was investigated (210, 211) with
687 bacteriocin levels in the range of 20–35 µg/mL and 80 µg/mL,
688 respectively.

689 Investigation of the antimicrobial properties of preservatives
690 from animal sources and their possible potential in food applica-
691 tion is still in its infancy, with few published studies available as
692 described above. A common conclusion that could be drawn
693 from these studies is the fact that the significant potential of
694 antimicrobials from animal sources is not being exploited.

695 Some other applications in foods that got attention in previous
696 years are the use of bioactive packaging technologies. These
697 systems can be applied for all of the discussed antimicrobials,
698 i.e., plant, animal, and microbial origin agents either by adding a
699 sachet (or possibly by encapsulating the agents (212)) into the
700 package, dispersing bioactive agents in the packaging, coating
701 bioactive agents on the surface of the packaging material, or
702 utilizing antimicrobial macromolecules with film-forming prop-
703 erties or edible matrixes (213, 214). Film-coating applications
704 have been reported for meat, fish, poultry, bread, cheese, fruits,
705 and vegetables (215).

706 USE OF NATURAL ANTIMICROBIALS IN THE MULTIPLE- 707 HURDLE CONCEPT

708 Investigations based on combinations of natural antimicro-
709 bials with other nonthermal processing technologies within the
710 multiple-hurdle concept are warranted to counteract any poten-
711 tial organoleptic or textural effects on food products as well as

712 optimizing microbial inactivation. The preservative action of
713 bacteriocins alone in a food system is unlikely to ensure compre-
714 hensive safety. This is of particular significance with regard to
715 Gram-negative pathogenic bacteria that are protected from the
716 antimicrobial action of bacteriocins by the presence of an outer
717 membrane. When the outer membrane is disrupted by agents
718 such as the food grade chelating agent ethylene diamine tetra-
719 acetate (EDTA), which acts by binding to Mg²⁺ ions in lipopo-
720 lysaccharide, the outer membrane of Gram-negative bacteria are
721 rendered sensitive to the antimicrobial action of bacterio-
722 cins (181). Potential synergistic effects may be found with other
723 chemical or physical inactivation technologies including dense
724 phase carbon dioxide, ultrasound, pulsed-electric field, high
725 pressure, and ozone treatment. As a consequence of applying
726 these nonthermal methods, bacterial cell membranes can weaken
727 or become susceptible to additional antimicrobial agents such as
728 bacteriocins, causing lethality. The use of bacteriocins in combi-
729 nation with organic acids or other antimicrobials can similarly
730 result in enhanced inactivation (216). Studies reporting the
731 effective use of nisin against Gram-negative organisms and fungi
732 are those in which nisin was used in combination with traditional
733 food preservatives such as organic acids and chelating
734 agents (217). Rajkovic et al. (218) found that the activity of nisin
735 combined with carvacrol was enhanced in a potato puree by
736 comparison with BHI broth and that more obvious effects against
737 *B. cereus* and *B. circulans* were observed at higher temperatures.
738 The application of bacteriocins in combination with treatments
739 that could enhance their effectiveness in foods requires investiga-
740 tion. Examples of the synergistic effects that can be obtained
741 using mild traditional preservation techniques in conjunction
742 with novel food processing technologies are better studied in
743 vitro but require further investigation in food products to ensure
744 successful practical application. The antibacterial activity of
745 inhibitory compounds, such as nisin, enterocin, monolaurin,
746 and the lactoperoxidase system (LPS), can be enhanced if applied
747 in combination (219–221), with chelating agents (182, 222, 223) or
748 with preservative treatments such as high hydrostatic pressure,
749 pulsed electric field, low pH, or freeze/thaw cycles (224–228). The
750 combination of plant EOs with modified atmosphere packaging
751 for control of spoilage species was reported by Skandamis and
752 Nychas (229) and Matan et al., (230). Seydim and Sarikus (231)
753 also investigated the use of EOs in an active packaging system
754 based on an edible whey protein film and concluded that oregano
755 was the most effective EO against a range of food pathogens.
756 Allyl isothiocyanate was successfully applied to chopped, refrig-
757 erated, nitrogen packed beef for the control of *E. coli* at levels in
758 excess of 1000 ppm.

759 **Conclusions and Future Trends.** Interest in natural antimicro-
760 bials has expanded in recent years in response to consumer
761 demand for greener additives. During the last two decades,
762 natural preservatives have been investigated for practical applica-
763 tions. These technologies have been shown to inactivate micro-
764 organisms and enzymes without significant adverse effects on
765 organoleptic or nutritional properties. Reported studies have
766 demonstrated that natural antimicrobial agents described in this
767 review may offer unique advantages for food processing. In
768 addition to improving the shelf life and safety of foods, natural
769 antimicrobial agents may allow novel food products with en-
770 hanced quality and nutritional properties to be introduced to the
771 market.

772 The applications of natural antimicrobial agents are likely to
773 grow steadily in the future because of greater consumer demands
774 for minimally processed foods and those containing naturally
775 derived preservation ingredients. More complex considerations
776 arise for combinations of technologies, particularly with respect

777 to optimization of practical applications. Intelligent selection of
778 appropriate systems based on detailed, sequential studies and
779 quantitative approaches to evaluate the efficiency of antimicro-
780 bials is necessary. The impact of product formulation, extrinsic
781 storage parameters, and intrinsic product parameters on the
782 efficacy of novel applications of combined nonthermal systems
783 requires further study.

784 ABBREVIATIONS USED

785 Abu, amino butyric acid; Ala, alanine; asn, asparagine; Dha,
786 dehydroalanine; Dhb, dehydrobutyrine (β -methyldehydroala-
787 nine); Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine ;
788 Lys, lysine ; Met, methionine ; Pro, proline; Ser, serine; Val, valine.

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