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Use of Natural Antimicrobial Agents: A Safe Preservation Approach

Farhan Saeed, Muhammad Afzaal, Tabussam Tufail and Aftab Ahmad

Abstract

Microorganism contamination at various stages of food chain is one of the major causes for food spoilage that ultimately leads to food waste, increasing food insecurity issues and substantial economic losses. Various synthetic chemical preservatives are being used to control microbial food spoilage and to extend product shelf life. Researchers and consumers are discouraging the use of synthetic preservatives due to their negative health impacts. Naturally occurring antimicrobials have gained attention among researchers and food manufacturer due to their safety and nontoxic status. Natural preservatives are easy to obtain from plants, animals and microbes. These naturally occurring antimicrobial agents can be isolated from indigenous sources using various advanced techniques. Natural preservatives such as nisin, essential oils, and natamycin have effective potential against spoilage and pathogenic microorganisms. The regulations regarding the use of these naturally occurring preservatives are not well defined in some developing countries. This chapter focuses on source and their potential role, antimicrobial mechanism in food preservation, and current knowledge on the subject.

Keywords: natural antimicrobials, food safety, regulations

1. Introduction

The world population is increasing tremendously and food security is a highly-managed issue as approximately one-third of all produced food for human consumption is either lost or wasted [1]. Due to the lack of advanced handling technologies, developing countries have more postharvest losses as compared to developed countries. It is still an alarming situation in many developing countries as maximum amount of the produce is lost during post-harvest [2], while in industrialized countries one third of the food waste occurred at the retail or consumer levels [1]. The detail of the losses of differ category is as follows; root crops (40–50%), fruits, and vegetables (35%), fish and seafood (30%), cereals (20%), meat, oil seed, and dairy products (20%) [2].

There are many reasons for this massive global food loss but microbial spoilage is the leading cause for this loss. This leads to food spoilage and enhance the food insecurity issues worldwide. Microbial contamination not only causes food loss but also has undesirable effects on the organoleptic product quality.

Among spoilage microorganisms, bacteria, fungi (yeast and mold) are the major concerns. Fungi are the major cause for food spoilage at any stage of the food chain because of their ability to grow in low moisture and stress physiological conditions [3]. Fungi not only cause the food spoilage they have ability to produce secondary metabolites like mycotoxins that cause serious health issues in humans. These mycotoxins are able to withstand various harsh food processing conditions, the impact of microbial contamination at each level of food chain (Farm to Fork) noticeably lead to huge economic losses for not only producer as well as consumer [1]. Three main stages of microbial contamination routes are; the field (water, soil, and air), raw materials (crops, meats, and milk) and food processing and manufacturing levels. Various methods and technologies can be used to control the contamination at each stage [4].

Chemical preservatives (benzoate, propionate, sorbate, nitrate, nitrite, and sulfites) are widely used to control the microbial growth. Several traditional food-preservation techniques (freezing, chilling, reduction of water activity, modified atmosphere packaging, acidification, nutrient restriction, fermentation, and nonthermal physical treatments or the addition of synthetic antimicrobials) have been utilized to control food spoilage microorganisms [5]. In recent era, it has been noticed that synthetic preservative have raised many health concern issues. Consumers are increasingly aware of the relationship of health issues and the food they consume. The awareness of the consumers are being increasing about the synthetic-based antimicrobials in the food formulations. These additives have serious impacts on human health as their long run use causes liver damage, asthma, many allergic reaction, and even cancer. and these concerns increasing the use of natural Antimicrobials [6].

The use of synthetic preservative is being discouraged by food scientists and consumers due to their harmful impacts on human health [7]. Therefore, scientists are inspired to find alternative natural antimicrobial agents for the preservation of foods. There are two major categories of antimicrobials that are naturally occurring; 1. Combination of different compounds that extracted from different plants and animals or microorganisms with special antimicrobial characteristics. Essential oils, bacteriocins, protamine, endolysins, lysozyme, lactoferrin, flavor compounds, phenolic compounds, chitosan, isothiocyanates and bacteriocins have been applied to fresh and processed fruits and vegetables. The use of probiotics to show antagonistic effect in pathogens have been shown in different in VIVO studies [8].

