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Prospects and Challenges of Essential Oils as Natural Food Preservatives - A Review

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ABSTRACT

Food is the prime requirement of all living organisms, including human beings. Storage of food items has become essential for society in order to minimize the time to obtain a particular food as well as to maintain its nutritional quality. The earliest evidence of food preservation can be traced back to post glacial era. Luis Pasteur proved for the first time that microorganisms are associated with foods and are the major cause of food spoilage, during storage. In early days people used heat, boiling, smoking, drying, salting, and other methods for food preservation. A major development in this field was noticed during the 1940s due to the availability of low cost refrigerators and freezers. During the last two to three decades fermentation as a process and fermented foods increased the shelf life of various food items and revolutionized the techniques of food preservation, too. Later on, with the advancement of food science and technology, various modern techniques such as artificial drying, vacuum packaging, irradiation, and chemical preservatives were employed for long-term storage of food. However, these physical and chemical methods have their own limitations and various side effects. Now-a-days consumers are more concerned about the synthetic, harmful chemicals used as preservatives. Hence, human civilization has renewed its interest for use of natural products, more specifically plant products in food preservation. In the present review I try to review the history and development of food preservation techniques over time. The limitations of different modern methods of food preservation are discussed briefly, leading in search of natural compounds as food preservatives. The nature, extraction, chemical composition and various biological properties of essential oils have been elucidated. Plant essential oils with antimicrobial, antioxidant and other properties makes them a suitable candidate for use in food processing as natural food preservatives.

Keywords: antimicrobial activities, biological activities, chemical composition, essential oils extraction, food preservation

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INTRODUCTION

Food is the material that enables man and other organisms to grow, maintain and reproduce themselves. Food is composed of different chemicals in various combinations and is as highly essential to life as water and oxygen. The demand of food and its production dates back to the dawn of civilization, and of course there is a growing demand for food production all over the globe due to an increase in population. Food includes prepared food, grains, fruits, vegetables, all of which have a self-life and are more prone to spoilage, as the food items are made with different organic and inorganic constituents that provides a suitable nutritional medium for microorganisms. Food spoilage can be defined as any change in natural constituents and/or state of food that lessens its desirability for aesthetic or health reasons (Jay 1987). Food items undergo different types of spoilage resulting in abnormal colours, flavours, taste and other changes in its consistency. In general these changes are of a biological nature brought about by the growth of the microorganisms or are autolytic changes, brought about by enzymes produced within the tissue itself, in the case of living food materials like cereals, grains, fruits, and vegetables. In some cases changes result from oxidations. The first man to appreciate and understand the presence of the role of microorganisms in food was Pasteur, in 1837 who showed that the souring of milk was caused by microbes. Microorganisms that are associated with food spoilage mainly include bacteria (*Acetobacter*, *Acenetobacter*, *Aeromonas*, *Alcaligens*, *Alteromonas*, *Bacillus*, *Campylobacter*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Erwinia*, *Escherichia*, *Flavobacterium*, *Listeria*, *Micrococcus*, *Moraxella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Vibrio*, *Yersinia*), yeasts (*Brettanomyces*, *Candida*, *Debaryomyces*, *Endimycopsin*, *Hansenula*, *Kloeckera*, *Saccharomyces*, *Saccharomycopsis*, *Torulopsis*, *Trichosporon*), molds (*Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Cephalosporium*, *Colleotrichum*,

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Fusarium, *Geotrichum*, *Gloeosporium*, *Mucor*, *Penicillium*, *Rhizopus*, *Sporotrichum*, *Trichothecium*). The enzymes that are responsible for spoilage of food mainly include cytase, diastase, lipase, protease, pepsin, ptyalin, trypsin, zymase, among others. Food spoilage due to chemical changes and enzymatic degradations are less harmful in comparison to spoilage by different microorganisms (Jay 1987). Consumption of contaminated food or the presence of different toxic materials secreted by microorganisms results in loss of life due to food poisoning. Therefore, preservation of food has become an integral part of human life, science and research and development program.

HISTORY OF FOOD PRESERVATION

Food preservation technology has developed sufficiently to preserve a wide variety of foods for a considerably long time. In the early stages people used heat, boiling, drying, smoking, pickling, salting methods for food preservation long before they had any knowledge of the cause of food spoilage. Salted meat, fish, fats, dried skin, wheat and barley were preserved for a long time by Sumerians of about 3000 BC (Goldblith 1971). Between 3000 BC and 1200 BC the Jews used salt from the Dead Sea in preservation of various foods. The Chinese and Greeks are credited for passing this technique to the Romans. Mummification and preservation of foods seems to have developed by this time. Wines are known to have been prepared by the Assyrians by 3500 BC and fermented sausages were prepared and consumed by the ancient Babylonians and the people of ancient China as far back as 1500 BC. Another method of food preservation that apparently arose during this time was the use of edible oils such as olive and sesame oils. Canning as a method of food preservation had its beginning during 1975, when French Government offered a prize of 12,000 Francs for the discovery of a practical method of food preservation. In 1809 a Persian confectioner Nicholas Appert succeeded in preserving meat in glass bottles by keeping in boiling water for variable time period. A year later, during 1810, Nicholas Appert was issued a patent for this process and the technique was made public. This of course, was the beginning of canning as it is known and practiced today, though in 1665, L. Spallanzani showed that beef broth that had been boiled for an hour and sealed remained sterile and did not spoil and disproved the doctrine of spontaneous generation of life. Of course, during 1860s Pasteur used heat for the first time scientifically to destroy undesirable microorganisms in wine and beer that led to the development of the technique pasteurization.

