ESSENTIAL OILS AS NATURAL FOOD ADDITIVES: STABILITY AND SAFETY

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ABSTRACT: Essential oils are volatile aromatic liquids derived from the secondary metabolism of plants, consisting of a complex mixture of different active compounds. Several medicinal plants are sources for the extraction of essential oils, being recognized as safe (GRAS) by the FDA (Food and Drug administration). The use of essential oils as possible food preservatives has been gaining ground in research due to their antioxidant and antibacterial activities, important characteristics in food preservation and also to the population's interest in consuming healthier products. Essential oils are a promising alternative for the food industry, as they act to control pathogenic and deteriorating bacteria. Compared to synthetic chemical preservatives, essential oils are generally safer, but have low stability, so encapsulation is a way to protect them from adversities. This review aims to demonstrate the effectiveness, stability and safety of essential oils and their use in food matrices.
KEYWORDS: Antibacterial Activity; Natural Compounds; Preservatives; Foodborne Diseases; Encapsulation.

ÓLEOS ESSENCIAIS COMO ADITIVOS ALIMENTARES NATURAIS: ESTABILIDADE E SEGURANÇA

RESUMO: Os óleos essenciais são líquidos aromáticos voláteis derivados do metabolismo secundário das plantas, sendo constituído por uma mistura complexa de diferentes compostos ativos. Diversas plantas medicinais são fontes de extração de óleos essenciais sendo reconhecidos como seguros (GRAS) pelo FDA (Food and Drug administration). A utilização de óleos essenciais como possíveis conservantes em alimentos vem ganhando espaço nas pesquisas devido a suas atividades antioxidantes e antibacterianas, características importantes na preservação de alimentos e também ao interesse da população em consumir produtos mais saudáveis. Os óleos essenciais são uma alternativa promissora para a indústria de alimentos, pois atuam no controle de bactérias patogênicas e deteriorantes. Em comparação com os conservantes químicos sintéticos, os óleos essenciais geralmente são mais seguros, porém apresentam baixa estabilidade, deste modo a encapsulação é uma forma de protegê-los de adversidades.

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Esta revisão tem como objetivo demonstrar a efetividade, estabilidade e a segurança dos óleos essenciais e seu uso em matrizes alimentares.
PALAVRAS-CHAVE: Antibacteriano; Compostos Naturais; Conservantes; Doenças Transmitidas por Alimentos; Encapsulação.

ACEITES ESENCIALES COMO ADITIVOS ALIMENTARIOS NATURALES: ESTABILIDAD Y SEGURIDAD

RESUMEN: Los aceites esenciales son líquidos aromáticos volátiles derivados del metabolismo secundario de las plantas, constituidos por una mezcla compleja de diferentes compuestos activos. Varias plantas medicinales son fuentes para la extracción de aceites esenciales, siendo reconocidas como seguras (GRAS) por la FDA (Administración de Alimentos y Medicamentos). El uso de aceites esenciales como posibles conservantes de alimentos ha ido ganando terreno en las investigaciones debido a sus actividades antioxidantes y antibacterianas, características importantes en la conservación de alimentos y también al interés de la población por consumir productos más saludables. Los aceites esenciales son una alternativa prometedora para la industria alimentaria, ya que actúan para controlar las bacterias patógenas y deteriorantes. En comparación con los conservantes químicos sintéticos, los aceites esenciales son generalmente más seguros, pero tienen baja estabilidad, por lo que la encapsulación es una forma de protegerlos de las adversidades. Esta revisión tiene como objetivo demostrar la eficacia, estabilidad y seguridad de los aceites esenciales y su uso en matrices alimentarias.
PALABRAS CLAVE: Antibacteriano; Compuestos Naturales; Conservantes; Enfermedades Transmitidas por Alimentos; Encapsulación.

1. INTRODUCTION

Foodborne diseases occur through the consumption of contaminated food and/or water, and the World Health Organization estimates that annually approximately one in ten inhabitants in the world become ill from this cause (FAO, 2019). Foodborne illness costs society US$ 7.4 million annually in lost productivity, overburdening health systems and reducing economic development (PAHO, 2022). To avoid contamination by micro-organisms in food, synthetic preservatives are commonly used, but they can cause toxic effects depending on the amount ingested and the organism’s susceptibility (BENSIDE *et al.*, 2020).

