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Natural peservatives: Current insights and applications

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ABSTRACT

To preserve food in a state that is appetizing, nutritious, safe and unspoiled, need of a preservative is must. People are trying to stay away from everything synthetic including preservatives, as much as possible in the current scenario. This is due to increasing complications arising from the use of the synthetic ingredients. These consumers led trends have fuelled a renewed interest in the development of more 'natural preservatives' for extending the shelf life and maintaining the safety of foods. This review focuses natural preservatives and other substances of natural origin which have potential to act as preservatives for foodstuff.

Key Words: Preservative, anti-browning agent, bacteriocin, Maillard reaction.

INTRODUCTION

Natural Preservatives

In recent years there has been a dramatic increase in the number of reported cases of food-borne illness[1]. Consequently, there is considerable interest in approaches to stop this increasing trend and reduce the incidences of food poisoning.

Now-a-days, people are trying to stay away from everything synthetic including preservatives, as much as possible. This is due to increasing complications arising from the use of the synthetic ingredients as carcinogenicity, terratogenicity, liver, kidney, heart, respiratory or nervous systems problems and other disorders[2]. At this juncture of time, consumers are demanding partial or complete removal of chemically synthesized preservatives from foodstuffs. Also, there is an increased demand for convenient and packed foods with long shelf lives. These consumer led trends have fuelled a renewed interest in the development of more **'natural preservatives'** for extending the shelf-life and maintaining the safety of foods. Natural substances are considered safe, more acceptable and reliable due to their fewer side effects, local and easy accessibility and eco-friendly nature[3]. Although the antimicrobial properties of many compounds from plant, animal and microbial sources have been reported, their potential for use as natural food preservatives has not been fully exploited yet. Potential natural preservatives under intensive trial include lytic enzymes, bacteriocins from lactic acid bacteria and plant antimicrobials, for a variety of foods and beverages including juices, milk, hard-cooked cheese and fresh fruit slices[4]. Plant extracts, including their essential oils and essences have been reported to possess significant antimicrobial properties against bacteria, moulds and yeasts[5-8]. However, only recently much attention has been given to their potential applications as food preservatives[1].

Preservative is a natural or synthetic substance that is added to products such as foods, pharmaceuticals, paints, biological samples etc. to retard spoilage due to whether, microbial growth or undesirable chemical changes.

Preservatives may also be defined as the substances, which are capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food or of masking any of the evidence of putrefaction. These are the substances with antiseptic properties, which inhibit growth of microorganisms without necessarily destroying them.

A completely adequate preservative should have the following properties:-

- It must not under any reasonable condition injure the health of the consumer.
- It must not allow the utilization of unfit raw materials.
- Its use must not make possible the employment of careless and imperfect methods of manufacture.
- It must be non-irritant.
- It must be efficient in its action.
- It must not retard the action of digestive enzymes.
- It must have no tendency to decompose within the body into substances, which have a greater toxicity than that of the preservative itself.
- It must lend itself to simple methods of determination and thus simplify the control problem[9].

There are basically three types of preservatives used in foods:

(i) Antimicrobials
(ii) Antioxidants
(iii)Antibrowning agents

1. Natural Antimicrobials

The antimicrobials with E and INS number ranging from 200 to 290 are used as food preservatives to check or prevent the growth of microorganisms. These play a major role in extending the shelf life of numerous snacks and convenience food stuffs, which have come into even greater use in recent years, as microbial food safety concerns have increased[10]. There are a number of natural antimicrobial substances that act as natural preservatives. Some of them are as follows:

