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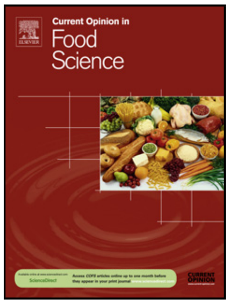

Title: Application of Encapsulated Essential Oils as Antimicrobial Agents in Food Packaging<!–<RunningTitle>Essential Oils Applied to Food Packaging</RunningTitle>–>

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Application of Encapsulated Essential Oils as Antimicrobial Agents in Food Packaging

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Highlights

- Essential oils have great potential as natural antimicrobial agents in food packaging.
- Micro and nanoencapsulation techniques improve the biological activities of essential oils.
- Essential oils nanoparticles applied to food packaging comprise a new class of effective antimicrobial nanocomposites.

Abstract

In recent years, natural antimicrobial compounds have attracted a great deal of attention from the food industry. Innovations in food packaging have also been focused on the incorporation of these active additives in polymer matrices with the purpose of extending foods shelf life. Essential oils (EOs) from aromatic plants are potent antimicrobial agents and many EOs are known to be powerful agents against *E. coli*, *L. monocytogenes* and other pathological bacteria. However, their volatility, their low solubility in water, and susceptibility for oxidation limits their use. Micro and nanoencapsulation of EOs is one of the options to reduce these effects and to improve their biological activities. In this review, the relevance of the encapsulation of EOs as antimicrobial agents and their incorporation into food packaging are discussed. Application of active antimicrobial packaging, based on encapsulated EOs, to foods is also addressed to evaluate their influence in food shelf life.

Introduction

Several studies have been focused on new packaging technologies due to the increasing population and the limited natural resources [1]. Active packaging, with the aim of releasing antimicrobials agents to foods is getting much attention due to their role in increasing foods shelf–life, minimizing or even eliminating the presence of food borne microorganisms [2–4].

Essential oils are volatiles compounds from aromatic plants with several biological properties, including antimicrobial activity. There is great interest in the use of EOs because they are natural products and have been considered as GRAS (Generally Recognized as Safe) food additives by the Food and Drug Administration

[5]. Nanotechnologies have emerged to support advances in preservation of foods [6]. For instance, the use of biodegradable materials is many times conditioned due to the lack of barrier (to oxygen, light or water vapor) or the poorer mechanical properties of these materials when compared with conventional materials. The reinforcement of biodegradable materials with nanofillers allows improving their properties and extends their range of applications [7]. Nanoparticles of nisin, silver and zinc oxide have been successfully used to obtain antimicrobial nanopackaging [8–11]. Food industry is evaluating the best approaches to include essential oils, as natural antimicrobials, in polymeric materials in order to have their advantages and to limit their drawbacks. Encapsulation is one of these techniques, consisting in a process where small particles are enclosed by a coating in order to form a capsule [12–14].

This review focuses on EOs as potent antimicrobials against different bacteria and on their encapsulation with the purpose of their incorporation in active food packaging, The advantages and drawbacks of this technique are discussed and the incorporation into different polymeric matrices is reviewed. Moreover, the application of these packaging to foods is also addressed to evaluate their effectiveness. in terms of food shelf life extension.

Antimicrobial food packaging

New ways to protect foods against internal and external factors and to prolong foods shelf-life have encouraged the food industry to develop new packaging concepts [15]. In line with technology advances, active and intelligent packaging has emerged. Active packaging interacts with the product, absorbing or releasing compounds from/to the food or food headspace [16,17]. This allows extending food products shelf-life, maintaining or improving the properties of the packaged food. Intelligent packaging is designed to monitor certain conditions of the environment at which the food is exposed [16,17]. In addition to the extended protection of foodstuffs, these packages can be made from biodegradable and environmental-friendly materials.

Among active packaging, active antimicrobial packages incorporated with essential oils are being developed and evaluated. These packages are formed by a polymer matrix that contains the additives that will protect foods against food pathogens [18]. These new active packaging materials can be applied to food as coatings or films [1].

With the increase of world population and consumer and industrial concerns regarding the limited resources and environment protection, the use of materials from renewable resources and/or biodegradable have gained a special attention. For instance, carbohydrates, proteins and lipids are being used as polymeric matrices to develop food packages in detriment to synthetic materials [1].

The packaging materials can be designed in order to incorporate certain components with the aim of being released during food transportation and storage. Thus, the substances responsible for the active function of packaging might be directly incorporated into the packaging material, and may interact directly with the food surface [19].