In this regard plants are being considered as most important and rich natural source of antimicrobial substances like saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes, lactones, terpenoids and phorbol esters [9]. These plant substances act as antimicrobial, antioxidants, flavor and color enhancer. These properties of the plant agents do not only extend the shelf life of the product but also enhance the organoleptic acceptability of the products. These substances play a vital role in preventing the growth of foodborne pathogens and thus reduce the chances of illness [10]. Additionally, most plant-derived extracts are generally recognized as safe (GRAS) and Qualified Presumption of Safety (QPS) status in the USA and EU, respectively [11]. Natural antimicrobials have wide application and can be applied as preservatives in fruits and vegetables to ensure safety, protect the quality and extend shelf life. These agents can be obtained from animal, plant, and microbial sources, where they play a role in the natural defense system of their hosts.

Due to potential adverse effect of certain synthetic fungicides and preservatives on environment and health there is a strong societal manufacturer and regulatory authorities demand for less processed and synthetic preservative-free foods and the use of natural preservative as alternatives. Such alternatives mainly include natural preservatives from plants, animals, and microorganisms themselves and their

metabolites. The use of such natural agents will build the consumer confidence regarding the consumption of food products. These alternatives are complementary to hurdle technologies. However, their use of natural agents for food preservation is not regulated in many countries, and ingestion of some of them raises questions about health effects, especially when consumed over period of time. The objective of this chapter is to focus on the availability of natural antimicrobial agents, their sources, action mechanism, food applications and regulations.

2. Sources and types of natural antimicrobial agents

Natural preservatives can be derived from various sources. However, plants, animals and microorganisms are considered as major source of these essential substances. These derived compounds have wide application to fresh and processed

Plant-based products	Target microorganisms	References
Sage	<i>E. coli</i> ,	[49]
Coriander	<i>Clostridium botulinum</i> ,	
Allspice	<i>Listeria</i>	
Mustard	<i>monocytogenes</i> ,	
Clove	<i>Pseudomonas spp.</i> ,	
Oregano	<i>Clostridium spp.</i> ,	
Cinnamon	<i>S. aureus</i> ,	
Rosemary	<i>Yersinia</i>	
Turmeric	<i>enterocolitica</i> ,	
Ginger	<i>Bacillus spp.</i> ,	
Cardamom	<i>Salmonella</i>	
Seeds of muscadine	<i>E. coli</i> O157:H7,	[50]
Celery seed	<i>Campylobacter</i>	
Poppy	<i>Jejuni</i>	
Seeds of Anise	<i>L. monocytogenes</i> ,	
Grape seeds	Typhimurium	
Bell pepper	<i>S. aureus</i> ,	[51]
Chives	<i>Enterococcus</i> ,	
Broccoli as a vegetable	<i>Pseudomonas</i> ,	
Kale	<i>Bacillus</i> ,	
Rind	<i>L. monocytogenes</i>	
Peel of lemon	<i>S. aureus</i> ,	[52]
Cassia	<i>Salmonella infantis</i> ,	
Peel and extract of pomegranate	<i>E. coli</i>	
Pomegranate	<i>E. coli</i> O157:H7, <i>Salmonella</i>	[53]
Strawberry		
Pears		

Table 1.
 Some selected plant products and their natural antimicrobial potential.

fruits and vegetables to prevent spoilage, shelf life extension and to assure safety of the products. Plants, herbs, and spices have been found to be rich sources of aldehydes, ester terpenoids, phenolics, and sulfur-containing compounds. These natural occurring agents commonly found in roots, flowers, leaves, seeds and bulbs and in other parts of the plants. These substances are produced in defensive mechanism and are helpful for inactivation or inhibition of many microorganisms (bacteria, yeast and molds) [12]. Essential oils (EO) that is obtained from different plants have wide application as food additives are considered as good alternatives to synthetics. A large variety of antimicrobial agents can be obtained from spices [13]. Different parts of plants like flowers, bark, herbs, wood leaves, seeds, buds, twigs, fruits, and roots are good sources of volatile oils. Volatile oils can be obtained from plants and spices by various methods [11]. Boiling water or hot steam is the most commonly used method for commercial production of essential oils. However, other extraction techniques, like the use of microwaves or liquid carbon dioxide, could be also used [14] (**Table 1**).