In view of the possible harmful health effects related to the consumption of synthetic preservatives, consumers are increasingly searching for foods with little or no negative impact on health (JACKSON-DAVIS *et al.*, 2023). Thus, the use of natural products such as essential oils in food preservation has gained prominence in the scientific community, aiming at the total or partial replacement of synthetic chemical additives (FREDERICO *et al.*, 2021).

Essential oils are volatile aromatic liquids derived from the secondary metabolism of plants, consisting of a complex mixture of different active compounds (terpenes, phenolic compounds, alcohols), and due to their composition, essential oils have anti-inflammatory, antibacterial, antifungal and antioxidant activity (FALLEH *et al.*, 2020).

The essential oil composition may vary according to intrinsic (soil, climate, plant development stage) and extrinsic factors (extraction methods) (DIFHI *et al.*, 2016; RAHAL *et al.*, 2022). Hydrodistillation is the most used technique due to its practicality and low cost, where the plant material is immersed in water under heating until boiling, resulting in the formation of vapors containing volatile compounds, which, after condensation, are separated from the aqueous phase by decantation (PRINS *et al.*, 2006; LALONDE *et al.*, 2019).

Most essential oils from medicinal plants and spices are generally recognized as safe (GRAS) for use in food by the Food and Drug Administration (FDA) (Food and Drug administration/CRF21, 2016). However, although essential oils are safe for use in food, there are limitations in their application as food preservatives due to interactions with other components of the food matrix, their high volatility, and flavor changes in the product (CHRISTAK *et al.*, 2021). Due to these drawbacks, essential oil encapsulation techniques can be used to improve its stability, solubility, facilitate handling, promote controlled release and hide undesirable taste and odor (REIS *et al.*, 2022).

This study contributes to the understanding of the stability and safety of using essential oils with potential as food preservatives, highlighting gaps in knowledge and providing insights for future research efforts in this field. From a practical perspective, the article delves into the applications of essential oils, demonstrating their ability to inhibit the growth of pathogenic microorganisms and extend the shelf life of various food products. Thus, considering the use of essential oils as food preservatives, this review article aimed to demonstrate the effectiveness and safety of their use in food matrices, as well as the advantages of encapsulation.

2. METHODOLOGY

In the present study, a bibliographic review was carried out using PubMed, Science Direct, Scopus and Scielo platforms as scientific databases. The keywords used as descriptors were: "essential oils", "essential oils from medicinal plants", "food preservative", "antibacterial", "safety of essential oils in food". Articles published in the period from 2018 to 2023 were selected. Articles from previous years were maintained at the base of the article, since the reported information was considered important for the general discussion.

2.1 Food Safety: the Importance of Preservatives and Their Possible Harmful Effects

Foodborne diseases are caused by ingestion of food and/or water contaminated by microorganisms such as bacteria, fungi, viruses or parasites, as well as physical agents or chemicals (SHARIF *et al.,* 2018). These diseases have emerged as a growing economic and public health problem worldwide (NELLURI; THOTA, 2018). According to the World Health Organization, every year, approximately 600 million people worldwide become ill due to foodborne diseases (WHO, 2015).

Several studies have indicated that 66% of foodborne disease outbreaks are of bacterial origin (BHALLA; SHEETAL, 2019). The main bacterial species that result in foodborne diseases include *Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Cronobacter sakazakii, Escherichia coli, Listeria monocytogenes, Salmonella spp., Shigella spp.* and *Staphylococcus aureus* (ÜNUVAR, 2018). When someone eats food contaminated with toxins produced by bacteria, it results in intoxication, whereas infection is when food containing and infectious agent is ingested (SHARIF *et al.*, 2018).

There is no way to completely eliminate the risk of consuming contaminated food, as contamination can occur at different stages of the production chain, but there are ways to prevent the proliferation of pathogenic and deteriorating microorganisms, such as the use of preservative substances that are added to food in order to preserve its quality and extend its shelf life (NELLURI; THOTA, 2018). However, the exacerbated use of preservatives can cause harmful effects to the consumer. Santos and Lourival warn for the emergence of possible adverse effects related to the consumption of synthetic chemical preservatives (SANTOS; LOURIVAL, 2018).

