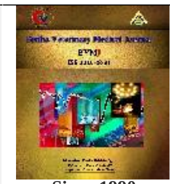
Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/



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Essential oil as microbial reductant

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ARTICLE INFO

Keywords

Antimicrobial

Antioxidative

Essential oils

shelf- life

Received 09/08/2020
Accepted 24/09/2020
Available On-Line 20/01/2021

ABSTRACT

Essential oils are concentrated liquids of complex mixtures of volatile compounds and can be extracted from several plant organs. It is a good source of several bioactive compounds, which possess antioxidative and antimicrobial properties. In addition, some essential oils have been used as medicine. Furthermore, the uses of essential oils have received increasing attention as the natural additives for the shelf- life extension of food products, due to the risk in using synthetic preservatives. Essential oils can be incorporated into packaging, in which they can provide multifunction termed “active or smart packaging.” Those essential oils can modify the matrix of packaging materials, thereby rendering the improved properties. This review covers up- to- date literatures on essential oils including sources, chemical composition, extraction methods, bioactivities, and their applications, particularly with the emphasis on preservation and the shelf- life extension of food products.

1. INTRODUCTION

Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants, for example, leaves, peels, barks, flowers, buds, seeds, and so on, but also widely found in bryophytes, such as the liverworts (Abdelhady et al., 2012). They can be extracted from plant materials by several methods, steam distillation, expression, and so on. Among all methods, for example, steam distillation method has been widely used, especially for commercial scale production (Cassel and Vargas 2006; Di Leo Lira et al., 2009).
Essential oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. They can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved. Almost any part of a plant may be the source of the oil, which could be extracted and fully exploited for food applications or others. (Bakkali et al., 2008).
Although essential oils are only slightly soluble in water, the aqueous solubility of individual essential oil components varies with respect to polarity (magnetic activity). Generally, components with more polar functional groups are expected to be more soluble in water relative to other components. (Aguirre et al., 2013). Essential oils are most commonly produced using hydrodistillation; however, prior to this, individual components of the whole essential oil are present within the source tissue, either in the same molecular form or as a heat labile precursor. The process of hydrodistillation involves heating in the presence of water to temperatures higher than boiling point, to produce mixed gases that expand and travel into a condenser. A variation of this is steam distillation, which places the source tissue (leaves, stem or bark) in the path of steam and not in the boiling water itself, as in hydrodistillation (Amarni et al., 2010). Volatile oils have been produced by using microwave-assisted distillation or hydrodistillation technology should correctly be called essential oils because of the chemical alteration of heat labile constituents that become part of an essential oil with both natural ingredients and these derived “artefacts”. This is clearly an area of contention and the essential oil industry may need to embark upon the development of a new system for communicating information related to the distillation method used to produce essential oil products, to establish consumer awareness of potential qualitative differences.

2. MATERIAL AND METHODS

2.1. Sources and Chemical Composition

Several plants contain essential oils, however, parts of plants, which serve as the major source of essential oil can be different. Those include roots, peels, leaves, seeds, fruits, barks, and so on. Plant essential oils are usually the complex mixture of natural compounds, both polar and nonpolar compounds (Masango, 2005). Dominant compounds in various essential oils. In general, the constituents in essential oils are terpenes (monoterpenes and sesquiterpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids) (Bakkali et al., 2008; Mohamed et al., 2010). Compounds and aroma of essential oils can be divided into 2 major groups: terpene hydrocarbons and oxygenated compounds.

2.1.1. Terpene hydrocarbons

The hydrocarbons are the molecule, constituting of H and C atoms arranged in chains. These hydrocarbons may be

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acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5 carbon base units, C_5), which are the combinations of 2 isoprene units, called a "terpene unit." Essential oils consist of mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which are hydrocarbons with the general formula $(C_5H_8)_n$, The diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) exist in essential oils at low concentration (Mohamed et al., 2010). Terpenoids (a terpene containing oxygen) is also found in essential oils (Burt, 2004). Essential oils mostly contain monoterpenes and sesquiterpenes, which are $C_{10}H_{16}$(M_W 136 amu) and $C_{15}H_{24}$ (M_W 204 amu), respectively. Although sesquiterpenes are larger in molecules, structure and functional properties of sesquiterpenes are similar to the monoterpenes (Ruberto et al., 2000). For diterpenes, triterpenes, and tetraterpenes, they have the larger molecule than monoterpenes and sesquiterpenes, but they are present at very low concentration in essential oils (Bakkali et al., 2008).

2.1.2. Oxygenated compounds

These compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from the terpenes, in which they are termed "terpenoids." Some oxygenated compounds prevalent in plant essential oils are shown as follows:

- *Phenols*:
  Thymol, eugenol, carvacrol, chavicol, thymol, and so on.
- Alcohols:
  Monoterpene alcohol: borneol, isopulegol, lavanduol, α-terpineol, and so on.
  Sesquiterpenes alcohol: elemol, nerolidol, santalol, α-santalol, and so on.
- *Aldehydes*:
  Citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, and so on.
- *Ketones*:
  Carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, and so on.
- *Esters*:
  Bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, and so on.
- *Oxides*:
  1, 8- cineole, bisabolone oxide, linalool oxide, sclareol oxide, and so on.
- *Lactones*:
  Bergaptene, nepetalactone, psoralen, aesculatine, citroptene, and so on.
- *Ethers*:
  1, 8- cineole, anethole, elemicin, myristicin, and so on.

