The use of natural antimicrobial compounds in food has gained much attention by the consumers and the food industry. This is due primarily to two major factors. First, the misuse and mishandling of antibiotics has resulted in the dramatic rise of a group of microorganisms including foodborne pathogens that are not only antibiotic resistant but also more tolerant to several food processing and preservation methods. In addition, increasing consumers’ awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products in foods. This has prompted the food industry to look for alternative preservatives that can enhance the safety and quality of foods. Compounds derived from natural sources have the potential to be used for food safety due to their antimicrobial properties against a broad range of foodborne pathogens. This article reviews the antibacterial activity of natural components from different sources including plants, animals, bacteria, algae and mushrooms, and their potential use in food systems.

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1. Introduction

Foodborne illnesses are a major concern for consumers, the food industry, and food safety authorities. In recent years, considerable effort has been made to find natural antimicrobials that can inhibit bacterial and fungal growth in foods in order to improve quality and shelf-life. Similarly, consumers have become concerned about the safety of synthetic preservatives used in food. As a result, there is increasing demand for natural products that can serve as alternative preservatives. In addition to being used in food to impart flavor, pungency and color, herbs and spices also have antioxidant, antimicrobial, nutritional and pharmaceutical properties (Lai & Roy, 2004). Plant-derived compounds are mostly secondary metabolites, most of which are phenols or their oxygen-substituted derivatives. These secondary metabolites possess various benefits including antimicrobial properties against pathogenic and spoilage microbes (Hayek et al., 2013). Major groups of compounds that are responsible for antimicrobial activity from plants include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Ciocan & Bâră, 2007; Lai & Roy, 2004). Variations in the structure and chemical composition of these compounds result in differences in their antimicrobial action (Savoia, 2012). (Fig. 1).

The structural diversity of plant-derived compounds is immense, and, the impact of antimicrobial action they produce against microorganisms depends on their structural configuration. Phenolic compounds possess great structural variations and are one of the most diverse groups of secondary metabolites. The hydroxyl (–OH) groups in phenolic compounds are thought to cause inhibitory action (Lai & Roy, 2004) as these groups can interact with the cell membrane of bacteria to disrupt membrane structures and cause the leakage of cellular components (Xue, Davidson, & Zhong, 2013). The presence of –OH group in the phenolic compounds plays an important role in the antimicrobial activity of carvacrol and thymol. Active group such as –OH promotes the delocalization of electrons which then act as proton exchangers and reduce the gradient across the cytoplasmic membrane of bacterial cells. This will cause the collapse of the proton motive force and depletion of the ATP pool and ultimately leading to cell death (Ultee, Bennik, & Moezelaar, 2002). Similarly, Farag, Daw, Hewidi, and El-Baroty (1989) reported that these –OH groups can easily bind the active site of enzymes by altering the cell metabolism of microorganisms. This action demonstrates the importance of –OH groups in antimicrobial activity. Phenolic compounds also act as antioxidants. The presence of a free –OH group in phenolic compounds results in the antioxidant properties. This property has been reported to inhibit the generation of reactive oxygen species, as well as the scavenging of free radicals thereby reducing the redox potential of the growth medium (Cueva et al., 2010; Stojkovic et al., 2013). This lowering of redox potential may further restrict the growth of undesirable microorganisms.

As reported by Dorman and Deans (2000), the position of the –OH group also influences the components antimicrobial effectiveness. For example, the structure of thymol is similar to that of carvacrol; however, difference in antimicrobial effectiveness between thymol and carvacrol against Gram-positive and Gram-negative bacteria was observed when tested in agar medium. This difference has been attributed to the –OH group located at the meta position in thymol compared to the ortho position in carvacrol. Alcaraz, Blanco, Puig, Tomas, and Ferrer (2000) also reported the importance of the –OH group at position 5 of flavanones and flavones for activity against methicillin-resistant Staphylococcus aureus strains. Ultee et al. (2002) emphasized the importance of the –OH group and its system of delocalized electrons present in carvacrol against foodborne pathogens. Their study compared the activity of carvacrol to menthol, carvacrol methyl ester, and cymene. The authors concluded that the growth of Bacillus cereus was not inhibited due to the lack of –OH groups in carvacrol methyl...
ester, and cymene. The strong antimicrobial effect of carvacrol was therefore hypothesized to be due to the presence of \(-\text{OH}\) groups and the presence of a system of delocalized electrons. The specific mechanism involved here is the destabilization of the cytoplasmic membrane by compounds with phenolic \(-\text{OH}\) groups which act as proton exchangers, thereby reducing the pH gradient across the cytoplasmic membrane and thus leading to cell death. The authors further illustrated that even though menthol has an \(-\text{OH}\) group, it does not show high antimicrobial activity due to its lack of a system of delocalized electrons (double bonds). This deficit thereby prevents the \(-\text{OH}\) group from releasing its proton.

The importance of the number of double bonds in relation to antimicrobial effectiveness is described by Gochev, Dobreva, Girova, and Stoyanova (2010). Among citronellol, geraniol, and nerol, citronellol was found to be less effective due to the presence of only one double bond whereas geraniol and nerol with two double bonds, showed higher antimicrobial activity against tested bacteria (\textit{B. cereus}, \textit{Escherichia coli}, \textit{S. aureus}) and yeast (\textit{Candida albicans}). The antimicrobial activity of two pairs of isomeric phenolic compounds, eugenol-isoeugenol, has also been shown to differ solely due to the position of the double bond in the aliphatic side chain. Friedman, Henika, and Mandrell (2002) found eugenol to be about 13-fold more active than isoeugenol against \textit{Listeria monocytogenes} \textit{(Friedman et al., 2003; Ramos-Nino, Ramirez-Rodriguez, Clifford, & Adams, 1998)}.

Cueva et al. (2010) grouped phenolic acids based on their chemical structure. According to these authors, the position of \(-\text{OH}\) substitution in the aromatic ring and the length of the saturated side chain were influencing factors for antimicrobial activity. The high antimicrobial activity of phenolic compounds also depends on the size of added alkyl or alkenyl group. Pelczar, Chan, and Krieg (1988) reported stronger antimicrobial activity with the presence of larger size of added alkyl or alkenyl groups. The high activity of phenolic compounds could be thus due to the alkyl substitution in the phenol nucleus (Pelczar et al., 1988). The aldehyde group associated with carbon to carbon double bond has high electronegativity, thus increasing its antibacterial activity (Kurita, Miyaji, Kurane, Takahara, & Ichimura, 1981; Moleyar & Narasimhan, 1986). One possible explanation for this could be that the electron-negative compounds interfere with electron transfer and react with proteins and nucleic acids, thereby inhibiting the growth of microorganisms (Dorman & Deans, 2000).
Studies investigating the antimicrobial activity of EOs in plants and the structural relationships between their compounds and their antimicrobial activity have been reported (Dorman & Deans, 2000; Ultee et al., 2002). These studies demonstrated that phenolic structure plays an important part of the antimicrobial activity. Active groups in the phenolic structure are very critical for activity. For example, –OH groups and allylic side chains enhance volatile oils efficacy. Carvacrol with the –OH group had more activity than modified carvacrol with methyl ester. Limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) is more effective compared to p-cymene. This is likely attributable to the type of alkyl group in nonphenolic compounds indicating that alkyl has more influence on antimicrobial activity than alkyl (Dorman & Deans, 2000). Acetate moieties in volatile oils compounds may also positively influence the activity of several plant extracts. For example, Geranyl acetate has been found to be more active against a range of Gram-positive and negative bacteria than Geraniol (Dorman & Deans, 2000). Because antimicrobial activity depends not only on chemical composition but also on lipophilic properties, the potency of functional groups or aqueous solubility (Dorman & Deans, 2000) and the mixture of compounds with different biochemical properties may increase the efficacy of EOs. This action involves membrane disruption by lipophilic compounds resulting in inhibition of electron transport, protein translocation, phosphorylation, and other enzymatic activity (Dorman & Deans, 2000) which ultimately destroy the cell membrane integrity resulting in the death of microorganisms. This antimicrobial activity of plant antimicrobials could also vary depending on the type of microorganisms, extraction method, culture medium, size of inoculum, and method of determination (Tajkarimi et al., 2010).

