Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 2078-2082
Received: 15-09-2017
Accepted: 17-10-2017

Fiza Nazir
Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Rehana Salim
Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Nargis Yousf
Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Mohsin Bashir
Department of Food Science and Technology, Islamic University of Science & Technology, Awantipora, Kashmir, Jammu and Kashmir, India

HR Naik
Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Syed Zameer Hussain
Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Correspondence
Fiza Nazir
Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Natural antimicrobials for food preservation

Fiza Nazir, Rehana Salim, Nargis Yousf, Mohsin Bashir, HR Naik and Syed Zameer Hussain

Abstract
Many nontraditional preservation techniques are being developed to satisfy consumer demand of nutritious food. Generally, foods are thermally treated with temperatures varying from 60 to 100 ºC for few seconds or a minute to destroy vegetative microorganisms. The energy transferred to the food during thermal treatment can affect the organoleptic and nutritional attributes. Ensuring food safety and at the same time meeting such demands for retention of nutrition and quality attributes has resulted in increased interest in alternative preservation techniques for inactivating microorganisms and enzymes in foods. Flavour, odor, color, texture, and nutritional value of a food are its important quality attributes. Use of natural preservatives derived from plants, animals, or microflora is being increasing studied to fulfill consumer demands. Use of natural or controlled microflora, mainly lactic acid bacteria (LAB) and/or their antibacterial products such as lactic acid, bacteriocins, and others to enhance products safety or storage life is called biopreservation. Typical examples of antimicrobials are casein, whey (milk), lysozyme (egg white, figs), saponins and flavonoids (herbs and spices), bacteriocins (LAB), and chitosan (shrimp shells). Shelf life of unprocessed or processed food is extended by reducing the microbial growth rate or viability. Some of spices and herbs are also known to contribute antimicrobials.

Keywords: Bacteriocins, Enzymes, Flavour, Herbs, Spices

1. Introduction

Major concern of consumers, food industry, and food safety authorities are foodborne illnesses. Finding natural antimicrobial agents that inhibit bacterial and fungal growth for better quality and shelf-life has been of considerable interest in recent years. There is a concern about synthetic preservatives that are used in food among consumers. As a result, natural products that can serve as alternative food preservatives are showing increasing demand (Tajkarimi *et al*., 2010). This, in turn, has led to a search for antimicrobials derived from a variety of natural sources. Different sources from which natural antimicrobials are obtained include plants, animals, bacteria, algae and fungi. Several plant antimicrobials related studies have demonstrated the efficacy of plant-derived compounds in food applications, as well as factors influencing this effectiveness (Cowan, 1999; Gyawali and Ibrahim, 2012; Hayek *et al*., 2013; Tajkarimi *et al*., 2010). However, there has been limited research related to the structure function relationship of these compounds. As a result, the importance of the chemical composition of plant derived compounds regarding their antimicrobial activity is still not well understood. Polyphenolic compounds have great structural diversity and variations in chemical composition among several plant-derived compounds and thus show difference in their antibacterial effectiveness against pathogenic microorganisms. Phenolic compounds or other hydrophobic components in the essential oils (EOs) may be responsible for antimicrobial activity of plant extracts (Dorman and Deans, 2000). Research on possible antibacterial activity of by-products generated from fruits and vegetables is very limited. These by-products can act as sources of antibacterial compounds (Baydar *et al*., 2006; Jayaprakasha *et al*., 2003; Matsumoto *et al*., 2004) and thus could be a potential source of low-cost natural antimicrobials. Algal and fungal (mushrooms) antimicrobial compounds have recently received considerable attention as a new source of novel antimicrobial substances against pathogens (Bhagavathy *et al*., 2011; Ramesh and Pattar, 2010). There are many unexplored sources of such potential compounds that could be used as natural preservatives in food applications.

2. Antimicrobials of animal origin

Few potential animal origin antimicrobials which could be used as food additives are discussed below.