Though these older methods are entirely adequate for preservation of certain types of foods, they have inherent disadvantages which limit their usefulness. These older methods sometimes bring about severe changes in appearance, taste, and odour, time limit storage; therefore, in many cases they are objectionable. So some other means of preservation techniques must be utilized to maintain the quality as well as long term preservation of the food items.

Modern techniques of food preservation

Since the development of science, in this modern era of biotechnology, various novel techniques of food preservation have evolved. These involve the application of different physical and chemical agents (Tortora *et al.* 2006). Physical methods mainly include drying, application of low and high temperatures, irradiation, and regulating the pH of food items. Further, fermentation as a process has gained much attention in production of fermented foods with a quality and preservation strategies. The preservation of foods by drying is based on the fact that microorganisms and enzymes need water in order to be active. In preserving foods by this method one seeks to lower the moisture content of foods to a point where the activity of food spoiling and food poisoning microbes are inhibited. Such foods are commonly known as dried, desiccated or low moisture food with a moisture content of 25% and water potential between 0.00-0.60, which is generally achieved through application of heat, desiccations or freeze drying. The use of low temperature to preserve foods is based in the principle that the activities of the food borne microorganisms can be slowed down and/or stopped at temperatures above freezing and generally stopped at subfreezing temperatures. However, psychrophilic food borne pathogens pose a great problem in food preservation through this technique (Gunderson and Rose 1948; Ingrahm 1951; Ingrahm and Stokes 1959). The use of high temperature to preserve food is based on their destructive capabilities on microorganisms. High temperatures commonly affect the enzyme system, denature the cellular proteins, affect the membrane and membrane permeability, etc., thus killing the microorganisms. Three common techniques: pasteurization (mild heating to destroy the pathogens and other organisms that cause spoilage), sterilization (the killing or removal of all microorganisms in a material or in food items) and tyndallization (fractional sterilization; sterilization with intermediate incubation to kill sporulating microorganisms) are applied on the basis of food and presence of microorganisms killing the vegetative cells and spores (Xezones and Hutchings 1965; Stumbo 1973; Horner and Anagnostopoulos 1975; Madigan and Martinko 2004; Prescott *et al.* 2006). It is important to mention that temperature affects the relative humidity (RH) and as well as pH that prevents the metabolic activities and growth of microorganisms in food items too. Radiation may be defined as emission and propagation of energy through a medium. The type of radiation of primary interest in food preservation is electromagnetic, specifically the ionizing radiations such as α-particles, β-rays, γ-rays, x-rays, cosmic rays with a wave length of 200 nm or less. However, ultra violet radiation is a powerful non ionizing bactericidal agent with the most effective wave length about 260 nm. In addition to this microwave energy may be used for microbial eradication of specific food items (Goldblith 1963; Ley 1987). In practice radiation itself has its own limitations: costly to generate, its activity is mainly based on the penetrating capacity and radiation in many cases bring chemical changes.

Besides these physical methods of food preservation a number of chemical compounds are used in different food industries for preservation, which is based on the fact that a large number of chemical compounds, including antibiotics and chemotherapeutic agents, are used with great success in the treatment of various diseases of man, animals and plants. While a large number of chemicals have been described to have antimicrobial potential, as food preservatives, only a relatively small number are allowed in food preservation. This is because of their toxicity nature at definite concentrations, different side effects and strict rules of safety adhered by the Food and Drug Administration (FDA). The common chemical preservatives (Wilkins and Board 1989; Brewer *et al.* 1994) generally recognized as safe (GRAS; section 201(32)(s) of the U. S. Federal Food Drug and Cosmetic Act) include propionic acid/propionates, sorbic acid/sorbates, benzoic acid/benzoates, SO_2/sulfites, ethylene or propylene oxides, sodium nitrite, sodium diacetate, caprylic acid, ethyl formate, etc. and used in preservation of different food items. Besides their antimicrobial action, chemical compounds are also added to the food items at a definite concentration as stabilizers, emulsifiers, defoaming agents buffer or sequestrant, antioxidants, etc. (Barnen *et al.* 1980; Gilani and Fung 1984; Fung *et al.* 1985). Chemical compounds such as butylated hydroxyanisole (BHA), butylated hudroxytoluene (BHT), *t*-butylhydroxyquinoline (TBHQ), propyl gallate (PG), ethyldiamine tetraacetic acid (EDTA), sodium citrate, lauric acid, monolaurine, menthol, phenylacetaldehyde, carvone, vanillin, ethylene/propylene oxides, sodium nitrites, etc. are generally used for the above said purposes. Though, these chemical compounds are widely used the food industry many are reported to be carcinogennic/mutagenic (BHT, BHA, ethylene/propylene oxides, nitrites, EDTA), non-degradable (SO_2/sulfites), and residues are accumulated in the environment (TBHQ, EDTA), and once released to the ecosystem enter the food chain and result in biomagnification, with a number of side effects, more importantly pose a physiological pressure on the pathogens for development of resistance towards these chemicals. In addition, now-a-days consumers are concerned about the use of synthetic chemicals such as colourants and preservatives in food and there is a resulting trend towards less processed foods (Soomro *et al.* 2002); moreover, people prefer foods with less chemical preservatives (Daeschel 1993). Therefore, pressure mounts on the researchers to search for natural biological compounds for food preservation which are eco-friendly, degradable, without any side effects and more precisely less costly in comparison to the chemical preservatives.