1.1 Essential Oils

The antimicrobial action of essential oils in model food systems or in real food is very well established and is well documented in the scientific literature[11-13]. Although the majority of essential oils are classified as **G**enerally **R**ecognized **A**s **S**afe (GRAS)[14], yet their use in foods as preservatives is often limited due to flavour considerations. Since, effective antimicrobial doses may exceed organoleptically acceptable levels. Therefore, there is an increasing demand for

accurate knowledge of the minimum inhibitory concentrations (MIC) of essential oils to enable and establish a balance between the sensory acceptability and antimicrobial efficacy[15].
In vitro studies have demonstrated antibacterial activity of some essential oils (EOs) against *Listeria monocytogenes, Salmonella typhimurium, E. coli, Shigella dysenteria*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10 µl ml^{-1}. Gram-negative organisms are slightly less susceptible than gram-positive bacteria. A number of EO components such as Carvacol, thymol, eugenol, perilladehyde, cinnamaldehyde and cinnamic acid possess minimum inhibitory concentrations (MICs) of 0.05-5 µl ml^{-1}. *In vitro* studies with fresh meat, meat products, fish, milk, dairy products, vegetables, fruits and cooked rice have shown the concentration needed to achieve a significant antibacterial effect is around 0.5-20 µl g^{-1} in foods and about 0.1-10 µl ml^{-1} in solutions for washing fruits and vegetables[16].
Mode of Action: Little is known about mechanism of action of organo essential oils (OEO). Generally different bacteria or microorganisms are inhibited by specific effect of OEO constituents such as carvacol and thymol[17-21]. According to Conner and Beuchat the antimicrobial action of essential oils may be due to impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis[22]. General studies on the mechanism of action of essential oils have indicated a common methodology that attempts to illustrate deleterious effects on cellular membranes, i.e. permeability and proton motive force[17-19,21-24].

1.2 Polysaccharide

1.2.1 Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed □-(1,4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan is produced commercially by deacetylation of chitin which is the structural elements in the exoskeleton of crustaceans (Crabs, shrimp, etc.).
The antimicrobial properties of chitosan glutamate, a derivative of chitin, were investigated in laboratory media/apple juice against different yeasts and moulds associated with food spoilage in order to assess the potential for using chitosan as a natural food preservative. Growth inhibition and inactivation of filamentous moulds and yeasts, were found to be concentration, pH and temperature dependent. It was inferred that Chitosan is worthy candidate for further study as a natural preservative for foods prone to fungal spoilage[25,26].

1.3 Bacteriocins

Interest in novel biological preservation methods has increased during recent years. Also, research indicating that antagonistic microorganisms or their antimicrobial metabolites may have some potential as natural preservatives to control the growth of pathogenic bacteria in foods[27] has further strengthened this interest. Bacteriocins are compounds that are produced by bacteria and antagonistic to other bacteria[28].

1.3.1 Nisin

Nisin is the only bacteriocin that has found practical application in some industrially processed food. Nisin was first introduced commercially as a food preservative in the UK, approximately 30 years ago. The established use of nisin as a preservative is found in processed cheese, various pasteurized dairy products and canned vegetables. Many other bacteriocins from lactic acid bacteria have recently been characterized[27]. Because of potential usefulness as natural food preservatives, increased interest has been found on bacteriocins from lactic acid bacteria. Bacteriocin producing (Bac+) lactic acid bacteria (LAB) detected in retail foods indicates that the public is consuming a wide variety of Bac + LAB. This suggests a greater role for bacteriocins as biopreservatives in foods[29].

1.3.2 Mesentericin Y105

Mesentericin Y105 is a small non-lantibiotic bacteriocin (Class II) encoded within a 35 Kb plasmid from *Leuconostoc mesenteroides* Y105 and it is active against *Listeria monocytogenes*[30].

1.3.3 *Pediococcus pentosaceus*

Pediococcus pentosaceus L and S were isolated from two strains of *Pediococcus pentosaceus.* These may inhibit food borne pathogens and have potential to be used as natural preservatives[31].

1.3.4 Propionicin PLG-1

Propionicin PLG-1 is produced and released by *Propioni bacterium* thoenii P-127[35]. Class II bacteriocins namely enterocin A, mesentericin Y105 divercin V41 and Pediocin ACH and nisin A have tremendous potential and may be used as natural food preservatives[32-34].
Mode of Action: Bacteriosin acts by dissipation of proton motive force which was identified as the common mechanism for the lethal activity of LAB bacteriocin[35].