In indiscriminate amounts, preservatives can cause the following health problems: benzoic acid (asthma attacks, they are carcinogenic and neurotoxic), sulfur dioxide (asthma attacks, gastric irritation, skin diseases, fetal anomalies), ethylenediaminetetra acetic acid - EDTA (diarrhea, vomiting and urinary disorders), phosphoric acid and tartaric acid (reduced calcium absorption), sulphites (allergies, gastric irritation, asthma attacks and hives), nitrites and nitrates (type 1 diabetes, irritate the digestive system) butylhydroxytoluene - BHT and terbutylhydroxyanisole - BHA (carcinogenic, eczema and angioedema, skin irritation, increased amounts of lipids and cholesterol in the bloodstream) (BENSID *et al.*, 2020). In view of the above, studies related to the use of natural preservatives such as essential oils have been carried out with a view to producing healthier products, with the clean label concept (MARUYAMA; STRELETSKAYA; LIM, 2021).

2.2 Essential Oils and Their Antimicrobial and Antioxidant Mechanism of Action

Essential oils are volatile aromatic low-viscosity liquids composed of a complex mixture of substances derived from plants which can be extracted from leaves, flowers, fruits, seeds, bark, branches, roots and stems (LIANG *et al.*, 2023).

The chemical composition of essential oils results from the secondary metabolism of plants resulting from genetic factors and environmental stimuli such as temperature, luminosity, seasonality, development stage, harvesting time, nutrition, water and extraction technique (DHIFI *et al.*, 2016; MORAIS, 2009). According to the International Standardization Organization (ISO) 9235:2021 item 3.11, essential oils are products obtained from natural raw materials of vegetable origin by steam distillation, mechanical processes from the epicarp of citrus fruits or by dry distillation, after separation of the aqueous phase – if any – by physical processes (ISO 9235:2021).

Structurally, the chemical constituents of essential oils can be classified into four groups: terpenes, terpenoids, phenylpropanoids and other constituents, which bioactive compounds have a wide range of biological activities (anticancer, antimicrobial, anti-inflammatory, antioxidant and antiallergic) (MASYITA *et al.*, 2022).

The biological activities of essential oils are related to their content of phenolic compounds. The antibacterial activity is related to the ability of the essential oil to penetrate the bacterial membrane, where Gram-positive bacteria are more susceptible than Gram-negative ones, due to their cell wall composition, essential oils can interact with lipid bilayers and disrupt plasma membrane functionality (ÁLVAREZ-MARTINEZ *et al.*, 2021). With alteration of its structure, pore formation may occur, changing the electrical charge, changing polarity, increasing permeability, modifying fluidity, displacing membrane proteins, inhibiting the synthesis of proteins, DNA and RNA and metabolic pathways, causing leakage of important cellular contents (ÁLVAREZ-MARTINEZ *et al.*, 2021; FALLEH *et al.*, 2020). In terms of antioxidant activity, these substances act to prevent the initiation of the oxidative chain by neutralizing free radicals, preventing the continuation of the oxidative process, or acting to eliminate free radicals (KYUNGMI; EBELER, 2008; RODRIGUEZ-GARCIA *et al.*, 2016).

2.3 Essential Oils as Food Preservatives

Essential oils can be added directly to food matrices through active packaging produced from biodegradable polymers, in edible films or even in the preservation of seeds.

Antifungal film (pomegranate bark fiber 2.77%, pectin 1.23%, polyvinyl alcohol 1.94%, glycerol, clove and thyme essential oil at concentrations of 0.5, 1 and 2%) for the post-harvest coating of mangoes was effective against *Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides*, with inhibition percentage of 66% and 22%, respectively (NANDHVATHY *et al.*, 2021).

Martins *et al.* evaluated the effect of adding oregano essential oil to rice starch films and its application as packaging for fish fillets. Fillets packed with the active film showed increased shelf life, less oxidation (1.65 mg malonaldehyde/kg of sample) and lower microbiological growth, 10^7 CFU/g in 6 days of storage, while fish fillets packed in standard film without the addition of essential oil showed greater oxidation (1.88 mg malondialdehyde/kg of sample) and greater microbiological growth, 10^8 CFU/g (MARTINS *et al.*, 2020).

