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Natural Preservatives: An Alternative for Chemical Preservative Used in Foods

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***Abstract*—**Microbial degradation of foods is defined as a decrease of food safety due to microorganism activity. Organic acids, sulfur dioxide, sulfide, nitrate, nitrite, dimethyl dicarbonate and several preservative gases have been used as chemical preservatives in foods as well as natural preservatives which are indigenous in foods. It is determined that usage of herbal preservatives such as blueberry, dried grape, prune, garlic, mustard, spices inhibited several microorganisms. Moreover, it is determined that animal origin preservatives such as whey, honey, lysosomes of duck egg and chicken egg, chitosan have antimicrobial effect. Other than indigenous antimicrobials in foods, antimicrobial agents produced by microorganisms could be used as natural preservatives. The antimicrobial feature of preservatives depends on the antimicrobial spectrum, chemical and physical features of material, concentration, mode of action, components of food, process conditions, and pH and storage temperature. In this review, studies about antimicrobial components which are indigenous in food (such as herbal and animal origin antimicrobial agents), antimicrobial materials synthesized by microorganisms, and their usage as an antimicrobial agent to preserve foods are discussed.

***Keywords*—**Animal origin preservatives, antimicrobial, chemical preservatives, herbal preservatives.

I. Introduction

MICROBIAL spoilage of foods defines as the decrease of food safety due to several microorganism activity. Among the subjected microorganism groups, saprophyte and pathogen microorganisms are important for food safety, which cause food spoilage, food poisoning and infection [1]. Nutritional ingredients of food such as protein, carbohydrate, mineral, vitamin and water provide a suitable environment for growth of bacteria, yeast and mold. Inhibitory substances, pH, water activity and oxidation-reduction potential of food are as important as nutritional ingredients for growth of microorganisms. Furthermore, moisture, temperature and composition of environment gas play an important role in food spoilage [2], [3].

Chemical preservatives such as organic acids, sulfur dioxide, sodium, potassium and calcium salts of hydrogen sulfide, disulfide and sulfide ion, dimethyl carbonate, nitrate and nitrite are used in foods as well as natural preservatives which found naturally in foods [4]-[8]. Antimicrobials synthesized by microorganisms are used as preservative in foods, except for natural preservatives which found naturally in food [9], [10].

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Today, bacteria associated with food fermentation are known as synthesizing antimicrobial metabolites. It is not ignored that some of these antimicrobial metabolites have not been defined yet [10]. Some metabolites synthesized by bacteria are shown in Table I. Interest in increasing stability and conservation of not fermented food by usage of these metabolites is increasing day-by-day.

TABLE I
SOME ANTIMICROBIAL METABOLITES SYNTHESIZED BY FOODBORNE BACTERIA

Metabolite	Effect
Organic acids: Lactic acid, acetic acid, propionic acid	Against bacteria and mold
Aldehyde, ketone and alcohol: Acetaldehyde, diacetyl, ethanol	Against bacteria, mold and bacteriophage
Hydrogen peroxide	Against bacteria, mold and virus
Reuterin	Against Gram-positive bacteria
Bacteriocin	Against Gram-positive bacteria

In this review, antimicrobial agents, used to preserve foods, and which are naturally found in food, are summarized, such as herbal and animal origin preservatives and antimicrobial agents produced by microorganisms.

II. Herbal Origin Preservatives

A. Blueberry

In a research made by Wu et al. [11] antimicrobial effect of blueberry extracts on *Listeria monocytogenes* and *Salmonella* Enteritidis were determined. According to the results, blueberry extracts inhibited *L. monocytogenes* growth more than *S.* Enteritidis.

B. Raisin

In studies made to determine the antimicrobial effect on raisin, it was reported that raisin inhibited pathogen bacteria growth. Compounds containing in raisin such as oleanolic acid, oleanolic aldehyde, inoleic acid, linolenic acid, betulin, betulinic acid, 5-(hydroxymethyl)-2-furfural, β-sitosterol and β-sitosterol glucoside were determined as having inhibition effect. It is reported that oleanolic acid inhibited biofilm production of *Streptococcus mutans* [11], [12].