1.4 Antimicrobial Enzymes

1.4.1 Lysozyme

Lysozyme (1,4-α-N-acetyl muramidase) is a 14,600 Da-enzyme present in avian eggs, mammalian milk, tears and other secretions[10]. Although tears contain the greatest concentration of lysozyme, dried egg white (3.5%) is the commercial source[36]. The antimicrobial effects of lysozyme against common food spoilage and food borne disease causing bacteria were investigated. It was identified that lysozyme can be used as an effective antimicrobial[37]. Lysozyme is one of the few naturally occurring antimicrobials approved by regulatory agencies for use in foods specially for its preservative properties[10].
Mode of Action: Lysozyme catalyse the hydrolysis of the α-1,4 glycosidic bonds between N-acetylmuramic acid and N-acetyl-glucosamine of the peptidogycan of the bacterial cell wall. This causes cell wall degradation.

1.4.2 Lactoperoxidase

Lactoperoxidase is an enzyme that occurs in raw milk, colostrum, saliva and other biological secretions. This enzyme reacts with thiocyanate (SCN^-) in the presence of hydrogen peroxide and forms an antimicrobial compound which is termed the lactoproxidase system (LPS). It means LP enzyme, an oxidizable substrate and hydrogen peroxide all three component must be present for the system to exert its antibacterial effect[38]. The naturally occurring LP system is primarily active against hydrogen peroxide producing bacteria such as *lactococci* and *lactobacilli*[39]. However recent work has shown that LPS is more active against gram-negative bacteria including *Pseudomonas* than the gram-positive bacteria[38-40]. LP system has been applied of preservation of some foods including milk, infant milk formula and liquid whole egg[41-44].

Mode of Action: The enzyme (LP) catalyzes the oxidation of thiocyanate (SCN^-) by hydrogen peroxide to hypothiocyanous acid and hypothiocyanate ($OSCN^-$). At neutral pH, these two products, the major products (hypothiocyanous acid and hypothiocyanate) of the LP system, exist in equilibrium and are the major active antimicrobial compounds. Other products (e.g. HO_2SCN and HO_3SCN) may also be formed and contribute to antimicrobial activity[45]. The $OSCN^-$ and $HOSCN^-$ are highly reactive oxidizing agents and react with sulphydryl groups and reduce nicotinamide nucleotides (NADH and NADPH) of microbial cells. This interaction accounts for the mode of action of the LP system and gives the LP system a broad spectrum of activity. Through the oxidation of these cellular components, cytoplasmic membranes, carbohydrates and amino acid transport systems, glycolytic pathways are impaired or damaged.

Such an impairment or damage may kill the microbial cells or inhibit growth and/or other cellular metabolic activity[46].

1.5 Antimicrobial Protein/Peptide

1.5.1 Citropin 1.1 and Protegrin 1

The increasing resistance of microbes to antimicrobials and preservatives has necessitated search of new targets such as antimicrobial peptides. Numerous antimicrobial peptides have been isolated from insects, amphibians, mammals, plants and bacterial species. Two animal peptides citropin 1.1 and protegrin 1, alone or in combination have been found active against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. The antimicrobial preservative effectiveness of the substances is comparable to benzalkonium chloride[47]. So they may be explored for their potential to be employed very effectively as preservatives.

1.5.2 Conalbumin

Conalbumin is an iron-binding protein that constitutes at least 10-12% of the total egg white solid[48,49]. Both gram-negative and gram-positive organisms are inhibited by conalbumin. Some yeasts are also sensitive to conalbumin[42].

Mode of Action : Conalbumin inhibits microbial growth by making iron unavailable to the microorganisms. Conalbumin is not expected to destroy the microorganism. It simply extends the lag phase of growth and decrease the rate of multiplication[46,50-52].