Control of *Fusarium verticillioides* in corn seeds using ginger essential oil at concentrations of 0, 1, 2, 3, 4 and 5%, where doses of 1, 4 and 5% provided lower incidence of *Fusarium verticillioides* (COSTA; GONÇALVES; MACHADO, 2020). Microencapsulated *Lippia turbinata* essential oil in the preservation of peanut seeds showed significant antifungal effect, reducing between 59 and 77% of its mycoflora (GIRARDI *et al.*, 2017).

The action of *Zingiber officinale* essential oil encapsulated in ultrafine protein fibers added to packaging aiming at the control of *Listeria monocytogenes* in cheese was evaluated. After the third day of storage, *L. monocytogenes* bacterial counts decreased from 4.39 log CFU/g $^{-1}$ to 3.62 log CFU/g aw3szx\$^{-1}$ (SILVA *et al.*, 2018).

The addition of essential oil in edible films for preserving fruits and vegetables increases the shelf life of these products (PERUMAL *et al.*, 2022). Other studies on the application of free, encapsulated, nanoemulsion essential oils in different food matrices are reported in Table 1.

Table 1. Application of free or encapsulated essential oil as antibacterial in food

Plant	Major components	Essential oil	Food matrix	Microorganism	Reference
Origanum vulgare	Carvacrol (71%), thymol (3%), p-cimene 3,5%, β-cariofilene (4%)	Free	Minas cheese	*E. coli, S. aureus*	Campos *et al.*, 2022
Eucalyptus globulus	Eucalyptol (94%), α-pinene (2,93%), γ-terpinene (1,93%)	Nanoemulsion	Fruit juice (orangine)	*S. aureus* , *B. cereus* and *E. coli*	Boukhatem *et al.*, 2020
Origanum vulgare e *Cinnamomum*	-	Encapsulated	Salami	*L. monocytogenes*	Gottardo *et al.*, 2022
Origanum vulgare e *Thymus vulgaris*	Thymol and carvacrol	Encapsulated	Bread	*L. monocytogenes, S. aureus, E. coli, S. Typhimurium*	Rosa *et al.*, 2020
Salvia officinalis	Epirosmanol (20%), viridiflorol (18%), camphor (13%), α-thujone (10%)	Free	Fresh pork sausage	*Salmonella spp., E. coli, L. monocytogenes*	Šojic *et al.*, 2018
Citrus essential oils (orange, mandarin, grapefruit, and lemon)	-	Nanoemulsion	Fish fillets (Rainbow trout)	Mesophilic, psychrophilic and coliform bacteria	Durmus, 2019

Source: Elaborated by the authors, 2023.

2.4 Encapsulation of Essential Oils for the Stabilization of Active Compounds

Although essential oils have been presented as alternative to synthetic chemical additives, they still have limitations (low solubility, high volatility, intense odor and flavor) regarding their direct application in food (FALLEH *et al.*, 2020). To avoid these drawbacks, techniques such as encapsulation can be applied. There are several encapsulation techniques: extrusion, lyophilization, fluidization, solvent removal, spray drying, coacervation, ionic gelation, liposomes, emulsification (REIS *et al.*, 2022). The encapsulation technique consists of involving a substance by a coating wall, promoting protection against adverse conditions and controlled release of the active substance (EGHBAL; VITON; GHARSALLAOUI, 2022).

Studies have demonstrated that encapsulation is effective by increasing the physical stability of the active substances of essential oils, minimizing possible deleterious effects, protecting them from interactions with food ingredients (REIS *et al.*, 2022; ZHU *et al.*, 2021).

Melo *et al.* (2020), evaluated the effectiveness of adding *Cymbopogon flexuosus* essential oil (at concentration of 0.25%) encapsulated with gum arabic and maltodextrin in different proportions (gum arabic + maltodextrin (3:1); and gum arabic + maltodextrin (1:1)) by the spray drying technique in cheese against coliforms and coagulase-negative *Staphylococcus aureus* during 21-day storage period. On the 21st day of storage, the encapsulated essential oil showed greater reduction in *S. aureus* count (0 log CFU/ml^{-1}) when compared to cheese with the addition of free oil (6 log CFU/ml^{-1}) and control sample (7 log CFU/ml^{-1}).