2.2. Plant by-products

During food processing, oftentimes large amount of by-products are generated including fruit peomace, seeds, peels, pulps, unused flesh, and husks. Although these by-products typically have been considered waste, some studies have shown that the peels, seeds, husks and kernels are promising sources of valuable components such as phenolic compounds (polyphenols, tannins, and flavonoids) and many other bioactive components that have several functionalities including antimicrobial activity (Balasundram, Sundram, & Samman, 2006; Engels et al., 2009; Tiwari et al., 2009). By-products of fruits and vegetables are potentially good sources of minerals, organic acid, and phenolics that have a wide range of antimicrobial properties (Chanda, Baravalia, Kaneria, & Rakhohiya, 2010). Ayala-Zavala et al. (2011) reported that by-products can have a similar or even higher proportion of bioactive compounds than the usable parts of produce. For example, the total phenolics in peels of lemons, oranges and grapefruits have been found to be 15% higher than the level found in the peeled fruits (Gorinstein et al., 2005). Therefore, commercial exploitation of these by-products and their major components as antimicrobials is an important by-product, which accounts for almost 25–35% of processed fruits (Schieber et al., 2003). Pomace consists of seeds, skins, and stems, and is an important by-product that is a rich source of phenolic compounds, flavonoids and non-flavonoids, polyphenols, minerals, and dietary fiber (Figueroa, Hurtado, Estévez, Chiffelle, & Azenjo, 2005; Sudha, Baskaran, & Leelavathi, 2007). These phenolic compounds have antibacterial activity against a wide range of microorganisms (Sagdic, Ozturk, Yilmaz, & Yetim, 2011). Ethanolic extract of grape pomace at 10% has been shown to inhibit the growth of Enterobacteriaceae, S. aureus, Salmonella, yeasts and molds in beef patties during 48 h of storage at 4 °C indicating that pomace can be a good choice for extending the microbial shelf-life of food products (Sagdic et al., 2011). Similarly, spoilage and pathogenic bacteria including Aeromonas hydrophila, B. cereus, Enterobacter aerogenes, Enterococcus faecalis, E. coli, E. coli O157:H7, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella Enteritidis, Salmonella Typhimurium, S. aureus, and Yersinia enterocolitica have been inhibited by grape pomace extract (Özkan, Sagdıç, Göktürk Baydar, & Kurumahmutoglu, 2004). The antimicrobial properties of olive pomace and olive juice powder were investigated by Friedman, Henika, and Levin (2013) using a quantitative bactericidal activity assay against foodborne pathogens E. coli, L. monocytogenes, S. enterica, and S. aureus. The results indicated that olive pomace showed a similar inhibitory pattern to olive juice powder due to the presence of phenolic compound oleocanthal. This was also confirmed by Cicereala, Conlan, Barnett, and Keast (2011), who reported that olive pomace waste is also a valuable source of phenolic oleocanthal that is responsible for the antimicrobial action. Fruit skin contains various sugars and nutrients and also serves as a fermentation medium. Salem et al. (2009) demonstrated the production of lactic acid from apple skin waste using lactic acid bacteria. This could be a promising method for the utilization of fruit skin waste for the production of lactic acid which can be used as a preservative in a wide range of food applications.

The antimicrobial activity of fruit peels is well documented. For example, pomegranate fruit peels have been widely used in herbal remedies for treating several diseases (Al-Zorkey, 2009). Pomegranate fruit peels extracts were shown to inhibit the growth of several foodborne pathogens including L. monocytogenes, S. aureus, E. coli, Y. enterocolitica, and B. cereus (Agourram et al., 2013; Al-Zorey, 2009; Kanatt, Chander, & Sharma, 2010). Pomegranate peel extract was more effective against Gram-positive bacteria even at a concentration of 0.01%. However, in the case of Gram-negative bacteria, extract was effective against Pseudomonas spp. at a higher concentration of 0.1% and less effective against E. coli and S. Typhimurium at the same concentration (Kanatt et al., 2010; Negi & Jayaprakasha, 2003). It has also been reported that pomegranate

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Estimated resulting wastes of selected fruits and vegetables (Adapted from Oreopoulou &amp; Tzia, 2007).</td>
</tr>
<tr>
<td><strong>Fruits/vegetables</strong></td>
</tr>
<tr>
<td>Apple</td>
</tr>
<tr>
<td>Carrot</td>
</tr>
<tr>
<td>Cooking banana</td>
</tr>
<tr>
<td>Grape</td>
</tr>
<tr>
<td>Kiwifruit</td>
</tr>
<tr>
<td>Orange and other citrus fruits</td>
</tr>
<tr>
<td>Peach (canned)</td>
</tr>
<tr>
<td>Pear</td>
</tr>
<tr>
<td>Potato</td>
</tr>
<tr>
<td>Tomato</td>
</tr>
</tbody>
</table>

*Not available.*
peel extracts have some potential as preservatives in food products. Kanatt et al. (2010) assessed the shelf-life of chicken products using pomegranate peel extract. The results showed that the addition of 0.1% extracts in chicken meat reduced the total bacterial count, coliform, and S. aureus and enhanced the shelf-life by 2–3 weeks during chilling temperature storage. In addition, pomegranate peel inhibited the growth of S. aureus in inhibitory effect was less effective at higher temperatures (7°C and 12°C) indicating that the inhibitory effect of the extract was temperature dependent. The presence of active inhibitors in peels, phenolics, flavonoids, and tannins exhibit antimicrobial activity (Machado et al., 2003; Voravuthikunchai et al., 2005). Among several polyphenols present in pomegranate peel, its antimicrobial activity has been largely attributed to its hydrolyzable ellagitannins. Recently, the antilisterial effect of tannin-rich fraction from pomegranate rind was investigated by Li et al. (2014). Results from an agar dilution assay showed that the minimum inhibitory concentration (MIC) of tannin-rich fraction was 1.25 μg/ml for E. coli O157:H7, S. Typhimurium, and Yersinia enterocolitica. The MIC of this fraction for L. monocytogenes was 5.0 μg/ml for E. coli O157:H7, S. enterica, and S. aureus. Friedman et al. (2013) demonstrated that these tannin fractions impaired the cell membrane structure, leading to a loss of cell homeostasis.

