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Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties

Alejandra Acevedo-Fani, Laura Salvia-Trujillo, María Alejandra Rojas-Graü, Olga Martín-Belloso*

Department of Food Technology, University of Lleida – Agrotecnio Center, Av. Alcalde Rovira Roure 191, 25198, Lleida, Spain

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ABSTRACT

Edible films including active ingredients can be used as an alternative to preserve food products. Essential oils (EOs) exhibit antimicrobial activity against pathogenic microorganisms but their low water solubility limits the application in foods. To improve water dispersion and protect EOs from degradation, nano-sized emulsions emerge as a viable alternative. Nanoemulsions containing EOs and polysaccharides could be used to form edible films with functional properties. This study was focused on the evaluation of physical, mechanical and antimicrobial properties of alginate-based edible films formed from nanoemulsions of EOs. Nanoemulsions containing thyme (TH-EO), lemongrass (LG-EO) or sage (SG-EO) oil as dispersed phase and sodium alginate solution as continuous phase were prepared. The average droplet size of nanoemulsions was reduced after the microfluidization treatment exhibiting multimodal size distributions. The ζ-potentials of nanoemulsions were between −41 mV and −70 mV depending on the type of EO used. The lowest whiteness index was found in SG-EO nanoemulsions, whereas those containing TH-EO showed the highest value. Films formed from SG-EO nanoemulsions exhibited higher transparency, water vapor resistance and flexibility than films formed from TH-EO or LG-EO. Edible films containing TH-EO were those with the strongest antimicrobial effect against inoculated *Escherichia coli*, achieving up to 4.71 Log reductions after 12 h. Results obtained in the present work evidence the suitability of using nanoemulsions with active ingredients for the formation of edible films, with different physical and functional properties.

1. Introduction

Edible films have been proposed as an alternative of food packaging to improve the quality and safety of food products. This technology protects foods from dehydration and acts as gases barrier with the surrounding media. In addition, edible films may serve as carriers of active compounds such as antimicrobials, antioxidants and texture enhancers, among others. Sodium alginate is a polysaccharide isolated from marine brown algae, widely used in the food industry as thickening agent, which also has film-forming properties (Krochta, Baldwin, & Nisperos-Carriedo, 1994). Essential oils (EOs) are aromatic oily liquids extracted from plant materials and commonly utilized as flavoring in foodstuffs (Burt, 2004). Their antimicrobial properties against several pathogenic microorganisms involved in foodborne illness have been demonstrated in previous investigations (Alboofetileh, Rezaei, Hosseini, & Abdollahi, 2014; Oriani, Molina, Chiumarelli, Pastore, & Hubinger, 2014). For this reason, the scientific community and food industry are considering the use of EOs as potential preservatives of natural origin. Nevertheless, their incorporation in food systems is mainly limited by flavor considerations, since effective antimicrobial doses may exceed organoleptic acceptance levels (Lambert, Skandamis, Coote, & Nychas, 2001). In addition, EOs have low-water solubility, meaning their dispersion within aqueous-based products is rather difficult.

Nanoemulsions are being increasingly used to encapsulate, protect and deliver lipophilic ingredients to liquid foods or minimally processed fruits and vegetables (Bhargava, Conti, da Rocha, & Zhang, 2015; Donsì, Cuomo, Marchese, & Ferrari, 2014; Kim, Ha, Choi, & Ko, 2014). Literature refer nanoemulsions as emulsions with very small droplet size, below 100 nm (McClements, 2011; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). The small particle size in nanoemulsions has two important consequences: i) the possibility of enhancing physicochemical properties and

* Corresponding author.
E-mail address: omartin@tecal.udl.es (O. Martín-Belloso).

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stability; and ii) the ability of improving biological activity of lipophilic compounds by increasing the surface area per unit of mass (McClements & Rao, 2011). This fact also allows using lower doses of active ingredients. Recent studies have shown an enhancement of the physical properties of EOs-loaded nanoemulsions regarding to their equivalent conventional emulsions (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2013). In addition, it has been also observed a higher antibacterial activity in nanoemulsions containing EOs (Buranasuksombat, Kwon, Turner, & Bhandari, 2011; Liang et al., 2012; Severino et al., 2015).

In this sense, nanoemulsions based on polysaccharides such as alginate and EOs as antimicrobial agents may be used for edible films formation, which could be considered a new generation of edible packaging. The particular properties observed in nanoemulsions regarding to the conventional emulsions could be extrapolated to the physicochemical and functional properties of the edible films formed from them. Previous studies have evaluated the formation of films based on nanoemulsions prepared by low-energy methods and high-energy techniques such as ultrasounds or high-speed homogenization (Bilbao-Sáinz, Avena-Bustillos, Wood, Williams, & McHugh, 2010; Otoni, Moura, et al., 2014; Otoni, Pontes, Medeiros, & Soares, 2014). However, up to our knowledge, systematic studies have not been reported about the properties of polysaccharide-based films made from EOs-loaded nanoemulsions prepared by microfluidization. Therefore, the purpose of this study was to evaluate the antimicrobial, physical and mechanical properties of edible films obtained from alginate-based nanoemulsions loaded with EOs (thyme, TH-EO; lemongrass, LG-EO or sage, SG-EO), as well as characterizing the physicochemical properties of the EO nanoemulsions relating them with the edible film properties.

