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Avocado seed: Modeling extraction of bioactive compounds

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ABSTRACT

Avocado seed is a by-product that contains a large amount of extractable polyphenols, which have attracted the attention of food and cosmetic industries due to their high antioxidant capacity. This fact makes it a promising candidate for the cheap and sustainable extraction of such compounds. This work aims to evaluate the effect of ultrasound power (0–104 W) and temperature (20–60 °C) on the extraction of total polyphenols from avocado seed using water as a green solvent. Increasing temperature and ultrasound power resulted in extracts with higher polyphenol content and antioxidant capacity. Different mathematical models (Peleg's, empirical, film theory and Fick's law) were also used to find the one that best fit the extraction kinetics. Models based on film theory and Fick's law were able to predict the ultrasound-assisted batch and continuous extractions, respectively, at 95% accuracy. Using a model based on Fick's law, diffusion coefficients of polyphenols in both fast and slow stages were calculated for the extractions. In addition, a linear relationship between total polyphenolic content and antioxidant capacity was proposed.

1. Introduction

The food industry is generating many organic by-products and it is well known that the food wastes such as seeds, hulls, wood, bark, roots and leaves are potential sources of antioxidants compounds (Rubilar et al., 2006; Soong and Barlow, 2004; Velioglu et al., 1998). In this work, avocado seed is proposed to be a potential candidate for polyphenol extraction. There are several studies about avocado and its high antioxidant capacity, as well as its large amount of extractable polyphenols, from quantifying its total polyphenolic yield until how it prevents food matrices or emulsions oil-water from oxidation (Logaraj et al., 2008; Rodríguez-Carpena et al., 2011; Soong and Barlow, 2004; Wang et al., 2012, 2010).

In the other hand, the intake of polyphenols as natural substances through the diet, such as food products enriched with them, is a fact whose popularity has greatly increased in recent years. It is widely accepted that high intakes of fruit and vegetables prevent people from some diseases due to the presence of various antioxidants (Johnson, 2004).

Natural antioxidants, particularly polyphenols, have potential to be used in pharmaceutical and food industries because of their numerous benefits like reducing the risk of inflammatory diseases and preventing lipid oxidation (Moure et al., 2001). Furthermore, the use of synthetic antioxidants, like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), are restricted because concern is expressed about the possible negative effects on human health (Jayaprakasha et al., 2003).

In addition, it is necessary to point out that processes, such as solid-liquid extraction, can be modeled and simulated thanks to the numerical methods and computational advances. A model for the extraction of polyphenols and the estimation of their effective diffusivity is required in order to analyze and design an extraction process in industry (Guerrero et al., 2008; Pinelo et al., 2005; Rodríguez-Fernández et al., 2007). Unfortunately, most of the studies about these extraction processes are scarce and their point is not from engineering (Guerrero et al., 2008).

The solid-liquid extraction of polyphenols is a multi-phase and unsteady-state transfer mass operation, where the concentration of the solute inside the solid varies continuously. Experimental studies about kinetics extraction are required to estimate effective diffusivity. The liquid phase concentration as a function of time is used to fit the experimental data to theoretical models, which are under some hypothesis and specific kinetics parameters (Mantell et al., 2002).

A large number of mathematical models can be applied to extraction kinetics. These models are generally based on modifications of Fick's law such as the film theory, which has been used to model the extraction of bioactive compounds from plants. However, it is worth to mention that empirical equations like

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Peleg's model can also provide a proper fitting to extraction processes.

Several experimental studies about estimating effective diffusivities and mass transfer coefficients of polyphenols (or specific phenolic compounds) from a food by-product have been carried out for these years. Diffusivities and models are estimated by expressions from Fick's second law of diffusion (Amendola et al., 2010; Cacace and Mazza, 2003; Espinoza-Pérez et al., 2007; Guerrero et al., 2008; Pinelo et al., 2006). In these works, many variables such as temperature, ultrasound power and extraction time in batch extractions were studied (Bucić-Kojić et al., 2013; Capparucci et al., 2011; Dibert et al., 1989; Gironi and Piemonte, 2011; Guerrero et al., 2008; Pinelo et al., 2006).

The aim of this work is to model the solid-liquid ultrasound-assisted extraction in batch and continuous of total polyphenols from avocado seed, as well as estimate the effective diffusivity of polyphenols and its influence with temperature and ultrasound power. Antioxidant activity was also studied to find its relationship with total polyphenol yield.

2. Materials and methods

2.1. Sample preparation

Avocado (*Persea gratissima*) seeds from domestic consumption were used. The seeds were manually separated from avocado. The fresh seeds were ground by using a Moulinex mill (A5052HF, Moulinex, Lyon, France). Afterwards, the ground seeds were sieved to particle size of 2.00–2.36 mm. The average particle diameter of the samples was estimated to be 2.19 mm. Finally, the ground seeds were stored in a dark bottle under refrigeration at 4 °C until use.