3. Plant extracts as natural antimicrobial agents

Extracts of plants, herbs and spices are GRAS products that are used for centuries in the food products, for longevity and as a means of flavor. Plants and spice extracts have greatest antimicrobial activity. Antimicrobial activity potential of clove, oregano, cinnamon, and thyme essential oils and components, cinnamaldehyde, eugenol, carvacrol, and thymol have been reported in numerous literature. [12]. Essential oils obtained from spices contain active compounds that exhibit the great antimicrobial potential, like 3-phenylprop-2-enal, 5-isopropyl-2-methylphenol, etc. [15]. The above-mentioned compounds show antimicrobial activity against *Aspergillus* spp., *Escherichia coli*, *Listeria monocytogenes*, *Shigella sonnei*, and *Shigella flexneri*. *E. coli* and enterohemorrhagic *E. coli* are found more sensitive to garlic extract than what? Garlic extract has good antimicrobial potential against *S. aureus* and *Salmonella typhimurium*. Other essential oils or extracts from, basil, eucalyptus citrus, bay, lemongrass, rosemary, savory, and tea plants have demonstrated antimicrobial activities against selected microorganisms [16]. The composition of essential oils is affected by geographical areas and the time of harvest. Some plants consist of 85% essential oil; while few plants have only traces [17]. The minor components present in essential oils play a vital role as antimicrobial agents through synergistic effects. The essential oil and minor components presently affect the cell membrane of the bacterial cell. The hydrophobic nature of essential oil makes it an effective agent to inactivate the growth of microorganisms. The release of the bacterial cell contents make it unable to grow and reproduce.

Extracts/compounds obtained from plants	Target spoilage and foodborne microorganisms	References
Thyme EO	<i>L. monocytogenes</i>	[54]
Grape seed extract	<i>S. aureus</i>	[55]
Cranberry extract	<i>S. aureus</i>	[56]
Lemongrass EO	<i>Salmonella enteritidis</i>	[57]
Garlic extract	<i>Salmonella</i> spp.	[58]

Table 2. Antimicrobial effect of plant-based preservatives against selected spoilage and foodborne microorganisms.

Essential oil cause inactivation of essential enzymes, coagulation of cytoplasm, disturbance of genetic material and ultimately affect cell viability [13] (Table 2).

4. Main antimicrobials from plants

4.1 Eugenol

Eugenol is a volatile phenolic compound. Eugenol is the main extracted constituent (70–90%) of cloves and is responsible for clove aroma. Main sources include clove essential oil, buds, and leaves mainly harvested in Euglenol play a prominent role in dental and oral hygiene preparations. Eugenol is used as flavor, irritant, and sensitizer and can produce local anesthesia. Eugenol-producing dental materials are used in clinical dentistry and are effective against *Salmonella Shigella*, *Clostridium botulinum*, *Listeria monocytogenes*, and *E. coli* [18].

4.2 Thymol

Thymol is one of the most important essential oils found in thyme. The main monoterpene phenol found in thyme essential oil. It has immunomodulators, antioxidant, antibacterial anti-inflammatory, and antifungal properties Thymol is active against *Salmonella* and *Staphylococcus* bacteria. Inhibition effect is due to damage to membrane integrity of the microorganism which further affects pH homeostasis and equilibrium of inorganic ions [19].

4.3 Aldehydes

Fruits and vegetables contain (hexanal, 2-(E)-hexenal, trans-2-hexenal, and hexyl acetate) lipoxygenase pathway plant products for preservation. These are effective against Gram-negative and Gram-positive bacteria. α,β -unsaturated aldehydes have a broad antimicrobial spectrum and show similar activity against Gram-positive and Gram-negative microorganisms [20].