NATURAL BIOLOGICAL COMPOUNDS AND FOOD PRESEVATION

A definite solution to this dilemma is the use of plants and their secondary metabolic products in food preservation. Higher plants and plant parts are being used in food presservation since the dawn of agriculture. To quote one example, dried leaves of *Azadirachta indica* and *Vitex nigundo* are commonly used for the storage of pulses to prevent insect attack in the Indian agriculture system. Spices and condiments in food preparations not only enhance the food quality in terms of flavour, colour and taste but also help in preservation, as a large number of spices proved to be antimicrobial in nature. Further, a large number of plant products and/or plant extracts from different parts have been reported in literature to have antimicrobial potential (Morris *et al.* 1977; Shelef 1983; Zaika 1988; Rath 1991; Sahoo 1998; Smith-Palmer *et al.* 1998; Hammer *et al.* 1999; Padhi 2006; Patra 2006; Teixeira da Silva 2006). Because of the antimicrobial properties of the plants and development of drug resistance among the pathogens to commonly used antibiotics and chemotherapeutic agents, the herbal medicinal system has gained a renewed interest all over the globe. Recently the World Health Organization (WHO) has compiled a least of 20,000 plants that are used for phytotherapy in herbal systems of medicine all over the world (Manavalan and Manian 2001). Amongst these medicinal plants, over 100 botanicals are reported to be consistently in large demand and are utilized in major drug markets the world over. This indicates that the full potential of these groups of plants has not been scientifically explored. In addition to this, the plant-based remedies are also popular in other countries like India, France, China, Netherlands, Japan, Pakistan, Bangladesh, Korea and South Africa (Bhatt 1998). This provides an idea that the plant products promise a great potential for use in the food industry. In this regard medicinal and aromatic plants and their essences are in the front line of choice. Foremost, among these are the volatile compounds and essential oils (EOs). Below, I try to review the potentiality of plant products, more specifically the EOs, in food preservation.

EOs and food preservation

A number of EOs have been proved to possess antimicrobial activities (Maruzella and Securella 1960; Deans and Ritchie 1987; Pattnaik 1994; Lis-Balchin 1995; Rath *et al.* 1999a, 1999b, 1999c; Devi 2001; Rath *et al.* 2001a, 2001b; Gupta 2002; Mohapatra 2002; Rath *et al.* 2002a, 2002b; Mishra 2003; Singh 2003; Gupta *et al.* 2004; Rath *et al.* 2005a, 2005b; Mohapatra *et al.* 2006). Rath (2007) reviewed and listed the broad spectrum antimicrobial activity of 122 plant EOs against bacteria and fungi. Many EOs are already in use as flavourings in the food industry (Lis-Balchin 1998a; Muyima 2005; Burt 2004; Senhaji *et al.* 2007). The combination of flavouring and antimicrobial potential, often with antioxidant (Barrata *et al.* 1998a, 1998b) properties of EOs would be of great benefit to the food industry, as more natural food ingredients are now favoured in the food trade, above the synthetic antioxidants, potential carcinogenic preservatives e.g. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etc. (Lis-Balchin *et al.* 1998a). However, an extensive knowledge on EOs, their extraction procedures, chemical constituents, action patterns, system processes are vital before their use in the food industry as preservatives. I will explore these in the next part of this review.

EOs of plant origin are characterised by their colour, pleasant taste with a strong aromatic smell or odour. Another main property of these oils is their evaporation or volatilization when they come in contact with air. EOs occur in all aromatic plants. They have been found to be present in about 60 families, particularly Compositae, Lamiaceae, Myrtaceae, Apiaceae, Lauraceae, Umbelliferae, etc. Almost any organ of the plant may be the source of the oil: root (ginger, *Gingiber officinale*, Zingiberaceae), rhizome (turmeric, *Curcuma longa*, Zingiberaceae), bark (cinnamon, *Cinnamomum zeylanicum*, Lauraceae), wood (cedar, *Cedrus deodora*, Pinaceae-Laricoideae), leaves (mint, *Mentha* spp. Lamiaceae), flowers (rose, *Rosa* spp., Rosaceae), fruits (orange, *Citrus amurantiu*, *Citrus* spp., Rutaceae), seeds (cardamom) and stem (geranium, *Pelargonium graveolens*, Geraniaceae). EOs accumulate in specialised structures in the plant organs such as cittae (Apiaceae) oil cells, secretory tissues (lisogenous and schizogenous cavities, Myrtaceae, Rutaceae, etc.), glandular trichomes (Lamiaceae) and oil and resin ducts (Pinaceae). The degree of production of EOs in plants is very low. An entire plant when distilled might produce only a single drop of EO, as these are highly concentrated extracts.

Extraction of EOs

EOs are the secretory, secondary metabolites of plants, present in varied quantities in species. Depending on the quantity and stability, of the constituents EOs are extracted by different methods. Basically there are three principal techniques used for extraction of EOs: (i) distillation, (ii) expression, and (iii) extraction by solvents.

