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Infusion of essential oils for food stabilization: Unraveling the role of nanoemulsion-based delivery systems on mass transfer and antimicrobial activity

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ABSTRACT

Solid food preservation with essential oils requires the use of suitable carriers, such as nanoemulsions, which are able not only to promote dispersion in the aqueous part of foods, but also to enhance mass transfer within the food matrix.

The impact of emulsion formulation and mean droplet size on the rate of infusion of carvacrol in food matrices was investigated through: (a) image analysis of micrographs of histological sections of zucchini cylinders upon infusion with different emulsions stained with fluorescent dyes, and (b) microbiological assays in zucchini as well as in cooked meat sausages. The simplified geometry enabled the derivation of the effective diffusivities of the different emulsions in the food structure and their correlation with microbial inactivation.

Results showed that emulsions of nanometric droplet size, below the characteristic size of inter- and intracellular interstices, exhibited a significantly enhanced effective diffusivity, which promoted a more efficient antimicrobial action of carvacrol.

Industrial relevance: The growing interest towards "greener" food products, where safety is ensured without the use of synthetic additives, has stimulated the study of essential oils as antimicrobial compounds. However, in order to overcome the limitations related to their lipophilic nature, the use of essential oils requires their encapsulation in a suitable carrier. The objective of this study is to investigate the fundamental aspects of the use of nanoemulsion-based delivery systems for essential oils, and in particular the impact of their composition and morphological characteristics on the mass transfer in solid food products, in order to enable their rational application at the industrial scale in a wide range of vegetable and animal products.

1. Introduction

Essential oils (EOs) extracted from plants and fruits encompass different components with significant biological activity, such as anti-inflammatory, expectorant, carminative, psychoactive, pesticide and above all antimicrobial properties (Saad, Muller, & Lobstein, 2013). Therefore, in addition to more traditional applications such as their use in flavorings (i.e. bergamot, peppermint), perfumes (i.e. rose, vanilla) or nutraceuticals (i.e. cloves, thyme), EOs are increasingly exploited as antibacterial additives of natural origin for food preservation (Burt, 2004), replacing artificial compounds to meet the growing needs of consumers for "greener" products.

The mechanism of antibacterial action of EOs is mainly based on the hydrophobicity of their constituent molecules. Indeed, the EOs with higher antibacterial properties contain a high percentage of phenolic compounds, capable of interacting with the cytoplasmic membrane, causing its irreversible damage (Burt, 2004; Ceylan & Fung, 2004; Di Pasqua et al., 2007; Di Pasqua, Hoskins, Betts, & Mauriello, 2006).

For example, carvacrol, a phenolic compound contained in large amounts in the extracts of thyme and rosemary, is reported to act as a carrier of protons across the lipid bilayers, causing the dissipation of the proton motive force (Ultee, Bennik, & Moezelaar, 2002), as well as to alter the permeability barrier of cytoplasmic membrane (Helander et al., 1998), causing leakage of various other substances, such as ions, ATP, nucleic acids and amino acids (Lambert, Skandamis, Coote, & Nychas, 2001).

The direct incorporation of EOs in foods is limited by several technological challenges, which concern their adequate dispersion in the food matrix, the control of their interaction with the other ingredients, as well as the preservation of their activity for the required time (Donsì, Annunziata, Sessa, & Ferrari, 2011; Donsì, Annunziata, Vincensi, & Ferrari, 2012). A possible approach to overcome such challenges is based on the encapsulation of EOs in appropriate delivery systems, which can contribute (a) to improve the protection of the bioactive compounds from chemical degradation as well as (b) the dispersion in the aqueous part of the food, where microorganisms proliferate, (c) to reduce the impact of EOs on sensorial properties and (d) to enhance

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their biological activity through the promotion of mass transport (Weiss, Gaysinksy, Davidson, & McClements, 2009).

O/W food nanoemulsions are ideal candidates as carriers for EOs, due to the affinity of their core for EO components, their wide food compatibility, the simple industrial scalability of their manufacturing process, as well as their ability to cross biological membranes (McClements, Decker, & Weiss, 2007; McClements & Rao, 2011).

Recently, the encapsulation of EOs in nanoemulsions has been increasingly investigated (Donsì, Annunziata, et al., 2012; Liang et al., 2012; Salvia-Trujillo, Rojas-Grau, Soliva-Fortuny, & Martin-Belloso, 2013; Terjung, Loffler, Gibis, Hinrichs, & Weiss, 2012; Ziani, Chang, McLandsborough, & McClements, 2011), specifically addressing the issues related to the role played by nanoemulsions on EOs antimicrobial activity. In particular, it has been questioned if the encapsulation of EOs in nanoemulsions, altering the localization of the active components and their mass transfer rate across the microbial cell membrane, might significantly impact on the mechanisms of antimicrobial action.

Two separate and independent studies clearly showed that the enhancement of antimicrobial activity of EOs encapsulated in emulsions can be positively correlated to the increase of EO concentration in the aqueous phase in partition equilibrium with the emulsion oil phase. In particular, higher antimicrobial activity was observed for those systems, where the partition of EO components in the aqueous phase was promoted either by using small-molecule emulsifiers or by increasing the mean droplet size of emulsions. In fact, experimental data showed that when small-molecule emulsifiers, such as polysorbates, monoolein and sugar esters, are used, higher aqueous phase concentration of EOs is observed than when large-molecule emulsifiers, such as proteins or lecithins, are used, because of the occurrence of micellization processes (Donsì, Annunziata, et al., 2012). Similarly, experimental results proved that EOs are preferentially localized at oil–water interfaces, and therefore, the reduction of the specific interfacial area of emulsions by increasing the mean droplet size caused the increase of EO concentration in aqueous phase (Terjung et al., 2012).

