Food Chemistry 141 (2013) 3664–3669

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Food-compatible method for the efficient extraction and stabilization of cranberry pomace polyphenols

Diana E. Roopchand [a,*], Christian G. Krueger [b,c], Kristin Moskal [d], Bertold Fridlender [e], Mary Ann Lila [f], Ilya Raskin [a,*]

[a] Rutgers, The State University of New Jersey, School of Environmental and Biological Sciences, Foran Hall, 59 Dudley Road, New Brunswick, NJ 08901, USA
[b] Complete Phytochemical Solutions, LLC, 317 South Street, Cambridge, WI 53523, USA
[c] University of Wisconsin-Madison, Reed Research Group, Dept. of Animal Sciences, 1675 Observatory Drive, Madison, WI 53706 USA
[d] Nutrasorb LLC, 675 US Highway 1, North Brunswick, NJ 08902, USA
[e] Hadassa Academic College, 37 Hanevi'im Jerusalem, Israel
[f] Plants for Human Health Institute, North Carolina State University, 600 Laureate Way, Kannapolis, NC 28081, USA

ARTICLE INFO

Article history:
Received 18 January 2013
Received in revised form 2 May 2013
Accepted 12 June 2013
Available online 21 June 2013

Keywords:
Cranberry pomace
Polyphenols
Proanthocyanidins
Anthocyanins
Soy protein isolate

ABSTRACT

Cranberry pomace is a byproduct of cranberry processing and is comprised of seeds, skins and stems of the cranberry fruit. While cranberry pomace contains beneficial polyphenols, including proanthocyanidins and anthocyanins, it is not a palatable source of these compounds and is typically discarded. In this study, we have developed and optimized a method to extract polyphenols from cranberry pomace using aqueous ethanol, a food grade solvent. Biochemical characterization of the pomace extract showed the presence of a broad range of polyphenols also present in cranberry juice concentrate. By co-drying cranberry pomace extract with a protein-rich food matrix, such as soy protein isolate (SPI), we have developed a method to produce a cranberry polyphenol–SPI complex (CBP-SPI) containing 10% cranberry polyphenols. Unlike dried cranberry pomace extract alone, proanthocyanidins, anthocyanins and total polyphenols were found to be highly stable at 37 °C in the CBP-SPI powder. The extraction and stabilization of cranberry pomace polyphenols using SPI provides an innovative approach for utilizing pomace in the development of novel food ingredients.

1. Introduction

Cranberry fruits (*Vaccinium macrocarpon* Ait) are a rich source of type A proanthocyanidins, anthocyanins, flavonols, phenolic acids, benzoates, hydroxycinnamic acids, terpenes and organic acids (Pappas & Schaich, 2009). Research has associated several health benefits with the consumption of cranberry. Cranberries components are antimicrobial against *Staphylococcus*, *Salmonella* and *Escherichia*, which are responsible for food-borne illness and human disease (Puupponen-Pimia, Nohynek, Alakomi, & Oksman-Caldentey, 2005). Cranberry polyphenols elicit antimicrobial activity via mechanisms independent of the acidic pH (Lacombe, Wu, Tyler, & Edwards, 2010). Cranberry is used as a prophylactic for urinary tract infection (UTIs) (Barbosa-Cesnik et al., 2011; Epp et al., 2010; Jepson & Craig, 2008) and the presence of A-type proanthocyanidins has been associated with preventing the adhesion of uropathogenic *E.coli* to uroepithelial cells (Howell et al., 2005; Sobota, 1984). *In vitro* studies have also shown that cranberry can prevent the adhesion of *H. pylori* to gastric mucosal cells (Burger et al., 2000; Burger et al., 2002; Shmuely et al., 2004) and in a clinical study the daily consumption of cranberry juice cocktail for 90 days was shown to decrease the *H. pylori* infection in adults compared to placebo (Zhang et al., 2005).