In the evaluation of the effect of *Thymus capitatus* essential oil on milk contaminated by *Staphylococcus aureus* (24 hours of inoculation), bacterial growth reached 202×10^3 CFU/ml in the presence of the free essential oil, while for oil encapsulated by nanoemulsion (70% *T. capitatus* essential oil + 30% soybean oil), bacterial growth was 132×10^3 CFU/ml (JEMAA *et al.*, 2017).

The nanoencapsulation of thyme essential oil in chitosan-gelatin nanofibers by electrospinning and its application for nitrite reduction in sausages was evaluated. Nanofibers were prepared using gelatin/chitosan in proportions of 1:6, 1:8 and 1:10 and thyme concentrations of 20 and 40%. *Clostridium perfringens* bacteria at concentration of 10^5 CFU/gr were inoculated into sausages and the number of colonies was analyzed after 0, 2, 8 and 20 days of storage, and after 8 days, and the number of colonies reached 0 for nanofibers containing essential oil at concentration of 40%, while sausage containing nanofibers without essential oil showed no significant change in the number of colonies ($P > 0.05$). The results indicated that encapsulated thyme oil had bactericidal effect against *C. perfringens*, with no significant adverse impact on the color and sensory properties of sausages compared to sample containing 120 ppm nitrite (VAFANIA *et al.*, 2019).

Radunz *et al.* (2020), evaluated the effect of thyme essential oil (*Thymus vulgaris*) encapsulated with casein and maltodextrin on beef burgers and found that the encapsulated oil showed reduction in thermotolerant coliforms and *E. coli* from 23 MPN/g on the first day to 15 MPN/g thermotolerant coliforms and 0 MPN/g *E. coli* on the fourteenth day, which may be related to the controlled release of active substances by encapsulation that occurred during the storage period, acting on microorganisms (RADUNZ *et al.*, 2020).

2.5 Safety in the Use of Essential Oils as Food Additives

Most essential oils from medicinal plants and spices are generally recognized as safe (GRAS) for use in food by the FDA (Food and Drug administration), according to the list provided in the Code of Federal Regulations, Title 21 - number 182.20 (Food and Drug administration /CRF21, 2016). Table 2 shows the essential oils generally recognized as safe by the FDA.

Table 2. GRAS essential oils (generally recognized as safe) by FDA (Food and Drug administration)

Common name	**Scientific name**
Sweet basil	*Ocimum basilicum*
Bergamot	*Citrus bergamia*
Black pepper	*Piper nigrum*
Chinese cinnamon	*Cinnamomum cassia*
India cinnamon	*Cinnamomum zeylanicum*
Sage	*Salvia sclarea*
Clove bud	*Eugenia caryophyllata*
Coriander	*Coriandrum sativum*
Cumin	*Cuminum cyminum*
Common fennel	*Foeniculum vulgare*
Rose-Scented Geranium	*Pelargonium graveolens*
Ginger	*Zingiber officinale*
Grapefruit	*Citrus* x *paradisi*
Common Juniper	*Juniperus communis*
Lemon	*Citrus limon*
Cochin grass	*Cymbopogon flexuosus*
Sweet marjorum	*Origanum majorana*
Lemon balm	*Melissa officinalis*
Oregano	*Origanum vulgare*
Peppermint	*Mentha piperita*
Bitter orange	*Citrus aurantium*
Roman chamomile	*Anthemis nobilis*
Rosemary	*Rosmarinus officinalis*
Mint	*Mentha spicata*
Tangerine	*Citrus reticulata*
Thyme	*Thymus vulgaris*

Sweet orange	*Citrus sinensis*
Ylang Ylang	*Cananga odorata*

Source: Food and Drug administration, 2016.

The Flavor and Extract Manufacturers Association (FEMA) panel of experts has operated since the 1960s to evaluate flavoring ingredients for use in foods and beverages in the United States (COHEN *et al.*, 2021). The International Organization of the Flavor Industry (IOFI) is an association that brings together regional and national flavor industry associations globally, including: Australia, Brazil, Canada, Colombia, Indonesia, Japan, Mexico, Africa, Singapore, South Africa and the United States, as well as the European regional flavor association. This association created the code of good practices that provides for the use of essential oils as natural flavorings in food (IOFI, 2010).