Both studies suggested that the main mechanism of action of EOs is via the dissolution of active compounds in the aqueous phase and the consequent direct attack on the cell membrane; therefore it can be hypothesized that nanoemulsions act mainly as “nano-tanks” for the EOs, improving their dispersion in aqueous phase and ensuring a sustained concentration of the active compounds over an extended period of time, being new active molecules released from the emulsion droplets when those dispersed in the aqueous phase are depleted.

Despite the numerous efforts to clarify the in vitro behavior of EO nanoemulsions, their behavior in product to date has been only marginally approached for beverages (Donsì et al., 2011) and can be still considered at a pioneering development stage for solid foods, where EOs were applied only through their dispersion in aqueous solutions directly sprayed on the products (Karabagias, Badeka, & Kontominas, 2011; Karagozlu, Ergonul, & Ozcan, 2011).

The aim of this work is to study the fundamental issues related to the infusion of EO nanoemulsions in solid foods to naturally improve their microbiological stability. A model EO component, carvacrol, and a model food matrix, zucchini (*Cucurbita pepo*) are selected, in order to unravel the role of emulsion formulation and mean droplet size on the kinetics of infusion of nanoencapsulated EOs into a solid biological matrix, and their potential for microbiological stabilization of foods. The effects of emulsion formulation and mean droplet size are then studied also for the microbiological stabilization of a cooked meat product, in order to extend the reach of the discussion.

2. Materials and methods

2.1. Materials

The EO component used in this work was carvacrol ≥98%, purchased from Sigma-Aldrich s.r.l. (Milan, Italy). Carvacrol was mixed in different proportions with peanut oil, purchased from Sagra (Lucca, Italy) used as carrier oil. Emulsions were stabilized with fluid soy lecithin Solec IP, a kind gift from Solae Italia s.r.l. (Milan, Italy), polysorbate Tween 20 and monoolein (rac-1-oleoyl-glycerol: 40%, diglyceride: 20–40%, triglyceride: 20–40%), purchased from Sigma-Aldrich s.r.l. (Milan, Italy), sugar ester P-1670 (sucrose palmitate), a kind gift from Prodotti Gianni (Milan, Italy) and the pea protein isolate NUTRALYS® F85M, a kind gift from Roquette (Milan, Italy). Tween 20 and monoolein were used at 50:50 weight ratio, according to a previous validation (Donsì et al., 2011).

Zucchini (*C. pepo*) were purchased from a local market, which ensured during the experimental campaign the consistency of origin and quality.

Commercial cooked sausages (Würstel di Suino Classici, Salumificio Fratelli Beretta S.p.A., Italy) were purchased from a local market, with the package being stored at 4 °C and opened immediately before the treatment with essential oils.

2.2. Emulsion preparation

Oils in water emulsions and nanoemulsions were prepared using the high pressure homogenization (HPH) technique. Pre-emulsions were obtained by mixing the ingredients and dispersing them by high shear mixing (HSM) with an Ultra Turrax T25 (IKA Labortechnik, Jahnke und Kunkel, Germany) at 24,000 rpm for 5 min, maintaining the samples in an ice bath. Subsequently, the pre-emulsions were processed 5 times by HPH, in an in-house developed system, equipped with a 80 μm diameter orifice valve (model WS1973, Maximator JET GmbH, Schweinfurt, Germany), operated at 200 MPa through an air-driven Haskel pump model DXHF-683 (EGAR S.r.l., Milano, Italy). The inlet temperature of the process fluid was maintained at 5 °C through a jacketed inlet tank, while the outlet temperature was quickly reduced to 5 °C in a heat exchanger placed immediately downstream of the orifice valve.

Carvacrol and peanut oil concentrations were varied in a wide range. In the study of the effect of carvacrol on emulsion properties, carvacrol concentration was varied between 0% and 3% wt, maintaining the total oil phase (carvacrol + peanut oil) at 4% wt. In infusion studies, carvacrol concentration was 2% wt and peanut oil concentration 8% wt (EM-1 through EM-4), while in EM-5 carvacrol concentration was 0.5% wt, and peanut oil concentration 3.5% wt, with the detailed formulations being given in Table 1.

2.3. Droplet size distribution

The droplet size distribution of emulsions was determined at 25 °C by dynamic light scattering (DLS), using a high performance particle sizer (HPPS 3.3, Malvern Instruments, Alfatest, Roma, Italy) with polystyrene cuvettes, and was expressed in terms of hydrodynamic diameter (d_H) or z-diameter and of polydispersity index (PDI), as previously described (Donsì, Senatore, Huang, & Ferrari, 2010; Donsì, Sessa, & Ferrari, 2012). Prior to any measurements being taken, the samples were diluted with distilled water to a suitable concentration (1:10) to avoid multiple scattering effects. Each measurement was conducted at least on two independent samples, with the means and the standard deviations being calculated.

In the case of HSM-treated pea protein emulsions, which exhibited a mean droplet size larger than instrument sensitivity, measurements were conducted by laser diffraction (Mastersizer 2000, Malvern Instruments, Malvern, UK), with d_H being expressed in terms of the Sauter diameter $d_{3,2}$, calculated as previously reported (Donsì et al., 2010).

2.4. Emulsion turbidity

Emulsion turbidity was evaluated through the measurement of the optical density of suitably diluted emulsion samples at two specific wavelengths, 560 nm and 600 nm, using a V-650 UV–vis spectrophotometer (Jasco Instruments, USA).