Separation and Purification Technology 149 (2015) 389–397

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Nanofiltration and reverse osmosis as a platform for production of natural botanic extracts: The case study of carob by-products

Mohammad Almanasrah [a,1], Carla Brazinha [a,*], Mari Kallioinen [b], Luís C. Duarte [c], Luísa B. Roseiro [c], Rafal Bogel-Lukasik [c], Florbela Carvalheiro [c], Mika Mänttäri [b], João G. Crespo [a]

[a] LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[b] Laboratory of Membrane Technology and Technical Polymer Chemistry, Department of Chemical Technology, Lappeenranta University of Technology, P.O. Box 20, Lappeenranta FIN 53851, Finland
[c] Laboratório Nacional de Energia e Geologia – Unidade de Bioenergia (LNEG-UB), Edifício K2, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 6 June 2015
Accepted 9 June 2015
Available online 10 June 2015

Keywords:
Nanofiltration
Diafiltration
Carob
Phenolic compounds
Natural extract

ABSTRACT

Carob kibbles are a low-cost and renewable source of economically relevant phenolic compounds (high value catechin and its derivatives and gallic acid) and abundant in small sugars. This work aims at producing two distinct natural extracts from carob kibbles, one extract enriched in catechin and its derivatives for the nutraceuticals market and an extract enriched in sugars for the food industry. This valorisation strategy involves an integrated process based on membrane technology that fulfils the zero discharge principle and may be applied to other agro-industrial by-products. Different aqueous extraction schemes were considered (a one-step process and a two-steps approach). The aqueous extracts obtained were fractionated by diananofiltration and the fractions obtained were evaluated in terms of their content in target products. An integrated scheme for production of fractionated extracts is proposed based on the experimental work developed assuring, simultaneously, a minimal use of resources and emission of waste.

1. Introduction

Carob by-products from the carob seed gum industry (carob kibbles), have a high content in marketable sugars and phenolic compounds, but are currently only used in low value applications such as animal feed and regional confectionary. The development of a valorisation strategy for carob kibbles should take into consideration these two classes of compounds. Besides the high concentration of mono- and disaccharides (glucose, fructose and sucrose), several studies reported that carob kibbles have a high variety of antioxidant phenolic compounds comparable to olive residues [1]. According to Avallone et al. [2] and Corsi et al. [3], water extractable carob phenolic compounds are mainly gallic acid and also (−)-catechin and its derivatives: (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate, also present in green tea. Furthermore, carob derived phenolic compounds have also been shown to present anti-tumural activities [4,5].

The valorisation strategy of carob kibbles should involve an effective separation between (more valuable) phenolic compounds and sugars, increasing the economic value of their crude extracts. The production of an extract from carob kibbles enriched in catechins and its derivatives is very attractive for the profitable nutraceuticals market. Additionally, the production of a natural extract enriched in mono- and disaccharides has also economical interest to the food industry in general. Nevertheless, this fractionation is a demanding task because sugars are much more abundant than phenolic compounds in carob kibbles extracts and the target compounds have similar molecular mass.

The release and recovery of phenolic compounds from carob kibbles was carried out by extraction with water, a common biocompatible solvent [3,6]. In our previous work [7], a one-step aqueous extraction from carob kibbles was optimised aiming the recovery of phenolic compounds. However, the extract obtained presented a high concentration in mono- and disaccharides and gallic acid. Therefore, a sequential two-step extraction process was developed in order to obtain two different fractions: a first extract enriched in sugars and gallic acid with a reduced concentration in phenolic

Abbreviations: c_i, concentration of compound i; D, number of diafiltration volumes; GAE, gallic acid equivalents; J_v, volumetric flux; L_p, permeability; MWCO, molecular weight cut-off; NF, nanofiltration; p, pressure; R_i, apparent rejection of compound i; RO, reverse osmosis; VRF, volume reduction factor; V_w, volume of solvent added to the retentate; subscript perm, permeate.

* Corresponding author.
E-mail address: c.brazinha@fct.unl.pt (C. Brazinha).
[1] Permanent address: Laboratory of Membrane Technology and Technical Polymer Chemistry, Department of Chemical Technology, Lappeenranta University of Technology, P.O. Box 20, Lappeenranta FIN 53851, Finland.

http://dx.doi.org/10.1016/j.seppur.2015.06.008
1383-5866/

compounds, and a second extract enriched in higher molecular mass phenolics, almost free of sugars and gallic acid. Actually, this process made possible the production of a first step extract enriched in sugars, free of valuable phenolic compounds, despite a minor contamination in gallic acid. A second step extract, enriched in valuable phenolic compounds was also obtained, with a minor contamination in gallic acid, but still with a significant presence of sugars (although less contaminated in sugars than the extract produced with the one-step extraction protocol).

This work discusses the process developed for further improving the separation between valuable phenolic compounds from sugars and gallic acid. In order to separate the target phenolic compounds from sugars and gallic acid (which have lower molecular mass) it was decided to select a highly retentive membrane for the phenolic compounds. The strategy was to retain as much as possible the high molecular mass species. This approach allows for obtaining permeates essentially free of phenolic compounds and assure the quantitative retention of these valuable target compounds. Additionally, if economically relevant, the permeating species may be further processed in order to concentrate them, using reverse osmosis.