Based on extraction technology, the EOs are broadly classified into four categories: (i) absolute (Only essences), (ii) expressed, (iii) steam distilled and, (iv) solvent extracted. Steam distillation is the oldest, simplest and most traditional method of EO extraction. According to the degree of volatile nature of the oil it is subjected to hydro distillation, water and steam distillation, or direct steam distillation. Steam distillation is advantageous over water distillation, as in steam distillation decomposition of oil is less, requires less fuel, less time and hence yield is more. The quality of EO also depends on storage, charge of plant material and pH of water. The citrus oils are usually obtained by expression method. The rinds of citrus fruits such as tangerines, grape fruits, lemons and oranges are crushed and their oils are extracted under high pressure. Technically it should be considered as expressed oils rather than EOs. Many of the EOs contain a number of organic compounds which are less stable and may undergo chemical changes when subjected to high temperature. In this case organic solvent extraction method is used to ensure no decomposition or changes have occurred during extraction which would alter the aroma, fragrance and other properties of the end product. A number of factors of organic solvents(solvent property, polarity, boiling temperature, latent heat of vapourization, reactivity, viscosity, stability to heat, O_2, light, safety to use, ready availability, low cost, suitable to reuse) should be considered before extraction for better recovery of EOs. The organic solvents more frequently used are propane, butane, hexane, methanol, ethanol, propanol, acetone, methyl acetate, dichloromethane, dichloroethane, etc. (Lang and Wai 2001; Smith 2002; Dung and Thang 2005a, 2005b).

The main disadvantage of solvent extraction is poor selectivity of most solvents and final extracts are often coloured and viscous. Now-a-days newer methods of EO extraction have been developed, such as use of supercritical

CO_2 which yield very high quality of oils and fragrances are commercially used but are less common. Liquid CO_2 has several advantages over most conventional organic solvents namely, non flammability, gaseous at ambient temperature, easily removable, colourless, odourless, easy availability, low cost, extracts (oils) with absolute characters, extracts are free of residues from the extraction solvent etc. Furthermore, novel methods such as Microwave-assisted Extraction (MAE), Solvent-Free Microwave Extraction (SFME), Solid-Phase Micro-Extraction (SPME), Continuous Subcritical Water Extraction (CSWE) have been used recently for extraction of high grade EO. However, the specific extraction method to be employed is dependent on the plant material to be distilled and the derived end product (Tiwari 1996; Baser 2003; Senatore 2005; Teixeira da Silva 2006).

Chemistry of EOs

EOs are highly complex substances, a mixture of many different natural chemicals of plant origin. The average EO may contain any where from 80-400 known chemical constituents. These chemicals are secondary metabolites produced by and stored in plants for several biological processses. The aromatic constituents of EOs are built from hydrocarbon chains. They are normally joined together in ring like structures. Oxygen, hydrogen, nitrogen, sulphur and other carbon atoms attached at various points of the chain to make up different oil. The main constituents of EOs are open- chain or cyclic hydro carbons of the general formula $(C_5H_8)_n$, called terpenes. The chemical structures of about 500 constituents of EOs have been studied (Lahlou 2005). On the basis of these analyses EOs can be divided into 4 main groups: a) Terpenes related to isoprene or isopentene; b) Straight chain carbon compounds not containing any side chain; c) Benzene derivatives; d) EOs not included above. Isoprene is the five carbon compound (C_5H_8) building block of many EOs that makes up the terpenoids (monoterpenes, triterpenes and sesquiterpenes). Phenolic derivatives like thymol, carvacrol, eugenol, and gaiseol, account for the major components in EOs. In addition to these, aromatic aldehydes (cinnamaldehyde, cuminal, phellandral), terpene, alcohols (linalool, geraniol, thujanol, borneol, menthol, citronellol, terpinene-1-ol 4, α-terpene-ol, farnesol, limonene), ketones, esters and flavanoids constitute and contribute to the quality and biological activity of different EOs (Lahlou 2004; Jirovetz *et al*. 2005; Lahlou 2005).

Chemical constituents of EOs may have two different paths of origin. The terpene derivatives are formed through acetate mevalonic acid pathway, whereas the aromatic components are formed through shikimic acid-phenylpropanoid pathway. Further, the compounds appearing in EOs may be classified as follows, acids (benzoic, cinnamic, myristic, isovaleric, salicylic), alcohols (borneol, carveol, cedrol, citronellol, geraniol, linalool, menthol, nerol, spathulenol, terpineol), aldehydes (benzaldehyde, cinnamaldehyde, citral, citronellal, myrtenal, neral, safranal, vanillin), ketones (atlantone, camphor, carvone, jasmone, menthone, pulegone, verbenone, vetivone), esters (of different acids like acetic, benzoic, cinnamic, salicylic), hydrocarbons (azulene, cymene, naphtalene, styrene), lactones (coumarin), oxides (ascaridol, eucalyptol, caryophyllene oxide, linallol oxide, pinene oxide, rose oxide), phenols (carvacrol, chavicol, eugenol, thymol), terpenoidic hydrocarbons (caryophyllene, germacrene, limonene, myrcene, pinene, sabinene, santalene, trycyclene).

Though EOs differ in their chemical composition, they have physical properties in common such as: They have common characteristic of aromatic odours, insoluble in water but miscible in organic solvents, characterized by a high refractive index and all of them are optically active. Further, their density is higher than water, generally white to pale yellow in colour, and on long-term storage may oxidize or resinify further darkening in colour. The production and characteristics of various EO in plants may be affected by different external factors such as; soil quality, cultivation practices, climatic and seasonal variations, harvest time and extraction procedures, etc. (Elamrani *et al*. 2000, 2005).