2.1 Lactoferrin

Lactoferrin (Lf), an iron-binding compound of milk, has been shown to possess antimicrobial activity against a wide range of bacteria and viruses (L€onnerdal, 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 (USDA-FSIS, 2010; Juneja *et al*., 2012) [10, 14, 30]. Lf shows antimicrobial effect against foodborne microorganisms including Carnobacterium, L. monocytogenes, E. coli, and Klebsiella (Al-Nabulsi and Holley, 2005; Murdock *et al*., 2007) [15, 39, 16]. Lf hydrolysate is capable of limiting the growth or reduce the population of pathogenic bacteria in a dairy product. There are several explanations that may accounts for Lf's antimicrobial action. One is that microbial access to nutrients it limited via iron chelation, resulting in an iron deficient medium. (Gonz_alez-Ch_avez *et al*., 2009) [17]. Another possibility is destabilization of the outer membrane of Gram-negative bacteria, resulting in liberation of lipopolysaccharides with increased membrane permeability (Orsi, 2004) [18]. Capability of Lf to provide iron for the growth of Bifidobacterium spp. is another possibility. However, under defined environmental condition such as lack of minerals, Lf with bifidobacterial strains acts indirectly against the growth of pathogens. The mechanism enabling this appears to involve microbial iron uptake (Bezkorovainy *et al*., 1996) [19]. Iron acts as a growth promoting factor for bifidobacteria, and bifidobacteria can bind iron, thus making it unavailable to pathogens thus negatively impacting their growth (Bezkorovainy and Miller-Catchpole, 1989) [20]. Under low iron condition, these probiotic bacteria start producing antimicrobial 'bifidogenic' compounds (Ibrahim, 2005) [21] that can inhibit the growth of other harmful microorganisms. Thus, Lf rich medium with bifidobacterial strains can be used to improve microbiological safety and quality.

2.2. Chitosan

Chitosan is one of the antimicrobials that has received considerable interest for commercial food application. It is a polycationic biopolymer present naturally in the exoskeletons of crustaceans and arthropods (Tikhonov *et al*., 2006) [22]. Insolubility of chitosan at neutral and higher pH limits its application as a food preservative and for other uses. Application of chitosan as a food preservative can improve with improvement in its solubility (Du *et al*., 2009) [23]. Water soluble chitosan derivatives prepared by Maillard reactions against S. aureus, L. monocytogenes, B. cereus, E. coli, Shigella dysenteriae, and S. Typhimurium have shown antibacterial activity and are promising commercial substitute for acid-soluble chitosan (Chung *et al*., 2011) [24]. Similarly, the native chitosan solubility limitation has been overcome by using N-alkylated disaccharide chitosan derivatives exhibiting antibacterial activity against E. coli and S. aureus (Yang *et al*., 2005) [25]. The antibacterial activity of chitosans has been examined against several Gram-negative (E. coli, P. fluorescens, S. Typhimurium, Vibrio parahaemolyticus) and Grampositive bacteria (L. monocytogenes, Bacillus megaterium, B. cereus, S. aureus) (No *et al*., 2002).

2.3. Lysozyme

Lysozyme is an enzyme 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 antimicrobial application in food products (Tiwari *et al*., 2009) [28] and commonly used as a preservative for meat, meat products, fish, fish products, milk and dairy products, and fruits and vegetables (Cegielska-Radziejewska *et al*., 2009) [29]. Ability to hydrolyze the β-1, 4 linkages between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of the microbial cell wall accounts for lysozymes antimicrobial activity (Juneja *et al*., 2012) [14, 30]. Lysozyme has been used primarily to prevent late blowing defect in cheeses, caused by Clostrodium tyrobutyricum. Lysozyme shows highest antimicrobial activity against Listeria innocua and Saccharomyces cerevisiae with an inhibition zone of 19.75 and 17.37 mm, respectively.