C. Garlic

Garlic showed antimicrobial effect on *Staphylococcus aureus*, *Salmonella* Enteritidis, *Aspergillus niger, Penicillium cyclopium* and *Fusarium oxysporum* growth [13]. Allicin is a component of garlic and obtained by crushed garlic. It has reported that allicin has antimicrobial effects on most of the

Gram-negative and Gram-positive bacteria and multi-drug resistant strains of *Escherichia coli*. It is reported to have a partially antifungal effect on *Candida albicans* and has an antiparasitic effect on *Entamoeba histolytica* and *Giardia lamblia,* which are found in the human intestinal system [14]. In a study made by Sallam et al. [15], the antimicrobial effect of fresh garlic, garlic powder and garlic oil were determined during the storage of chicken sausages. It has been reported that fresh garlic reduced aerobic bacteria load and increased shelf life of product.

D. Mustard

It has been reported that mustard oil damaged *E. coli* O157:H7 and *S. typhi* cell membranes. Mustard oil effected the concentration of compounds in the cell. According to the results obtained from this study, mustard oil could be used as an antimicrobial agent in foods [16]. In a study made by Kang et al. [17], mustard showed an antimicrobial effect on *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium strains.

E. Fruit Seed

It was determined that hawthorn, pear, black mulberry, cranberry, pomegranate have an antimicrobial effect. Blackberry, raspberry and strawberry extracts have a strong inhibition effect on *Salmonella* growth. Blackcurrant has the lowest antimicrobial effect on Gram-negative bacteria growth [18].

F. Spices/Essential Oils

In a previous study, flaxseed showed inhibition effect on *Penicillium chrysogenum,* Aspergillus flavus, *Fusarium graminearum,* and *Penicillium* spp. growth isolated from fresh pasta. According to the results obtained, flaxseed could be used as multi-functional food additive [19]. Lignan, extracted from flaxseed, showed inhibition effect on Gram-positive and Gram-negative bacteria but did not inhibit *Candida albicans* and *Aspergillus niger* growth [20].

Edible film enriched with thyme oil used in a study to determine the antibacterial effect of thyme oil on *E. coli* O157:H7, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Lactobacillus plantarum* growth in steaks covered with the film during storage at 4°C. It reported that thyme oil significantly inhibited *E. coli* O157:H7 and *Staphylococcus aureus* growth [21]. Thyme oil showed weak inhibition effect on *L. monocytogenes* growth [22].

The findings in a study maintained by Fyfe et al. [23] determined that clove oil had shown antibacterial effect on *Campylobacter jejuni, Salmonella* Enteritidis*, Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* growth. In an another study, clove and cinnamon oil inhibited *Aspergillus flavus, Penicillium roqueforti, Mucor plumbeus* and *Eurotium* spp., *Debaryomyces hansenii*, *Pichia membranaefaciens*, *Zygosaccharomyces rouxii* and *Candida lipolytica*, *Staphylococcus aureus* and *Pediococcus halophilus* growth. The inhibition effect was increased as the amount of oil increased [24]. It reported that clove and rosemary oil showed significantly antimicrobial effect on *S. aureus, E. coli* and *C. albicans* growth [25]. Hosseini et al. [26], studied on thyme, clove and cinnamon oil supplemented in edible film. They reported an inhibition effect on Gram-positive and Gram-negative bacteria growth.

According to the studies to determine antimicrobial effect of tea, *Staphylococcus aureus, Staphylococcus epidermidis*, *Salmonella typhi, Salmonella typhimurium, Salmonella Enteritidis, Shigella flexneri, Shigella dysenteriae*, and *Vibrio* spp. growth inhibited. It determined that green tea extract inhibited *Streptococcus mutans* and other pathogenic bacteria in mouth flora growth. Tea extracts inhibited *Clostridium* spp., *Erwinia* spp. and *Pseudomonas* spp. growth [27]. *Polyphenolic catechin* compounds in green tea inhibited most of Gram-negative and Gram-positive bacteria growth [28].

Johnson et al. [29] reported that hop inhibited *L. monocytogenes* growth and they indicated that hop could be used to control *L. monocytogenes* load in food products. In another study, the antimicrobial effect of hop was determined. It inhibited *Streptococcus salivarius, Staphylococcus aureus, Bacillus megaterium, Escherichia coli* and *Bacillus subtilis* growth [30].

Coffee was regarded as has an antimicrobial effect on bacteria growth [31]. In a study maintained by Murthy and Manonmani [32], water soluble extracts of coffee showed antimicrobial activity against foodborne pathogen. Highest inhibition determined on *E. coli, Yersinia* spp. and *Listeria* spp. respectively. Fungal isolates showed more resistance to the inhibition effect of water soluble coffee extracts than bacteria. Koga-Ito et al. [33] studied about antimicrobial effect of coffee solutions on *Streptococcus mutans.* It determined that, adhesion of *Streptococcus mutans* on a glass surface was significantly reduced, but the *Streptococcus mutans* load did not change.