1.5.3 Lactoferrin

Lactoferrin is an iron-binding glycoprotein that participates in the antimicrobial activity of milk. Lactoferrin has also been termed as lacto transferrin or lactosiderophilin[54]. Lactoferrin is reported to be active against *L. monocytogenes, B. subtilis, B. stearothermophilus, S. aureus, Klebsiella* and *E. coli*[54-56]. It was reported that an antimicrobial peptide was produced by pepsin digestion of bovine lactoferrin, which has been termed as lactoferricin[57]. It is used as a natural antimicrobial agent to preserve ground beef.

Mode of Action: The inhibitory activity of lactoferrin apparently results from the binding of essential iron which is needed for growth of the microorganisms. The mode of action of lactoferrin is as yet unclear, however the high number (eight) of basic amino acid residues contained in the peptide suggests that the peptide acts at the cell surface to increase membrane permeability[46].

1.5.4 Avidin

Avidin is a glycoprotein present in egg albumen. Avidin strongly binds the cofactor biotin at a ratio of four molecules of biotin per molecule of avidin and makes it unavailable for the growth of certain organisms[50].

Mode of action : Avidin strongly inhibits growth of some bacteria and yeasts that have a requirement for inhibition, with the primary mechanism being nutrient deprivation. Another potential mechanism is that, avidin binds porin proteins in the outer membrane of *E. coli.* Thus, avidin may inhibit microorganisms *in vitro* by interfering with transport through porins[58].

1.6 Plant Extracts

1.6.1 Neopein and improved biopein

Neopein and improved biopein is a proprietary blend of botanical extracts. The antimicrobial activity of improved biopein and neopin were tested against an array of microorganisms. It was found to act as effective natural alternatives to commonly used synthetic ingredients in appropriate formulations for product preservation[2].

1.6.2 *Aristolochia bracteata*

Aristolochia bracteata (Aristolochiaceae) root extract demonstrates a broad spectrum of antibacterial activity. The ethyl acetate extract was found to be most effective. Thus there is a potential for replacement of synthetic preservative by the use of natural extracts[59].

1.6.3 Plants of Allium Species

Plant of the Allium species, namely garlic and onion, have been extensively studies for their antimicrobial properties. Allicin, the major antimicrobial component from garlic bulbs, was isolated by steam distillation of ethanolic extracts[60-62]. It was also reported that garlic juice is a very potent antimicrobial agent against both bacteria as well as pathogenic yeasts[62,63].
Mode of action: It was also reported that allicin, disrupts microbial cell metabolism primarily by inactivation of –SH proteins by oxidation of thiols to disulfides. So, there is competitive inhibition of the activity of sulfydryl components such as cysteine and glutathione by binding with them[64,65]. It indicates that key metabolic enzymes of several food-borne yeasts were likely inhibited in this manner[66].

1.6.4 Vanillin

Vanillin (4-hydroxy-3-methoxy benzaldehyde) is a major constituent of vanilla beans, the fruit of an orchid (*Vanilla planifola, Vanilla pompona*). Vanillin is most active against molds and gram positive bacteria[40,67]. It may be used as a natural food preservative. Vanillin was found to possess significant activity against *A. flavus, A. niger, A. ochraceus* and *A. parasiticus*. *G*rowth on potato dextrose agar was found to possess a significant activity[68,69].
Mode of action: Vanillin is primarily a membrane active compound, resulting in dissipation of ion gradients and the inhibition of respiration[70].

1.6.5 Pepper fruit

Phenolic and essential oil extracts of pepper fruit (*Dementia tripetala*) showed antifungal activity against *Saccharomyces cerevisiae*, *Candida species*, *Aspergillus niger* and *Fusarium* species. Being an edible fruit, pepper fruit extracts may prove a useful natural preservative in food processing[71].

1.7 Flavonoids

1.7.1 Baicallin

Baicallin, is a flavonoid from the root of *Scutellaria baicalensis*, a perennial plant of Lamiaceae family. It seems to follow the criteria of biological preservatives. Baicallin, when used as a preservative lowers the microbiological counts during the entire period of storage[72].