2.4. Milk-derived peptides

Casein and whey proteins are some of the milk-derived bioactive compounds that have been found to have multifunctional properties including antimicrobial properties (Phelan *et al*., 2009; Schanbacher *et al*., 1998) [31, 32]. These peptides have shown antimicrobial effect against wide range of pathogenic microorganisms such as E. coli, Helicobacter, Listeria, Salmonella, Staphylococcus, yeasts, and filamentous fungi (Fadaei, 2012) [33]. 80% of total milk protein is casein. It is a rich source of bioactive peptides. Antibacterial peptides have been identified from aS1-casein and aS2-casein (McCann *et al*., 2006) [34]. Casocidin is a compound released by Hydrolysis of aS2-casein by chymosin. This has shown antibacterial properties against Staphylococcus spp., Sarcina spp., B. subtilis, Diplococcus pneumoniae, and Streptococcus pyogenes (Szwajkowska *et al*., 2011) [35]. Another casein derived peptide known as Isracidin has been found to be effective against S. aureus, L. monocytogenes, and Yak milk kcasein hydrolyzate at 0.5 mg/ml was also found to be effective against E. coli (Cheng *et al*., 2013) [36]. The whey protein fraction of bovine milk consists mainly of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA). Digestion of β-lactoglobulin with trypsin release biologically active antibacterial. These peptides demonstrated antimicrobial activity against foodborne pathogens such as S. aureus, L. monocytogenes, Salmonella spp. and E. coli O157.

3. Antimicrobials Derived from Plants

Herbs and spices have been recognized to contain a broad spectrum of active constituents that are responsible for antibacterial, antifungal, antiparasitic, and/or antiviral activities. Essential oils have been used for centuries as part of natural traditional medicine. They are plant material's aromatic oily liquids (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). The principal components responsible for essential oils effective antimicrobial activity include saponins, flavonoids, carvacrol, thymol, citral, eugenol, linalool, terpenes, and their precursors (Burt, 2004). Alkenyl thiosulfinates are mainly responsible for antimicrobial activity of alliums. Allium-derived antimicrobial compounds inhibit microorganisms by reacting with the sulfhydryl (SH) groups of cellular proteins. In olive oil, distinctive antimicrobial compounds include oleuropein, oleuropein aglycon, elenoic acid and oleocanthal (in addition to hydroxytyrosol and tyrosol) (Cicerale *et al*., 2012) [38]. Essential oils and other plant extracts having antibacterial activities have attracted great attention for application of the crude extracts or their bioactive components in food biopreservation (Holley and Patel, 2005; Richard and Patel, 2005; Tajkarimi *et al*., 2010) [15, 39]. In the concentration range of 0.05–0.1%, essential oils have demonstrated activity against pathogens, such as *S.* Typhimurium, *E. coli* O157:H7, *L. monocytogenes*, *B. cereus* and *S. aureus*, in food systems.

However, activity against various microorganisms on food products might be higher than the concentration applied for flavoring purposes. This might result in food tainting and/or adverse sensorial effects to food products (Bagamboula *et al*., 2004) [41]. Masking the odor with other approved aroma compounds can help to overcome the adverse sensorial effects of essential oils on food products (Gutiérrez *et al*., 2009) [42]. Antimicrobial agents derived from essential oils are interesting candidates for development of activated films or packagings. Plants also produce a variety of antimicrobial peptides, many of which can be grouped in different classes: thionins, defensins, lipid transfer proteins, cyclotides and snakins (Padovan *et al*., 2010) [43]. Some of them could possibly be exploited for food biopreservation. Plants can act as excellent source of antifungal proteins and peptides, including chitinases, glucanases, thaumatin-like proteins, thionins, and cyclophilin-like proteins (Ng 2004).

4. Microbial origin antimicrobial agents

Bacteria produce many compounds that are active against other bacteria, which can be harnessed to inhibit the growth of potential spoilage or pathogenic microorganisms. These include fermentation end products such as organic acids, hydrogen peroxide, and diacetyl, in addition to bacteriocins and other antagonistic compounds such as reuterin (Daeschel, 1989) [45]. Bacteriocins are produced by both Gram- negative and Gram-positive bacteria. Bacteriocins are proteinaceous antibacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides (De Vugst and Vandamme, 1994) [46]. Bacteriocin production can be exploited by food processors to provide an additional barrier to undesirable bacterial growth in foods.

