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Use of avocado phospholipids as emulsifier

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ABSTRACT

Avocado lipid fraction is rich in phospholipids; however these compounds are frequently discarded with the residual pulp during industrial oil extraction processes. In this study, emulsions were prepared using phospholipids from avocado pulp oil and characterized. First, emulsions were prepared with 1 wt% phospholipids and between 20 and 70 vol% oil phase. Then, emulsions with different phospholipid concentrations (1 and 2 wt%) and pH levels (3 and 7) were prepared. Emulsions prepared with 1 wt% phospholipids present phase inversion between 60 and 70 vol% oil phase. The emulsions with 50 and 60 vol% oil phase have higher stability against creaming than the others. Phospholipid concentration presented a higher influence on stability and droplet size distribution than pH. Emulsions with 2 wt% phospholipids are pseudo-plastic fluids and present typical gel-like behavior. Therefore, the avocado phospholipids can be used as emulsifier to form stable oil-in-water emulsions.

1. Introduction

Avocado is a tropical fruit rich in oil: up to 32% (Galindo-Tovar, Ogata-Aguilar, & Arzate-Fernández, 2008; Qin & Zhong, 2016, pp. 1–8; Tango, Carvalho, & Soares, 2004). Production and consumption of avocado oil have increased in recent years mainly due to its health benefits and flavor. This oil is often seen as a substitute for olive oil due to its similar composition of total fatty acids (Barros, Grimaldi, & Cabral, 2017; Pacetti, Boselli, Lucci, & Frega, 2007) and applications in the medical (Ding, Chin, Kinghorn, & D'Ambrosio, 2007) and cosmetic industries (Athar & Nasir, 2005; Berdick, 1972). However, during the enzymatic extraction processes, which are widely used industrially, the phospholipids are discarded together with the residual pulp. The phospholipid has a high added value due to its interfacial properties in addition to the growing interest in the use of natural surfactants by the food industry (Bai, Huan, Gu, & McClements, 2016; Dickinson, 1993; McClements & Gumus, 2016).

Phospholipids, or glycerophospholipids, are modifications of triacylglycerols, in which the phosphate is found (Nelson & Cox, 2013). Common sources of phospholipids are egg and soybean, besides milk, sunflower kernels, and rapeseeds (Ozturk & McClements, 2015; Pichot, Watson, & Norton, 2013). The avocado pulp lipid fraction is rich in polar lipids, such as phospholipids (Pacetti et al., 2007; Takenaga, Matsuyama, Abe, Torii, & Itoh, 2008). Some phospholipids found in the avocado are phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and phosphatidylglycerol (PG) (Dickinson, 1993; Takenaga et al., 2008).

Phospholipid is a naturally occurring surfactant that is widely used in practice to prepare oil-in-water (O/W) and water-in-oil (W/O) emulsions in products such as chocolate, cookies, mayonnaise, and others (Comas, Wagner, & Tomás, 2006; Pan, Tomás, & Añón, 2002; Rydhag & Wilton, 1981; Van Nieuwenhuyzen, 1981). The interfacial region that separates the oil phase from the aqueous phase constitutes only a small fraction of the total volume of an emulsion, but it has an important and direct influence on the physicochemical and sensory characteristics of such emulsions (Delacharlerie et al., 2016). Furthermore, the concentration of each phospholipid will influence the emulsions' characteristics. While

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PC tends to facilitate the formation of O/W emulsions, PE and, to a lesser extent, PI tend to facilitate the formation of W/O emulsions (McClements, 1999).

Hence, the objective of the present study was to investigate the preparation and characterization of emulsions stabilized by avocado phospholipids, a natural surfactant, taking into account the factors that influence the properties of these emulsions, namely the oil phase concentration, phospholipid concentration, and pH. The emulsion characterization was carried out by optical microscopy, conductivity, stability, droplet size distribution, and rheology analyses.

2. Materials and methods

2.1. Materials

The emulsions were prepared using commercial soy oil. The aqueous phase was distilled water with 1 wt% NaCl (Vetec, Rio de Janeiro, Brazil), in addition to NaOH (0.275 N) (Vetec, Rio de Janeiro, Brazil) and HCl (0.325 N) (Vetec, Rio de Janeiro, Brazil), to correct the pH (3 and 7). The avocados used for phospholipid extraction were purchased from CEASA (Curitiba, Brazil) in September 2013 and were all of the Margarida variety. For phospholipid extraction, analytical-grade chemicals were used: methanol (Vetec, Rio de Janeiro, Brazil), chloroform (Merck, Darmstadt, Germany), and acetone (Synth, São Paulo, Brazil). For analysis by liquid chromatography, *n*-hexane and 2-propanol chromatographic grade were employed (Merck, Darmstadt, Germany); ultrapurified water was obtained by the Milli-Q system (Millipore, USA); and chloroform, methanol, glacial acetic acid, and triethylamine (Merck, Darmstadt, Germany) were of analytical grade. The standard phospholipids were PC, LPC, PE, PI, PA, PG, and phosphatidylserine (PS) (Sigma-Aldrich, St. Louis, USA).