Melanoidin in coffee was reported to show antimicrobial activity against pathogens [34]. It was reported that coffee extract showed an antimicrobial effect on enterobacter strains. Caffeine concentration in coffee extract showed antimicrobial effect against *Salmonella enterica* growth [35]. In a study maintained by Pischetsrieder et al. [31], H_2O_2 in coffee is one of the compounds that give the antimicrobial property to coffee. For this reason, coffee or coffee extracts could be used as a natural preservative.

Propolis has been known as having an antimicrobial effect against several microorganisms such as bacteria, fungi and virus. In addition to this, it has anti-inflammatory, anesthetic, healing, antioxidant, antitumor, antiulcer properties and protective effects on liver [36]. In studies made about the microorganism growth inhibition effect of propolis, it was determined that propolis showed antimicrobial effect all of the microorganisms used (Gram-positive cocci and bacilli, Gram-negative bacteria and yeast) in these studies [37], [38]. Drago et al. [39] reported that propolis did not have any inhibition effect on *Enterobacteriaceae* growth.

III. Animal Origin Preservatives

A. Whey

Verma et al. [40] studied whey enriched with pediocin PA-1. They used it as a bio-preservative to increase the shelf life of buffalo milk. Total bacteria, *S. aureus* and lactic acid bacteria load reduced significantly. Coliform and yeast-mould number did not change. It was reported that whey containing pediocin PA-1 could be used to reduce total bacteria numbers in buffalo milk. In another study, whey protein film containing *Lactobacillus sakei* used. *E. coli* and *L. monocytogenes* inoculated to meats and they covered with whey protein film. It was determined that film inhibited *E. coli* and *L. monocytogenes* growth [41].

B. Honey

Many compounds found in foods naturally have antimicrobial effects and flavonoids are one of them. Flavonoids are abundant in fruits, vegetables, hazelnuts, walnuts, seeds, and flower and stem part of plants, tea, wine, propolis and honey. Flavonoids have antifungal, antiviral and antibacterial activity [42].

Honey inhibits most of the microorganism growth which cause disease and infections. In many studies, it was determined that honey is effective against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium. Some honey samples have reported as naturally effective against pathogen and food spoilage microorganism growth. Therefore, the antimicrobial and antioxidant effect of honey should be researched in several food products [43].

In a study made by Aksoy and Dığrak [44], the antimicrobial effect of honey and propolis extracts were determined. *Klebsiella pneumoniae* 13883, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* 9027, *Staphylococcus aureus* 6538, *Bacillus subtilis* IMG 22, *Bacillus megaterium* DSM 32, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatis* RUT, *Bacillus brevis* FMC 3, *Enterobacter aeruginosa* ATCC 27859, *Corynebacterium xerosis* ATCC 373, *Kluyveromyces marxianus* 332, *Rhodotorula rubra* 116, *Candida albicans* 30114 were used in this study. It was determined that honey and propolis extracts showed antimicrobial effect.

C. Egg White Lysozyme

Egg white lysozyme has antibacterial effect on *L. monocytogenes*, *Clostridium botulinum* which cause problems in terms of food safety. Some food spoilage bacteria such as *Clostridium tyrobutyricum*, *Bacillus stearothermophilus* and *Clostridium thermosaccharolyticum* (which are thermophilic bacteria) were reported as susceptible to lysozyme. Many Gram-positive and Gram-negative pathogens isolated form foods (such as *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni, Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica*) were determined as resistant to lysozyme. It was reported that lysozyme could be used against spore forming thermophilic bacteria [45].

D. Chitosan

Chitosan has been used in several process applications in the food industry, especially to increase the quality and shelf life of food products. The antimicrobial effect of chitosan plays an important role to increase the shelf life of products. Many studies that maintained the antimicrobial effect of chitosan, and determined that chitosan inhibited many microorganism growths [46].