### Table 2
Plant by-products as antimicrobials.

<table>
<thead>
<tr>
<th>By-products</th>
<th>Major component</th>
<th>Target organisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate fruit peels</td>
<td>Phenolics and flavonoids</td>
<td>L. monocytogenes, S. aureus, E. coli, Yersinia enterocolitica, P. fluorescens</td>
<td>Agourram et al. (2013); Al-Zoreky (2009)</td>
</tr>
<tr>
<td>Pomegranate juice byproducts</td>
<td>Phenolics, flavonoids, tannins</td>
<td>MRSATCC 43300 and E. coli ATCC 35218</td>
<td>Reddy, Gupta, Jacob, Khan, &amp; Ferreira (2007)</td>
</tr>
<tr>
<td>Apple peels</td>
<td>Polyphenolic compounds</td>
<td>S. aureus, P. fluorescens</td>
<td>Agourram et al. (2013)</td>
</tr>
<tr>
<td>Almond skin extracts</td>
<td>Polyphenols</td>
<td>S. aureus, L. monocytogenes</td>
<td>Mandalari et al. (2010)</td>
</tr>
<tr>
<td>Coconut husk</td>
<td>Phytochemical including phenolics and tannins</td>
<td>L. monocytogenes, S. aureus and V. cholera</td>
<td>Wonghirundecha and Sumpavapool (2012)</td>
</tr>
<tr>
<td>Green tea waste</td>
<td>Tannins</td>
<td>S. aureus, E. coli</td>
<td>Sung et al. (2012)</td>
</tr>
<tr>
<td>Acorn, chestnut, and persimmon hull</td>
<td>Tannins</td>
<td>L. monocytogenes, Bacillus coagulans, Shigella flexneri</td>
<td>Hayrapetyan, Hazelegger, and Beumer (2012)</td>
</tr>
<tr>
<td>Tomato seeds</td>
<td>Metabolites such as fatty acids, carotenoids, saponins, phenolic compounds</td>
<td>S. aureus, S. epidermidis, Micrococcus luteus, E. faecalis, B. cereus, Candida albicans</td>
<td>Taveira et al. (2010)</td>
</tr>
<tr>
<td>Quince fruit peel</td>
<td>Polyphenolic compounds such as chlorogenic acid, catechin, quercetin and kaempferol</td>
<td>S. aureus, P. aeruginosa, E. coli, C. albicans</td>
<td>Fattouch et al. (2007)</td>
</tr>
<tr>
<td>Potato peels</td>
<td>Chlorogenic, caffeic, gallic, and protocatechuc acids</td>
<td>Bacteriostatic effect on E. coli and S. Typhimurium</td>
<td>Sotillo, Hadley, and Wolf-Hall (1998)</td>
</tr>
<tr>
<td>Walnut green husk</td>
<td>Antioxidants such as phenolic compounds</td>
<td>S. aureus, B. cereus, B. subtilis</td>
<td>Oliveira et al. (2008)</td>
</tr>
<tr>
<td>Mango seed kernel extract</td>
<td>Phenolic compounds, saturated fatty acids, mono-unsaturated oleic acid, tocopherols, squalene, and different sterol fractions</td>
<td>Inhibited total bacterial count, coliforms, and E. coli</td>
<td>Abdalla et al. (2007)</td>
</tr>
<tr>
<td>Peels, seeds, and pulp of mexican lime</td>
<td>Phytochemicals: flavonones, methylated flavonones, tannins</td>
<td>E. coli O157:H7, S. Typhimurium, Shigella sonnei</td>
<td>Mathur et al. (2011); Orue, Garcia, Feng, and Heredia (2013)</td>
</tr>
<tr>
<td>Grape pomace</td>
<td>Phenolic acids, flavonoids, stilbenes</td>
<td>S. aureus, Salmonella, Enterococci, Total aerobic mesophilic and psychrotrophic bacteria, yeasts and molds</td>
<td>Sagdic et al. (2011)</td>
</tr>
<tr>
<td>Olive pomace</td>
<td>Phenolic compounds including oleocanthal, deoxylignocyclic acid lauryl ester</td>
<td>E. coli O157:H7, S. enterica, L. monocytogenes, and S. aureus</td>
<td>Friedman et al. (2013)</td>
</tr>
<tr>
<td>Beet root pomace extract</td>
<td>Phenolics, flavonoids betacyanins, betaxanthins</td>
<td>S. aureus, B. cereus E. coli, P. aeruginosa</td>
<td>Djilas and Markov (2011)</td>
</tr>
<tr>
<td>Buckwheat hull extracts</td>
<td>Phenolics, flavonoids, antioxidants comprising tocopherols, rutin, quercetin derivatives</td>
<td>Gram-positive (B. cereus, S. aureus, Enterococcus faecalis) and Gram-negative bacteria (Salmonella Choleraesuis, E. coli and Proteus mirabilis)</td>
<td>Cabarkapa et al. (2008)</td>
</tr>
<tr>
<td>Legume hulls (Vigna radiate, Cicer arietinum and Cajanus cajan)</td>
<td>Polyphenolic compounds, flavonoids</td>
<td>B. cereus, S. aureus</td>
<td>Kanatt et al. (2011)</td>
</tr>
<tr>
<td>Grapefruit seed extracts</td>
<td>Phenolic compounds such as catechins, epicatechin, epocetachin-3-O-gallate, dimeric, trimeric and tetrameric procyanidins</td>
<td>Pseudomonas spp.</td>
<td>Bevilaqua, Ficelo,Corbo, &amp; Sinigaglia (2010)</td>
</tr>
<tr>
<td>Oriental mustard (Brassica juncea L.) seed meal extracts</td>
<td>Phenolic compounds (sinapic acid and several sinapoyl conjugates)</td>
<td>B. subtilis, E. coli, L. monocytogenes, Pseudomonas fluorescens, and S. aureus</td>
<td>Engels et al. (2012)</td>
</tr>
<tr>
<td>Coffee pulp</td>
<td>Polyphenols such as flavan-3-ols, hydroxycinnamic acids, flavonoids and anthocyanidins</td>
<td>Total viable count, coliform, E. coli, and fungal count</td>
<td>Adebowale et al., (2012); Ramirez-Coronel et al. (2004); Ulioa Rojas et al. (2003)</td>
</tr>
</tbody>
</table>
bacteria (E. coli, S. Typhimurium, V. cholera). Hulls removed prior to the utilization of the seeds are also rich in bioactive components. The antimicrobial properties of flavonoid-rich fractions derived from almond skins, a by-product of the almond processing industry, were investigated by Mandalari et al. (2010) in culture medium. Almond skin fractions were found to have antimicrobial activity against L. monocytogenes and S. aureus in the range 0.25–0.5 mg/ml. The highest antimicrobial potential was for natural almond skins compared to that of blanched skins. This could be explained by the amount of flavonoids being 10 fold higher in natural almond skins compared to blanched skins. As a result, natural skins at 0.5 mg/ml were able to inhibit Gram-negative S. enterica var. Typhimurium. The hulls from buckwheat grains are more abundant in total phenolics and flavonoids in comparison to other parts of grain. According to Cabarkapa, Sedej, Sakac, Saric, and Plavšič (2008), Gram-positive (B. cereus, S. aureus, E. faecalis) and Gram-negative (Salmonella Choleraesuis, E. coli, Proteus mirabilis) bacteria were sensitive to all buckwheat hull extracts. The antimicrobial activity of different legume hulls was tested against common food spoilage and pathogenic bacteria showing the potentiality of legume hulls to be a novel natural additive (Kanatt, Arjun, & Sharma, 2011). This study showed >2 log CFU reductions of B. cereus at 0.05% of Cicer arietinum (Bengal gram) and complete inhibition at 0.1% of hull extracts when tested in 0.85% saline solution. Similarly, a one log reduction of S. aureus was achieved by 0.2% extract of C. arietinum and Cajanus cajan (pigeon pea) extract while Vigna radiata (mung bean) extract was ineffective against S. aureus. The presence of high polyphenols in these hull extracts could account for the microbial inhibitory effect.

Coffee is one of the most important agricultural commodities in the world. Coffee husks, peel and pulp, which comprises nearly 45% of the cherry, are one of the main by-products of coffee processing (Esquivel & Jiménez, 2012). Similarly, tea is another popular beverage consumed worldwide. Tea extract powder, a major by-product of tea processing also contains large amounts of phenolic compounds. These by-products contain active components such as caffeine and other polyphenols including tannins, flavonols, flavanols, and phenolic acids (Pandey et al., 2000; Ulloa Rojas, Verreth, Amato, & Huisman, 2003) and have the potential to be used as natural food preservatives (Gyawali, Adkins, Minor, & Ibrahim, 2013). In the last few decades, research interest has focused on the antibacterial properties of coffee and tea extract. The antioxidative and antimicrobial effects of coffee pulp were investigated against rancidity and microbial growth on smoked fish samples. Coffee pulp smoke has shown antioxidative effect on rancidity in smoked fish and suppression of microbial load (coliform, fungal count) during three weeks of storage at room temperature (Adebowale, Ogunjobi, Olubamiwa, Olusola-Taiwo, & Omidiran, 2012). However, studies on the antibacterial activity of coffee by-products including coffee pulp and tea waste have been very limited.

These studies have provided useful information on the utilization of by-products (peels, seeds, pulps, & husks) of different fruits and vegetables as natural antimicrobials in foods. These by-products could be of great benefit from economic and environmental perspectives as sources of low cost natural antimicrobials. In addition, the waste produced by the processing industry could be incorporated into antimicrobial packaging or utilized as edible antimicrobial films (Taveira et al., 2010).

### 3. Antimicrobials of animal origin

Some of the potential antimicrobials of animal origin which could be used as food additives are discussed below. In addition,
other antimicrobials derived from animal sources which are not discussed in this section are listed in Table 3.

3.1. Lactoferrin

Lactoferrin (Lf), an iron-binding glycoprotein present in milk, is reported to possess antimicrobial activity against a wide range of bacteria and viruses (Lönnerdal, 2011). Recently, Lf has been approved for application on beef in the United States and has been applied as an antimicrobial in a variety of meat products (Junea et al., Dwivedi, & Yan, 2012; USDA-FSIS, 2010). The antimicrobial properties of Lf against foodborne microorganisms including *Carnobacterium*, *L. monocytogenes*, *E. coli*, and *Klebsiella* have been reported (Al-Nabulsi & Holley, 2005; Murdock, Cleveland, Matthews, & Chikindas, 2007). Al-Nabulsi et al. (2009) reported a reduction of 4 log CFU/ml of *Cronobacter* spp. in the presence of 2.5 mg/ml Lf in 0.2% peptone water within 4 h incubation at 37 °C. The effects of Lf alone and in combination with nisin on the microbiological quality of meatballs were evaluated by Colak, Hampikyan, Bingol, and Aksu (2008). Meatballs treated with a mixture of Lf (0.2 mg/g) and nisin (0.1 mg/g) showed a significant reduction in spoilage bacterial counts (total aerobic bacteria, coliform, *E. coli*, total psychrophilic bacteria, *Pseudomonas* spp., yeast and molds) and extended shelf-life of up to 10 days. Lf hydrolyzate has also been shown to reduce the population of *E. coli* O157:H7 and *L. monocytogenes* by approximately 2 log in ultra-high temperature milk (Murdock & Matthews, 2002). The results suggest that Lf hydrolyzate can limit the growth or reduce the population of pathogenic bacteria in a dairy product. Shimazaki (2000) investigated the addition of Lf to rehydrated powder infant milk formula as a means of inhibiting pathogenic microorganisms.

There are several explanations that may account for Lf’s anti-microbial action. One is that it limits microbial access to nutrients via iron chelation, which thereby produces an iron deficient medium. (González-Chávez, Arévalo-Gallegos, & Rascón-Cruz, 2009).

Another possibility is that Lf is capable of destabilizing the outer membrane of Gram-negative bacteria, which results in liberation of lipopolysaccharides with increased membrane permeability (Orsi, 2004). Support for this latter explanation is provided by Jønsson and Hancock (2009), who demonstrated that Lf can increase the antibacterial effect of commercial drugs like rifampicin by damaging the bacterial membrane.

Various published works have shown that Lf can provide iron for the growth of *Bifidobacterium* spp. However, under defined environmental condition such as lack of minerals, Lf with bifidobacterial strains acts indirectly against the growth of pathogens (Gyawali & Ibrahim, 2012). The mechanism enabling this appears to involve microbial iron uptake (Bezkorovainy, Kot, Miller-Catchpole, Halofitis, & Furmanov, 1996). Iron acts as a growth promoting factor for bifidobacteria, and bifidobacteria have the ability to bind iron, thus making it unavailable to pathogens thus negatively impacting their growth (Bezkorovainy & Miller-Catchpole, 1989). Under low iron condition, these probiotic bacteria start producing antimicrobial ‘bifidogenic’ compounds (Gyawali & Ibrahim, 2012; Ibrahim, 2005) that can inhibit the growth of other harmful microorganisms. Thus, an Lf rich medium with bifidobacterial strains can be used to improve microbiological safety and quality.