4.4 Carvacrol

Carvacrol, a phenolic compound, is considered one of the main components of certain EOs that employ antimicrobial activity. Its sources include savory, thyme, and oregano. Carvacrol is reported to have disruptive action on the plasma membrane of intracellular ATP content of *E. coli* O157:H7. In different studies, the importance of the hydrophobicity and its antimicrobial effectiveness has been identified [21].

4.5 Vanillin

Vanillin, a phenolic compound present in vanilla pods. It holds tremendous industrial applications in food, pharmaceuticals, beverages, perfumes and as nutraceuticals. Inhibitory activity against several fungi and pathogenic and food spoilage bacteria including species from *Escherichia*, *Klebsiella*, *Salmonella*, *Bacillus*, *Serratia*, *Staphylococcus*, and *Listeria*, these compounds may be used as preservatives in fruits and vegetables, applied as vapors in storage operations or in modified atmosphere packaging [22].

4.6 Allicin

Allicin has biological properties. It is a sulfur-containing natural compound. It has typical smell and taste in freshly cut or crushed garlic. For industrial purpose it is mainly extracted from garlic.

The major physiological role of garlic are its antimicrobial, antioxidant, anticancer, antifibrinolytic, and antiplatelet aggregatory activity has been observed [23].

4.7 Cinnamaldehyde

Cinnamaldehyde is the organic and major active constituent in cinnamon. Cinnamaldehyde has yellowish appearance and is mainly present in the essential oils of cinnamon.

4.8 Alkaloids

Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms [24].

4.9 Anti-microbial peptide

Plant antimicrobial peptides act as natural defense compounds against many pathogens (pAMPs) and were discovered in 1942. Potato defensin, hevein, thionines, snakins are the examples of the plant antimicrobial peptides. These act as membrane-active antifungals, antibacterials, and antivirals.

4.10 Citral

Citral is a terpenoid that is oxygenated derivative of terpenes, which is compound of a mixture of two isomers. The trans isomer is known as geranial or citral A. The cis-isomer is known as neral or citral. It has antifungal properties. The antifungal effects of citral and eugenol, has been studied in many research [25].

4.11 Saponins

Saponins are high molecular weight glycosides that are present in a diversity of plants and some marine organisms. They act as an antiviral, as an antimicrobial, as an anticancer drug and when included in animals feeds as a growth stimulatory supplement [25].

4.12 Flavonoids

Flavonoids have been extensively researched are hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. These mainly occur in green teas. The term flavonoid includes the polyphenols, flavanones, flavones, flavan-3-ols, flavonols and anthocyanins. Flavonoids are secondary metabolites well documented for their biological effects, in vitro to be effective antimicrobial substances against a wide array of microorganisms. Flavonoids have good antimutagenic, anti-inflammatory, anticancer, and antiviral, activities. The antimicrobial activity of polyphenols found in fruit, vegetables, and medicinal plants has been extensively investigated against a wide range of microorganisms [26].

4.13 Quinones

Quinones are aromatic rings with two ketone substitutions. They are highly reactive and ubiquitous in nature. Quinones may also render substrates unavailable to the microorganism. Tertiary butylhydroquinone (TBHQ) are approved as food antioxidants to prevent rancidity in fats, oils, and lipid foods.

4.14 Tannins

Polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Ellagitannin with molecular weights ranging from 500 to 3000 is an excellent example of tannin. Ellagitannin is a general descriptive name for a group of tannin. Ellagitannins are almost found in different parts of the plants including bark, wood, leaves, fruits, and roots. Condensed tannins have shown antimicrobial activities against *E. coli*, *S. aureus*, *Salmonella typhimurium*, *B. subtilis*, *Shigella sonnei*, MDR *E. coli*, *C. albicans*, and *K. pneumoniae*. They act by binding the cell wall of bacteria and inhibit their growth.

4.15 Coumarins

Coumarins are phenolic substances made of fused benzene and an alpha pyrone ring. Their antimicrobial activity is directed against fungi, but they also have an effect on bacteria.

