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Development and characterization of whey protein isolate active films containing nanoemulsions of *Grammosciadium ptrocarpum* Bioss. essential oil

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ABSTRACT

Whey protein isolate (WPI) based films containing emulsions and nano-emulsions of *Grammosciadium ptrocarpum* Bioss. essential oil (GEO) (0.5, 1 and 1.5% of WPI) were investigated. The formation of new interactions between WPI and GEO nano-droplets was confirmed by FT-IR. According to XRD results, the crystalline nature of the film was completely preserved by GEO addition. The FE-SEM images revealed that the incorporation of GEO nano-emulsions would improve the film integrity. The nano-emulsion incorporation improved the mechanical properties and caused to decrease water vapor permeability (WVP) (3.5×10^{-7} g/m.s.Pa to 3.21×10^{-7} g/m.s.Pa and 4.10×10^{-7} g/m.s.Pa to 3.92×10^{-7} g/m.s.Pa for concentrations of 0.5 and 1.5% respectively). The antioxidant potential (20.86% for nano-emulsion of 1% compared to 16.80% of 1% emulsion) and antimicrobial activity of the films containing GEO nano-emulsions was higher than emulsion incorporated films. Based on release studies, the release rate of GEO in water was increased by decreasing droplet size. The results showed a potential of WPI-GEO nano-active films for novel food protection.

1. Introduction

Considering the rising problem of so-called white pollution; which is an interesting issue in environmental area nowadays, a novel research field in biodegradable packaging has been provided. This area of research has directed a large part of the studies in focusing on presenting innovative ideas for edible coatings and films (Oleyaei, Almasi, Ghanbarzadeh, & Moayedi, 2016). Due to the serious disadvantages of chemically synthesized polymeric films, in terms of not being biodegradable, their petrochemical-base, and environmental concerns, they have been met by a decreasing superiority in novel packaging apart from their availability, affordability, durability, etc. (Ghanbarzadeh, Almasi, & Entezami, 2011). Among a variety of biodegradable biopolymers used for film formation, proteins and especially whey protein isolate (WPI) have been applied since long time ago (Fairley, Monahan, German, & Krochta, 1996; Kaya & Kaya, 2000; McHugh, Aujard, & Krochta, 1994; McHugh & Krochta, 1994), mainly because they have proved to have acceptable functional and film producing properties (Gounga, Xu, & Wang, 2007); which has led in forming transparent, flexible, colorless and odorless films (Fairley et al., 1996; McHugh & Krochta, 1994). Whey proteins are globular proteins with nutritional value, and the ability of forming gels, emulsions and foams (Zhou, Wang, & Gunasekaran, 2009).

With regard to the 'Green consumerism', a trend by which consumers demand for natural antimicrobial compounds (Imran et al., 2012), there has been an increasing necessity for consuming plant based materials. These organic materials may serve as non-toxic, environmentally safe and act effectively in suppressing microorganisms. *Grammosciadium ptrocarpum* Bioss. is one of the native plants; that, is famous for its flavor, antibacterial and antioxidant characteristics (Sonboli, Eftekhar, Yousefzadi, & Kanani, 2005a). Its spatial distribution is east, south and central Anatolia, and north-west of Iran. Linalool (68.4%) and β-pinene (22.0%), were found to be the main constituents of fruit volatiles and caryophyllene oxide (55.1%) and β-caryophyllene (15.3%) are the main components of essential oil extracted from aerial parts of this plant (Küçükboyacı, Demirci, Adıgüzel, Bani, & Başer, 2015). The essential oils (EOs) of plants have showed their role in protecting against foodborne pathogens and spoilage bacteria; yet, considering their hydrophobic nature and therefore their low solubility in polar solutions, it is necessary to envelope them with a hydrophilic material to accelerate their efficiency. Encapsulation of essential oils within nanoscale delivery systems has been recently adapted in behalf of the elevated efficiency resulted from their higher bioavailability in cells (Severino et al., 2015). Due to the obtained higher efficiencies of encapsulated essential oils, the applied dose of the oil would have be decreased. Target delivery, is another advantage of applying nanoscale

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encapsulation (Severino et al., 2015). The incorporation of nanoencapsulated EOs and herbal extracts in the form of nanoliposomes and nanoemulsions in various biopolymer-based active films such as alginate (Acevedo-Fani, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2015; Salvia-Trujillo, Rojas-Gracu, Soliva-Fortuny, & Martın-Belloso, 2015), corn starch and sodium caseinate (Jimenez, Sanchez-Gonzalez, Desobry, Chiralt, & Arab Tehrany, 2014), fish gelatin (Wu et al., 2015) and chitosan (Almasi, Zandi, Beigzadeh, Haghju, & Mehrnow, 2016; Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016) has been described. According to Acevedo-Fani et al. (2015), edible films containing nanoemulsions of thyme, lemongrass, or sage could be used as active packaging. Alginate based nano-emulsion films containing these active ingredients, showed zeta potential of −41 to −70 mV and thyme treated samples had the highest whiteness values and antimicrobial effect against *Escherichia coli* (4.71 log reduction after 12 h). In another study, Jimenez et al. (2014), studied the antimicrobial potential of orange essential oil and limonene into soy and rapeseed nanoliposomes. Based on the obtained results, liposome incorporation into polymeric matrix decreased the mechanical strength of the film and the antimicrobial activity was not observed in films may be due to the inhibition effect of encapsulation. In a similar study, nano-emulsions of nettle extract (0, 0.5, 1 and 1.5% w/w) were adopted within chitosan films. Results showed that nano-emulsions showed lower antioxidant activities in comparison to free extracts and it could delay the induction period (60 days) of soybean oil oxidation (Almasi et al., 2016).