2.1.1. Phospholipids

The avocado phospholipids were obtained according to Vianna, Pires, and Viana (1999) with some modifications. The oil was extracted from avocado mesocarp with methanol/chloroform and acetone. First, 10 vol% water was added to the avocado oil and the mixture was heated to 60 °C. To form an emulsion, this mixture was stirred in a Polytron PT 3100 D homogenizer (Kinematica AG, Luzern, Switzerland) at 10,000 rpm for three minutes. The emulsion was centrifuged in an Excelsa II Centrifuge (Fanem, São Paulo, Brazil) at 5632 g for 3 min to separate its water and oil phases. After separation, the oil was removed and the water phase was lyophilized in a Liobras L101 lyophilizer (Liobras, São Paulo, Brazil) to remove the water from the phospholipids. The determination of phospholipids concentration was performed according to AOCS Recommended Practice Ca 19–86 (2009).

2.2. Phospholipid characterization

The phospholipid characterization was performed according to ISO 11701 and the Bligh and Dyer method (Bligh & Dyer, 1959). Approximately 400 mg of phospholipid sample was placed in a 50-ml volumetric flask and diluted in a 30-ml *n*-hexane: 2-propanol (80: 20) solution with stirring until complete homogenization and passed through filters with a pore diameter of 0.22 μm. The phospholipid composition was determined by high-performance liquid chromatography (HPLC) with injection of 20 μL of sample. An Agilent 1200 series chromatograph (Santa Clara, USA) with a 1260 Infinity evaporative light scattering detector (ELSD) and Merck LiChrospher 100 Diol column (250 × 4.0 mm, 5.0 μm) was used. The oven temperature was 55 °C. The detector temperature was 50 °C. Binary gradients A [*n*-hexane: 2-propanol: glacial acetic acid: triethylamine (81.4: 17.0: 1.5: 0.08 v/v/v/v)] and B [2-propanol: ultrapure water: glacial acetic acid: triethylamine (84.4: 14.0: 1.5: 0.08 v/v/v/v)] were used. The gradient started at 95% A, decreased to 0% in 15 min, was then held for 150 s, then reached 95% A in 0.1 min, and was held for 12.5 min. The flow rate was 1.0 ml min^{-1}. Phospholipids were identified by comparing retention times between the phospholipids and the respective standards.

2.3. Emulsion preparation

This procedure was divided into two parts. In the first part, emulsions were prepared with several concentrations of oil phase in order to determine the inversion interval and phase concentration with more stability. In the second part, emulsions were prepared in different pH conditions and phospholipid concentrations in order to evaluate the behavior of avocado phospholipids as emulsifiers. These procedures are described below.

In the first experiment, emulsions were prepared with oil phase concentrations of 20, 30, 40, 50, 60, and 70 vol%. Then, 1 wt% avocado phospholipids was added to the oil phase preparation in commercial soy oil and the mixture was stirred for 60 min at 60 °C to complete the homogenization. The aqueous phase was distilled water with 1 wt% NaCl at pH 7.0 ± 0.2. The aqueous and oil phases were mixed and stirred in a Polytron homogenizer PT3100 D (Kinematica AG, Switzerland) at 10,000 rpm for three minutes. The experiments were performed in triplicate. These emulsions were analyzed for stability, conductivity, optical microscopy, and droplet size distribution (DSD). All analyses were repeated three times (n = 3). The only exception was the optical microscopy, which was performed five times (n = 5) for each sample.

Considering the results of this first analysis, a concentration of 60 vol% oil phase was chosen for the preparation of emulsions. A 2^2 experimental design was used with pH 3 and 7 and concentrations of 1.0 and 2.0 wt% avocado phospholipid. These pH values were chosen because 7 is neutral and is found in many food emulsions, for example milk and non-fermented and non-acidified milk products, while pH 3 occurs in several acid foods. NaOH 0.275 N and HCl 0.325 N were added to adjust the pH.

Stability, optical microscopy, DSD, and rheological behavior analyses of the formed emulsions were carried out. The rheological behavior was analyzed only for stable emulsions one hour after preparation.

2.4. Emulsion characterization

2.4.1. Stability

The stability of the emulsions was determined by evaluating the phase separation of 10-ml samples placed in a conical graduated tube after 10, 20, 30, 60 min and 4 and 24 h at 25 °C (Züge et al., 2015) and reported as the percentage separated volume, denoted as *VS*(%), which was calculated according to Equation (1),

$$VS(\%) = \frac{V_f}{V_0} \times 100 \quad (1)$$

where V_0 is the initial volume of emulsion (10 mL) and V_f is the volume of separated aqueous phase.

2.4.2. Type of emulsion and inversion interval determination

The emulsion type and the inversion interval were evaluated on a conductivity meter (Shoot Lab 970, SI Analytics GmbH, Germany). High conductivity values indicate that water is the continuous phase and oil is the dispersed phase in an O/W emulsion, while low conductivity values indicate a W/O emulsion (Thakur, Villette,