Different constituents in essential oils exhibit varying smell or flavor (Burt , 2004). Also, the perception of individual volatile compounds depends on their threshold.

2.2. Extraction of Essential Oils

Essential oils can be extracted from several plants with different parts by various extraction methods. The manufacturing of essential oils, and the method used for essential oil extraction are normally dependent on botanical material used. State and form of material is another factor used for consideration. Extraction method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil. This results in the loss in bioactivity and natural characteristics. For severe case, discoloration, off- odor/flavor as well as physical change such as the increased viscosity can occur. Those changes in extracted essential oil must be avoided (Babu et al., 2005; Durling et al., 2007).

2.2.1. Solvent extraction

2.2.1.1. Solvent

Conventional solvent extraction has been implemented for fragile or delicate flower materials, which are not tolerant to the heat of steam distillation. Different solvents including acetone, hexane, petroleum ether, methanol, or ethanol can be used for extraction (Areias et al., 2000; Pizzale et al., 2002; Kosar et al., 2005). For general practice, the solvent is mixed with the plant material and then heated to extract the essential oil, followed by filtration. Subsequently, the filtrate is concentrated by solvent evaporation. The concentrate is resin (resinoid), or concrete (a combination of wax, fragrance, and essential oil). Essential oil with antioxidant activity from Ptychotisverticillata was extracted using solvent extraction method by El Ouariachi et al. (2011). The oil was dominated by phenolic compounds (48.0%) with carvacrol (44.6%) and thymol (3.4%) as the main compounds.

2*.2.1.2. Supercritical carbon dioxide*

Conventional methods including solvent extraction and steam distillation have some shortcomings such as long preparation time and large amount of organic solvents (Deng et al., 2005). Moreover, the losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds, and toxic solvent residue in the extract may be encountered (Jimenez- Carmona et al., 1999; Glišića et al., 2007 ; Gironi , Maschietti et al., 2008). Therefore, supercritical fluids have been considered as an alternative medium for essential oil extraction. Carbon dioxide (CO_2) is the most commonly used supercritical fluid because of its modest critical conditions (Hawthorne et al., 1993; Jimenez- Carmona et al., 1999; Senorans et al., 2000). Under high- pressure condition, CO_2 turns into liquid, which can be used as a very inert and safe medium to extract the aromatic molecules from raw material.

2.2.1.3. Subcritical water

The subcritical water or pressurized hot water has been introduced as an extractant under dynamic conditions (pressure high enough to maintain water under liquid state and temperature in the range of 100 to 374 °C). (Jimenez-Carmona et al., 1999) reported that the efficiency (in terms of volume of essential oil/1 g of plant) of continuous subcritical water extraction was 5.1 times higher than HD method. This method is quicker (15 min compared with 3 h), provides a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes), and allows substantial savings of costs, in terms of both energy and plant material.

2.3. Role of Essential Oils as Food Additives

Essential oils from plants have been known to act as natural additives, for example, antimicrobial agents, antioxidant, and so on. Their activities vary with source of plants, chemical composition, extraction methods, and so on. Due to the unique smell associated with the volatiles, this may limit the use of essential oil in some foods since it may alter the typical smell/flavor of foods (Oussalah et al., 2004)

2.4. Use of packaging for meat and meat products

Microorganisms are responsible for meat spoilage. Most essential oils are classified as generally recognized as safe (GRAS). However, their use as food preservatives is often limited due to flavoring considerations (Zinoviadou et al., 2009). The effectiveness of bioactive films containing essential oils against the spoilage or pathogenic bacteria in food system has been studied. (Zinoviadou et al., 2009) studied the antibacterial effects of Whey protein isolates film containing oregano oil (0.5% and 1.5% w/w of Film forming solution [FFS]) against total variable bacteria count, *Pseudomonas* spp. and lactic acid bacteria on beef cuts. The use of films containing the highest level of oregano oil (1.5% w/w of FFS) resulted in a significant reduction of total variable bacteria count and *Pseudomonas* spp. population during 12 d of refrigeration storage (5 °C). The total variable bacteria population of the samples wrapped with films containing the high essential oil level at day 8 was 5.1 log CFU/cm^2, while the control had population of 8.4 log CFU/cm^2. Since microbial loads higher than 7 log CFU/cm^2 are usually associated with off- odors (Ercolini et al., 2006), it may be suggested that the use of WPI films containing 1.5% (w/w) oregano oil could double the shelf life of fresh beef stored under refrigerated condition.