In France, the Directorate General for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) issued a list of EOs whose use is considered traditional in human food (DGCCRF, 2019). In Brazil, essential oils can be added to foods as flavoring additives according to RDC No. 725, of July 1, 2022 (essential oil: volatile natural flavoring of vegetable origin obtained by distillation process, by extraction with water steam, by reduced-pressure distillation or by another suitable physical process, which may be isolated or mixed with another essential oil, including rectified, terpeneless and concentrated) (ANVISA, 2022).

Although they are GRAS, essential oils cannot be considered innocuous, as they are natural compounds derived from the secondary metabolism of plants, being affected by extrinsic and intrinsic factors as well as the plant part used for extraction; thus, variations in their chemical composition may occur, and may contain substances that promote toxicity depending on the organism sensitivity and the amount ingested (TAMBURLIN *et al.*, 2021). According to EFSA (2012), terpenoids and ketones may be associated with neurotoxicity and abortive properties, phenols with liver toxicity, monoterpenoids associated with kidney toxicity, pinene derivatives with increased risk of endocrine disruption and furocoumarins have phototoxic properties (EFSA, 2012).

Information on the safe dosage of essential oils for human consumption is scarce. Tamborlin *et al.* (2021), evaluated the toxicological safety of essential oils used as food supplements to establish recommended safe oral doses, and concluded that an essential oil dose is considered safe when the safety margin (ratio between toxicological reference value and systemic exposure) for all constituents is at least equal to 1 (TAMBURLIN *et al.*, 2021).

Cohen *et al.* (2018), presented the revised procedure for the safety assessment of natural flavor complexes used as food ingredients, which study is an update of the publication by Smith *et al.* (2005). In this procedure, information related to the estimated intake, metabolism and toxicology of natural flavoring complexes and their constituent congeneric groups are systematically analyzed in 14 steps (COHEN *et al.*, 2018; COHEN *et al.*, 2021).

Cohen *et al.* (2019), evaluated the oral toxicity of sweet orange oil in male and female Sprague Dawley rats. In their study, animals received doses of 0 (vehicle), 240, 600 and 1500 mg/kg bw/day of sweet orange oil in methylcellulose via gavage for 28 days. The no observed adverse effect level (NOAEL) for sweet orange oil in rats is 600 mg/kg bw/day (COHEN *et al.*, 2019).

In the study by Cohen (2021), *Origanum vulgare* essential oil (56% carvacrol, 5% thymol, 15% p-cymene, 4% γ-terpinene with other minor terpenoid constituents) was administered to Wistar rats. The oregano oil concentrations administered were 0 (neutral gelatin), 1000, 2000 or 4000 mg/kg of feed, providing dose of 0, 50, 100 or 200 mg/kg body weight per day, and no mortality or abnormal clinical observations were observed during the study period. The study authors determined NOAEL (no observed adverse effect level) of 200 mg/kg body weight per day of oregano essential oil in rats.

3. CONSIDERAÇÕES FINAIS

Essential oils exhibit antibacterial, antifungal, and antioxidant activities, which are important characteristics in food preservation. The use of essential oils from medicinal and culinary plants as preservatives in food shows promising results. However, while essential oils have emerged as an alternative to synthetic chemical additives, they still have limitations in terms of safety and stability.

This research demonstrated that the instability of essential oils can be rectified through encapsulation techniques, and most essential oils from medicinal plants and spices are generally recognized as safe for use in food. However, they cannot be considered entirely benign because they are natural compounds derived from the secondary metabolism of plants, which can vary in composition and may contain substances that promote toxicity. The study in question contributes to the clarification that not all essential oils can be ingested, and even those recognized as safe should be used with caution, as they can potentially lead to intoxication depending on the dosage used and individual susceptibility. This research identified a limitation in the scarcity of scientific articles addressing the safety of ingesting essential oils and their recommended usual dosage. Further exploration on this topic is suggested, with more detailed information on the potential toxic effects of ingesting essential oils, as well as their feasibility and use on an industrial scale.

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