1.8 Isothiocyanates

Allyl isothiocyanates (AITC), a group of natural compounds in plants belonging to the family Cruciferae, has been shown to have antimicrobial activity in liquid media as well as in its vapour form. Isothiocyanates (R–N=C= S) are inhibitory to fungi, yeasts and bacteria[73].

Mode of Action: The mechanism by which isothiocyanates inhibit cells may be due to inhibition of enzymes either by direct reaction with disulfide bonds or through thiocyanates (SCN^-) anion reactions which lead to inactivate sulfhydryl enzymes[40]. It was suggested that AITC inhibited *Salmonella* and *E. coli*[74]. Allyl isothiocyanate was most similar to polymixin B with respect to its antibacterial effect on cell membranes and on leakage of cellular metabolites[40]. The effectiveness of AITC in inhibiting bacteria at all the growth stages and its strong activity in vapour phase supports its effective applications in food preservation[74].

1.9 Phenols and Phenolic Acids

Phenolic compounds include monophenols (e.g., p-cresol), diphenols (e.g. hydroquinone) and triphenols (e.g. gallic acid). Practical use of simple phenols for preservation is found in the application of wood smoke.

The phenolic acids including derivatives of p-hydrobenzoic acid (protocatechuic, vanillic, gallic, syringic, ellagic) and o-hydroxybenzoic acid (salicylic acid) may be found in plants and foods. Phenolic compounds have broad spectrum antimicrobial activity. Resins from the flowers of the hopvine (*Humulius lupulus* L.) are composed of α-bitter acids, including humulone, cohumulone and adhumulone and α-bitter acids, including lupulone, colupulone, xanthohumol and adlupulone. Both types of bitter acids possess antimicrobial activity.

Mode of action: Their action involves interaction of the cytoplasmic membrane and the activity is selectively increased against gram-positive bacteria and fungi. Hop bitter acids inhibit growth of beer spoilage bacteria by dissipating trans-membrane pH gradient[40].

1.10 Organic Acids

Organic acids occur naturally in foods and have been used in many food products as preservative, because they inhibit the growth of many microorganisms[64]. This is due to their solubility, flavour and low toxicity. Acids commonly used as preservatives are acetic acid, citric acid, lactic acid, pyropolyphosphoric acid, sorbic acid and malic acid[25].

1.10.1 Acetic acid

It is the major component of vinegar and its salts are widely used in foods as antimicrobials. Acetic acid is more effective against yeast and bacteria than against moulds[10,76].

1.10.2 Lactic acid

It is a primary end product of the lactic acid bacteria and serves to assist in preservation of many fermented dairy, vegetable and meat products[10,77].

1.10.3 Citric acid

It is derived from citrus fruits by fermentation of crude sugars. It is used as a preservative and to adjust acid-alkali balance[78].

1.10.4 Sorbic acid

Sorbic acid was first isolated from berries of the mountain ash try (rowanberry). It occurs naturally in ripe barriers, cherries, plums, pears and apples. It is used as a natural preservative[79].
Mode of action: In the undissociated form, organic acids penetrate the cell membrane lipid bilayer. Inside the cell, organic acid is dissociated because of a higher pH inside the cell than the exterior. Since bacteria must maintain their internal pH near neutrality, protons generated from dissociation of the organic acid must be extruded to the exterior. Therefore, the protons generated by organic acid inside the cell, must be extruded using energy in the form of ATP. A constant influx of these protons will eventually deplete cellular energy and will result in death of the microorganisms. Undissociated form of the acid is primarily responsible for its antimicrobial activity[80-82].

2. Natural Antioxidants

Natural antioxidants are primarily extracts of herbs or plant materials with inordinately high concentration of particular polyphenolics having good-electron donating and chain breaking properties[10].