Biological activities of EOs

EOs and flavour compounds of the EOs have been widely used as anti-microbial agents or helped in the development of aromatherapy (Rath 2006) since time immemorial. The development of drug resistant patterns in infectious pathogens prompted human civilization to screen the natural products including EOs as a source of alternate medicine. Aromatic compounds like alcohols, acetone, aldehyde, phenols have been proved to be antimicrobial earlier. Since EOs are a mixture of organic aromatic compounds, they have been tested and proved to have anti-microbial (acting against bacteria, fungi, protozoa, viruses) properties. The susceptibility of microorganisms to EOs depends on both the properties of EOs and the type of microorganisms. It is commonly observed that Gram$^+$ bacteria are more susceptible towards EOs in comparison to Gram$^-$ ones. Similarly fungi are more susceptible to EOs in comparison to bacteria, although, exceptions exist to this rule. The effectiveness of antibacterial and antifungal activity of EOs strictly depends on oil composition and active constituents present on it. On the basis of different investigations throughout the world, the activity gradation of EO components have been set as phenols > aldehydes > alcohols > ketones > ethers > hydrocarbons (Lahlou 2005; Kalemba and Kunicka 2005). Besides their antimicrobial properties, insecticidal antifeedant (Saxena and Rhodendory 1974; Dale and Saradamma 1981; Koul *et al*. 1990; Isman *et al*. 1990; Laurent *et al*. 1997, 2001), antiparasitic and antiviral (Larshini *et al*. 1999; Mikus *et al*. 2000; Lahlou 2003; Lahlou *et al*. 2003), molluscicidal activity (Lahlou *et al* 2001) and antinooiceptive effect (Santos *et al*. 1998) of EOs have been reported.

Escherichia coli is a common food-borne pathogen. The control of bacterial cells is an important factor to reduce food-borne diseases due to *E. coli*. Different EOs have been reported to have bactericidal activity against *E coli* including ETEC, EPEC (Rath *et al*. 2001b, 2002a; Gupta *et al*. 2004; Rath *et al*. 2005b; Senhaji *et al*. 2007). Senhaji *et al*. (2007) reported the inactivation of *E. coli* and *E coli* 0157: H7 by EO from *Cinnamomum zeylanicum*. The EO showed a higher and stronger antibacterial activity than streptomycin used as control in the study. EOs of pepper fruit (*Dennetia tripetala* G. Annonaceae) showed antibacterial activities against common food borne microorganisms such as *Staphyloccus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Proteus* sp., *E. coli*, *Enterococcus faecalis*, *Serratia* sp., *Bacillus* sp., *Clostridium* sp., *Penicillium* sp., *Aspergillus flavus* isolated from various food products (Ejechi and Akpmedage 2005). The results of this study shows that EOs of pepper fruit can play a significant role in food preservation and protection against common food borne pathogens as the oil retarded the growth of these microbes in fresh, boiled and roasted beef (it was observed for seven days).This may have potential applications in the distribution of fresh meat to rural areas in different parts of the world where refrigeration is not available. In another study, Nanasombat and Lohasupthawee (2005) reported the antibacterial activity of 14 spice essential oils (clove, cumin, coriander, cardamom, nutmeg, mace, turmeric, lemongrass, kaffir lime pills, nutmeg, ginger, holy basil, garlic, kaffir lime leaves) against twenty serotypes of *Salmonella* and other members of enterobacteriaceae including species of *Citrobacter*, *Enterbacter*, *Klebsiella*, *Serratia* and *E. coli*. The degree of antibacterial activity against Salmonellae was observed to be: clove > kaffir lime peels > cumin > cardamom > coriander > nutmeg > mace > ginger > garlic > holy-basil > kaffir lime leaves > turmeric > mace > lemongrass. *Salmonella typhimurium* and *E. coli* were more sensitive to most of the spice oils among salmonellae and other non salmonellae strains tested. The study suggests that, these essential oils can be used as potentially anti-*Salmonella* and anti-*E coli* agents in fermented meat products and other foods. However, a major objection to use of these EOs is the problem of imparting colour to meat, which may raise objections among the consumers. A combination of low concentrations of these oils and other mild preservatives such as acid, salt, sugar and other food preservatives may solve the problem of colour in accordance with the concept of hurdle technology (Listner 1999; Leistner and Gould 2002). To supplement this investigation Hammer *at al.* (1999) reported the antibacterial activity of 52 plant EOs against food borne pathogens of both Gram -ve and Gram +ve bacteria and yeasts such as *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus*, *Salmonella* sp. and *Candida albicans*.

The antimicrobial activity of some EOs and their components against food-borne pathogens including mycotoxin producing fungi have also been tested (Bullerman *et al.* 1977; Kim *et al.* 1995; Ultee *et al.* 2000; Nevas *et al.* 2004). Behura *et al.* (2001) reported the fungitoxicity of turmeric EOs against rice sheath blight fungus *Rhizoctonia solani*. Many publications have documented the antimicrobial activity of EOs of various spices against many food-borne pathogens where eugenol, cinnamic aldehyde, carvacrol and thymol are present as major antimicrobial components (Senhaji *et al.* 2007).