Once the highly retentive membrane was selected, two different operating approaches could be followed: fractionation by nanofiltration, namely using a cascade approach suggested by Refs. [8,9]; or a diananofiltration using water as a washing solvent, where sugars and gallic acid are soluble, being removed through the permeate. Both approaches could be considered, but in this work it was decided to use a diananofiltration approach because the feed stream is highly viscous due to the elevated concentration of sugars. Diananofiltration with addition of fresh washing solvent allows for operation under relatively mild and controlled viscosity conditions, enabling for operation under suitable external mass transfer conditions, with advantage over nanofiltration. When this restriction imposed by the viscosity of the feed stream is not relevant, the use of nanofiltration cascades with recycle of the retentates should be taken into consideration [8,9].

Diananofiltration is commonly used for the removal of genotoxins from active pharmaceutical ingredients [10]. Several examples of diananofiltration in agro-industrial applications are also reported in literature, namely in the partial removal of the monovalent salts from oligosaccharides in a model solution of soybean [11] and from high value protein and lactose in a cottage cheese whey [12], or to remove mainly aliphatic acids and furfural derivatives from sugars (xylose, arabinose and glucose) in olive pomace aqueous extracts [13]. Nevertheless, the use of diananofiltration for the recovery/purification of valuable phenolic compounds has only been reported, to our knowledge, in the removal of pesticides and recovery of bioactive steryl esters from deodorizer distillates [14,15].

This work aims for recovering, fractionating and purifying valuable phenolic compounds (catechin and its derivatives) from small sugars (glucose, fructose and sucrose) present in carob by-products. The valorisation strategy was defined with the objective of producing one purified extract rich in catechin and its derivatives for the profitable nutraceuticals market and one purified extract rich in small sugars for the food industry, increasing the overall value of carob extracts. This strategy involved the development of an integrated process based on membrane technology that fulfils the zero discharge principle. The approach followed may be easily extended to other agro-industrial by-products.

2. Experimental

2.1. Materials

Carob (*Ceratonia siliqua* L.) kibbles (chopped and deseeded carob pods) were obtained from a local de-seeding factory (Algarve, Portugal), and then stored in plastic containers at room temperature in a dark and dry place prior to use. Screening of the kibbles using selected sieves (Retsch, Germany) with different pore sizes and an appropriate sieve shaker (EVS1, Endecotts, England) showed that 27% was larger than 8 mm, 40% of the material was between 8 and 4 mm, 21% between 2 and 4 mm, and only 11% of the kibbles was smaller than 2 mm. Aqueous extracts from carob kibbles were produced as described previously [7]. The membranes used in this work are described in Table 1, with specifications obtained from the producers. Desal 5 DK, which is a hydrophilic nanofiltration membrane, was chosen in order to enable the permeation of the hydrophilic sugars and gallic acid. A membrane with a higher molecular mass cut-off was not chosen in order to assure a total rejection of hydrophobic catechin (molecular mass of 290.3 Da) and its derivatives with molecular mass between 400 and 500 Da. The membrane SW30, a reverse osmosis membrane, was chosen in order to assure a total rejection of mono- and disaccharides (glucose and fructose with a molecular mass of 180 Da and sucrose 342 Da).

2.2. Membrane processing

The membrane experiments performed are summarised in Table 2. The one-step extract was centrifuged (12,000 rpm, 10 min at 4 °C) and the 2nd extract of the two-steps extraction process was pre-filtered with a 20 μm fabric filter for 15 min. In order to exhibit a viscosity value similar to water at 20 °C (1 mPa s) and hence enabling membrane processing, the one-step extract was diluted with water at a mass dilution ratio of 3.9 and the extracts used in both diafiltration processing (see Table 2), were heated at 50 °C. High temperature and relatively low transmembrane pressure values were chosen during diananofiltration experiments, in order to assure the lowest possible apparent rejection values of gallic acid and sugars and, consequently, enhance their separation from catechin and its derivatives.

The one-step experiment was carried out with the membrane unit operated in a dead-end mode with a gas control unit (METCell, Membrane Extraction Technology, UK) at a rotor speed of 300 rpm, using a Desal 5DK flat sheet membrane. The temperature of the retentate was maintained constant by a controlled temperature bath. The 2nd extract of a two-steps extraction process was operated in a cross-flow mode. The membrane modules used had a spiral wound configuration with dimensions 2.5″ diameter and 40″ length: a Desal 5 DK 2540 C1076 nanofiltration module and a SW30 2540 reverse osmosis module. The temperature of the retentate was maintained constant using a heat exchanger.

In each membrane experiment (diananofiltration, nanofiltration and reverse osmosis experiments), the initial and the final feed solution, cumulative permeate samples (permeate accumulated up to a defined time, t) and "instantaneous" permeate samples collected along time (instant permeate samples collected at time "t") were characterised in terms of content in sugars (glucose, fructose, sucrose), in total phenolic compounds and in gallic acid (see below). The apparent rejections of total phenolic compounds TP, glucose, fructose, sucrose, and gallic acid, R_i (%), were calculated during the diananofiltration experiments through Eq. (1):

$$R_i = 1 - \frac{c_{i,perm}}{c_{i,feed}} \quad (1)$$

where $c_{i,perm}$ (g/L) and $c_{i,feed}$ (g/L) are respectively the (instant not accumulated) concentration of compound i under study in the permeate and the feed (retentate) compartments.

During diafiltration, the volume of water added to the retentate, V_w (L), is the volume required to maintain the retentate volume at a constant value since the beginning of the experiment, V_{feed} (L). The number of diafiltration volumes, D (–), is the ratio between the