Industrial Crops and Products 70 (2015) 443–450

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction

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

[a] Unidade de Bioenergia, Laboratório Nacional de Energia e Geologia, I.P. (LNEG), Ed. K2, Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal
[b] REQUIMTE/CQFB, Department of Chemistry, FCT, Universidade Nova de Lisboa, P-2829-516 Caparica, Portugal
[c] Laboratory of Separation Technology, LUT Chemtech, Lappeenranta University of Technology, P.O. Box 20, Lappeenranta, FIN-53851, Finland

ARTICLE INFO

Article history:
Received 10 August 2014
Received in revised form 21 January 2015
Accepted 22 February 2015
Available online 6 April 2015

Keywords:
Aqueous extraction
Biorefinery
Fermentable sugars
Glucose fructose syrup
Scale-up

ABSTRACT

Carob kibbles are an important renewable source of valuable compounds, such as fermentable sugars and phenolic compounds. However, the selective recovery of these compounds is not a trivial task. In this work, a strategy was developed to enable the recovery of both classes of compounds by means of a water-based extraction.

One-step extraction recovered only approximately 20% of the phenolic compounds, corresponding to an extraction yield of 0.6 g Gallic acid equivalents (GAE)/100 g dry mass of carob kibbles. The obtained extract contained a significant amount of carbohydrates (110 g/L). The alternative two-step extraction developed enabled higher compound selectivity together with an increase in the yield of the phenolic compounds to about 70%, corresponding to 1.9 g_{GAE}/100 g carob dry matter.

The two-step extraction was easily scaled-up and is an effective method to obtain significantly separated carbohydrates and polyphenol-rich streams that can be further processed, e.g., in biorefineries or food industries, respectively.

1. Introduction

Agro-food industry wastes, such as olive, grape and carob residues, can be exploited as raw materials for various valuable products, and these agro-food residues have received considerable attention as an abundant and inexpensive renewable resource for chemical, biotechnological and pharmaceutical applications. Carob kibbles, in particular, are recognized as having great potential, not only due to its high content of easily fermentable sugars, but also due to its phenolic content. Carob kibbles are the result of mechanical treatment of carob pods, using a kibbler, after the high-value seeds (~10% of the carob pod weight) have been extracted for the production of locust bean gum (Albergaria et al., 1999). Currently, carob kibbles are mainly used as animal feed, despite its valuable composition. The soluble sugars in carob kibbles can reach up to 50% on a dry basis (Roseiro et al., 1991a; Petit and Pinilla, 1995; Avallone et al., 1997) and the kibbles can be used in a wide range of applications, including the production of ethanol, citric acid (Roukas, 1998), xanthan (Roseiro et al., 1991b) and mannitol (Carvalheiro et al., 2011), as well as the specialty chemical pinitol (Macias Camero and Sanjuan Merino, 2003). Several studies have drawn attention to this residue as a good source of valuable phenolic compounds (Owen et al., 2003; Makris and Kefalas, 2004).

The phenolic compounds are a group of very diverse chemicals that include e.g., phenolic acids and aldehydes, hydroxycinnamic acids and its derivatives, flavonoids, lignans, or tannins (Manach et al., 2004). Many of these compounds have been shown to present useful traits that support their use as bioactive compounds for human health. As such, the study of their selective recovery is a significant scientific and industrially applied relevant topic. Moure et al. (2001) reviewed various extraction and recovery methods of antioxidant compounds (mainly phenolic compounds) from agricultural and industrial residues, and this has been complemented by other studies, e.g., considering residues such as olive seeds and olive mill waste water (Paraskeva and Diamadopoulos, 2006; Marco et al., 2007), grape seeds (Casazza et al., 2011), potato peels (Singh and Saldaña, 2011), carob (Turhan et al., 2006), and many other biomass residues (Junior et al., 2010).

* Corresponding author. Tel.: +351 210924600; fax: +351 969069117.
E-mail address: luis.duarte@lneg.pt (L.C. Duarte).
[1] Permanent address: Laboratory of Separation Technology, LUT Chemtech, Lappeenranta University of Technology, P.O. Box 20, Lappeenranta FIN-53851, Finland.

http://dx.doi.org/10.1016/j.indcrop.2015.02.051
0926-6690/

In carob kibbles, the major phenolic compounds present are condensed tannins (Marakis, 1996), but many other phenolic compounds can be recovered by water extraction. For instances, different forms of gallic acid, including the free form and its derivatives (gallotannins and methyl gallate); catechin, flavonol glycosides, myricetin rhamnoside, eriodictyol glycoside, quercetin glycoside and quercetin rhamnoside have been described (Avallone et al., 1997; Corsi et al., 2002; Papagiannopoulos et al., 2004). As such, relevant bioactivities have been described in carob, namely antioxidant activity (Kumazawa et al., 2002; Makris and Kefalas, 2004), together with others e.g., anti-proliferative activity for special cancer lines (Roseiro et al., 2011).