Nowadays, especially due to consumers' demands, there is a tendency to search for natural additives. Besides, synthetic additives have been associated with negative side effects to human health [20].

In line with this, in the last few years, alternative sources of antimicrobials were investigated, being extracts and EOs derived from plants, such cinnamon (*Cinnamomum zeylanicum* Blume), rosemary (*Rosmarinus officinalis* L.), sweet fennel (*Foeniculum* *vulgare* var. dulce (Mill.) Batt. & Trab) and oregano (*Origanum vulgare* L.), great candidates and have been successfully incorporated in polymeric matrices [18,21].

In a study carried out by Gómez-Estaca *et al*. (2010) a gelatine-chitosan film incorporated with clove EO was applied to fish during chilled storage and the growth of microorganisms was reduced, allowing to extend the fish shelf-life [22].

Essential oils

Essential oils (EOs) are aromatic substances produced naturally as secondary metabolites by a specific plant species. EOs are mainly composed by terpenoids, phenolic and aromatic compounds and their composition can vary, depending on the edaphoclimatic characteristics of the plant, part of the plant (flower, seed, leaves, fruits, stems and others) used for the extraction and the extraction method. These are the factors that determine the biological function of the EOs. Since ancient times, EOs are used for aromatic and flavouring purposes and also in traditional medicines such as Ayurveda (Indian medicine), Jamoo (Indonesian medicine) and Zhong Yo (Chinese medicine) [23–25].

In the literature, the antimicrobial activity of different EOs have been reported For instance, in the research study developed by Santos *et al*. (2016), cellulose acetate films incorporated with mixtures of oregano, cinnamon and sweet fennel EOs, showed high antimicrobial activity against *Penicillium* spp and *Staphylococcus aureus*. The film incorporated only with oregano EO showed the highest antimicrobial activity against *S. aureus* and the film incorporated with a mixture (1:1) of oregano and cinnamon EOs showed the highest antimicrobial activity against *Penicillium* spp [18]. Rather *et al*. (2012) proved the antimicrobial activity of common walnut (*Juglans regia* L.) EO against *Escherichia coli*, *Salmonela typhi*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Shigella dyssenteriae* [26]. Ribeiro-Santos *et al*. (2017) showed that *Cinnamomum cassia* EO has a great antimicrobial activity against *E. coli*, *S. aureus* and *Penicillium* spp as well as, the *Cinnamomum zeylanicum* EO against *Penicillium* spp [27]. In the review carried out by Raut and Karuppayil (2014), the authors recognized antimicrobial activities of several EOs, such as clove (*Syzygium aromaticum* L.), basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum zeylanicum* Blume), rosemary (*Rosmarinus officinalis* L.) ginger (*Zingiber officinale* Rosc.) and lemongrass (*Cymbopogon citratus* (DC.) Stapf.), against several microorganisms, such as *E. coli*, *S. aureus*, *S. aureus* (MRSA), *Shigella dyssenteriae*, *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Baillus subtilis*, *Enterococcus faecalis*, *Alternaria alternate*, *Aspergillus parasiticus* and *Candida albicans* [25], contributing to the increase of food safety and to extend shelf life.

In Table 1 the effect of different EOs against several microorganisms is compiled and in Table 2 the application of EOs, as antimicrobials, to food packaging is summarized.

Nanoencapsulation

Encapsulation is a technique in which droplets or particles of an active substances (core material) are polymeric coated by extremely small capsules (wall material) [28]. Generally, encapsulation can be classified according to their size: Microencapsulation particles are range in a size between 1 and 1000 μm and can be formed by natural or synthetic materials. However, nanomaterials are range in a size between 1 and 100 nm and are currently being used in various areas such as the food industry. The polymer or nanocapsule acts as a protective film, isolating the inner core containing the active agent and avoiding the effect of the inadequate exposure [29].

An important point in the production of a nanoencapsulated product is the choice of the wall material, generally it is a polymer selected depending on the physicochemical properties of the active agent and the intended application. A wide variety of natural and synthetic polymeric matrixes have been used such as: polyethylene, carbohydrates (starch, cellulose and chitosan), proteins (casein, albumin and gelatin), lipids (fatty acids, wax, and paraffin) and gum (alginate, carrageenan, and arabic gum) [28].