2. Materials and methods

Food grade sodium alginate was supplied by FMC Biopolymers Ltd (Scotland, UK). Nonionic surfactant (Tween 80) was purchased from Scharlau (Spain), and plasticizer (glycerol) was provided by Fisher Scientific (UK). Thyme (TH-EO; *Thymus vulgaris*), lemongrass (LG-EO; *Cymbopogon citratus*) and sage (SG-EO; *Salvia officinalis*) essential oils were purchased from Essential arôms (Spain).

2.1. Preparation of Film-Forming Nanoemulsions (FFN)

Sodium alginate dispersions were prepared by dissolving 3% w/v in double distilled water at 70 °C. A coarse emulsion was obtained by mixing alginate dispersions at room temperature, glycerol (2% v/v), Tween 80 (3% v/v) and TH-EO, LG-EO or SG-EO (1% v/v) in a high-speed blender at 17,500 rpm for 2 min (Ultra-turrax, Jake & Kunkel, Staufen, Germany). To prepare nanoemulsions, coarse emulsions were pumped into the microfluidizer (M110P, Microfluidics, Massachusetts, USA) at 150 MPa for three cycles. Temperature of nanoemulsions during processing was maintained below 15 °C with an external coiling coil immersed in an ice-water bath and placed at the exit of the interaction chamber. All samples were prepared using ultra-pure water obtained from a Milli-Q filtration system. Alginate films (ALG) were also made without the addition of EO (alginate 3% w/v, Tween 80 3% v/v and glycerol 2% v/v) to be considered as control.

2.2. Characterization of Film-Forming Nanoemulsions (FFN)

2.2.1. Particle size and ζ-potential

Particle size, polidispersity index and ζ-potential were analyzed with a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). Particle size was measured by dynamic light scattering (DLS) at 633 nm, 25 °C and using a backscatter detector of 173°. FFN were first diluted with ultra-pure water to 1:20 to avoid multiple scattering effects. The average droplet size (z-average) and polidispersity index were reported. Polidispersity index (PDI) value is a measure of heterogeneity in the droplet size distribution. PDI values close to 0 indicate homogenous size distributions, whereas PDI values close to 1 indicate heterogenous size distributions. The surface charge at the interface of oil droplets within FFN (ζ-potential) was measured by phase-analysis light scattering (PALS).

2.2.2. Whiteness index and viscosity

The color of the FFN was determined with a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan), using an illuminant D_{65} and the 10° observer angle. The device was calibrated in a white standard plate (Y = 94.00, x = 0.3158, y = 0.3222). A crystal flat-faced cuvette was filled with FFN and then placed at the top of the measuring device of the colorimeter. CIE L^*, a^* and b^* values were utilized to calculate whiteness index (WI) throughout Eq. (1) (Vargas, Cháfer, Albors, Chiralt, & González-Martínez, 2008):

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^{*2} + b^{*2})} \quad (1)$$

The viscosity of FFN was measured in aliquots of 10 ml using a vibro-viscometer SV-10 (A&D Company, Tokyo, Japan), vibrating at 30 Hz and constant amplitude.

2.3. Film formation

Nanoemulsions and alginate film-forming solutions were treated with a vacuum pump to remove air bubbles and avoid the presence of micro-holes in film structure. Then, oil-containing and control films were formed by casting in crystal plates of 30 × 40 cm, previously covered with Mylar paper. Films were dried at room temperature for 24 h and peeled off from Mylar paper for further determinations.

2.4. Characterization of edible films

2.4.1. Scanning Electron Microscopy

Aluminum stubs with films were dried in a heater at 60 °C for 48 h. Films were fixed with carbon and metalized with evaporated gold in a Blazers SCD 050 sputter coater (Balzers Union AG, Liechtenstein) to grant electrical conductive properties. Microstructure of films surface were examined using a Scanning Electron Microscope with an acceleration voltage of 10 kV and a working distance of 10 mm (DSM 940 A, Zeiss, Germany).

2.4.2. Color and opacity

Film color and opacity were measured with a colorimeter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan), using an illuminant D_{65} and the 10° observer angle. The instrument was calibrated with a standard white plate (Y = 94.00, x = 0.3158, y = 0.3222). Measurements were performed by placing film squares of 30 mm × 30 mm onto a white background. Chromaticity coordinates CIE L^*, a^*, b^* were recorded to obtain the color difference (ΔE^*), which was calculated using Eq. (2) (Pires et al., 2013):

$$\Delta E^* = \left((L^* - L_o)^2 + (a^* - a_o)^2 + (b^* - b_o)^2\right)^{0.5} \quad (2)$$

where L^*, a^*, b^* are the color coordinates of the films, and L_o, a_o, b_o values are those corresponding to the white background ($L_o = 90.97$, $a_o = 0.08$, $b_o = -0.28$).