Cranberries are commonly consumed as dried fruit or juice adulterated with large amounts of sugar. The sugar used to mask the tartness of cranberry adds excess calories and can counteract the beneficial effects of the fruit. Earlier, we developed a technology that uses protein-rich legume flours or powders, such as soy protein isolate (SPI), to sorb, concentrate and stabilize mid-polarity range cranberry phytochemicals from cranberry juice concentrate while separating them from excess water, sugar and lipid components (Roopchand et al., 2012). Cranberry pomace, a byproduct of the cranberry processing industry, is composed of dietary fiber from skins, seeds and stems (White, Howard, & Prior, 2010) and is typically discarded. Due to its acidity and low protein content it has limited use as animal feed and disposal in landfills poses an environmental problem due to low pH. Cranberry pomace still

Abbreviations: SPI, soy protein isolate; CBP-SPI, cranberry polyphenol-SPI complex; ACN, anthocyanin; PAC, proanthocyanidin.

* Corresponding authors. Tel.: +1 (848) 932 8165; fax: +1 (732) 932 6535 (D.E. Roopchand), tel.: +1 (848) 932 6267 (I. Raskin).
E-mail addresses: roopchand@aesop.rutgers.edu (D.E. Roopchand), raskin@aesop.rutgers.edu (I. Raskin).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.050

contains an assortment of beneficial phytochemicals, such as polyphenols, which have a natural affinity for proteins. Soy protein isolate (SPI) made from soybeans is an inexpensive readily available protein that is frequently incorporated into foods and supplements. Compared to animal proteins such as casein, SPI was shown to be hypocholesterolemic (Nagata, Ishiwaki, & Sugano, 1982), decrease the activity of lipogenic enzymes (Iritani, Nagashima, Fukuda, Katsurada, & Tanaka, 1986), and reduce body fat in diet-induced obese rats and mice (Aoyama, Fukui, Takamatsu, Hashimoto, & Yamamoto, 2000). Therefore we have developed a food-compatible method for the extraction of cranberry pomace and subsequent stabilization of cranberry phytochemicals via complexation to SPI to produce a novel phytochemical-enriched protein that can be used as a food ingredient.

2. Materials and methods

2.1. Optimization of method for cranberry pomace extraction

Frozen depectinized cranberry pomace (provided by BNK, Wisconsin Rapids, WI) was used in all analyses. Cranberry pomace (30 g wet wt.) was pureed in a Vitamix blender with 300 ml of water or 50% ethanol (10:1 extraction). Each puree was aliquoted into 40 ml volumes in 50 ml tubes and the pH was adjusted from 4 to 2, 3 or 5 with HCl or NaOH to investigate the effect of pH on extraction efficiency. The material was then extracted in loosely capped tubes in an 80 °C water bath for 2 h. The samples were centrifuged at 4000 rpm for 10 min to separate solids from the liquid extract. The concentration of total polyphenols and proanthocyanidins in each extract was determined using the Folin–Ciocalteu (Singleton & Rossi, 1965) and 4-(dimethylamino)cinnamaldehyde (DMAC) methods (Prior et al., 2010), respectively.

Three independent lots of frozen depectinized cranberry pomace were extracted to compare solvent to pomace ratio and percentage of ethanol on polyphenol extraction efficiency. A 10 g (wet wt.) sample was removed from each lot and the dry weight of each was determined by freeze drying. A 100 g (wet wt.) sample of each lot of cranberry pomace was pureed in a Vitamix blender with 1 l of 50% ethanol (10:1 extraction) or 500 ml of 50% ethanol (5:1 extraction). Each puree was adjusted to pH 2 with HCl and the material was then extracted in a rotary evaporation flask (without vacuum) in an 80 °C water bath for 2 h (Buchi, Switzerland). Any evaporated solvent collected in the catch flask was added back to the extraction flask. The samples were centrifuged at 4000 rpm for 10 min to separate the solids from the extract, which was then filtered through miracloth (Calbiochem). Similarly, 100 g (wet wt.) of each lot of cranberry pomace was also extracted with 1 l of 75% ethanol (10:1 extraction) or 500 ml of 75% ethanol (5:1 extraction). The volumes of extract recovered were measured and the concentrations of total polyphenols and proanthocyanidins in each extract were quantified as described above. The dry weight of each extract was determined by vacuum drying 1 ml aliquots of liquid extract. These data were then used to calculate the percentage of total polyphenols and proanthocyanidins in the dried extract. Proanthocyanidins as a percentage of total polyphenols was also calculated.