The spray drying technique process is one of the unit operations most frequently used during the nanoencapsulation process [30]. This technique involves the formation of an emulsion, containing an essential oil and wall material. Then, the emulsion is transformed into a large number of small droplets that fall into the spray chamber concurrently with circulating hot air. The water evaporates, instantly, when in contact with the hot air, and these droplets become solid particles [31].

Encapsulation of essential oils

New technologies, as nanoencapsulation of bioactive compounds with antimicrobial activity, can be applied in order to protect the EOs during the incorporation process in the polymeric matrix.

A successful incorporation of the EOs into processed foods or food packaging is however limited. Due to the fact that EOs are volatile compounds, present low solubility and are high susceptible to oxidation. Therefore, encapsulation techniques can increase their stability to oxidation, light-induced reactions, moisture and high temperatures and therefore, protect their antimicrobial activity, which is imperative [30,32].

The nanoencapsulation of EOs can also improve their biological properties, by increasing their bioavailability due to increase surface to volume ratio by reducing particle size into nano-range [33]. In fact, due to the interest of this subject, several research studies can be found in the literature regarding the encapsulation of several EOs (Table 3).

Beyki et al. [12] showed an enhanced antimicrobial activity of *Mentha piperita* EO encapsulated in chitosan-cinnamic acid nanogels.

Components present in the EOs responsible for their antimicrobial activity have also been encapsulated. Some studies were performed with eugenol, carvacrol, limonene, cinnamaldehyde and thymol [34,35].

When applied into to food packaging, Wen et al. [13] demonstrated that polyvinyl alcohol/cinnamon essential oil/β-cyclodextrin (PVA/CEO/β-CD) antimicrobial nanofibrous film can effectively prolong the shelf-life of strawberry, indicating its potential for the application in active food packaging.

Biddeci et al. [14] prepared a functional bionanocomposite film by filling a pectin matrix with modified halloysite nanotubes (HNT) containing peppermint EO. This biofilm exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Conclusion

Essential oils have proven to have antimicrobial activity that can be used to increase foods shelf life. EOs can be used either in food or food packaging, however, their low solubility in water, volatilization and susceptibility to oxidation limits their use. Encapsulation technique is helpful in improving the stability of EOs and consequently their efficiency. The use of natural antimicrobial agents, such as essential oils applied with new nanotechnologies techniques, allows the successful development of new antimicrobial packages for food preservation. These new techniques are gaining global interest across the food industry and research community. However more studies and regulations are required to assure the safety of nanofoods and nanofood packaging.

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Table 1. Antimicrobial activity of essential oils against fungi and bacteria.

Essential Oil	Antimicrobial Action	Ref.
Mentha rotundifolia (L.) Huds	*Aspergillus flavus, Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*; Fumigant and contact toxicity properties against *Rhyzopertha dominica* (F.).	[36]
Thymus vulgaris L.	*Pantoa* sp. and *Escherichia coli.*	[37]
Mentha rotundifolia L.	*E. coli*, *Salmonella typhimurium*, *S. aureus*, *Bacillus cereus*, *Aspergillus niger* and *C. albicans.*	[38]
Ocimum basilicum L.	*S. aureus, E. coli, B. subtilis, Pasteurell amultocida* and pathogenic fungi *A. niger, Mucor mucedo, Fusarium solani, Botryodiplodiatheobromae, Rhizopussolani*.	[39]
Cinnamomum zeylanicum; *Citrus aurantium*; *Pelargonium graveolens*; *Rosmarinus officinalis*; *Citrus sinensis*; *Citrus limon*; *Eugenia caryophyllus*	*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *S. aureus* and *B. subtilis*	[40]
Cinnamomum osmophloeum	*E. coli, P. aeruginosa, Enterococcus faecalis, S. aureus, Staphylococcus epidermidis, S. aureus*, *K. pneumoniae, Salmonella sp*., and *Vibrio parahemolyticus.*	[41]
Eucalyptus radiata	*P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Salmonella Typhimurium* and *Acinetobacter baumannii*.	[42]

Table 2. Examples of antimicrobial food packaging containing essential oils or their main constituents