Several methodologies have been used for the extraction of phenolic compounds from carob residues. Papagiannopoulos et al. (2004) found that acetone-water extraction produces a higher yield of phenolic compounds than water extraction. Owen et al. (2003) claimed that Soxhlet extraction using methanol gives higher yield and greater variety in phenolic compounds content than water batch extraction at room temperature. Aqueous acetone extraction was found to be highly efficient in the recovery of phenolic compounds from carob pods. The extraction was performed as two room-temperature water extractions to remove carbohydrates from the carob pods before subjecting the solid residues to boiling water extraction (Kumazawa et al., 2002). Recently, supercritical CO_2 extraction has also been tested for the recovery of phenolic compounds from de-sugared carob kibbles (Bernardo-Gil et al., 2011). Some of these methods have demonstrated good performance at lab-scale; however, their conversion to a larger scale is not without challenges. Water-extraction based processes can overcome some of these problems and performs well at a larger scale, in both economic and environmental terms.

The present work aims to develop a sustainable, efficient and selective water-based extraction procedure for the recovery of phenolic compounds and sugars from carob kibbles that is suitable for scale-up from lab-scale to industrial scale. Two different strategies are tested, a single-step and a two-step procedure. The fractionation should support further application of the carbohydrates and phenolic compounds independently, for example, as a biorefinery substrate or as raw materials for the food and pharmaceutical industries.

2. Materials and methods

2.1. Carob kibbles

Carob (*Ceratonia siliqua* L.) kibbles (chopped and deseeded carob pods) were obtained from a local de-seeding factory in Algarve, Portugal, and then stored in plastic containers at room temperature in a dark and dry place prior to use. The moisture content of the carob kibbles was 10–13%. 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.

2.2. One-step extraction

Carob kibbles were subject to aqueous extraction by mixing with water at liquid-to-solid ratios (LSR) between 2 and 50, w/w. To enable optimization of the LSR, five extractions were performed in duplicate using an appropriate amount of carob kibbles (100 g, 50 g and 25 g, for higher ratios) and purified water based on the tested LSR. The extractions were carried out for 5 h in an orbital incubator (Infors Unitron HT, Switzerland) set at 50 °C.

To enhance the extraction yield, the liquid extract was separated from the remaining solids using a manual hydraulic press (Sotel, Portugal) up to 200 bar. Suspended solids in the extract were removed by centrifugation using a Heraeus Sepatech centrifuge (12,000 rpm, 10 min at 4 °C) and the liquid extract was stored in a freezer until further use.

The extraction conditions were further optimized by studying the effects of temperature (30–100 °C) and time (20–300 min), based on previous knowledge and the scale-up potential/practicality. The assays were carried out either in the orbital incubator described above or in a water bath (for temperatures >80 °C).

A Doehlert experimental design (Doehlert, 1970) consisting of 7 sets of experiments A–G (Table 1) was applied to establish the experimental conditions. All assays were done at least in duplicate to provide a measure of the inherent experimental error.

The model used to express the responses was a second-order polynomial model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \epsilon \quad (1)$$

where Y is the response, X the independent variables, and the subscripts 1 and 2 refer to extraction temperature and time, respectively. β_0 is the regression coefficient at the center point; β_1 and β_2 are the coefficients of the variables 1 and 2 (main effects), respectively; β_{12} is the two-factor interaction coefficient between variables 1 and 2; and β_{11} and β_{22} are the quadratic coefficients for variables 1 and 2; ϵ is independent random errors, assumed to be normally and independently distributed. The linear multiple regressions to Eq. (1) and its analysis of variance (ANOVA) were carried out using the Microsoft® Excel 2010 regression tool pack. All replicates were used. The best water extraction conditions were determined using the Microsoft Excel® 2010 Solver tool based on the best-fit equation using a constrained model. Coded representation of the variables was used for all calculation purposes (X_1 = temperature; X_2 = time).

2.3. Two-step extraction

In the first step, the extraction temperature was set at 30 °C. Two different LSR (10 and 20) and extraction times (210 min and 300 min) were tested in order to achieve the highest carbohydrates yield together with the lowest total phenolics removal.

After definition of the operating conditions for the first water extraction step, the two-step water extraction was tested using 100 g and 1.2 kg of carob kibbles. After the first step, the liquid fraction was separated from the remaining solids by pressing using the hydraulic press described above. The pressed solids were recovered and sampled (1–2 g) to rapidly quantify moisture content in an automatic AMB 50 moisture balance (Adam Equipment, CT), in order to determine the amount of water needed to achieve the proper LSR for second step extraction. The optimized operation conditions from the one-step extraction experiments were used in

Table 1
Codified matrix for the Doehlert experimental design for two variables and the corresponding experimental matrix. Each row represents an experimental trial.

Variables Coded			Real	
Trial	X_1	X_2	Temperature (°C)	Time (min)
A	−1.00	0.00	30	150
B	0.00	0.00	65	150
C	1.00	0.00	100	150
D	−0.5	−0.866	47.5	20
E	−0.5	0.866	47.5	280
F	0.5	−0.866	82.5	20
G	0.5	0.866	82.5	280