The Journal of Supercritical Fluids 138 (2018) 7–16

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Subcritical water extraction of flavanones from defatted orange peel

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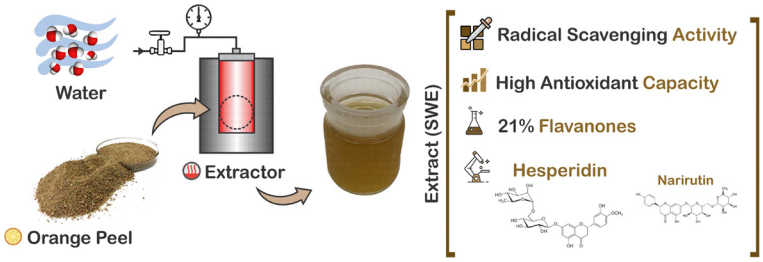
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Defatted orange peel
Subcritical water extraction
Bioactive compounds
Flavanones
Antioxidant capacity

ABSTRACT

Subcritical water extraction (SWE) is a green process that uses water as a solvent for extracting non-polar flavonoids by varying the temperature-dependent dielectric constant. Flavanones, including hesperidin and narirutin, constitute the majority of the flavonoids that occur naturally in citrus fruits. The effects of operating parameters temperature (110 − 150 °C) and water flow rate (10 − 30 mL/min) on the SWE of flavonoids from defatted orange peel (DOP) were studied. The maximum yields of hesperidin (188.74 ± 0.51 mg/g extract) and narirutin (21.98 ± 1.39 mg/g extract) were obtained at 150 °C and 10 mL/min. These yields accounted for approximately 21% of the total amount of these flavanones in the extracts, leading to the purest extracts. SWE was compared with three conventional extraction methods and the results demonstrated that, compared to conventional extractions, SWE is a highly efficient method for the recovery bioactive compounds with high antioxidant activity.

1. Introduction

Brazil is the world's largest producer of oranges [1]. In 2016, the orange production in the country was 15.9 Mt, being the main use for the juice production. After juice's extraction, 50% of the fruit is discarded as residue representing approximately 2% of all agricultural residues produced in Brazil [2], which is commonly used as an animal food supplement.

Therefore, orange peel (OP) is the main waste by-product of the juice extraction industry. Nonetheless, OP is an attractive source of bioactive compounds, which include phenolic compounds as the flavonoids. Flavonoids' concentrations in citrus fruits are higher in the peel and seeds [3–6]. Various reports have shown that flavonoids may have positive health effects, including antiproliferative, cardioprotective, anti-inflammatory and antioxidant effects [7–9]. Two flavonoids are found in large amounts in OP: the hesperidin and the narirutin. The

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https://doi.org/10.1016/j.supflu.2018.03.015
Received 31 January 2018; Received in revised form 19 March 2018; Accepted 21 March 2018
Available online 21 March 2018

hesperidin is a flavanone, which is a type of flavonoid [10]. Hesperidin is being widely used in pharmaceutical industries because it is associated with low risk of spills and certain types of cancer [11], besides its antimicrobial and antioxidant capacity. According to Sigma-Aldrich, up to 102.0 thousand tons of hesperidin could be obtained from the amount of orange bagasse generated per year in Brazil, which could represent a return of USD 2704.5 trillion [12].

Flavanones from orange peel can be obtained by different extraction methods, such as Soxhlet extraction, pressurized liquid extraction, ultrasound assisted extraction and, microwave assisted extraction, where organic solvents are commonly used. However, these solvents can be toxic and environmentally prejudicial, requiring severe solvent removal procedures before the extracts are ready to be consumed or exposed to potential environmental risks. Moreover, the solvents are usually expensive to purify and, generally cannot be easily discarded, taking both time and money. Therefore, the employment of efficient extraction techniques using non-toxic solvents are required, mainly when extracting compounds for people's ingestion, such as bioactive compounds [13].

In this context, a convenient extraction technique using water as solvent is interesting, since water is non-flammable, non-toxic, cheap and environmentally safe. Subcritical water extraction (SWE) takes place at temperatures between the boiling point and critical point of water (100 °C at 1 bar and 374.1 °C at 221 bar), at pressures high enough to keep water in the liquid state. Under these conditions SWE could be a viable method for the extraction of nonpolar flavonoids, by varying its temperature-dependent dielectric constant (ε). When the temperature of water increases, its physicochemical properties, in particular its relative ε, change. The ε, representing polarity, decreases as temperature increases (e.g., from $\varepsilon = 53$ at 110 °C to $\varepsilon = 41$ at 150 °C), which is close to that of methanol ($\varepsilon = 33.6$ at 25 °C) and ethanol ($\varepsilon = 24.5$ at 25 °C), which are traditionally used extraction solvents. Thus, subcritical water can be used to extract nonpolar flavonoids [5,10,14]. The extraction reactions in subcritical water depend strongly of temperature. However, extract degradation is not only dependent upon temperature, but also the exposure time (resident time), flow rate and solute chemical structure [15]. Degradation of the extracts or the sample matrix, as well as some technical issues involved with SWE, can also affect the extraction's efficiency.