However, any former study has not used WPI as carrier of nanoencapsulated natural preservatives for preparation of nano-active packaging materials. Moreover, to the best of our knowledge, there are no reports on the using of *Grammosciadium ptrocarpum* Bioss. EO (GEO) in the fabrication of active films and coatings based on WPI. The aim of this work was to characterize morphological, water vapor barrier, mechanical, some physical properties (water absorption, color, …), antimicrobial, antioxidant and release properties of films cast from mixtures of WPI solution and various concentrations of *Grammosciadium ptrocarpum* Bioss. EO-loaded nanoemulsions. Moreover, the influence of unencapsulated form incorporating of GEO compared with nanoliposomes was assessed in order to establish a possible enhancement of GEO functionality due to the encapsulation.

2. Materials & methods

2.1. Materials

Whey protein isolate (WPI, ca. 90% Kjeldahl, N × 6.38) was obtained from Arla company (Denmark). *Grammosciadium ptrocarpum* Bioss. essential oil was obtained by direct distillation of the growing parts of the *Grammosciadium ptrocarpum* Bioss. plant in a Clevenger apparatus at 1:5 ratio with distilled water. Glycerol (Gly) and all the other chemicals were of analytical grade and purchased from Merck or Sigma Aldrich companies (Germany).

2.2. Preparation and characterization of nanoemulsions

The nanoemulsion was formulated based on GEO, non-ionic surfactant Tween 80 (HLB-15) and water. Due to the high hydrophilic and lipophilic balance value and its non-ionic nature, Tween 80 was preferred mainly because it stabilizes emulsion droplets via stearic stabilization. It is also reported that, the low molecular weight of this surfactant, enhances minimization of the obtained droplet sizes. Coarse emulsion was acquired by mixing essential oil and surfactant in 4:1 ratio, followed by water addition and mixing for 20 min at 1200 rpm and 25 °C. For nanoemulsion fabrication, the prepared coarse emulsion was treated with ultrasound (20 kHz, Hielscher, Germany) at maximum power output (750 W). The sonicator probe was symmetrically dipped into the sample and the process of sonication lasted for 4–5 min with time intervals of 5 s (30 s sonication 5 s interval). The experiments were conducted in room temperature.

The average diameter and particle size distribution of the produced nano-emulsions were determined by Dynamic Light Scattering (DLS) using Malvern Zetasizer (Worcestershire, UK Malvern instruments) (Zhang et al., 2012). Samples were diluted with distilled water (1:100) before measurements. All the measurements were carried out at 25 °C and three replicates and the results were reported as the average of volume diameter. For zeta potential measurement, the test samples were also diluted with deionized water prior to be applied in the Zetasizer. Zeta potential measurement was done using a Malvern Zetasizer (Worcestershire, UK Malvern instruments) at 25 °C and zeta run of 12. The Zetasizer was equipped with a 4 mW He/Ne laser emitting 633 nm, measurement cell, photomultiplier and correlator.

2.3. Film preparation

Film preparation was accomplished by dissolving 5 g of WPI powder in 100 mL of double distilled water. The mixture was stirred (1 h) on a magnetic stirrer to obtain a homogeneous solution. Then the pH of the solution was adjusted on 8 (using 0.1 N NaOH) and heated for 40–45 min at 70 ± 2 °C in order to denature whey proteins. The temperature of the solution was then lowered to the room temperature and glycerol was added at the ratio of 2:1 (WPI:Gly). Afterwards, free and nanoemulsion forms of GEO were added at 3 levels of 0.5, 1 and 1.5% of WPI to the film solution. 25 g of the prepared solutions was poured over 10 cm diameter glass plates according to the casting method and were let to dry at ambient temperature for 24 h (Fernández-Pan, Royo, & Ignacio Maté, 2012; Gounga et al., 2007).

2.4. Characterization of films

2.4.1. Thickness

Film thickness was measured using a manual digital micrometer (Mitutoyo, Japan) with an accuracy of 0.01 mm at ten random positions. The obtained values were used in calculations of water vapor permeability and mechanical properties.

2.4.2. Water vapor permeability (WVP)

Water vapor permeability (WVP) test was performed according to the ASTM (1995) standard with some modifications (Mali, Grossmann, Garcia, Martino, & Zaritzky, 2006). Glass vials with an average diameter of 2 cm and 4.5 cm height, were used to determine WVP of the films. Films were cut into disc shapes with a diameter of slightly larger than the diameter of the test vial and were then put on top of the vials containing 3 g of anhydrous $CaSO_4$. Relative humidity (RH) of 0 was obtained using anhydrous $CaSO_4$ in the vial. Vials were placed in a desiccator containing saturated K_2SO_4 solution. Saturated K_2SO_4 solution in the desiccator provided a constant RH of 97% at 25 °C. The desiccator was placed in an incubator at 25.0 ± 0.1 °C and the vials were weighed every 24 h. Water vapor transport was determined by the weight gain of the vial. Weight changes of the vials were recorded as a function of time and the slopes were calculated by linear regression (weight change vs. time). The water vapor transmission rate (WVTR) was defined as the slope (g/h) divided by the transfer area (m^2). WVP ($g\,m^{-1}\,h^{-1}\,Pa^{-1}$) was calculated as:

$$WVP = \frac{WVTR}{P(R_1 - R_2)}X \quad (1)$$

Where, P is the saturation vapor pressure of water (Pa) at the test temperature (25 °C), R_1 is the RH in the desiccator, R^2, the RH in the vial and X is the film thickness (m). Under these conditions, the driving force [P ($R_1 - R_2$)] is 3073.93 Pa. All the measurements were carried out in three replicates.

2.4.3. Swelling ratio

Swelling ratio of the film samples was determined on 2 × 2 cm