In response to perceived desire by consumers for less chemically processed food ingredients, several naturally occurring chain-breaking antioxidants accomplished essentially the some effects as BHA (Butylated hydroxy anisole), Butylated hydroxy toluene (BHT)[83]. Some of the common natural antioxidants which are used as preservative are as follows:

2.1 Tocopherols

Tocopherols are the most active chain-breaking antioxidants and there is an explicit dietary requirement for tocopherols. Basis for the essentiality of tocopherol lies in its ability to prevent oxidative damage. It is obtained from vacuum distillation of edible vegetable oils. Plant phenolics inhibit lipid acid formation catalysed by metals, radiation and heme compounds[84,85]. They also scavenge peroxy alkoxy and hydroxy radicals and singlet oxygen[86,87]. This is a powerful antioxidant used in preserving oils and preventing them from getting rancid.

2.2 Honey

Honey from different floral sources has been evaluated for antioxidant content and for their ability to inhibit enzymatic browning in fruits and vegetables. Soy honey was particularly effective when compared to clover honey. Honey has great potential to be used as a natural source of antioxidants to reduce the negative effects of polyphenol oxidase (PPO) browning in fruit and vegetable processings. Bacteria can’t live in honey, which demonstrates its natural preservative properties[88].

2.3 Bee propolis

Bee propolis is a mixture of various amounts of beeswax and resins collected by honey bee from plants, particularly from flowers and leaf buds. It is rich in phenolic compounds which act as natural antioxidant[89].

2.4 Phytic acid

Phytic acid is abundant in edible legumes, cereals and seeds. It forms an iron chelate which greatly accelerates Fe^{2+} mediated oxygen reduction. It also blocks iron-driven hydroxyl radical generation and suppresses lipid peroxidation. High concentration of phytic acid prevents browning and putrefaction of various fruits and vegetables by inhibiting polyphenol oxidase. This suggests that phytate may be a substitute for presently employed preservatives, many of which pose potential health hazards[90].

2.5 Citric acid

Citric acid is derived from citrus fruits by fermentation of crude sugars. Citric acid is found to possess antioxidant properties and thus is used as a natural preservative. At least two free carboxylic acid groups are necessary for its free antioxidant potency[78].

2.6 Nigella sativa

Seeds of *Nigella sativa*, a dicotyledon of the Ranunculaceae family, have been employed for thousand of years as a spice as well as a food preservative. Both oil and its active ingredients, particularly thymoquinine, possess reproducible anti-oxidant effects through enhancing the oxidant scavenger system[91]. It is used as a fruit preservative due to its antioxidant properties.

2.7 Thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosin

These are natural products that have received attention as possible food antioxidants. These are obtained from different essential oils[92] and used in preserving vegetable oils.

2.8 Carotenoids
Carotenoids especially beta-carotene, may be prepared on a commercial scale, from carrot, yarn and other sources. Carotenoids are excellent scavengers of singlet oxygen and are used mainly in foods that are exposed to light, such as vegetable oils packaged in clear bottles. Red palm oil is dominated by □-carotene but it also contains beta-carotenoid and minute amounts of other carotenoids including lycopene, the major source of which is tomato. The beneficial health effects of carotenoids, including their oxygenated derivatives are very well documented[93, 94].

2.9 Thai leaves extract
The leaves of the thai vegetables (*Cratoxylum formosum* Dyer) possess antioxidant properties. Its main component is cholorogenic acid. This plant is a promising source of natural food antioxidants and may be used as a natural food preservative[95].

2.10 Mushrooms extract
Antioxidant capacity and antimicrobial activities of *Laetiporus sulphureens* extracts possess antioxidant activity. Positive correlation was found between total phenolic contents in mushroom extracts and their antioxidant activities. Edible mushrooms have potential as natural antioxidants and as future preservative[96].

2.11 Star fruit
Star fruit (*Averrhoa carambola*) is a good source of proanthocyanidine and polyphenolics which are its major antioxidants. It is used for tarts, preserves, chutney and stewed fruits[97].

2.12 Green tea extract
Extract of green tea was found to be one of the most effective natural antioxidants. The crude extracts contain at least 25% catachins dominated by epigallocatechin gallate (48-55%) followed by equal amounts of epigallocatechin and epicatechin gallate (each at 9-12%) as well as epicatechin (5-7%) gallocatechin (3.5%), catechin (0.3-0.6%) gallic acid (0.3-0.5%)[98].