4.16 Caffeic acid

Caffeic acid (3,4-dihydroxycinnamic acid) is a simple phenolic acid derived from the hydroxycinnamic acid, with some interesting biological properties, such as antibacterial, fungicide, and antioxidant. The antibacterial activity against *S. epidermidis*, *S. aureus*, and *K. pneumoniae*, has been observed 3.3. **Main.**

5. Main antimicrobials from animal origin

5.1 Chitosan

Chitosan is obtained from partial deacetylation of chitin and sometimes known as deacetylated chitin. It is a natural polycationic linear polysaccharide mainly found in shells of marine crustaceans [27]. Due to its nontoxicity biodegradability and low allergenicity have wide application. It has antitumor, antifungal, antimicrobial antioxidant activities [28]. It is effective against Gram-negative bacteria like *Bacteroides fragilis*, *cholera*, *Shigella dysenteriae*, *E. coli*, and *Vibrio*. Chitosan has good antimicrobial resistance to swelling and antioxidant potential.

5.2 Defensin

These are small cationic peptides and are primarily known for their antimicrobial activities mainly antibacterial and antimycotic. These are found in all mammal s cells and tissues abundant in leukocytes [29].

5.3 Lactoperoxidase

Lactoperoxidase (LP) belongs to peroxidase family, and its primary function is to catalyze the oxidation of certain molecules. It is a group of natural enzymes, widely distributed in nature and found in plants and animals, including man. Lactoperoxidase (LP) secreted by ductal epithelial cells of the mammary gland. The level of LP in bovine milk is about 20 times higher than that of human milk and changes constantly during the postpartum period. Thiocyanate, which is present in significant amounts in saliva, milk, and airway secretion system, is required for the antimicrobial activity. Bacteria including *salmonellae*, *Shigella*, *pseudomonads*, and *coliforms* are not only inhibited by lactoperoxidase (LP) but may be killed [30].

5.4 Lysozyme

It is a single chain polypeptide of 129 amino acids naturally present in bodily secretions such as tears, saliva, and milk. It has good antimicrobial effectiveness and cause death of bacteria by cleaving a glycosidic linkage of bacterial cell walls peptidoglycan. Lysozyme is an important defense mechanism and is considered a part of the innate immune system in most mammals [31], and is also an important component of human breast milk [32]. Large amounts of lysozyme can be found in egg white [33].

5.5 Lactoferrin

Lactoferrin (LF) is iron-binding and bioactive glycoprotein also termed as lactosiderophilin or lactotransferrin. Lactoferrin is present in many body secretions (reproductive, digestive, and respiratory) such as those from the systems. It is present in large amount in bovine colostrum than in mature milk, lactoferrin shows strong antimicrobial effects against various Gram-negative and -positive bacteria, fungi, and parasites. It has been shown to have direct effects on different pathogenic microorganisms including bacteriostatic. [34].

5.6 Avidin

Avidin is a positively charged glycoprotein which is present in egg. Egg also contains biotin. Avidin binds biotin (avidin-biotin system as a diagnostic tool in immunoassays) and makes it unavailable for the use of microorganism. The activity of *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *P. aeruginosa* can be controlled [35].

5.7 Pleurocidin

It is an antimicrobial peptide consisting of 25 amino acids and is very active against bacteria both Gram-positive and Gram-negative. It has potential for use in food applications due to heat-stability, salt tolerant ability and other characteristics. In many studies it has been found to be effective against many pathogenic organisms like *E. coli* O157:H7, *Vibrio parahaemolyticus*, and *L. monocytogenes*. [36].

5.8 Protamine

Protamine is a cationic antimicrobial peptide (CAP), used as a natural food preservative. It is obtained from various kinds of fish. It has wide potential for food application due to high stability under heat and a preservative effect in neutral or

alkaline food. Protamine does not influence the sensorial characteristics (texture, smell, or taste) of the food to which it is added [37]. It is effective against any gram positive and negative bacteria also useful against yeast and mold as well [38].

5.9 Lactolipids

Lipids may serve to inhibit multiplication and proliferation of disease causing microorganism. The effects increased when used in combination with other antimicrobial agents like lactoglobulins, lactoferrin and lactoperoxidase [39]. Lipids derived from animal origin have good antimicrobial potential against wide range of pathogenic microorganisms. Free fatty acids have been shown to be effective against *S. aureus* and many Gram-positive bacteria like *S. aureus*, *C. botulinum*, and *L. monocytogenes*. The majority of the lipids derived from animal origin are considered as GRAS and effective for food applications. A projected application of these animal based antimicrobial lipids has been made in infant formulas. This provides protection after hydrolysis of the triglycerides in the gastrointestinal tract (GIT) following consumption [40].