3.2. Chitosan

Among several natural antimicrobials, chitosan has received considerable interest for commercial applications in food. Chitosan is a polycationic biopolymer naturally present in the exoskeletons of crustaceans and arthropods (Tikhonov et al. 2006). The application of chitosan as a food preservative and for other uses has been limited by its insolvability at neutral and higher pH. Improvement of its solubility could improve its application as a food preservative (Du, Zhao, Dai, & Yang, 2009). The antibacterial activity of water-soluble chitosan derivatives prepared by Maillard reactions against *S. aureus*, *L. monocytogenes*, *B. cereus*, *E. coli*, *Shigella* is discussed in this chapter.

### Table 3

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Major component</th>
<th>Major source</th>
<th>Target organisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin</td>
<td>Iron binding glycoprotein</td>
<td>Milk</td>
<td><em>E. coli</em> O157:H7, <em>L. monocytogenes</em>, <em>Salmonella</em>, <em>Pseudomonas</em></td>
<td>Lönnerdal 2011; Perez, Taylor, &amp; Taormina 2012</td>
</tr>
<tr>
<td>Lipids</td>
<td>Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)</td>
<td>Fish and shellfish</td>
<td><em>B. subtilis</em>, <em>L. monocytogenes</em>, <em>S. aureus</em> ATCC 6538, <em>S. aureus</em> KCTC 1916, and <em>Enterobacter aerogenes</em>, <em>E. coli</em>, <em>E. coli</em> O157:H7, <em>Pseudomonas aeruginosa</em>, <em>S. Enteritidis</em>, and <em>S. Typhimurium</em></td>
<td>Shin, Bajpai, Kim, and Kang 2007</td>
</tr>
<tr>
<td>Defensins</td>
<td>Cationic peptides</td>
<td>Mammalian epithelial cells of chickens, turkeys, Raw milk, colostrum, saliva and other biological secretions</td>
<td>Gram-positive and Gram-negative bacteria, fungi, viruses</td>
<td>Tiwari et al. 2009</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>Glycoprotein</td>
<td></td>
<td><em>Salmonella</em>, <em>E. coli</em>, <em>S. aureus</em>, <em>L. monocytogenes</em>, <em>Yentericalitica</em></td>
<td>Perez et al. 2012</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Glutamic acid, aspartic acid</td>
<td>Hens’ eggs, mammalian milk</td>
<td><em>C. tyrobutyricum</em>, <em>Bacillus</em>, <em>Micrococcus</em>, <em>L. monocytogenes</em></td>
<td>Junea et al. 2012; Perez et al. 2012</td>
</tr>
<tr>
<td>Pleurocidin</td>
<td>Polypeptides</td>
<td>Myeloid cells and mucosal tissues of many vertebrates and invertebrates</td>
<td><em>L. monocytogenes</em>, <em>E. coli</em> O157:H7, and pathogenic fungi</td>
<td>Burrowes, Hadjicharalambous, Diamond, and LEE 2004; Jung et al. 2007</td>
</tr>
<tr>
<td>Protamine</td>
<td>Cationic antimicrobial peptides</td>
<td>Salmon</td>
<td><em>L. monocytogenes</em>, total bacteria, coliforms</td>
<td>Junea et al. 2012; Potter, Truelstrup Hansen, and Gill 2005</td>
</tr>
<tr>
<td>Casein and whey</td>
<td>Bioactive peptides (Isracidin nα, casein f1-23; Casocidin-I f150-188; Kappacin)</td>
<td>Milk protein</td>
<td><em>Enterobacter sakazakii</em> ATCC 12868, <em>E. coli</em> DPC5063, <em>S. aureus</em>, <em>L. monocytogenes</em>, <em>S. Typhimurium</em>, <em>B. subtilis</em></td>
<td>Hayes et al. 2006; Körhonen and Rokka 2010</td>
</tr>
</tbody>
</table>
*dyssenteriae*, and *S. Typhimurium* have shown to be a promising commercial substitute for acid-soluble chitosan (Chung, Yeh, & Tsai, 2011). Similarly, the solubility limitation of native chitosan has been overcome by using N-alkylated disaccharide chitosan derivatives that exhibited antibacterial activity against *E. coli* and *S. aureus* (Yang, Chou, & Li, 2005). In a study by Du et al. (2009), water-soluble chitosan at 2.0% showed strong inhibitory effects against *E. coli*, *B. subtilis*, and *S. aureus* with diameters of inhibition zones greater than 20 mm. Rhodes and Roller (2000) reported that mild hydrolysis of chitosan improved microbial inactivation in saline and a greater inhibition of growth of several spoilage yeasts in laboratory media. The addition of 0.3 mg of chitosan per ml eliminated yeasts entirely for the duration of 13 days in pasteurized apple-juice stored at 7 °C. The antibacterial activity of chitosan was examined against several Gram-negative (*E. coli*, *P. fluorescens*, *S. Typhimurium*, *Vibrio parahaemolyticus*) and Gram-positive bacteria (*L. monocytogenes*, *Bacillus megaterium*, *B. cereus*, *S. aureus*) by No, Young Park, Ho Lee, and Meyers (2002). Authors reported that chitosan inhibited the growth of most of the tested bacteria showing stronger bactericidal effects against Gram-positive bacteria than Gram-negative bacteria at a concentration of 0.1% in agar medium. Helder, Nurmiho-Lassila, Ahvenainen, Hronadék, and Knappe (2007) investigated the effect of chitosan concentration on Gram-negative bacteria (*E. coli* and *S. Typhimurium*) using electron microscopy. This study showed that chitosan at 0.025% caused extensive cell surface alterations and covered the outer cell membrane with vesicular structures, thus explaining the loss of the bacterial barrier function. Tsai, Wu, and Su (2000) investigated the antimicrobial activity of a chitosan against various food poisoning and food spoilage bacteria. The chitosan mixture retarded the growth of *Salmonella* spp. and reduced the population of *Staphylococcus* spp. in raw milk. A chitosan concentration of 5.0 mg/ml was used to determine the shelf-life of oysters stored at 5 ± 1 °C (Cao, Xue, & Liu, 2009). This study showed that chitosan treatment extended the shelf-life of oysters from 8–9 days to 14–15 days, indicating that chitosan has a great potential for seafood preservation. Our literature review indicates that chitosan has been applied mainly as an antimicrobial packaging films and coatings as discussed in this review under the section ‘incorporation of antimicrobials in food systems’.

### 3.3. Lysozyme

Lysozyme is an enzyme that is naturally present in avian eggs and mammalian milk and is generally recognized as safe (GRAS) for direct addition to foods (FDA, 1998). The white lysozyme of hen eggs is a bacteriolytic enzyme widely reported for its application as an antimicrobial in food products (Tiwari et al., 2009) and commonly used as a preservative for meat, meat products, fish, fish products, milk and dairy products, and fruits and vegetables (Cegelska-Radziejewska, Lesniewski, & Kijowski, 2009). The antimicrobial activity of lysozyme is due to its ability to hydrolyze the β-1, 4 linkage between N-acetylmuramic acid and N-acetylgalcosamine in the peptidoglycan of the microbial cell wall (Juneja et al., 2012). Lysozyme has been used primarily to prevent late-blowing defect in cheeses, caused by *Clostridium tyrobutyricum* (FDA, 1998). Lysozyme showed the highest antimicrobial activity against *Listeria innocua* and *Saccharomyces cerevisiae* with an inhibition zone of 19.75 and 17.37 mm, respectively (Rawdkuen, Suthaluk, Kamhangwong, & Benjakul, 2012). Peptides derived from hen egg lysozyme at a concentration of ≥0.01 mg/ml showed an inhibitory effect on both vegetative and spore forms of *B. subtilis*. This result may be useful in controlling the growth of *Bacillus* spoilage organisms as well as increasing the shelf-life of many food products (Abdou, Higashiguchi, Aboueleinin, Kim, & Ibrahim, 2007).

As reported by Tiwari et al. (2009), the cell wall of Gram-positive bacteria consists of peptidoglycan, which makes it susceptible to the activity of lysozyme. This susceptibility is contrary to that of Gram-negative bacteria which are generally resistant to lysozyme due to their lipopolysaccharidic layer of outer membrane which acts as a physical barrier. However, the susceptibility of Gram-negative bacteria to lysozyme can be increased by the use of detergents and chelators (EDTA) as membrane disrupting agents (Branen & Davidson, 2004). These authors reported the synergistic effect of lysozyme with EDTA against *E. coli*. The effect of different chelating agents (EDTA, disodium pyrophosphate, & pentasodium tripolyphosphate) in the presence of lysozyme on the inhibition of *E. coli O157:H7* was also investigated by Boland, Davidson, and Weiss (2003). Their results indicated that the inhibition of *E. coli O157:H7* occurred with each lysozyme–chelator combination. Simgaglia, Bevilacqua, Corbo, Pati, and Del Nobile (2008) also studied the effectiveness of lysozyme and EDTA against the growth of coliforms and *Pseudomonas* on the microbiological shelf-life of mozzarella cheese.