Essential oils	Food packaging	Food	Ref.
Oregano Pimento	Milk protein	Whole Beef Muscle	[43]
Winter savory Cinnamon Oregano	Alginate	Bologna and ham	[44]
Linalool or methylchavicol	Low-density polyethylene (LDPE)	Cheddar cheese	[45]
Oregano	whey protein isolate (WPI)	Fresh beef	[46]
Oregano	Cellulosic resin	Pizza	[47]
Clove	Gelatin Chitosan	Fish	[22]
Oregano Thyme	Soy protein	Fresh ground beef	[48]
Limonene constituent EO Lemongrass Oregano Peppermint Red Thyme	Chitosan	Strawberries	[49]
Rosemary	Cellulose acetate	chicken breast cuts	[50]

Cinnamon, Clove	Cassava starch	Bakery	[51]

Table 3. Encapsulation of essential oils

Essential oil	wall material	Encapsulation technique	Main Conclusion	Ref.
African Wormwood, Tasmanian blue gum, and tea-tree	Diastearoyl phosphatidylcholine and diastearoyl phosphatidylethanolamine liposomes	Reverse phase evaporation	With the exception of African Wormwood, microbial growth was inhibited at lower concentrations for the encapsulated formulations in comparison with the nonencapsulated oils.	[52]
Pepper-rosmarin	Chitosan and cashew gum nanogel	Spray dried	Gum:chitosan (1:1) presented highest loading (11.8%) and encapsulation efficiency (70%). Nanoparticles presented slow and sustained release	[53]
Chia	Whey protein concentrate (WPC) with mesquite gum; WPC+ gum Arabic	Spray dried	Encapsulation efficiency was independent of microcapsules particle size. The encapsulation efficiency was higher when core to wall material ratio was higher.	[54]
Oregano	Chitosan nanoparticles	Two-step: oil-in-water (o/w) emulsification, and ionic gelation	The *in vitro* release studies indicated that the content of oregano EO in the nanoparticles influenced its release rate from the chitosan nanoparticles.	[55]
Pepper mint	Chitosan-cinnamic acid nanogels	Crosslinking methods	Encapsulation considerably improved the performance of EO against *Aspergillus flavus*.	[12]

Table 3 (cont.)

Essential oil	Wall material	Encapsulation technique	Main Conclusion	Ref.
Thyme	Chitosan- benzoic acid nanogel	Cross-linking methods	*In vivo* analysis revealed significant anti-fungal properties of the encapsulated oils at concentrations above 700 mg/L. Nanogel encapsulation was found to have significantly increased half-life and the anti-fungal properties of thyme EOs.	[56]
Shirazi thyme	Chitosan	Ionic gelation	Better performance of the EO when encapsulated under both *in vitro* and *in vivo* conditions in comparison with nonencapsulated oils against *Botrytis cinerea* was found. *In vivo* experiment showed that the encapsulated oils at 1500 ppm concentration significantly decreased incidence of Botrytis-inoculated strawberries during 7 days of storage at 4 °C and 10 days at 20 °C.	[57]
Lemon ironbark	Cashew gum	Spray Dryer	Nanoencapsulated oil exhibited enhanced activity against *Salmonela Enteritidis*. Augmenting the proportion of the matrix there was an increase in encapsulation efficiency and a decrease in particle size.	[58]
Summer savory	Chitosan nanoparticles	Ionic gelation	High *in vitro* antimicrobial activity against *S. aureus*, *L. monocytogenes* and *E. coli*.	[59]
Walnut	Skim milk powder (SMP), SMP+Tween 80, and SMP+maltodextrin	Spray dried	Emulsion prepared using SMP and Tween 80 combination resulted in the highest microencapsulation efficiency (91.01%) at drying air temperature of 180 °C and feed atomization pressure of 3 bar.	[32]

Table 3 (cont.)

Essential oil	wall material	Encapsulation technique	Main Conclusion	Ref.
Oregano	Nanoemulsions: deionized water, Cremophor RH 40, Span 80 lipid phase (EO + sunflower oil)	Phase inversion temperature method	Incorporation of the nanoemulsion in chicken pâté was technologically feasible, as well as its antibacterial action against *E. coli* and *S. aureus*. Nanoemulsions were influenced by the different concentration of oregano EO.	[60]
Cardamom	chitosan nano-particles	Ionic gelation	Encapsulation efficiency was more than 90%. Antimicrobial potential against *Escherichia coli* and *Staphylococcus aureus*.	[61]
Shirazi thyme	Solid lipid nanoparticles	High shear homogenization and ultra sound	Antifungal efficacy of the nanoparticles was significantly more thannonencapsulated oil against *Alternaria solani*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Aspergillus níger*, *Aspergillus ochraceus*, *Aspergillus flavus*.	[62]