6. Main antimicrobials from microbial origin

6.1 Natamycin

Natamycin has been used food preservation against the food spoilage organisms particularly yeast or mold. Its molecular weight is 665.7 Da. Natamycin is produced by *Streptomyces natalensis* and is effective against almost all molds and yeasts. It has been observed that, however, natamycin has little or no activity against many pathogenic bacteria. Due to its antifungal nature, it has been used in various products like dairy, meats, and many others. Natamycin is effective for juices in both cases (unpasteurized and pasteurized) against growth of yeasts and molds (Table 3).

6.2 Reuterin

It is an antimicrobial compound produced by *Lactobacillus reuteri*. It is water soluble non proteinaceous with a broad antimicrobial range. It is effective against Gram-negative and Gram-positive bacteria filamentous (mold) and nonfilamentous (yeasts). It is active to wide range of pH and resistant to various enzymes like proteolytic and lipolytic. [41]. It exhibit bacteriostatic activity against many pathogenic bacteria particularly against *Listeria monocytogenes*.

6.3 Bacteriophages

Natural bio preservatives from animal and plant origin are considered as alternative to chemical preservatives because of the good hygienic quality, safety and extension of shelf life of food products [42]. Compounds of plants, animals, and microorganism origins are used as natural preservatives because of their cost-effective approach. Both bacteriophages and bacteria can be used as preservatives for food applications. These can be easily propagated. It has been observed that bacteriophage (phages) is favorable because phage has the ability to target specific bacteria [43] (Table 4).

Natamycin dosage levels	Method of application	Foods	References
1250–2000 ppm	<ul style="list-style-type: none"> • Surface treatment • Direct addition • Immersion • coating emulsion 	Cheese	[59]
1250–2000 ppm	<ul style="list-style-type: none"> • Immersion method • Surface treatment by spray 	Meat products	
5–10 ppm	<ul style="list-style-type: none"> • By surface treatment By direct addition 	Yogurt	
1250–2000 ppm	<ul style="list-style-type: none"> • Surface treatment by spray 	Bakery products	
7.5 ppm	<ul style="list-style-type: none"> • Direct addition 	Tomato purée/paste	
2.5–10 ppm	<ul style="list-style-type: none"> • Direct addition 	Fruit juice	

Table 3.
Application of natamycin in different foods as natural antimicrobial agents.

Effective concentration (mg/l) of chitosan	Selected microorganisms	References
2500	<i>P. aeruginosa</i>	[60]
150	<i>Listeria monocytogenes</i>	
50	<i>Staphylococcus aureus</i>	
1500	<i>Salmonella typhimurium</i>	
600	<i>E. coli</i>	[61]
600	<i>S. typhi</i>	
100	<i>Escherichia coli</i>	

Table 4.
Antimicrobial potential chitosan (animal-based antimicrobial) agent against different foodborne microorganisms.

6.4 Lactic acid bacteria (LAB)

Lactic acid bacteria are important probiotics that confer many health benefits including protective role in foods. They act as preservative and inhibit the growth of many pathogenic bacteria. They inhibit the growth of pathogen by producing antimicrobial agents like organic acids and bacteriocins (antimicrobial peptides) [44]. Various strains of the bacteria are effective against many pathogens (Table 5).

6.5 Bacteriocins

Main antimicrobial compounds produced by many gram positive bacteria. These are actually metabolites of the LAB that are produced during their growth. These polypeptides give the producing microorganism a competitive advantage over other type of microorganisms. Bacteriocins are classified based on their chemical nature and mainly produced by Gram-positive bacteria [45] (Table 6).