### 3.4. Milk-derived peptides

Milk-derived bioactive compounds such as casein and whey proteins have been found to have multifunctional properties including antimicrobial properties (Phelan, Ahern, FitzGerald, & O’Brien, 2009; Schanbacher, Talbou, Murray, Gherman, & Willett, 1998). These peptides have been found to be active against a broad range of pathogenic microorganisms such as *E. coli*, *Helicobacter*, *Listeria*, *Salmonella*, *Staphylococcus*, yeasts, and filamentous fungi (Fadai, 2012). In this section, we review the literature pertaining to antimicrobial peptides derived from major milk proteins such as caseins, α-lactalbumin and β-lactoglobulin upon hydrolysis either by digestive proteases or by fermentation with proteolytic lactic acid bacteria.

Casein accounts for 80% of total milk protein and is a rich source of bioactive peptides. Antibacterial peptides have been identified from αs1-casein and αs2-casein (McCann et al., 2006). Hydrolysis of αs2-casein by chymosin released a compound called casocidin. This has shown antibacterial properties against *Staphylococcus* spp., *Sarcina* spp., *B. subtilis*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes* (Szwarzkowska, Wolaniuk, Barowski, Król, & Litwińczuk, 2011). Isracidin is another casein derived peptide that has been found to be effective against *S. aureus*, *L. monocytogenes*, and *C. albicans* (Korhonen & Rokka, 2010). Hayes, Ross, Fitzgerald, Hill, and Stanton (2006) reported antimicrobial activity of peptides derived from milk proteins. Peptides such as casein A and B at 0.05 mM and 0.22 mM respectively, inhibited the growth of pathogenic strain *Enterobacter sakazakii* when tested using the agar well diffusion assay. This indicates the potential application of these peptides in milk-based formula, which has been linked to *E. sakazakii* infection in neonates. Peptides generated from αs1-casein, αs2-casein, and κ-casein have shown antibacterial effects against *E. coli* and *B. subtilis* (Elbarbary et al., 2012). Yak milk κ-casein hydrolyzate at 0.5 mg/ml was also found to be effective against *E. coli* (Cheng, Tang, Wang, & Mao, 2013).

The whey protein fraction of bovine milk consists mainly of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA). Biologically active antibacterial peptides are released during the digestion of β-lactoglobulin with trypsin. Szwarzkowska et al. (2011) reported that these peptides demonstrated antimicrobial activity against foodborne pathogens such as *S. aureus*, *L. monocytogenes*, *Salmonella* spp. and *E. coli O157*. The antibacterial activity of peptides derived from β-LG at concentration of 10–20 mg/ml was shown to inhibit
the growth of *L. monocytogenes* and *S. aureus* in culture medium (Demers-Mathieu et al., 2012). Similarly, peptides obtained by tryptic hydrolysis and those obtained by chymotryptic hydrolysis of bovine α-LA were identified as bactericidal to *B. subtilis* and *S. epidermidis* (Pellegrini, Thomas, Bramuz, Hunziker, & von Fellenberg, 1999). Pihlanto-Leppälä et al. (1999) also showed that bovine β-LG & α-LA hydrolyzate fractions enhanced bacteriostatic properties against *E. coli* JM103. Synergistic effects in inhibiting *E. coli*, *S. Typhimurium*, and *Shigella flexneri* resulted from peptide fractions containing α-LA and glycomacropeptide at concentrations of 0.25–0.05 mg/ml (Korhonen & Rokka, 2010). Cheng et al. (2013) reported peptide accumulation on the cell membrane surface can lead to increased permeability and accompanied leakage of cytoplasmic components, which ultimately results in cell death. However, the exact mode of action of antimicrobial peptides has not been established.

4. Antimicrobials of bacterial origin

Microbes, especially lactic acid bacteria (LAB) produce a wide range of chemicals with antimicrobial activity. Among these, the proteinaceous compounds called bacteriocins such as nisin have been shown to inhibit the growth and development of other microbial species. Similarly, reuterin produced from glycerol by some strains of *Lactobacillus reuteri* is another widely used broad-spectrum antimicrobial agent. Reuterin is also effective against many pathogenic and spoilage microorganisms. In this section, we discuss two widely recognized antimicrobials of bacterial origin: bacteriocin and reuterin as natural preservatives. Some other antimicrobials that are derived from microorganisms are listed in Table 4.

4.1. Bacteriocin

Nisin, the only bacteriocin approved for use as an antimicrobial is used in over 50 countries world-wide. It is produced by *Lactococcus lactis* and is active against Gram-positive and spore forming bacteria associated with food (Arquès, Rodríguez, Nuñez, & Medina, 2011; Lucera, Costa, Conte, & Del Nobile, 2012; O’Sullivan, 2012). Nisin acts as bactericide against Gram-positive foodborne pathogens or spoilage bacteria such as *S. aureus*, *M. luteus*, and *B. cereus* (Rajendrani, Nagappan, & Ramamurthy, 2013). It achieves this effect by permeating the cytoplasmic membrane causing leakage of intracellular metabolites and dissipation of membrane potential (Lucera et al. 2012). Changes in *Bacillus* morphology causing leakage of cytoplasmic contents were also reported in the presence of nisin (Hyde, Parisot, McNichol, & Bone, 2006). Davies, Bevis, and Delves-Bruheto (1997) conducted a shelf-life study of ricotta cheese and found that the incorporation of nisin at 0.0025 mg/ml inhibited the growth of *L. monocytogenes* during 8 weeks of storage.

Bacteriocin is known to be more effective against Gram-positive bacteria than Gram-negative bacteria. The resistivity of Gram-negative bacteria is explained by the presence of a protective outer membrane that forms the outermost layer of the cell membrane. Moreover, researchers have found that nisin’s activity against Gram-negative bacteria can be enhanced when used together with outer membrane disrupting agents (Belfiore, Castellano, & Vignolo, 2007). Natural hydrophobic compounds such as EOs are known to have outer membrane disrupting properties. Therefore, it has been suggested that combining bacteriocin with EOs may enhance the inhibitory effect against Gram-negative bacteria (Ghrair & Hani, 2013). Galvez, Abrioue, López, and Omar (2007) reported the enhancing effect of nisin by using exposure to chelating agents (EDTA), sub-lethal heat, and osmotic shock and freezing, because these treatments make the outer membrane of Gram-negative microorganisms more permeable and therefore more susceptible to nisin. Nisin in combination with EDTA has exhibited antimicrobial activity against some Gram-negative spoilage and pathogenic bacteria, such as *E. coli*, *P. aeruginosa*, and *S. Typhimurium* (Martín-Visscher, Van Bleulink, & Vederas, 2011). The antimicrobial activity against *S. Enteritidis* in minced sheep meat was found to be stronger when nisin at 500 IU/g was combined with oregano EO at 0.09% (Goveris, Solomakos, Pexara, & Chatzopoulou, 2010). Recently, Field et al. (2012) used a mutagenesis approach by altering the amino acid sequence to develop nisin derivatives with enhanced activity against both Gram-positive and Gram-negative bacteria such as *S. aureus*, *E. coli*, *S. enterica* serovar Typhimurium, and *Cronobacter sakazakii*. Nisin can be applied either alone or in combination with other natural compounds as a food preservative against foodborne pathogens and thus extend product shelf-life. Nisin in combination with natamycin significantly inhibited fungal growth (yeasts/molds) in table olives (Hondrodinou Kourkoutas, & Panagou, 2011) and in soft Galotyri cheese, extending the shelf-life (>28 days) compared to control samples (14–15 days) (Kallintiri, Kostoula, & Savvaidis, 2013). Pediocin is another type of bacteriocin produced by *Pediococcus* strains such as *P. acidilactici* and *P. pentosaceus*. Pediocins have been used as food preservatives in vegetables and meat based products (Papagianni & Anastasiadou, 2009). Most pediocins are thermostable peptides and function over a wide range of pH values. Pediocins have shown proven efficacy against both spoilage and pathogenic organisms, including *L. monocytogenes*, *E. faecalis*, *S. aureus*, and *Clostridium perfringens* (Juneja et al., 2012; Tiwari et al., 2012).