2.2. Biochemical characterization of cranberry pomace extract

Cranberry pomace (50 g wet wt.) was blended with 250 ml of 50% ethanol, mixture was adjusted to pH 2 and then extracted in a flask at 80 °C for 2 h on a shaking water bath. The cranberry pomace extract was filtered (0.22 μm) prior to analysis. Samples were separated and analyzed with a Shimadzu LCMS-2010A high performance liquid chromatography/electrospray ionization/single quadrupole. The LCMS is equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10ADvp), column oven (CTO-10ACvp), photodiode array detector (SPD-M10Avp), and a single quadrupole analyzer. The HPLC column was a Widepore C5, 5 μm, 2.1 × 150 mm (Supelco, Bellefonte, PA). The mobile phase consisted of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) at a flow rate of 0.2 ml/min. The gradient used was: initially 90% A and 10% B using an isocratic flow for first 5 min; 85% A and 15% B over 15 min, followed by isocratic elution at 85% A and 15% B for 5 min; 100% A and 0% B over 2 min, followed by an isocratic elution at 100% A and 0% B for 5 min. There was a 13 min equilibration interval between injections.

For characterization of proanthocyanidins in the cranberry pomace extract, mass spectra were collected on a Bruker ULTRAFLEX®III MALDI TOF/TOF mass spectrometer (Billerica, MA, USA) equipped with delayed extraction and a SmartBeam® laser. All analyses were performed in positive linear mode. Spectra were the sum of 8–10 different locations in each well, accumulating a total of 400–500 shots to minimize intra-well variability and avoid heterogeneous co-crystallization spots. Threshold laser power was used to achieve optimal isotope patterns. The matrix was 2, 5-dihydroxybenzoic acid (DHB) at a concentration of 50 mg/ml in ethanol. FlexControl and FlexAnalysis (Bruker Daltonik GmbH, Bremen, Germany, version 3.0) were used for data acquisition and data processing, respectively. mMass (version 3.9.0) was used for spectra analysis. Based on previously described structures an equation was developed to predict the mass distribution of PAC in cranberry products (Reed, Krueger, & Vestling, 2005). The equation is $290 + 288a - 2b + 23$, where 290 represents the molecular weight of the terminal catechin/epicatechin unit, a is the degree of polymerization of catechin/epicatechin units, b is the number of A-type interflavan bonds, and 23 is the molecular weight of sodium.

2.3. Production of cranberry polyphenol-SPI complex

Cranberry pomace (300 g wet wt.) was extracted as described in Section 2.1 and 2.4 l of liquid extract was collected after separating the cranberry pomace solids; the concentrations of total polyphenols and proanthocyanidins were quantified in the extract. Three 1 ml aliquots of extract were dried in a speed vacuum and the average dry weight per ml was determined. A calculated amount of soy protein isolate (SPI; ADM, Decatur, IL) was added to the liquid cranberry pomace extract so that after solvent evaporation the result is a cranberry polyphenol-SPI complex (CBP-SPI) containing 10% total polyphenols. The dry weight of the 2.4 l of cranberry pomace extract was 19.1 g containing 3.04 g of total polyphenols. The final amount of CBP-SPI was therefore calculated to be 30.4 g to have the cranberry polyphenol content standardized to 10%. Therefore, the amount of SPI to be added to the cranberry extract was calculated as follows: 19.1 g + x = 30.4 g; x = 11.3 g of SPI.

After SPI was added to the cranberry pomace extract, ethanol was removed by rotary evaporation under vacuum (Büchi, Switzerland) with temperature set at 40 °C. The remaining liquid was removed by freeze-drying to yield CBP-SPI powder.

2.4. Temperature stability of polyphenols in CBP-SPI compared to cranberry pomace extract

Cranberry pomace extract was prepared as described in Section 2.1. A portion was used to make CBP-SPI containing 10% total polyphenols as described in Section 2.3 and a portion was dried to make cranberry pomace extract powder (CBP extract). The CBP-SPI and CBP extract were placed in a 37 °C incubator and aliquots of each sample were removed at indicated times. The dried cranberry pomace extract (10 mg) or CBP-SPI complex (100 mg) was eluted 3 times with 1 ml of 50% acetone. Proanthocyanidins were quantified