All over the world in baking industry, bread occupies a unique position both in production and utilization as compared to other bakery products. The main type of microbial spoilage of bread is due to molds. The ingredients of bread are supportive to growth of microorganisms and multiplication at different stages of bread production, slicing and wrapping. Since bread is an important part of our daily diet ways should be explored to increase its shelf-life. To enhance shelf-life of bread several chemical antimicrobial agents have been employed but they are considered responsible for many carcinogenic and teratogenic attributes and residual toxicity (Skandamis *et al.* 2001). For these reasons demand for natural preservatives have been intensified in bakery industries too. In a study Rehman *et al.* (2007) determined the effect of citrus peel EO on microbial growth and sensory characters of bread. When *Citrus sinensis* peel EOs were applied on bread they significantly affected the sensory characters such as symmetry of form, aroma and grain of bread. The oil inhibited the fungal growth of *Aspergillus flavus*, *A. fumigatus*, *Penicillium* spp., *Rhizopus* and *Mucor* spp. Duccio *et al.* (1998) reported the antifungal activity of citrus EO and components on *Penicillium digitatum* and *Penicillum italicum*. Citrus EOs are mainly used for flavouring beverages, confectioneries, soft drinks, perfumes, soaps, cosmetics and household products. Since they possess antifungal, antibacterial and antiparasitic properties (Duccio *et al*, 1998; Burt and Reinders 2003; Moreira *et al.* 2005) they are suitable as natural preservatives in different food industries as discussed above.

A large number of reports have been documented on antimicrobial properties of various EOs. In a review, Rath (2007) enlisted 122 plant EOs showing different biological activities. Many reported to have antihelmintic and anticarcinogenic properties, too. Many EOs have been reported to possess antimicrobial activities against specific food-borne pathogens, and, as described here, suggest their possible utility in food industries as natural preservatives. However the food industries have observed a rather slow growth in using these EOs as natural preservatives as some studies have concentrated exclusively on one oil or one microorganism. While these data are useful, the reports are not directly comparable due to methodological differences such as choice of plant extract(s)/ EO(s), test microorganism(s) and antimicrobial test methods.

Most of the studies reveal the methodologies as the disc diffusion method or agar well method for testing the antimicrobial activities of EOs. The serial dilution method in agar/liquid medium has been applied for bacteria and fungi with many modifications. The serial dilutions are simultaneously checked for their nature of antimicrobial properties. The assay seems to be much more suitable for the real measurement of the EO's effectiveness (Kalemba and Kunicka 2005). Some factors describe the microbiostatic (the oil merely inhibiting the growth of the pathogen in the dilution tube) or bactericidal (oil kills the pathogens in dilution tubes) dose of EOs. The activity of EOs determined by the dilution method is represented by two means: a) the growth inhibition index defined as the percentage ratio to the control growth culture without EO; b) Minimal inhibitory concentration-MIC (or maximal inhibiting dilution MID) restraining microorganism growth (bacteriostatic/fungistatic) or minimal lethal concentrations-MLC (bactericidal/fungicidal assays).

The second reason why EOs in food preservation are inhibitory is the dose component. Different studies reveal that dose effectiveness of EOs is directly proportional to the constituents. EOs rich in phenols show better activities at low concentration. The most important phenols present in EOs are three monoterpene derivatives: thymol, carvacrol and eugenol. Thymol and carvacrol are the main constituents of the EOs of the Lamiaceae, common culinary herbs and spices used in the food industry. Besides these components EOs rich in eugenol also revealed better antimicrobial activities. The most important sources of eugenol (Hammer *et al.* 1999; Juliani and Simon 2002; Muyima *et al.* 2004; Kalemba and Kunicka 2005; Muyima 2005; Nanasombat and Lohasupthawee 2005; Rehman *et al.* 2007) are the EOs of clove (*Syzygius aromaticum*), cinnamon (*Cinnamomum zeylanicum*), bay-leaf (*Laurus nobilis*), nutmeg (*Myristica fragrans*) and some species of basil (*Ocimum basilicum*, *O. sanctum*, *O. gratissimum*). Mint oils from *Mentha piperata* (peppermint), *M. arvensia* (Japaneese mint), *M. spictata* (spear mint) with menthol have shown high or average activity against bacteria and fungi when compared with other EOs. Sage oil (*Salvia officinalis*) with α- and β-thujones, camphor, linalool also showed remarkable activities against bacteria, yeasts and moulds at a very low concentrations. Some aromatic grass oils are extensively used as fragrance components in perfumes and cosmetics. The most common and valuable are palmarosa oil (*Cymbopogon martini*), citronella oil (*C. nadus* and *C. winterianus*) and lemongrass oil (*C. citraues*, *C flexosus*, *C. pendulus*). Pattnaik *et al.* (1995) reported the antibacterial activity of lemongrass oil of *C. flexosus*, *C. winterianus* and *C. martini* against 18 bacterial strains with an MIC value ranging between 0.3 and 1.3 µl/ml. The study includes activity agains common food-borne pathogens such as *E. coli*, *Vibrio*, *Salmonella*, *Shigella* and *Bacillus* spp. Antifungal activity of these oils were also observed (Pattnaik 1994) with a higher MIC value in comparison to this. Another major source of EO is the oils from coniferous trees. The oils are usually cheap and are therefore used in fragrances in a variety of products. The most important commercial oils are obtained from the needles or resins of pine (*Pinus sylvestris*) as well as berries of juniper (*Juniperus communis*). Monoterpene hydrocarbons are the main component of these oils. In contrast, citrus oils and oils of coniferous trees containing limonene have shown high antimicrobial activities (Kalemba and Kunicka 2005). Rath *et al.* (2005b) observed the antibacterial activity of lime and juniper EOs against 32 strains of methicillin-resistant *Staphylococcus aureus*. The lime oil showed better antistaphylococcal activity (MIC <1.0 to 3.9 µl/ml) in comparison to juniper (MIC 1.9 to 7.81 µl/ml) EO. There is general agreement that various EOs and their constituents are effective against common food-borne pathogens when having an MIC value of <1.0 to 250 µl/ml or more in solutions. Direct application of EOs with food items (fresh meat, fried meat, roasted meat, boiled meat, fish, milk, dairy products, fruit, cooked rice, bread, vegetables) have shown that a higher concentration is needed to achieve a significant antimicrobial activity, as shown by various model systems (Tassou *et al.* 1995; Lis-Balchin 1998a).