Bacteriocins being cationic peptides that are either hydrophobic or amphiphilic, mostly target bacterial membrane. There are several groups of bacteriocins based on the producer organism and classification criteria. There are as many as five classes of bacteriocins proposed. The majority fall into classes I and II, which are the most intensively researched to date. The class I group, termed lantibiotics, are small peptides that are characterized by their content of several unusual amino acids. The class II bacteriocins are small, nonmodified, heat stable peptides (Nes and Holo, 2000) [47]. Another classification is with respect to the producing microorganism and is specifically named after the genus, species, or the group of microorganisms, e.g., lantibiotics for bacteriocins of lactic acid bacteria, colicins of E. coli, klebisins of Klebsiella pneumoniae (Riley and Chavan, 2006) [48]. Many bacteriocins have been isolated and characterized from lactic acid bacteria, and some have acquired a status as potential food preservatives because of their antagonistic effect on important pathogens. Many bacteriocins are active against food borne pathogens and spoilage bacteria. The important ones include nisin, diplococcin, acidophilin, bulgarican, helveticin, lactacin, and plantaricin (Nettles and Barefoot, 1993) [49]. Nisin produced by various Lactococcus lactis strains, is the most thoroughly studied bacteriocin to date, and is applied as an additive in food worldwide. While the antimicrobial polypeptide nisin and related compounds such as pediocin are the only bacteriocins widely used for food preservation, many other bacteriocins have been reported and have shown potential for food preservation and safety applications.

4.1. Reuterin

Reuterin (β-hydroxypropionaldehyde) is a water-soluble non-proteinaceous metabolite of glycerol. It is a broad spectrum antimicrobial compound produced by some strains of Lactobacillus reuteri, with recorded activity against Gram-negative and Gram-positive bacteria, yeasts, and filamentous fungi (Nom and Rombouts, 1992) [50]. Isolated, purified reuterin (Talarico and Dobrogoz, 1989) [51] is active over a wide range of pH values and resistant to the action of proteolytic and lipolytic enzymes. Reuterin is reported to exhibit bacteriostatic activity against Listeria monocytogenes. However, higher bactericidal activity has been reported against E. coli O157:H7, S. choleraesuis sub sp. Choleraesuis, Y. enterocolitica, A. hydrophila subsp. Hydrophila, and C. jejuni (Arques *et al*., 2004) [52].

4.2. Pediocin

Pediocin is produced by strains of Pediococcus acidilactici and P. pentosaceus and is designated generally recognized as a safe (GRAS). The organismis commonly isolated from and used in fermented sausage production. The bacteriocins produced by P. acidilactici are AcH, PA-1, JD, and those obtained from P. pentosaceus are A, N5p, ST18, and PD1. Most pediocins are thermostable proteins and function over a wide range of pH values. Pediocin AcH is effective against both spoilage and pathogenic organisms, including L. monocytogenes, Enterococcus faecalis, S. aureus, and Cl. Perfringens (Bhunia *et al*., 1988) [53].

4.3. Nisin

Nisin is the most widely used bacteriocin. To date, nisin is the only natural antimicrobial peptide approved by the FDA for use as a food preservative; however, it has a limited spectrum of activity, does not inhibit Gram-negative bacteria or fungi, and is only effective at low pH. Nisin is produced by fermentation of a modified milk medium by certain strains of lactic acid bacterium, Lactococcus lactis. Nisin functions by interacting with the phospholipids in the cytoplasmic membrane of bacteria, thus disrupting membrane function and preventing outgrowth of spores by inhibiting the swelling process of germination. It is highly active against many of the Gram-positive bacteria and specifically used by the cheese industry to control the growth of Clostridium spp. (Branby-Smith, 1992) [54]. Substantial research has evaluated the efficacy of nisin against various pathogens and its use for different food products. Nisin has been used to inhibit microbial growth in beef (Eckner, 1992) [55], sausages (2), liquid whole egg (Henning *et al*., 1986) [56], ground beef (Zhang and Mustapha, 1999) [57], and poultry (Delves-Broughton *et al*., 1992) [58]. It has also been reported to reduce initial levels of Listeria monocytogenes and suppress subsequent growth in ready-to-eat (RTE) meat products (Nassar and Farrag, 1995) [59].

5. 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. 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.

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