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Source</th>
<th>Target organisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td>Lactococcus lactis</td>
<td>Clostridium spp., <em>L. monocytogenes</em></td>
<td>Lucera et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Strains of Pediococcus acidilactici and <em>P. pentosaceus</em></td>
<td><em>L. monocytogenes</em>, Enterococcus faecalis, <em>S. aureus</em>, <em>C. perfringens</em></td>
<td>Tiwari et al. (2009)</td>
</tr>
<tr>
<td>Natamycin</td>
<td>Streptomyces natalensis</td>
<td>Fungi</td>
<td>Juneja et al. (2012)</td>
</tr>
<tr>
<td>Lactocin</td>
<td>Lactobacillus curvatus CRL705</td>
<td><em>E. coli</em> strains</td>
<td>Belfiore et al. (2007)</td>
</tr>
<tr>
<td>Bulgaricin</td>
<td>L. bulgaricus</td>
<td><em>L. monocytogenes</em>, <em>S. aureus</em>, <em>B. subtilis</em>, Helicobacter pylori</td>
<td>Siamansouri et al. (2013); Simova, Beshkova, &amp; Dimitrov (2009)</td>
</tr>
<tr>
<td>Helveticin</td>
<td>L. helveticus</td>
<td><em>L. monocytogenes</em>, Clostridium botulinum</td>
<td>O’Sullivan, Ross, and Hill (2002); Siamansouri et al. (2013)</td>
</tr>
<tr>
<td>Plantaricin</td>
<td>L. plantarum</td>
<td><em>L. monocytogenes</em>, <em>S. aureus</em>, <em>S. Typhimurium</em>, <em>E. coli</em></td>
<td>Gong, Meng, and Wang (2010); Siamansouri et al. (2013)</td>
</tr>
</tbody>
</table>
et al., 2009). The antimicrobial activity of pediocin against Oenococcus oeni and other wine bacteria has been reported by Diez et al. (2012).

4.2. Reuterin

Reuterin (β-hydroxypropionaldehyde) is a molecule with antimicrobial activity towards a broad spectrum of foodborne pathogens and spoilage organisms. Its high solubility in water, resistance to heat, proteolytic and lipolytic enzymes, and stability over a wide range of pH values makes reuterin ideal bio-preservative for foods. (Arquès et al., 2011; Vollenweider, Grassi, König, & Fuhin, 2003). Arquès et al. (2011) demonstrated the antimicrobial activity of reuterin in combination with different bacteriocins from lactic acid bacteria against foodborne pathogens in milk at refrigerated temperature. A synergistic effect of reuterin in combination with nisin was observed on L. monocytogenes and S. aureus. Reuterin from L. reuteri DPC16 showed a significant reduction in bacterial population against Gram-positive (S. aureus, L. monocytogenes) and Gram-negative (E. coli, S. Typhimurium) bacteria (Bian, Molan, Maddox, & Shu, 2011). In another study, the effect of reuterin from L. reuteri strain 12002 was tested against E. coli O157:H7 and L. monocytogenes in ground pork (El-Ziney, Van Den Tempel, Debevere, & Jacobsen, 1999). In this study, reuterin at 100 AU/g reduced a 5 log of E. coli O157:H7 after 1 day of storage at 7 °C. L. monocytogenes was reduced by 3 log after 7 days of storage under the same conditions. Likewise, reuterin in milk showed an inhibition of Gram-negative as well as Gram-positive pathogenic and spoilage bacteria (Stevens, Vollenweider, & Lacroix, 2011). The use of reuterin to control Gram-positive and Gram-negative pathogens has been investigated in milk, dairy, and in meat products (Arquès et al., 2011; El-Ziney et al., 1999). These studies have shown that reuterin has a higher antimicrobial activity on Gram-negative than on Gram-positive pathogenic bacteria. Although the mechanism of antimicrobial activity is not yet clear, a recent study reported that the aldehyde form of reuterin is the bioactive agent which causes an oxidative stress response by modifying thiol groups in proteins and small molecules (Langa et al. 2013).

5. Algae and mushrooms

In recent years, there have been many reports of algae and mushrooms as natural sources of bioactive compounds that have a broad range of biological activity, such as antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic, antimitotic activity and other health promotingbenefits (Bhagavathy et al., 2011; Demirel, Yilmaz-Koz, Karabay-Yavasoglu, Ozdemir, & Sukatar, 2009; Plaza et al., 2010; Willis, Iskhuemhen, & Ibrahim, 2007). However, there has been limited research devoted to the evaluation of the antimicrobial activity of algae and mushrooms in food preservation. The rich diversity of different algae and mushrooms species could therefore offer a potential source of novel antimicrobials. This section deals with potential applications of these species in controlling foodborne pathogens. Some examples of algae and mushrooms, their antimicrobial components, and action against pathogenic microorganisms are listed in Table 5.

5.1. Algae

The antimicrobial activity of different types of algae against pathogenic bacteria has been identified by several scientists as potential antimicrobial agents that may be useful in the food industry. Plaza et al. (2010) reported the antimicrobial activity of algae (Himanthalia elongate) and microalgae (Synechocystis spp.) against E. coli and S. aureus. Likewise, the antimicrobial activity of phlorotannins isolated from marine brown algae has been shown to inhibit the growth of foodborne pathogenic bacteria as well as antibiotic resistant bacteria (Eom, Kim, & Kim, 2012). The interactions between phlorotannins and bacterial proteins are considered to play an important role in bacterial inhibition. Salem, Galal, and Nasr El-deen (2011) tested the antibacterial effects of several marine algae against both Gram-positive (S. aureus NCIMB 50080 and B. cereus) and Gram-negative bacteria (E. coli NCIMB 50034, E. feacalis NCIMB 50030, Salmonella spp. and P. aeruginosa). Several photosynthetic pigments and derivatives have been isolated from marine algae and have been found to have bactericidal effects against the Gram-positive bacterium B. subtilis (Smith, Desbois, & Dyrynda, 2010). Yamada, Itoh, Murakami, and Izumi (1985) isolated halogenated compounds such as bromophenols from different types of algae that have antibacterial properties. Crude extracts of algae showed a strong inhibition against all tested pathogens. However, Gram-positive bacteria were found to be more susceptible to extracts producing a larger zone of inhibition than Gram-negative bacteria. In a similar study, Devi, Suganthi, Kesika, and Pandian (2008) reported a significantly higher zone of inhibition produced by seaweed extract Haligra spp. at 50 mg/ml concentration against Gram-positive food poisoning bacteria S. aureus (MTCC 96) than the standard antibiotic streptomycin benzate at 200 mg/ml. Authors also determined the minimum bactericidal concentration of Haligra spp. extract against S. aureus at a concentration of 5 mg/ml in skimmed milk, demonstrating the potential of this algae to be used in foods including dairy products, raw meats, poultry, salads, shrimp, and ham. Extracts of several microalgae were screened for their activity against common foodborne pathogens. Among the tested algae, extract of Scenedesmus obliquus showed strong inhibition against Gram-positive (S. aureus) and Gram-negative (Salmonella spp., E. coli, P. aeruginosa) bacteria (Catarina, Barbosa, Amaro, Pereira, & Xavier, 2011).

Compounds reported to be present in algae included terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulfides, and fatty acids that act as bactericidal agents (Watson & Cruz-Rivera, 2003). The presence of these compounds suggests a number of alternative mechanisms for antimicrobial action. For example, the presence of long chain fatty acids may act in a similar fashion as most of the plant extracts by promoting membrane damage that eventually permit cell leakage (Catarina, Barbosa, Amaro, Pereira, & Xavier, 2011). The antifungal activity of seaweed extracts can be explained by the presence of phenolic compounds and their impact on spore germination. Some algal extracts have also been shown to inhibit fungal enzyme activity due to the presence of bioactive metabolites (Gallal, Salem, & Nasr El-deen, 2011).

5.2. Mushrooms

Among fungi, mushrooms are commonly eaten as food and have been shown to possess antimicrobial properties (Gao et al., 2005; Ishikawa, Kasuya, & Vanetti, 2001; Turkgolu, Duru, Mercan, Kivrak, & Gezer, 2007; Willis, King, Iskhuemhen, & Ibrahim, 2009). The potential of several species of macrofungi to be used as a good source of natural antibiotics and antioxidants and as a possible food supplement was previously reported by Kalyoncu, Oskay, Saglam, Erdogan, and Tamer (2010). Many researchers have identified the antimicrobial activity of different species of these fungi due to the presence of several bioactive compounds. Varieties of Australian basidiomycetes macrofungi extracts were tested against Gram-positive (B. cereus, L. monocytogenes) and Gram-negative bacteria (P. aeruginosa, Acinetobacter baumannii). The ethanol extracts of all tested macrofungi inhibited the growth of one or more of these pathogens at 10 mg/ml (Bala, Aitken,
Antimicrobials from algae and mushrooms.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Source</th>
<th>Major component</th>
<th>Target organisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine algae</td>
<td>Gracilariaopsis longissima</td>
<td>Polyphenols, carotenoids, amino acids, and catechins (e.g., catechin, epigallocatechin, epigallocatechin).</td>
<td>Vibrio spp.</td>
<td>Cavallio et al. (2013);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Demirel et al. (2009)</td>
</tr>
<tr>
<td>Marine algae</td>
<td>Ecklonia cava, E. kurome, E.</td>
<td>Phlorotannins</td>
<td>S. aureus, MRSA, Salmonella spp., E. coli</td>
<td>Eom et al. (2012)</td>
</tr>
<tr>
<td>brown algae</td>
<td>stolonifera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-gilled</td>
<td>Cantharellus cibarius, Clavaria vermiculata, Ramaria Formosa Quel, Marasmus oredes, Pleurotus pulmonarius</td>
<td>Total phenols, flavonoid and ascorbic acid</td>
<td>S. aureus (ATCC 25923); B. subtilis (ATCC 6633); E. coli (ATCC 25922); P. aeruginosa (ATCC 27853)</td>
<td>Ramesh and Pattar (2010)</td>
</tr>
<tr>
<td>Fungal group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edible mushroom</td>
<td>Agaricus spp.</td>
<td>Fatty acids, β-carotene-linoleic acid, phenols, &amp; flavonoids</td>
<td>B. cereus, B. subtilis, P. aeruginosa, E. coli</td>
<td>Barros et al. (2007)</td>
</tr>
<tr>
<td>Edible mushroom</td>
<td>Lactarius deliciosus, Lactarius piperatus Sarcodon imbricatus and Tricholoma portentosum</td>
<td>Total phenols, flavonoids, ascorbic acid, β-carotene, lycopene</td>
<td>B. cereus, B. subtilis</td>
<td>Kitzberger, Smanio Jr, Pedrosa, and Ferreira (2007)</td>
</tr>
<tr>
<td>Edible mushroom</td>
<td>Lentinula edodes</td>
<td>Flavonoid components, palmatic acid, linoleic acid, ergosterol</td>
<td>Micrococcus luteus, B. cereus</td>
<td>Ren et al. (2014)</td>
</tr>
<tr>
<td>Edible mushroom</td>
<td>C. sinensis, P. australis</td>
<td>Polysaccharides, triterpenes</td>
<td>B. subtilis, S. epidermidis, P. aeruginosa, S. Enteritidis, E. coli, Y. enterocolitica, S. aureus, B. cereus</td>
<td>Turkoglu et al. (2007)</td>
</tr>
</tbody>
</table>