2.13 Verbascum macrurum
The antioxidant properties of methanolic extracts obtained from the aerial parts of *Verbascum macrurum* have been determined. They were identified as natural preservatives against oxidative rancidity. Acteoside, a polyhydroxylated phenylpropanoid glycoside derivative is the most potent free radical scavenger. This compound therefore represents a very interesting candidate for use in food preservation as natural preservative[99].

2.14 Ajowan
Ajowan (*Carum copticum*) extract was found to be a natural antioxidant because of presence of thymol and carvacrol as its constituents[100]. It is used in preserving oils and keeping them from getting rancid.

2.15 Rosemary oleoresin extract
Rosemary (*Rosmarinus officinalis*) extract is a powerful antioxidant. As a natural preservative, it prevents rancidity of vegetable oils. It is also used as anti-browning agent[2].

2.16 Grape fruit seed extract
Grape fruit (*Citrus grandis*) seed extract is made from dried, ground seeds and pulp of grape fruits. It is a natural antibiotic, antiseptic, disinfectant and preservative[2].

2.17 *Ocimum basilicum*

A major aroma constituent, 4-allylphenol present in extracts of basil leaves (*Ocimum basilicum* L.), is found to demonstrate strong antioxidant activities. It inhibits the oxidation of hexanal by completely for a period of 30 days at a concentration of 5 µg/ml. This compound has antioxidant activities comparable to those of the known antioxidants such as α-tocophenol and butylated hydroxy toluene (BHT)[101].

3. Anti browning agents

Browning is the process of spoilage and getting food brown when it is kept for some time after cutting. It is of two types i.e. enzymatic browning and non enzymatic browning. *Enzymatic browning* results from oxidation of polyphenols to quinines catalysed by enzyme polyphenol oxidase (PPO), tyrosinase, o-diphenol oxidase, catechol oxidase and subsequent further reaction and polymerization of the quinines. The occurrence of enzymatic browning can limit the shelf life of fresh cut fruits salad, vegetables, fresh mushrooms and prepeeled potato[102]. *Non enzymatic browning* results from classic Maillard reaction between carbonyl and free amino groups i.e. reducing sugars and amino acids[103] which produces melanoidin pigments in a wide variety of foods including diary, cereal, fruit and vegetable products[104, 105]. Anti browning agents are used to prevent both enzymatic and non-enzymatic browning in fruits and vegetables. Some common anti-browning agents are as follows:

3.1 Ascorbic acid

Ascorbic acid has been used as an anti-browning agent for more than five decades and is still the most widely used alternative to sulfating agent. This may be an outgrowth at the common kitchen practice of using lemon juice to delay browning during food preparation. The earliest scientific studies were reported by Tressler and DuBios[106]. These pioneering investigations added ascorbic acid or its isomer erythorbic acid to syrups or dips to control browning of fresh sliced and frozen apples and peaches.

3.2 Cysteine

The ability of cysteine to inhibit enzymatic browning is well established. It has been used commercially for a number of years[107]. Cysteine is also a component of browning inhibition treatment[108,109] and is used to delay browning during food preservation.

Mode of action: Cysteine reacts with quinine intermediates formed by phenol oxidase catalysed oxidation of polyphenols to yield stable, colourless compounds thereby blocking pigment formation[110]. Cysteine also directly inhibits the enzyme polyphenoloxidase[111].

CONCLUSION

Traditional and/or natural food preservatives such as antimicrobial agents, natural antioxidants and natural antibrowning agents are important tools in preserving foods and other related substances from the hazardous and detrimental effects of microorganisms as well as other spoilage processes. Therefore the future of preservatives lies in naturals. However, it must be emphasized that there is a need for systematic research endeavors to prove their potential, so that they can be explore for novel uses and can find considerable applications in food and pharmaceutical industries.

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