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Properties of active gelatin films incorporated with rutin-loaded nanoemulsions

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ABSTRACT

Physico-chemical, mechanical, barrier, release profiles and antioxidant properties of composite gelatin based-films incorporated with rutin-loaded oil-in-water nanoemulsion, at various concentrations (5, 10, 15, or 20% (based on the weight of the gelatin powder)) were studied. All the gelatin/rutin-loaded nanoemulsion films displayed higher tensile strength and higher elongation at break than the gelatin control film. The composite films did not show significant differences in thickness, color, brightness and transparency. The structural properties evaluated by FTIR showed that the rutin-loaded nanoemulsion achieved complete miscibility within the gelatin matrix. All the gelatin/nanoemulsion films exhibited compact and homogenous microstructure. In addition, these films showed high antioxidant activities monitored by DPPH radical scavenging and reducing power activities. The Korsmeyer-Peppas model described well the rutin release profile. Rutin release was mainly governed by Fickian diffusion with simultaneous interfering swelling and disintegration phenomena. These results indicate that nanoemulsions-in-gelatin systems can function as potential active packaging systems to enhance shelf life of food products and then to provide a high-quality products (fresh/safe).

1. Introduction

Edible films have provided an interesting and often essential complementary means for controlling the quality and stability of numerous food products. There are many potential uses of edible films (i.e. wrapping various products, individual protection of dried fruits, meat and fish, control of internal moisture transfer in pizzas, pies, etc.) which are based on the films properties (i.e. physical, mechanical, water and solute barrier) [1].

Edible films based on proteins have good gas barrier and mechanical properties, but poor water barrier properties. An approach to improving films functionality is to combine lipid materials with proteins to form composite films [2]. Current strategies to combine proteins and lipids include (i) the addition of lipids (i.e. essential oils) directly to the film forming solution [3,4], or (ii) the addition of lipids dispersed in aqueous phase (i.e. O/W emulsions) to the film-forming solution [2]. Since the lipid phase may act as a solvent of various food additives, the film may be used as active packaging due to the possibility of specific transport of lipophilic food additive from the film, into foodstuffs. This is of interest because the active films are biodegradable and could provide a wide range of activities, including antioxidants and antimicrobials [5].

Recently published studies had revealed the advantages of adding lipids material in the form of emulsions to films based on proteins. Otoni et al. [6] reported amelioration of mechanical properties of films made from isolated soy protein added with oil-in-water (O/W) emulsions (Acetem and Tween 60). The addition of emulsion had a direct impact on mechanical properties by acting as plasticizer [6]. The authors reported that emulsion addition with a reduced droplet size (~112 nm) improved the water barrier properties due to the hydrophobic nature of dispersed phase [6]. Alexandre et al. [7] developed films based on gelatin and loaded with montmorillonite (MMT) and nanoemulsioned ginger essential oil (GEO) and observed that the combined effect of MMT and GEO improved the elongation at break, puncture force and puncture deformation of gelatin-based films, and that these films presented antioxidant activity but not antimicrobial activity.

Sugumar et al. [8] reported that nanoemulsion of Eucalyptus oil reduced the moisture content of films based on chitosan and improve its antibacterial activity against *S. aureus*. Almasi et al. [9] developed an active films based on chitosan incorporated with nanoliposome loaded with nettle extract. Release studies indicated

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that the release profile of nettle extract encapsulated nanoliposome in 95% ethanol simulant significantly decreased by nanoliposome as vehicle of nettle extract. Moreover, the incorporation of nanoliposomes into the films decreased the effect of temperature on nettle extract release rate when storage temperature increased from 4 to 40 °C. Therefore, the addition of active compounds encapsulated into O/W emulsions, and then into protein matrices might bring about a two-fold advantage: the performance improvement of food packaging material and the provision of an additional active function.

The aim of this work was to prepare activated gelatin-based composite films by incorporating rutin-loaded nanoemulsions into the film-forming solution, and to evaluate the mechanical, water barrier, microstructural properties, antioxidant activities and the release profile of rutin of the films.

2. Materials and methods

2.1. Reagents

Rutin hydrate (R5143, purity ≥ 94.0%) was obtained from Sigma-Aldrich (São Paulo, Brazil). Soybean oil (dispersed phase) was purchased from Cargill Agricola S.A. (São Paulo, Brazil). Anhydrous glycerol (product number G1004.02.BJ), ethanol (99.5% v/v), Tween 80 and Span 80 were obtained from Labsynth Ltd. (São Paulo, Brazil). A pigskin gelatin's powder, Type A was purchased from Gelnex (São Paulo, Brazil). HPLC-grade acetonitrile and methanol were obtained from Labsynth (São Paulo, Brazil). The water used was of Milli-Q quality with electrical resistivity 17.8 MΩ.cm at 25 °C.

