# Essential oils as antimicrobial agents and their possible applications in food

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Essential oils are natural antimicrobial additives, rich in biologically active compounds, obtained mainly by distillation from various plant sources. The antimicrobial activity of essential oils has been studied and demonstrated against several microorganisms, mainly in vitro and by the use of natural substances. In foods, plants rich in essential oils, in addition to providing aroma and flavor, stand out for their antioxidant, antimicrobial and nutritional properties. In addition to the direct effects, the use of essential oils can extend to side effects such as the conservation of products, which is among the main objectives in food industrialization processes. In this context, in parallel with the needs of the food industry, there is the negative perception of consumers regarding the use of synthetic additives, as well as their increasing demand for natural products, which boosts the employability of essential oils with antimicrobial potential capable of replacing them, without impairing the effectiveness of microbiological control. Because of this the purpose of this chapter is to address the potential antimicrobial effects of essential oils and their applications for food.

**Keywords**: food preservation; essential oils

## 1. Essential oils

The industry is seeking new alternatives to the traditional chemical preservatives, such as the natural antimicrobial systems present in vegetal extracts and oils [1; 2]. Due to the growing demand for food free of synthetic additives and antimicrobials, even though they are considered safe for consumers, there is a growing need for natural substances that, in certain concentrations, would ensure the food safety. Simultaneously, the added natural substance should not modify the original sensory characteristics of product, unless this change is desirable, as the addition of condiments and spices, which enhance flavor and have volatile active principles known as essential oils with antimicrobial effect [3].

Essential oils are also known as volatile oils, ethereal oils or essences [4; 5]. They are volatile organic compounds of vegetable origin obtained by physical process (steam distillation, distillation under reduced pressure or another suitable method) [6; 7]. These oils are volatile, clear and typically noncolored liquids that are soluble in fat and organic solvents and generally have lower density than water [4]. Essential oils are known for their fragrance and antiseptic activity, such as bactericidal, fungicidal, antiviral and medicinal and can also be used as antimicrobials for food preservation, as analgesics, sedatives and anti-inflammatories [8].

Different factors can influence the essential oils chemical composition including plant species, geographic origin, harvest season and climatic conditions [9; 10; 11; 12; 13]. The chemical compounds capable of inhibiting or eliminating microorganisms that were obtained through essential oil extraction from raw plant materials have been extensively studied. [14]. Garlic, cinnamon, lemon grass, cloves, basil, marjoram, mint and menthol, oregano, rosemary and thyme essential oils were found to have significant antimicrobial potential [4; 15; 16].

Herbs such as cumin, oregano, rosemary, sage, thyme, among others have also been reported to possess significant antimicrobial inhibitory properties. On the other hand, black pepper, red pepper and ginger have little or no antimicrobial activity [17].

Various potential applications of essential oils are being investigated. Their use as ingredients in food products is of great interest to the industrial sector due to the consumers concerns about possible health damage caused by synthetic additives, which drives increased acceptance of the natural products [18; 19]. Aromatic plants that are rich in different compounds, such as terpenes and phenylpropanoids, can improve food quality by reducing lipid degradation and growth of microorganisms [20]. In addition, these compounds can confer good sensory properties and present low toxicity compared to synthetic preservatives [21].

Gas-chromatographic analysis of composition of some of the essential oils showed that the most active essential oils have a relatively high content of alcohols, phenols and aldehydes (eugenol, thymol, carvacrol, geranium), which are powerful antiseptic agents, and terpenes (alpha and beta pinenes and limonene), proving the existence of antimicrobial properties known for these chemical groups [22].

Essential oils are volatile and aromatic oily compounds, produced by secondary plant metabolism. They can be found in various parts of plants such as leaves, roots, rhizomes, bark, stems, flowers, fruits and seeds [13]. Essential oils are a mixture of chemical compounds belonging to different classes. The most representative ones are terpenes such as mono- and sesquiterpenic hydrocarbons, aldehydes, ketones, alcohols, esters, oxides and phenol derivatives [23].

The essential oils constituents are grouped into two chemical classes, according to their biosynthetic origin: terpenoids, synthesized through the mevalonic acid-acetate route and phenylpropanoids, formed through the shikimic acid route [24, 25]. Mevalonic acid forms isoprene, which represents the basic unit of terpenes and consists of a 5 carbon atoms (C5). Isoprene units (C5) bond head-to-tail forming different terpene structures. Monoterpenes, formed by two isoprene units (C10) and sesquiterpenes formed by three isoprene units (C15), are the main terpenoids present in essential oils [26].