Table 5

Antimicrobials from algae and mushrooms.

Cusack, & Steadman, 2012) indicating fungi as a potential source of antimicrobials. Edible mushroom extract from the non-gilled fungal group of *Aphyllophorales* were found to exhibit antimicrobial effects against pathogenic bacteria (Ramesh & Pattar, 2010). The extract of these fungi produced zones of inhibition in the range of 10–24 mm against tested Gram-positive (*B. subtilis, S. aureus*) and Gram-negative bacteria (*E. coli, P. aeruginosa*). The antimicrobial effects of methanol extract of edible mushrooms from *Agaricus spp.* were tested against Gram-positive and Gram-negative bacteria. These extracts were found to be effective on Gram-positive bacteria, especially against *M. luteus, Micrococcus flavus, B. subtilis,* and *B. cereus* (Ozturk et al., 2011). The antimicrobial properties of phenolic extracts of Portuguese wild edible mushroom species (*Lactarius deliciosus, Sarcodon imbricatus,* and *Tricholoma portentosum*) against pathogens were also investigated by Barros et al. (2007). These mushrooms showed a higher inhibition of Gram-positive bacteria (*B. cereus, B. subtilis*), while Gram-negative bacteria (*E. coli*) were found to be resistant. The antimicrobial properties of shiitake mushrooms (*Lentinula edodes*) have been shown against a wide range of Gram-positive and negative bacteria including *B. subtilis, B. cereus,* and *P. aeruginosa, E. coli* (Ozturk et al., 2011). The compound lentinan in shiitake mushrooms reportedly is the basis for its antimicrobial action. Recently, Ren et al. (2014) tested the antimicrobial effect of polysaccharide extracts of edible mushroom species. The polysaccharide extracts from *Cordyceps sinensis* inhibited the growth of Gram-positive strains *B. subtilis,* and *S. epidermidis.* Another mushroom species, *Pleurotus australis,* showed inhibition only against *S. epidermidis* while two other Gram-negative strains (*E. aerogenes, E. coli* 916) were resistant to mushroom extracts. The sensitivity of Gram-positive bacteria to mushroom extracts is explained by the absence of membrane bound periplasm. Instead, Gram-positive bacteria possess hydrophilic porus structure making cell walls more permeable. Similarly, Venturini, Rivera, Gonzalez, and Blanco (2008) also reported the resistivity of Gram-negative *E. coli* to extract of the mushroom *L. edodes.*

The antimicrobial activity of mushrooms may be attributed to the presence of various bioactive secondary metabolites, volatile compounds, some phenols, gallic acids, free fatty acids and their derivatives (Bala et al., 2012; Ramesh & Pattar, 2010). Thus, mushrooms producing these bioactive components could support applications in the food industry as an effective source of natural substances that could be used as preservatives in order to ensure food preservation and safety. Considering the wide biodiversity of mushrooms, they could easily become accessible sources of antimicrobial compounds. Further investigation into the potential use of mushrooms as food preservatives is warranted due to increasing interest in the search for more natural alternatives.
6. Incorporation of antimicrobials in food systems

Antimicrobials in foods can be added in different forms to control the growth of pathogenic and spoilage microorganisms. These can be either added directly or through slow release from packaging materials. In this section, we highlight three major methods of incorporating antimicrobials in food systems. These methods could play an important role in reducing harmful microorganisms and thus extending product shelf-life.

6.1. Direct applications

Researchers have demonstrated the antimicrobial activity of different natural compounds against a wide range of pathogenic microorganisms. There have been a number of studies conducted in culture media and tested in food products. These antimicrobial compounds have been directly applied in food systems either in the form of a powder or a liquid. In this section, we highlight the antimicrobial activity of compounds derived from plants in model food systems. However, only a few natural antimicrobials have found practical application in the food industry and their use in foods as preservatives is often limited due to the strong smell and taste they impart to these foods. In addition, natural antimicrobials' solubility in complex food matrices is another limitation (Sokovic et al., 2010).

Budka and Khan (2010) demonstrated the effect of EO's from basil, thyme, and oregano against B. cereus in rice-based foods. Carvacrol (EO of oreganoum and thyme) at 0.15 mg/g inhibited the growth of B. cereus on rice (Uitlee, Slump, Steging, & Smid, 2000).

Similarly, Smith-Palmer, Stewart, and Fyfe (2001) reported the inhibition of L. monocytogenes and Salmonella enteritidis in both low fat and full fat cheese in the presence of 1% clove, cinnamon, thyme, and bay oil. The antimicrobial effect of rosemary extract against L. monocytogenes populations by more than 2.0 logs in chicken meat (Piskernik, Klanclnik, Riedel, Brondsted, & Mozina, 2011). Xi, Sullivan, Jackson, Zhou, and Sebranek (2011) investigated the effect of cranberry powder against L. monocytogenes growth in meat model system. The results showed a 2–4 log CFU/g reduction in bacterial population at concentrations of 1–3% when compared to the control sample treated with nitrite. This showed a possibility of using natural ingredients such as cranberry powder instead of sodium nitrite to enhance the antibacterial quality and shelf-life of naturally cured meat.

Overall, plant extracts could be used as natural antimicrobial additives to prolong the shelf-life of foods. However, the level of these preservatives required for sufficient inhibition of microorganisms in foods may be considerably higher in comparison to laboratory media. Because this higher concentration may negatively impact the organoleptic properties of food, the use of natural compounds in combination with other natural preservatives or with other technologies could produce synergistic effects against foodborne pathogens.

6.2. Edible films and coatings

In recent years, food-packaging industries have shown an interest in edible films and coatings from natural antimicrobials. Edible films also improve food quality by providing barriers to moisture, and oxygen, and could serve as a barrier to surface-contaminating microorganisms (Cao et al., 2009; Jang, Shin, & Song, 2011; Joerger, 2007). In addition, edible films and coatings help reduce environmental concerns created by conventional plastic packaging. Various approaches have been proposed and demonstrated for the use of edible films and coatings to deliver antimicrobial compounds to a variety of food surfaces, including fruits, vegetables, and meat products (Devlieghere, Vermeulen, & Debevere, 2004; Ye, Neetoo, & Chen, 2008).

Ayala-Zavala et al. (2013) demonstrated the antimicrobial activity of an edible film formulated with cinnamon leaf oil that can be useful in preserving the quality of fresh-cut peaches. Similarly, Raybaudi-Massilia, Rojas-Grau, Mosqueda-Melgar, and Martin-Belloso (2008) reported the reduction of E. coli O157:H7 population by >4 logs on fresh cut Fuji apples with cinnamon, clove, and lemongrass oils at 0.7%, or their active compounds, cinnamonaldehyde, eugenol, and citral, at 0.5% incorporated into alginate films. In another study, the addition of grapefruit seed extract to the rape-seed protein–gelatin film inhibited the growth of E. coli O157:H7 and L. monocytogenes in strawberries (Jang, et al., 2011). The antimicrobial activity of the polypropylene/ethylene-vinyl alcohol copolymer (PP/EVOH) films with 5% oregano EO against pathogenic microorganisms E. coli, S. enterica and L. monocytogenes and natural microflora was recently investigated by Muriel-Galet et al. (2012) on packaged salads. The authors' findings showed a reduction in spoilage flora as well as inhibition of the growth of pathogens on contaminated salads. The antimicrobial activity of apple-based edible films containing plant antimicrobials (cinnamonaldehyde and carvacrol) was also investigated by Ravishankar, Zhu, Olsen, McHugh, and Friedman (2009). These films were shown to be effective against S. enterica and E. coli O157:H7 on poultry, and against L. monocytogenes on ham.