2.2. Preparation of rutin-loaded O/W nanoemulsions

O/W nanoemulsions were prepared using a two-step homogenization method, similar to the protocol from Tan and Nakajima [10]. Briefly, rutin was dissolved in soybean oil at a concentration of 0.1% (w/w) with heating at 90 °C for 1 h. Solution was cooled to room temperature, then Span 80 was added at concentration of 3.75% (w/w) and solubilized using magnetic stirrer for 15 min to form the dispersed phase.

To prepare the continuous phase, Tween 80 was dissolved in water at a concentration of 1.25% (w/w). One hundred grams of premix with a weight ratio of dispersed phase to continuous phase of 1:9 was homogenized using a rotor-stator homogenizer (Ultra-Turrax® IKA T25, Labotechnik, Germany) at 7000 rpm for 5 min. The resulting coarse O/W emulsion was homogenized in a three pass using a microfluidizer M-110Y (Microfluidics Co., Newton, USA) with a F20 Y (75 μm) interaction chamber at a pressure of 100 MPa. The O/W emulsion droplets loaded with rutin had a surface-averaged droplet size of 150 nm, measured by light scattering spectroscopy (PCS)-based ZetaPlus particle size analyzer equipped with a Brookhaven digital correlator (Brookhaven Instrument Co., Holtsville, USA), with refractive index of the dispersed phase (rutin-loaded soybean oil) was of 1.474 at 25 °C. O/W emulsion samples were stored in the dark at 4 °C for further addition to the film-forming solutions. The emulsion was physically stable in term of variations of droplet size, during 30 days of storage in the dark condition at 4 and 25 °C [11].

2.3. Preparation of films

To prepare the film-forming solutions, 5 g of gelatin was dissolved into 80 mL of distilled water at ambient temperature, then the temperature was increased to 70 °C using a hot water bath. The mixture was stirred for 30 min at this temperature. After cooling to 37 °C, glycerol was added (30 g/100 g of the gelatin) to film-forming solutions, acting as a plasticizer; and emulsion solutions were added with different concentrations (5, 10, 15, and 20%, based on the weight of the gelatin powder). Then, distilled water was added to complete to the final volume of 100 mL. Finally, the film-forming solutions were homogenized at 10,000 rpm with an Ultra Turrax (IKA T25, Labotechnik, Germany) for 5 min [12,13]. To remove the air bubble from film-forming solutions due to the high speed homogenization, vacuum condition (pressure max. 1.45 psi) was applied during 15 min with a diaphragm pump (model ME 1, Vacuubrand, Germany).

To produce films, around 25 mL of film-forming solutions were cast into polystyrene Petri dishes (150 × 15 mm, Pleion Co., São Paulo, Brazil), and dried in a ventilated climatic chamber (MA 035, Marconi, Brazil), at temperature and relative humidity fixed at 30 ± 1 °C and 40 ± 2%, respectively, for 12 h. Before characterization, all the film samples were conditioned at 25 ± 0.2 °C and 58 ± 1% of relative humidity, for at least 7 days. We have assumed that initial rutin incorporated into the films was chemically stable due to the previous stability studies on the emulsions which proven the stability of rutin during one week of storage [11].

2.4. Films characterizations

2.4.1. Thickness

The thickness of films was measured using a micrometer (model ID-C112PM, Mitutoyo, Japan). Ten random locations around each film sample were used for thickness determination.

2.4.2. Mechanical properties

Tensile strength (*TS*) and elongation at break (*EAB*) of films samples were determined according to ASTM standard method D882 [13], using the texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK). The tests were performed in a controlled room at 25 °C and 50 ± 5% RH.

Ten film samples (80 × 15 × 0.085 mm), with the initial grip length of 40 mm, were used for testing. The film samples were clamped and deformed under tensile loading using a 30 N load cell with the cross head speed of 1 mm/s until the samples were broken. The maximum load and the final extension at break were used for calculation of *TS* and *EAB*, respectively [14].

2.4.3. Film solubility in water

For film solubility in water determination, film samples (30 × 20 × 0.085 mm) were weighed and placed in 50 mL centrifuge tub containing 10 mL of distilled water with 0.1% (w/v) sodium azide, and agitated in a shaking incubator at a speed of 170 rpm and room temperature for 24 h. The remaining undissolved film was removed after centrifugation at 3000 × g for 10 min at 25 °C, and then dried at 105 °C for 24 h. Film solubility (*FS*) was calculated with the following equation:

$$FS(\%) = ((W_i - W_f)/W_i) \times 100 \tag{1}$$

where Wi was the initial weight and Wf was the weight of the undissolved film residue, both in dry basis. These tests were carried out in triplicate.

2.4.4. Water vapor permeability

The water vapor permeability (*WVP*) was determined gravimetrically, according to the standard ASTM E96 [15]. Film samples were placed in aluminum cells containing silica gel (0% *RH*) and placed in a desiccator containing distilled water (100% *RH*). Aluminum cells were weighed (± 0.01 g) daily for 10 days to guarantee the steady state permeation. *WVP* was calculated using Eq. (2):

$$WVP = \frac{\Delta g}{\Delta t}\left(\frac{x}{A.\Delta P}\right) \tag{2}$$