Different methods of extraction can be used for essential oil extraction depending on the plant part and intended application [27]. Distillation is the most often used method for commercial essential oils extraction. However, other techniques can also be applied including fermentation, high pressure, steam distillation, hydrodistillation, microwave assisted distillation, microwave hydrofusion and gravity, organic solvent extraction, $CO_2$ supercritical fluid extraction, ultrasonic and microwave extraction without the use of solvents or mechanical processing, such as pressing of citrus fruit pericarp [28; 29; 30; 31]. According to Cassel et al. [32] the essential oil composition may vary significantly depending on the method used for extraction.

## 2. Antimicrobials

Antimicrobial agents are synthetic or natural compounds that have the ability to inhibit the growth of microorganisms [33], including bacteria, fungi and viruses. Compounds, inhibiting microbial growth are classified as bacteriostatic, and, causing cell death as bactericidal [4]. Bacteriostatic agents act mainly on the bacterial cell membrane, causing its structural and functional damages [34; 35].

Among essential oil properties reported in the literature, their antimicrobial activity is considered one of the most important because of its broad spectrum of action with effect on variety of microorganisms, including species resistant to chemical antimicrobial agents [36; 37; 38; 39]. Essential oils have distinct mechanisms of action and cellular targets capable of preventing the bacteria ability to acquire resistance [40].

Studies involving antimicrobial agents from natural sources showed that in most cases, Gram-positive bacteria are more sensitive to essential oil action compared to Gram-negative bacteria [4]. Although the mechanisms of action are not fully elucidated it is believed to be related to the differences in interaction of apolar constituents of essential oils with cell walls of Gram-positive and Gram-negative bacteria [41; 42].

Gram-negative bacteria have a complex and rigid lipopolysaccharide outer membrane with an apolar character that restricts the passage of apolar components into the cell [43], whereas Gram-positive bacteria cell wall is formed by a layer of peptidoglycan with lipophilic ends, which facilitate the absorption of hydrophobic compounds [36; 44].

Verification of the microorganism resistance to antimicrobial agents can be done using methods of diffusion and dilution, which are carried out, in agar or broth, respectively [4]. Generally, the essential oils antimicrobial activity is evaluated by disk diffusion or well diffusion tests to determine its antibacterial spectrum of action. Different reservoirs can be used such as paper disks, culture medium and cylinders of porcelain or stainless steel. The disc diffusion test consists in sample diffusion in solid medium containing the microorganism, in the course of which a halo of inhibition with no microbial development is formed [4; 45].

After disk diffusion or wells analysis, bacteria sensitive to the essential oil are subjected to a more detailed analysis to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) [46]. Minimum inhibitory concentration is determined by subjecting bacterium to different concentrations of an antimicrobial agent. It corresponds to the least amount of antimicrobial agent capable of inhibiting the development of the microorganism [4].

MBC analysis is complementary to the MIC. It is performed sequentially by subculturing in agar plates aliquots of all dilutions used in MIC test that showed no cellular growth with the aim to find the lowest concentration of essential oil that promotes bacteria death after inoculation into a new culture medium without the antimicrobial agent [47; 4].

Essential oil antimicrobial activity can also be assessed in micro-atmosphere or vapor phase. This method consists of analyzing effect of the volatile compounds present in the essential oils without their direct contact with the bacterium and the culture medium. The bacteria are inoculated on the surface of agar plates and an amount of essential oil is deposited on sterile filter paper discs placed on the plates cover, plates are capped and incubated with the cover down [48]. The antimicrobial activity is expressed as a percentage of bacterial growth reduction, compared to a control plate [41].

*In vitro* studies of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils showed strong antimicrobial activity against strains of *Escherichia coli*, *Bacillus cereus*, *Salmonella Typhimurium*, *Listeria monoctytogenes* and *Staphylococcus aureus* [49; 50]. Longaray Delamare et al. [51] and Viuda-Martos et al. [52] reported antimicrobial activity of sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*) essential oils against *Staphylococcus* spp.