All EOs have been proved to possess antibacterial and antifungal activity, although the activity differs based on oil and strictly with its chemical composition. It has been observed that the EOs are effective against bacteria and fungi at a very low dose or concentration. Effect of EOs against microorganisms even at less than 1µl/ml has been reported by many workers (Pattnaik 1994; Pattnaik *et al*. 1995; Rath *et al*. 1999a, 2001a, 2002a; Gupta *et al*. 2004; Rath *et al*. 2005b; Mohapatra *et al*. 2006). The immediate effect of EOs (turmeric leaf and rhizome, coriander, carrot, juniper berry, citrus, zinger, mint, eucalyptus, lemongrass, palmarosa, jasmine, nutmeg, ani seed, etc.) have been observed against bacteria and fungi, which appears that coming in contact with the microbial cells, the oils induce an instant and irreversible damage in many cases (Rath *et al*. 1999a. 2001a, 2001b; Devi 2001; Gupta 2002; Mohapatra 2002; Rath *et al*. 2002a, 2002b; Mishra 2003; Singh 2003; Rath *et al*. 2005b). EOs are comprised by a large number of components and it is likely that their mode of action involves several targets in the bacterial cell. The hydrophobicity of EOs enables them to make partitions in the lipids of cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents resulting in death of microbial cells (Burl and Coot 1999). Senhaji *et al*. (2007) reported the antibacterial activity of thymol and carvacrol through outer membrane disintegration and increasing the permeability to ATP through cytoplasmic membrane against common food-borne pathogens such as *E. coli* o157:H7 and *Salmonella* sp. However, *trans*-cinnamic aldehyde exhibited neither outer membrane disintegrating activity nor depletion of intracellular ATP (Helander *et al*. 1998) against these pathogens. Cyclic terpene compounds have been reported to cause loss of membrane integrity and dissipation of proton motive force (Sikkema *et al*. 1995). In contrast, Wilkins and Board (1989) suggested that antimicrobial activity of natural compounds, including EOs is due to the impairment of a variety of enzyme systems involving in the production of energy or synthesis of structural components in microbial cells. The possible mode of action of different EOs (juniper, carrot, celery, turmeric leaf and rhizome, geranium) through protein synthesis, cell membrane and cell wall inhibition against food-borne pathogens has been reported (Rath *et al*. 2001a, 2001b, 2002b; Gupta *et al*. 2004; Rath *et al*. 2005b). The antimicrobial activity of different EOs have been reported even at very low temperatures, low pH and low oxygen level. Gupta *et al*. (2004) reported the activity of carrot and celery seed EOs even at 4°C against twenty one pathogenic bacteria including food-borne pathogens like *Salmonella*, *Shigella*, *Vibrio*, *Escherichia*, *ETEC*, *EPEC*, *Staphylococcus*, *Citrobacter*, *Bacillus* and members of Enterobacteriaceae. Pattnaik (1994) observed better antibacterial and antifungal activity of lemongrass, *Palmarosa*, and *Eucalyptus* EOs at low temperature i.e. at 4°C in comparison to at 37°C. Similarly, Rath *et al*. (1999b, 1999c, 2001b, 2002b, 2005a) reported better antifungal activity of rose scented geranium, Japanese mint, and turmeric EOs at low temperature. Further, Rath *et al*. (2001a) reported the antibacterial activity of heat-treated (heated at 100°C for 10 min) turmeric rhizome and leaf EOs against *E. coli*. Gupta *et al*. (2004) observed the antibacterial activity of autoclaved EOs of carrot and celery against twenty one bacteria. The synergistic effect of EOs has been well recorded in the literature (Rath *et al*. 2002). Synergism of EO components between carvacrol and its precursor *p*-cymene and between cinamaldehyde and eugenol has also been reported (Lachowicz *et al*. 1998; Burt 2004). This indicates the heat stable nature of the active compounds present in EOs. They further reported that the activities of both the EOs increased after being exposed to UV radiations for 8 h. Though polymerization of EOs occur on storage, antibacterial activity of turmeric rhizome and leaf EOs were reported even after 36 months (maximum time period tested) against *E. coli* (Rath *et al*. 2001a). This indicates that EOs once mixed with the substrate can retain their antimicrobial properties even for a longer period. These properties of different EOs find their suitability for use in food processing.