IV. Microorganism Origin Preservatives

A. Bacteriocins

Bacteriocins are protein or peptide compounds produced by bacteria. Most of them resistant to heat, active in acid foods and could be degradable by gastrointestinal system originated proteolytic enzymes. Bacteriocin spectrum of influence is limited with some strains. It is mostly effective against Gram-positive bacteria. Biochemical properties and influence spectrum of bacteriocin shows variation according to the producer microorganism. Although bacteriocin could be synthesized by several microorganisms such as *Lactococcus*, *Pediococcus*, *Leuconostoc* and *Staphylococcus*, most of bacteriocin used in foods are synthesized by lactic acid bacteria. Due to the antimicrobial effect against many microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum* and *Salmonella* spp., bacteriocin usage in food is increasing. It is reported that in order to be more effective in food preservation, bacteriocins should be used with other preservative agents or preservative processes [47].

a. Identification and Classification of Bacteriocins

Bacteriocins have different properties and their spectrum of influence, biochemical and genetic characteristics varies according to the microorganisms. Generally, it has low molecular weight and has hydrophilic and hydrophobic moieties [47]. Bacteriocins produced by Gram-negative bacteria are called as microcins. Microcins vary in terms of protein size, microbial target, mechanism of effect and resistance system [48]. There are different classifications of bacteriocins, but mostly Klaenhammer's classification scheme is used which based on Gram-positive bacteria. In this classification, their biochemical properties are considered and divided into four groups according to their molecule size, chemical structure, effect mechanism and heat stability [47]. Class I and Class II bacteriocins are reported as effective against Gram-positive bacteria. Colicin is produced by *E. coli* and it is reported as inhibits *Enterobacteriaceae* growth [49].

Class I

Class I bacteriocins are named as lantibiotics. They have uncommon amino acids such as lanthionine, β- methyl-lanthionine. Class I is divided into two groups as Class A and Class B according to their structural properties and antimicrobial activities. Class B lantibiotics inhibit host cell enzymes and are smaller than Class A lantibiotics [47]. Lacticin 3147, a lantibiotic, is reported as promising in preventing mastitis infections [49].

Class II

Bacteriocins in this group are small, have 30-60 amino acid residues, and resistant to heat and does not contain lanthionine. Class II bacteriocins are divided into groups. Lacticin F and lactrotoxin G, are in class A bacteriocin, and induce pores in the target cell membrane just in the same way as class B bacteriocins. Class C bacteriocins are peptides produced by secretory signals [48].

Class III

Bacteriocins in this group are big and heat susceptible. Helvetisin J and V, lactacin B are well known bacteriocins in this group [48].

Class VI

Glycoproteins (Lactocin 27) or lipoproteins in this group need non-protein structures to become active [48].

b. Bacteriocins Used in Foods

Bacteriocins can be used as therapeutic in agriculture and veterinary fields, and as a protective agent in food products to control many foodborne pathogens [50]. Bacteriocins could be added into foods directly. It was reported that *Clostridium* and *Listeria* growth inhibited in cheese. Nisin inhibits *C. botulinum* spore growth in cheese. Nisin is widely used in foods around the world [49]. Pediocin AcH, synthesized by *Pediococcus acidilactici*, has inhibition effect on foodborne pathogens such as *Staphylococcus aureus, Clostridium perfringens* and *Listeria monocytogenes* growth. It was reported that it showed bactericidal effect on susceptible cells and is reported to have a very rapid effect [51]. *Enterococcus faecium* P13, which is isolated from fermented sausages, produce a bacteriocin named enterocin P. Enterocin P was reported to have an inhibition effect on food spoilage pathogen bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridium botulinum* growth [52]. In a study made by Zhou et al. [53], a new bacteriocin named lactocin MM4, produced by *Lactobacillus alimentarius* FM-MM4, was purified, characterized and its antimicrobial properties were determined. It was reported that it has an antimicrobial effect against foodborne Gram-positive and Gram-negative pathogens.

B. Enterococcus

Enterococci have an important role in food industry. They are consumed as probiotic by human and animal. In addition to this, they are associated with food spoilage and food fermentation [54]. Enterococci are used in maturation and aroma development at traditional foods like sausages and cheese, especially produced in Mediterranean region [55]. Bacteriocins produced by enterococci are named as enterocin. Enterocins are in class II bacteriocins. It was reported that enterocin inhibited Gram-negative and Gram-positive bacteria growth. The results of a study maintain enterocin inhibited *Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter cloacae, Listeria monocytogenes* and *Proteus vulgaris* growth [56].