Trajano et al. [53] studied the antimicrobial activity of clove (*Eugenia caryophyllata*) and cinnamon (*Cinnamomum zeylanicum*) essential oils on strains of *B. cereus*, *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae*, *L. monocytogenes*, *Pseudomonas aeruginosa*, *S. aureus*, *Salmonella enterica*, *Serratia marcencens* and *Yersinia enterocolitica*. The essential oils under study showed antimicrobial activity with inhibition halos up to 30 mm in diameter [53].

Although some essential oils have major compounds, which may account for up to 85% w/w [54], it is difficult to elucidate a specific mechanism of action due to a large number of active molecules with distinct chemical structures [4].

## 3. Application of essential oil in food

Essential oils have proven bacteriostatic (*in vitro*) action against several microorganisms at low concentrations. However, their use as food preservatives is still limited, as high concentrations are required to have *in situ* bacteriostatic effect. This limitation is due to the characteristic aroma and flavor of the essential oils, which can change sensory characteristics of foods. Besides, the antimicrobial effect can be affected by the interaction between essential oils hydrophobic groups and some food constituents, such as fat [55; 56; 57].

The *in situ* evaluation of essential oils as antimicrobial agents applied to meats demonstrate that thyme essential oil at doses of 0.8 and 1.2 mL/100g inhibited the growth of *L. monocytogenes* in *Hypophthalmichthys molitrix* fish stored for 12 days at 4°C [58]. Oregano essential oil showed antimicrobial effect against *S. Enteritidis* at doses of 0.6 and 0.9% in minced mutton during storage at 4°C or 10°C. A decrease of bacterial count of 2.43 log CFU/g on the second day of storage was observed for meats treated with essential oils at doses of 0.6%, remaining unchanged until the end of the study. Samples treated with essential oils at doses of 0.9% showed microoganism count lower than 1 log UFC/g throughout the storage period [59]. The addition of oregano essential oil (0.02%) to sausages decreased aerobic bacteria growth during 24 days of storage at 4°C [60]. Moreover, rosemary, marjoram and thyme essential oils showed antimicrobial effect against aerobic bacteria when added to vacuum packaged Bologna sausages during 24 days of storage [60; 61] and also decrease *E. coli* count in ground pork after 24 hours of storage at 5°C [62]. Sage oil at doses of 3% showed bacteriostatic effect against *S. anatum* in red meat stored for 15 days, reducing log CFU/g by 2.6 in 3 days, while *Schinus molle* essential oil reduced log CFU/g by 2.3. Essential oils at 1% also showed bacteriostatic effect against *S. enteritidis* in red meat, reducing count by 2.8 log CFU/g at the end of storage [63].

Despite the antimicrobial effects observed for essential oils, their application to food is limited due to their characteristic taste and odor, as previously mentioned. Thus, alternatives capable to mask undesirable organoleptic effects are needed. Essential oil encapsulation or their application in active films are alternatives that increase the stability of the oil compounds, avoid interaction with the food matrix and increase antimicrobial activity by augmenting passive cellular uptake [64].

The application of edible films incorporating essential oils to the conservation of red meats, chicken and fish has been extensively studied. According to Matan [65], films incorporating anise essential oil at doses of 4 and 6% (v:v) promote microbial control of *Aspergillus flavus*, *Penicillium* sp. and *Staphylococcus aureus* for 28 days in dry fish. Oregano essential oil incorporated into films at dose of 1.5% led to a *Pseudomonas* counts reduction of 3.3 log CFU/g in meat burgers stored for 8 days [66]. Films incorporating fennel or thyme essential oils at doses of 0.5% reduced *Salmonella* sp. and *E. coli* growth in chicken breasts stored for 21 days [67]. Fish/chitosan gelatin films with added clove essential oil showed antimicrobial effect against *L. acidophilus*, *P. fluorescens* and *L. innocua* when applied as cover for cod [68].

Mechanism of the antimicrobial action of essential oils *in situ* is not completely understood yet and requires further research. Nonetheless, some authors suggest that encapsulation enhances the bactericidal effect. Cui et al. [69] concluded that the encapsulation of clove essential oil enhanced the bactericidal effect against *S. aureus* in tofu. Similar effect was reported for encapsulated rosemary essential oil against *L. monocytogenes* in pork liver sausage [70].

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