Several works in the literature indicate that it is possible to separate the orange peel essential oil by supercritical CO_2 extraction (SFE) [16–18], generating a defatted residue in the process. Considering the biorefinery context, that is, the total use of raw material, and the process variables, is possible to extract flavonoids in this matrix using SWE. In this work, we aimed to determine the optimal conditions for SWE of the flavanones hesperidin and narirutin from OP after supercritical CO_2 extraction, varying the extraction temperature (110–150 °C) and water flow rate (10–30 mL/min), and to compare the results with conventional extraction techniques.

2. Material and methods

2.1. Chemicals

The extraction solvents used in this work were distilled water and ethanol (Synth, São Paulo, Brazil). For flavanones analysis, water was obtained from a Purelab Flex 3 water purification system (Elga, UK). HPLC grade acetonitrile was obtained from JT Baker, USA and phosphoric acid from Synth. Standards of hesperidin and narirutin were purchased from Sigma-Aldrich (São Paulo, Brazil). Acetone (96%) supplied by Sigma was used for the determination of extractives. Sulfuric acid (96%), acetic acid, HPLC grade sodium chlorite (99%) and sodium hydroxide were purchased from Wako Pure Chem. Ind., Ltd., Osaka, Japan and used for quantification of holocellulose, α-cellulose and lignin content in the defatted orange peel (DOP). For analysis of total pectin, ethanol, sodium decahydrated tetraborate (99.5%), sulfuric acid (96%) and ethylenediaminetetraacetic acid tetrasodium salt were purchased for Synth (São Paulo, Brazil) and galacturonic acid and carbazol, for Sigma-Aldrich (Sao Paulo, Brazil). For the evaluation of antioxidant capacity *in vitro*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-etramethylchromane-2-carboxylic acid (Trolox), 2,2′−azobis(2-methylpropionamidine) dihydrochloride (APPH), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) and fluorescein were purchased from Sigma-Aldrich (São Paulo, Brazil).

2.2. Raw material

Orange peel (OP), as a model of juice processing by-product, was supplied by the company CPKelco, Limeira, SP, southeastern Brazil. The residue is named in this work as defatted orange peel (DOP), after SFE of essential oils at 40 °C, 35 MPa and S/F of 32 kg CO_2/kg dry residue. DOP was kept in absence of light at −18 °C in a domestic freezer until the extractions were performed.

2.2.1. Sample characterization

Moisture and ash content were measured using the methodology recommended by National Renewable Energy Laboratory, described in the technical report (LAP TP-5100-60956, 510-42618). Protein was determined from total Kjeldhal nitrogen − TKN (4500NORG B,C) using a calibration factor of 6.25 (Gnaiger & Bitterlich, 1984). Extractives were determined quantitatively in a Soxhlet apparatus using the following sequence of extraction solvents: acetone and ethanol for 3 and 6 h, respectively [19].

2.2.2. Chemical composition of orange peel

Holocellulose, α-cellulose, hemicellulose, and lignin content were measured in extractive-free basis. The holocellulose content was determined as the mass remaining after $NaClO_2$ delignification [20]. The delignified residue (holocellulose) was weighed after drying for 24 h in an oven at 105 °C [21]. Holocellulose (1 g) was then transferred to a flask with 25 mL of 17.5% NaOH aqueous solution and stirred for 40 min at 20 °C. After 5 min, the residue was filtered, and 40 mL of a 10% acetic acid aqueous solution was added to the residue. The residue was filtered again and washed with 1 L of boiling water. The α-cellulose residue was filtered, dried at 105 °C for 48 h in vacuum, and weighed. The amount of hemicellulose was calculated by subtracting the amount of α-cellulose and pectin from holocellulose.

The lignin content in the sample was determined by Klason's method based on acid hydrolysis [22]. This procedure uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The lignin fractionates into acid insoluble material and acid soluble material. The acid soluble lignin content was measured using a UV–vis spectrophotometer at 240 nm (Hach, modelo DR/4000U, São Paulo, Brazil).

For total pectin determination, a previous sugar-extraction in DOP was perfomed using percolation with 95% ethanol. For the pectin de-esterification, the residue was mixed with Versene solution and the pH was adjusted until 11.5 with 1.0 N NaOH solution, followed by pH adjustment 5–5.5 with acetic acid glacial and pectinase addiction (Pectinase from Aspergillus niger, 1 U/mg; Sigma-Aldrich; St. Louis, USA). After the enzymatic reaction, the assay was made according the carbazole reaction colorimetric method [23], using galacturonic acid as standard. The absorbance was measured at 530 nm using a microplate reader (Synergy HT, Biotek, Winooski, VT, USA), with Gen5 2.0 data analysis software spectrophotometer. In all cases, the composition of the raw material was measured in three independent experiments, and reported as mean ± standard deviation.

2.3. Subcritical water extraction

2.3.1. Extraction system

A schematic diagram of the semi-continuous SWE unit is shown in