Similarly, chitosan based films have proven to be very effective in food preservation. The shelf-life of carrot sticks coated with chitosan was evaluated. An edible coating containing 0.005 ml/ml chitosan applied to carrot sticks under modified atmospheric packaging over 12 days at 4 °C was shown to maintain quality and prolong the shelf-life (Simoes, Tudela, Allende, Puschman, & Gil, 2009). The antimicrobial effects of edible chitosan films containing niacin, peptide P34, and natamycin were investigated by Norena, and Brandelli (2012) against L. monocytogenes, B. cereus, S. aureus, E. coli, S. Enteritidis, C. perfringens, Aspergillus phoenicis, and Penicillium stoloniferum. These chitosan films were effective in controlling microbial growth in minimally processed pears and...
could thus be a feasible method for the biopreservation of food. Chitosan was also shown to possess a film-forming property, to decrease water vapor and oxygen transmission, diminish respiration rate, and increase the shelf-life of fruit (Jiang & Song, 2008). Nisin-incorporated polymer films could thus be a feasible method for the biopreservation of food. Incorporation of chitosan-coated films with green tea extracts (4%) inhibited the growth of L. monocytogenes on ham steak during storage at room and refrigerated temperature (Vodnar, 2012). Similarly, Leelu et al. (2011) reported the use of chitosan coatings to reduce bacterial contamination of egg contents resulting from trans-shell penetration by S. Enteritidis and other bacteria, such as Pseudomonas spp., E. coli, and L. monocytogenes.

The antibacterial activity of soy protein edible films incorporated with oregano or thyme EOs was tested on fresh ground beef patties at 4 °C. Films with 5% of EOs significantly inhibited the growth of Pseudomonas spp. and coliform counts (Emiroğlu, Yemiş, Coşkun, & Candogan, 2010). Increased concentrations of catechin in a film used during storage of sausages resulted in a decrease in E. coli O157:H7 and L. monocytogenes populations (Ku, Hong, & Song, 2008). Nisin-incorporated polymer films have shown to control the growth of undesirable bacteria, thereby extending the shelf-life and enhancing the microbial safety of meats (Cutter, Willett, & Siragusa, 2001). The effectiveness of active films using antibacterial peptides of Bacillus licheniformis Me1 against L. monocytogenes in dairy products was recently demonstrated by Nithya, Murthy, and Halami (2013). Their results showed that antimicrobial peptide from films diffused slowly into the food matrix (paneer) during the storage period, thereby extending the shelf-life of the product.

The incorporation of antimicrobials in food packaging as films and coatings could prevent surface growth in foods where a large portion of spoilage and contamination occurs. This approach also reduces the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. The gradual release of an antimicrobial compound from packaging films and coatings to the food surface could have an advantage over direct application of antimicrobials in food systems (Appendini & Hotchkiss, 2002). Franssen, Krochta, and Roller (2003) reported that food packaging prepared from edible antimicrobial coatings containing poly-peptides, such as lysozyme, peroxides, and lactoferrins have been shown to extend the shelf-life of food products and make them safer for human consumption, in addition to providing physical protection for the food. These studies suggest that the food industry and consumers could use these films and coatings to control surface contamination by foodborne pathogenic microorganisms.

6.3. Nanoparticles

Nanotechnology has been developing rapidly as one of the most significant technological advances of our time. Nanoscience and nanotechnology have already been applied in various fields including medicine and the food industry (Sozer & Kokini, 2009). In the last few years, the application of nanotechnology to food safety has attracted the attention of many researchers due to its considerable potential for the development of antimicrobial delivery systems (Zou et al., 2012). This technology could be used to improve antimicrobial stability and could be applied directly or as a coating or packaging in different food systems to inhibit the growth of foodborne pathogens. Applications of nanotechnology to deliver antimicrobials have been reported in several studies. However, the study of nanoparticles as antimicrobials in food models is very limited due to the complexity of food components. Some of the recent studies that have been effectively applied in food models using natural compounds are discussed in this section.

Zou et al. (2012) demonstrated the potential use of liposomal nanoparticles for enhancing the antimicrobial efficacy of nisin against L. monocytogenes and S. aureus in food systems. The antimicrobial activity of free nisin and nisin loaded solid lipid nanoparticles was also studied by Prombutara, Kulwatthanasal, Supaka, Sramala, and Chareonpornwattana (2012). The results of their study showed stable and longer antimicrobial activity of nisin loaded nanoparticles against L. monocytogenes DMST 2871 compared to free nisin, indicating that the nisin was released from nanoparticles throughout the storage period. In raw and cooked chicken meat systems, naturally occurring phenolic compounds delivered by nanoparticles were proven to be more effective against S. Typhimurium and L. monocytogenes at much lower concentrations than when delivered individually without nanoparticles (Ravichandran, Hettiarachchy, Ganesh, Rikke, & Singh, 2011). These findings demonstrate the potential for nanoparticles to be used for food safety applications such as the delivery of phenolic compounds for pathogen reduction.

EOs are widely used natural compounds of plant origin. However, the poor water-solubility of EOs makes it difficult to incorporate them into foods and reduces antimicrobial action (Weiss, Gaysinsky, Davidson, & McClements, 2009). Therefore, a higher concentration of EOs is required to achieve higher antimicrobial efficacy which could alter the sensory properties of foods. In a recent study, Shah, Davidson and Zhong (2012a, 2012b) reported a nanodispersion method to overcome this challenge. A follow-up study by Shah et al. (2012b) reported improved antimicrobial activity of nanodispersed eugenol against E. coli O157:H7 and L. monocytogenes in bovine milk. Thymol-containing nano-dispersions are also effective against pathogens in food applications. Shah et al. (2012a) also investigated the efficacy of thymol dispersed in whey protein isolate and maltodextrin nanocapsules to inhibit E. coli O157:H7 and L. monocytogenes in apple cider and 2% reduced-fat milk. More recently, Xue et al. (2013) demonstrated the higher antilisterial activity of nanoemulsified thymol in milk compared to free thymol. In this study, authors reported the reduction of L. monocytogenes from –5 log CFU/ml to below the detection limit in 6 h by nanoemulsified thymol in skim and 2% fat milk. In full fat milk, the bacterial population was reduced to undetectable limits after 48 h of incubation at room temperature. In all tested food systems, nano-encapsulated EOs were more evenly distributed even at higher concentrations above the solubility limit than free EOs, thus resulting in higher antimicrobial efficacy.

The application of antimicrobial compounds that have been widely applied to microbial control in food products and processing environments has met with several limitations including undesirable flavor, low solubility, and instability (Zou et al., 2012). The efficacy of such antimicrobial properties is exhausted due to interactions with food components (proteins and lipids), inactivation by enzymatic degradation or uneven distribution of antimicrobial compounds within the complex food systems (Prombutara et al., 2012). Nanoscale antimicrobial delivery systems could enhance the efficacy of antimicrobials by improving their solubility and dispersibility and thus improve the quality of food products. Nanotechnology is also being developed in the areas of food packaging. The incorporation of nanomaterials into food packaging has been shown to improve food quality in fresh fruits and vegetables, bakery products and confectionery by protecting food from moisture, lipids, gases, off-flavors and odors (Sozer & Kokini, 2009). Despite all of the potential applications, nanotechnology is still a new subject in the field of food safety. The specific properties and characteristics of nanomaterial used in food applications need to be carefully examined for any potential health risks.

7. Conclusion

Since, there is a growing demand for food that is free of synthetic chemicals as preservatives, it is necessary to examine and
identify alternative and safe approaches for controlling foodborne pathogens. Even though many natural products are currently being used for the preservation and extension of the shelf-life of foods, there are still many unexplored sources. The use of natural compounds from plant by-products, algae, and mushrooms could open up the possibility of using these compounds as novel antimicrobials. Utilization of these products could also be a more cost-effective way to produce antimicrobials. However, despite their potential, the use of natural antimicrobials in food systems remains limited mainly due to the side effects of undesirable flavor or aroma. Therefore, further research is needed to determine the optimum levels of antimicrobials that can be safely applied in food systems without unduly altering any sensory characteristics. An alternative approach would be to utilize one or more compounds that could produce synergistic effects at low concentrations without altering any sensory characteristics of the food.

In complex food matrices, active natural compounds could bind with other hydrophobic compounds such as some proteins and lipids. This interaction could limit the availability of the natural compound as an antimicrobial. In addition, processing steps could also reduce the antimicrobial activity of such compounds. Nanoparticles hold tremendous potential as an effective antimicrobial delivery medium and could be employed to incorporate natural antimicrobials that result in safer food products. However, the use of nanotechnology in food has raised a number of concerns over their safety to the consumers. Therefore, there continues to be a need to develop a more practical and effective delivery system for these antimicrobial compounds in food products. Furthermore, safety and health risks of natural antimicrobials need to be assessed before future applications in food products.

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References

Carvalho, S., Barbavara, Y., Kanaeri, M., & Rakhilova, K. (2010). Fruit and vegetable peels—strong natural source of antimicrobials. In Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology (Vol. 2); (paper presented at the 2nd Food Research Center.


USDA-FSIS. (2010). Safe and suitable ingredients used in the production of meat, poultry, and egg products. FSIS Dir. 7120.1 Revision 2.