3. RESULTS AND DISUCSSION

3.1. Antimicrobial activity

The ability of plant essential oils to protect foods against pathogenic and spoilage microorganisms has been reported (Lis- Balchin et al., 1998; Friedman 2006 ; Rojas- Graü et al., 2007). Among chemical components in several essential oils, carvacrol has been shown to exert a distinct antimicrobial action (Veldhuizen et al., 2006). Carvacrol is the major component of essential oil from oregano (60% to 74% carvacrol) and thyme (45% carvacrol) (Lagouri et al., 1993; Arrebola et al., 1994). It has a broad spectrum of antimicrobial activity against most Gram- positive and Gram- negative bacteria (Friedman et al., 2002). Carvacrol disintegrates the outer membrane of Gram- negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Burt, 2004). For Gram- positive bacteria, it is able to interact with the membranes of bacteria and alter the permeability for cations like H^+ and K^+ (Veldhuizen et al., 2006). In general, the higher antimicrobial activity of essential oils is observed on Gram- positive bacteria than Gram- negative bacteria (Kokoska et al., 2002; Okoh et al., 2010). Lipophilic ends of lipoteichoic acids in cell membrane of Gram- positive bacteria may facilitate the penetration of hydrophobic compounds of essential oils (Cox et al., 2000). On the other hand, the resistance of Gram- negative bacteria to essential oils is associated with the protecting role of extrinsic membrane proteins or cell wall lipopolysaccharides, which limits the diffusion rate of hydrophobic compounds through the lipopolysaccharide layer (Burt, 2004).

The dissipation of ion gradients leads to impairment of essential processes in the cell and finally to cell death (Ultee et al., 1999). The cytoplasmic membrane of bacteria generally has 2 principal functions: (i) barrier function and energy transduction, which allow the membrane to form ion gradients that can be used to drive various processes, and (ii) formation of a matrix for membrane- embedded proteins (such as the membrane- integrated F_0 complex of ATP synthase) (Sikkema et al., 1995; Hensel et al., 1996).

The activity of the essential oils is related to composition, functional groups, and synergistic interactions between components (Dorman and Deans et al., 2000). The removal of the aliphatic ring substituent of carvacrol slightly decreased the antimicrobial activity. 2- Amino- ρ- cymene has similar structure to cavacrol, except hydroxyl group. The lower activity by 3- fold of 2- amino- ρ- cymene, as compared to carvacrol, indicates the essential role of hydroxyl group in antimicrobial activity of carvacrol (Veldhuizen et al., 2006). The hydroxyl group present in the structure of phenolic compounds confers antimicrobial activity and its relative position is very crucial for the effectiveness of these natural components; this can explain the superior antimicrobial activity of carvacrol, compared to other plant phenolics (Veldhuizen et al., 2006).

Plant essential oils have been known as antimicrobial agents. Essential oil of rosemary (*R. officinalis*) exhibited both Gram- positive (*Staphylococcus aureus* and *Bacillussubtilis*) and Gram- negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria (Okoh et al., 2010). The major components of rosemary oil are monoterpenes such as α- pinene, β- pinene, myrcene 1,8- cineole, borneol, camphor, and verbinone (Santoyo et al., 2005; Okoh et al., 2010), which possess strong antimicrobial activity by the disruption of bacteria membrane integrity (Knobloch et al., 1989). It has been reported that oregano essential oil had higher antimicrobial activity against the Gram- positive bacteria (*S. aureus*) than Gram- negative (*E. coli* and *Pseudomonas aeruginosa*) (Aguirre., 2013; Burt, 2004; Pelissari et al., 2009). The main constituents of oregano essential oil are thymol, carvacrol, γ- therpinene, and ρ- cymene (Lambert et al., 2001; Burt, 2004; Aguirre et al., 2013). The greater resistance of Gram- negative bacteria toward essential oils may be attributed to the complexity of their double- layer cell membrane, compared with the single- layer membrane of Gram- positive bacteria (Hogg, 2005).

3.2. Antioxidant activity

Several compounds in essential oils have the structure mimicking the well- known plant phenols with antioxidant activity. Among the major compounds available in the oil, thymol and carvacrol were reported to possess the highest antioxidant activity (Dapkevicius et al., 1998). Essential oils have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts (Yildirim et al., 2000; Mao et al., 2006). With those functions, essential oils can serve as the potential natural antioxidants, which can be used to prevent lipid oxidation in food systems. Phenolics are organic compounds consisting of hydroxyl group (- OH) attached directly to a carbon atom that is a part of aromatic ring.

4. CONCULOSIONS

In conclusion, essential oils from different sources can be exploited as the natural additives in foods. Essential oils with other bioactivities or functions from new sources should be further searched. New technology for lowering the unique and undesirable smell of essential oil, which can limit their use in foods, such as encapsulation, and so on, must be implemented. Consequently, essential oil can be widely used without any negative effect on sensory property of foods. The development of release system for essential oil from packaging or fuming system inside packaging should be conducted to maximize the activity of active compounds in essential oils. Therefore, it can serve as the convenient packaging, which effectively extends the shelf life of foods.

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