C. Other Antimicrobial Substances Synthesized by Microorganisms

a. Organic Acid

Lactic acid bacteria have an antagonistic effect against other microorganisms due to their metabolites such as organic acids (lactic and acetic acid), H_2O_2, bacteriocin or bacteriocin-like substances, diacetyl, alcohol and CO_2. Lactic acid is one of the most commonly found acids in nature. Lactic acid and acetic acid are used in foods widely as preservative agents. Lactic acid has been used worldwide in cheese, butter, beer, bread dough, foods containing dried milk, cattle, and sheep and poultry carcasses as preservative agents. In a study, *P. acidilactici* and dextrose were used against botulinum toxin in chicken meat salads. In another study, it was used to reduce the microbial load of broiler carcasses. After the liver was washed with lactic acid solution and a lactic acid solution sprayed on to carcasses, it was determined that microbial load was reduced [57].

b. Diacetyl

Diacetyl is produced by certain strains of *Streptococcus, Leuconostoc, Lactobacillus* and *Pediococcus.* It is a liquid compound and has a butter smell. Diacetyl occurs in butter and other dairy products, red and white wine, brandy roasted coffee, silage and other fermented food products. According to studies, diacetyl inhibited Gram-negative and Gram-positive bacteria development [57].

c. Carbon Dioxide

Metabolites produced by certain lactic acid bacteria such as CO_2, could show inhibition effect on some microorganisms. In a study, it was determined that every 1 mM increase in extracellular CO_2 concentration, produced by *P. fluorescens,* causes a 0.03 unit reduction in intracellular pH. Nevertheless, it was determined that the main reason for the inhibition was not an intracellular pH decrease [57].

d. Hydrogen Peroxide

H_2O_2 addition in foods is a simple and effective method which inactivates many undesired microorganisms. The inhibition effect of H_2O_2 on cells effects its concentration, duration of treatment, microbial load, temperature, pH, inorganic ions, microorganism treatment with UV or other preservatives. In studies made before, H_2O_2 production from *L. bulgaricus* and *L. lactis* was inhibited *S. aureus* growth. Additionally, it was reported that lactobacilli inhibit *Pseudomonas* spp. growth in oysters as a result of H_2O_2 production [57].

e. Reuterin

Reuterin is produced by heterofermentative *Lactobacillus reuteri*. Reuterin has low molecule weight and high solubility. It has inhibition effect on *Salmonella, Shigella, Clostridium, Staphylococcus, Listeria, Candida* and *Trypanosoma* genus [58].

f. Antibiotics

Even though traditional antibiotics are produced from bacteria, they are considered as materials from bacteriocins. Generally, the difference between bacteriocin and antibiotics are their way of synthesizing, mechanism of effect, antimicrobial spectrum, toxic effect and different resistance mechanism. Bacteriocins are ribosomally synthesized, while antibiotics are synthesized by multi-enzyme complexes. Although bacteriocins could show an inhibition effect on target microorganisms at low concentration, antibiotics need higher concentration. Bacteriocins are generally produced in the exponential growth phase. Antibiotics are produced in the stationary growth phase as secondary metabolites [59]. Antibiotics produced by microorganism are shown in Table II.

TABLE II
ANTIBIOTICS PRODUCED BY MICROORGANISMS [60], [61]

Antibiotic	Microorganism
Streptomycin	*Streptomyces griseus*
Chloramphenicol	*S. venezuelae*
Neomycin B	*S. lavendulae*
Chlortetracycline	*S. aureofaciens*
Neomycin	*S. fradiae*
Oxytetracycline	*S. rimosus*
Nystatin	*S. noursei*
Erythromycin	*S. erythreus*
Tetracycline	*S.* sp.
Novobiocin	*S. spheroides, S. niveus*
Cycloserine	*S. orchidaceus, S. gaeryphalus*
Vancomycin	*S. orientalis*
Kanamycin	*S. kanamyceticus*
Paromomycin	*S. rimosus*
Gentamicin	*Micromonospora purpurea*
Tobramycin	*S. tenebrarius*
Sisomicin	*M. inyoensis*
Penicillin	*Penicillium chrysogenum*
Cephalosporin	*Cephalosporium acremonium*

V. CONCLUSION

Today, consumer demand is focused on foods which are natural and have beneficial effects on health instead of foods containing chemical additives. As a result of this demand, research studies have focused on natural preservatives. The search for natural preservatives, especially those that may be used in place of chemical protectors used in food, is important for healthier nutrition and survival. Increasing awareness of consumers about nutrition and increasing trends towards natural and healthy products enhance the popularity of lactic acid bacteria and ensures that these microorganisms are the focus of research [10].

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