LAB strain	Target spoilage and foodborne microorganisms	References
<i>Lactobacillus sakei</i>	Spoilage microorganisms	[62]
<i>Lactobacillus curvatus</i>	Spoilage microorganisms	[63]
<i>L. curvatus</i>	Food borne pathogens: <i>Escherichia coli</i> O157:H7	[64]
<i>Carnobacterium divergens</i>	<i>L. monocytogenes</i>	[65]
<i>Enterococcus faecium</i> , <i>Lactococcus lactis</i>	<i>Listeria innocua</i> and spoilage	[66]

Table 5.
 Antimicrobial effect of LAB against selected spoilage and foodborne microorganisms.

Classification	Target microorganism	References
Lactacin B enterococci	<i>L. acidophilus</i> Lactobacilli, <i>Lactococcus lactis</i>	[67]
Lacticin F	<i>L. acidophilus</i> , Lactobacilli	[68]
Nisin	<i>L. lactis</i> subsp <i>lactis</i>	[69]
Lacticin 3147	<i>L. lactis</i> subsp <i>lactis</i>	

Table 6.
 Lactic acid bacteria (LAB) as a source of bacteriocins.

6.6 Methods for the extraction of natural antimicrobial agents

The extraction of plant-based antimicrobial with the use of solvents (hydrochloric acid, ammonium chloride, ethanol, methanol, and alcohol) is time consuming and unwieldy. These methods required large amount of solvents and not cost effective regarding economic aspects. In addition the heat treatments can change the activity of bioactive agents [46]. These methods can also change the common natural characteristics, functionality, total content and activity of the compounds. The proposed methods are: direct, aqueous, and juice extraction—have been used widely to study the antimicrobial activity of plant extracts [47].

6.7 Mechanisms of action of natural antimicrobial agents

The action mechanism of natural preservative has not been fully understood. Different natural antimicrobial agents act in different way. In order to understand their mechanism below is the list of possible actions of the natural antimicrobial agents. They target the pathogenic microorganism in one or more of the following ways: membrane-disrupting compounds, direct pH reduction of the substrate, organic acids inhibiting NADH oxidation, organic acids interfering with membrane, and essential oils (EOs) producing structural and functional damage to the bacterial cell membrane.

6.8 Methods for application

Many factors can influence the application of natural antimicrobial agents to food including designing of food, physicochemical properties of food and agents, food composition, different processing operations storage conditions, target

microorganism. The application can also influence the sensory, quality and safety aspects of the subjected foods. Sometime natural antimicrobials agents from different sources can transfer odors and flavors to the food. It has been noted that different food constituents like proteins, lipids, complex carbohydrates, and sugars reduce antimicrobial activity. Many methods are available for the application of naturally occurring agents like Edible films, encapsulation and direct methods (spraying, dusting, and dipping).

6.9 Consumer concerns

Natural antimicrobial agents derived from plants, animals and microbial origins are considered safe as compared to synthetic preservatives. The growth of foodborne pathogens has not been observed with the use of natural preservatives. However, the optimal range of plant, animal and microbial based antimicrobials agents need to be defined to avoid any quality and safety issue. Many factors need to be addressed like temperatures, agents, food characteristics, and composition [48]. Preservation by lactic acid bacteria is being considered as natural solution for the preservation of the food commodities. Consumer, manufacturer and researcher giving it more consideration as these natural antimicrobial agents will be good way forward to extend the shelf life and in presentation of food spoilage. Several products composed of bacteria, fungi, and yeasts are currently commercialized worldwide.

7. Conclusion

With the increasing demand of fresh, semi processed, processed and raw food commodities their safety quality and preservation issues are need to be addressed. The regulation and new method of application of natural antimicrobials agents are important factors that should be addressed. Optimization of application methods and regulation will enhance the consumer confidence. The application methods for the natural antimicrobial agents to different food products required higher efficiency. The use of natural antimicrobials on fruits and vegetables without destructively affecting the sensorial characteristics is still a challenge for researchers. To inhibit the growth of spoilage or eliminate pathogenic bacteria, the required concentrations of natural antimicrobial agents is very high. These high concentrations can not only affects sensory qualities but can also have negative impact on human health. Research need to be done on the synergistic combinations of natural antimicrobial agents. A combination of different treatments strongly ensures the safety and quality issues of the food products.

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