2.2. Ultrasound-assisted batch extraction (UABE)

Batch extraction was carried out following the procedure described by Segovia et al. (2014b) with some slight modifications. About 8 ± 0.1 g (measured precisely) of avocado seeds were extracted in batch with 500 mL of water. The extractions were carried out in sealed flasks. These experiments were performed in triplicate at different temperatures (20, 40 and 60 °C). At specific intervals of time, 1000 μL of extraction were sampled and put in the fridge (away from light) at 4 °C. After two hours, the samples were analyzed to determinate total phenolic content and antioxidant capacity.

Ultrasound assisted extraction was performed in an ultrasonic bath (Type T 710 DH, 580VA, 40 KhZ, PROLABO, Germany) using the procedure mentioned above. The ultrasonic output ranged from 0 to 80% (100% equals 130 W).

2.3. Ultrasound-assisted continuous extraction (UACE)

The experimental extraction setup was similar to what Pinelo et al. (2006) reported. The column extractor was 0.75 cm of radius and 10 cm of height. The extractor was placed in the aforementioned ultrasonic bath under the same experimental conditions. Extraction was accomplished by continuous pumping of fresh water (4.17 mL/min) through the column. The solvent was pumped upward from the bottom. The outlet extract was sampled at specific intervals of time to record the polyphenol concentration and antioxidant capacity. At the end of the process, all these extracts were collected and stored as the final one.

2.4. Determination of total polyphenolics content (TPC)

Total phenol content of the extract was determined using the Folin–Ciocalteu reagent method with a slight modification (Segovia et al., 2014c). Samples were taken from the prepared extracts. The sample was placed in a plate by triplicate, adding 4% (v/v) of the Folin-Ciocalteu's reagent, 12% (v/v) sodium carbonate anhydrous solution (20%) and finally 80 μL of milli-Q water. Allowed to react for 1 h in the dark room, the absorbance was measured at 765 nm using a Fluorimetrics Fluostar Omega (Perkin–Elmer, Paris, France). The total phenolic content was expressed as mg Gallic Acid Equivalents (GAE)/l or mg GAE/fresh matter in final extract.

2.5. Determination of total antioxidant capacity (ORAC assay)

Antioxidant activities of avocado extracts were determined by the ORAC assay, as reported by Segovia et al., 2014b. The assay was carried out using a Fluorimetrics Fluostar Omega (Perkin–Elmer, Paris, France) equipped with a temperature-controlled incubation chamber. The incubator temperature was set at 37 °C. The extract samples were diluted 1:20 with milli-Q water. The assay was performed as follows: 20% of sample was mixed with Fluorescein (0.01 mM) and an initial reading was taken with excitation wavelength, 485 nm, and emission wavelength, 520 nm. Then, AAPH (0.3 M) was added and measurements were made for 2 h. This method includes the time and decrease of fluorescence. The area under the curve (AUC) was calculated. A calibration curve was made each time with the standard Trolox (500, 400, 250, 200, 100, 50 mM). The blank was 0.01 M phosphate buffered saline (pH 7.4). ORAC values were expressed as mmol Trolox Equivalents (TE)/mg of fresh matter in final extract.

2.6. Calculation of the diffusion coefficients

A model based on Fick's law was used in order to find the diffusion coefficients for each stage of the extraction:

$$\frac{C_\infty - C}{C_\infty} = \frac{6}{\pi^2}\left[f_1 exp\left(-\frac{\pi^2 D_1 t}{r^2}\right) + f_2 exp\left(-\frac{\pi^2 D_2 t}{r^2}\right)\right] \quad (1)$$

where f_1 and f_2 are the fractions of the solute, which are extracted with diffusion coefficients D_1 and D_2, respectively. C_∞ is concentration in equilibrium, C is concentration in time t and r the particle radius.

The next conditions were established to apply the above equation (Chan et al., 2013):

(1) Symmetrical and porous sample particles. The geometry of solid particles is assumed to be spherical with radius of r.

(2) The solid particle is assumed to be of a pseudo- homogeneous medium. The concentration of the active compounds in the solid particle depends on time and radius, r.

(3) Uniform distribution of active compounds in the sample matrix.

(4) Homogeneous mixing between solvent and plant sample particles. The concentration of the solute in the solvent only depends on time.

(5) The mass transfer of active compounds from the solid is a diffusion phenomenon in which the diffusion coefficient is independent of time.

(6) Diffusion of the solute and other compounds are in parallel and no interaction between them.

(7) External mass transfer resistance is negligible. The concentration of the solute in the solvent at the interior of the solid particle is equal to the concentration of the solute in the bulk solvent.

According to the method followed by Hojnik et al. (2008), in later stages of the extraction, only the second term on the right-hand side of Eq. (1) remains significant. The parameter D_2 is obtained from the slope and the parameter f_2, from the intercept of the curve where $ln[C\infty/(C\infty\text{-}C)]$ is plotted as function of time t. In earlier stages of the extraction, the second exponential term is close to unity and