EOs form the major volatile and aromatic components of many spices, indicating that EOs are also edible. Further, EOs have been used in aromatherapy for a long time in various hair products, skin care products, soaps, home care products, medicated creams or at times directly used for massage (Verlet 1993; Rath 2006). However, toxicity has not been reported for any EO any and their odour do not make even the most delicately flavoured food unpalatable (Lis-Balchin *et al*. 1998a). Evidences (Barrata *et al*. 1998a, 1998b) suggest that plant EOs possess strong antioxidant properties, which makes them a suitable candidate for use in the food industry to combat free radical mediated organoleptic deterioration of food. Antioxidants are compounds that protect cells against damaging effects of reactive oxygen species such as single oxygen, superoxide, peroxyl radicals, hydroxyl radicals, peroxynitrite, etc. An imbalance between antioxidants and reactive oxygen species results in oxidative stress leading to cellular damage. Therefore, presence of EOs or components in food items could protect the above said problems in *vivo* and provide a better health. Rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis*) oils are commonly used in food as natural flavouring, seasoning, as natural preservatives. Antioxidant properties of rosemary have been well documented (Bassaga *et al*. 1997; Srivastava 2002). Rosemary was considered as both a lipid antioxidant and metal chelator with a capacity to scavenge superoxide radicals. The antioxidant properties of rosemary on butter as well as filleted and minced fish during frozen storage have also been studied (Srivastava 2002). These investigators established that rosemary extracts and EOs inhibited the formation of polar substances, polymers and decomposition of polyunsaturated triglycerols and improved the sensory attributes of French fries. The antioxidant effect studies of rosemary EOs and oleoresin in Turkey breakfast sausages revealed that rosemary oils and oleoresins were as effective as the combination of BHA and/or BHT with citric acid suppressing oxidative rancidity (Srivastava 2002). It is thought that they act in synergy to provide antioxidant activity. Similarly sage is used in food for flavouring and seasoning. It is found that along with rosemary it has the best synergistic antioxidant activity among the numerous herbs, spices and teas tested. Both the plants possess common antioxidants like carnosol, carnosic acid, rosmanol, rosnadial and rosmarinic acid (Yanishlieva-Maslarova and Heinonen (2001). Since rosemary and sage belong to the Lamiaceae family EOs from other members of the family such as species of *Ocimum* could be novel sources for food preservation because of their antioxidant properties. Basils (*Ocimum* spp.) contain a wide range of EOs rich in phenolic compounds and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Phippen and Simon 1998, 2000). Juliani and Simon (2002) studied the antioxidant activity of EOs and extracts of basil cultivars including *O. basilicum*, *O. citrodorum* and *O. sanctum*. The EOs and phenolic extracts showed antioxidant properties, comparable with Greek oregano (*Oreganum vulgare*) and green tea (*Camellia sinensis*) which are recognized for their high antioxidant activity (Gramza-Michalowska and Bajerska-Jarzebowska 2007). Chemical analysis revealed that the antioxidant properties of basil EOs could be attributable to the presence of a high percentage of eugenol in these oils. Because of their antioxidant properties, these plants could constitute new sources of antioxidant phenolics in the diet providing 125 mg of gallic acid equivalents, 85-125 mg of Trolox or 106-140 mg of ascorbic acid equivalents per gm of dry weight (Juliani and Simon 2002). The antioxidant properties of different EOs (*Agathosma betulina*, *Artemisia afra*, *Lippia javonica*, *Pelargonium graveolens*, *Pteronia incana*, *Tagetes minuta*, *Curcuma longa*) have been reported in the literature (Muyima *et al*. 2004; Teixeira da Silva 2004; Muyima 2005; Teixeira da Silva *et al*. 2005).

For many years antioxidants have been added to processed foods to reduce the deterioration caused by the oxidation of unsaturated lipids and other ingredients. However many of the synthetic antioxidants are no longer acceptable to the conscious consumer who is looking for more natural products. Antioxidants are important to human health. Superoxides, hydrogen peroxide, singlet oxygen and hydroxyl radicals formed by different pathways are harmful to human health. This has led to a search for naturally-occurring antioxidants such as plant EOs as food preservatives. *Artemisia afra* oil, which has remarkable antibacterial, antifungal and antioxidative properties is a potential substitute as food preservatives (Mangena and Muyaima 1999; Burits *et al*. 2001). Similarly the efficacy of *Lipia javonica* oil in inhibiting *Pseudomonas aeruginosa* suggest that it has potential as food preservatives (Muyima *et al*. 2004). The use of *Pelargonium* EOs as antimicrobial agents in food processing has been shown to have considerable potential as there is no reported toxicity (Lis-Balchin 1998b). Lis-Balchin *et al*. (1998a) showed that its odour does not change, suggesting further its use in food industries along with clove, cinnamon, coriander, and thyme oils. In this context, the presence of antioxidant compounds at higher concentrations as in *Pteronia incana* (Bruns and Meiertoberens 1987) could be the first choice in the development of a novel antioxidant for the food industries.

CONCLUSION

From the above discussion it is clearly evident that, because of various limitations in food preservation through various physical and chemical methods, human civilization is in dire need of an alternate. Fragrance, antimicrobial properties, effectiveness at low concentrations, edibility, non toxicity nature, antioxidant properties, stability at different temperatures, pH and radiation of EOs make them a suitable candidate for food preservation. Though these natural biological compounds promise a great potential in food processing, still the chemical compounds are in use. It is mainly attributable to the complex situation related to demand with a great increase in industrial turn over. Therefore, the farmers, agronomists, microbiologists, technologists, chemists, perfumers, researchers, last but not the least the industrialists are to think upon the subject from an entirely different angle for both academic and commercial point of view, which can make EOs a definite alternate for food industries in future.

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