Industrial Crops and Products 63 (2015) 79–86

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Assessment of antioxidant and antibacterial potential of borojo fruit (*Borojoa patinoi* Cuatrecasas) from the rainforests of South America

Clemencia Chaves López [a], Giovanni Mazzarrino [a], Aida Rodríguez [b], Juana Fernández-López [c], José A. Pérez-Álvarez [c], Manuel Viuda-Martos [c,*]

[a] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via C.R. Lerici 1, 65023 Mosciano Stazione, TE, Italy
[b] Facultad de Ingeniería, Escuela de Ingeniería de Alimentos, Universidad del Valle Ciudad Universitaria Meléndez, Cali, Colombia
[c] IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana), AgroFood Technology Department, Miguel Hernández University, Orihuela, Spain

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 15 October 2014
Accepted 24 October 2014
Available online 10 November 2014

Keywords:
Borojo
Volatile profile
Antioxidant
Antibacterial activity

ABSTRACT

Borojo (*Borojoa patinoi*) is an endemic fruit of the rainforests of Colombia, Brazil, Peru, Ecuador and Panama that is listed for approval as novel food in the European Union. In this work the antioxidant activity (measured with DPPH, FIC and FRAP), the organic acid and sugar contents, the volatile profile and the antibacterial properties against 26 pathogenic and non-pathogenic strains were studied. Total phenol contents (TPC) ranged from 36.41 to 53.6 mg GAE/100 g fw while total flavonoids (TFC) from 88.45 to 49.83 mg RE/100 g fw. The extract showed also good antioxidant activity highly correlated to TPC and TFC. Among 21 volatile compounds detected by SPME–GC–MS, 2-nonanol represented the main component. The antimicrobial activity was in a decreasing order: *Salmonella enteritidis* > *Salmonella typhimurium* > *Listeria monocytogenes* > *Staphylococcus aureus* > *Brochotrix thermosphacta*. The biological properties of Borojo-fruit suggested that it is a promising new antioxidant and antibacterial agent.

1. Introduction

In South America, mainly Colombia, Brazil, Ecuador or Peru, a wide range of fruits from tropical and subtropical areas exist with different degrees of importance to the fruit sector, depending on their economic and social impact. Many of these fruits are produced and consumed in local markets and are rarely exported, often because of their perishability and the lack of knowledge of their sensory properties and nutritional value.

Borojo (*Borojoa patinoi* Cuatrecasas) is the name of a plant species belonging to the *Rubiaceae* family and endemic to the rainforests of Colombia, Brazil, Ecuador and Panama. The fruit is an edible berry 7–12 cm in length, generally flat at the apex, green in colour at the beginning and light brown at maturity; pulp constituted mesocarp and the endocarp, with no apparent separation from the shell, weight between 740 and 1000 g, with an average of 330 seeds per fruit. The pulp represents 88% of the total fruit weight, the water is about 64% and the water activity near to 0.91 (Sotelo et al., 2010). It exhibits an intense floral aroma and sweet acid taste and may be stored for refrigeration or at room temperature for up to 6 months (Diaz-Ocampo et al., 2012). Borojo pulp is used to prepare processed products like jelly, sauces, marmalades and juices. This fruit is also used in the traditional medicines with supposed antihypertensive, antitumoral, diuretic, healing and aphrodisiac effects (Gentry, 1988). It is also used by the local communities against bronchial diseases, gastritis and malnutrition.

Colombia produces nearly 17,000 t of borojo and the consumption of this fruit is increasing in both domestic and international markets due to the growing recognition of its value to human health (Contreras-Calderón et al., 2011); thus, the dynamic exportation of this fruit is linked to the new preferences of the international markets. In fact, this fruit has been famous for its health properties. For this reason, it is a special source of income for some of the native population who sell it in the local food markets in the main Colombian cities. Actually, it is listed for approval as novel food in the European Union. Studies have shown that borojo pulp has a pH of 2.9 and contains fat (0.15%), protein (0.69–0.78%), dietary fibre (23.58%), vitamins (C, B2, B3) and minerals (P, Fe, Ca, K) (Mosquera et al., 2010; Diaz-Ocampo et al., 2012,). Moreover, the presence of organic acids such as ascorbic, oxalic acid in the pulp has been also reported (Contreras-Calderón et al., 2011). On the other hand, triterpenes, flavonoids and phenols have been reported, in particular the total phenol content is ranged between 28 mg of GAEs/100 g and 253 mg of GAEs/100 g (Sotelo et al., 2010; Contreras-Calderón

* Corresponding author at: AgroFood Technology Department, Escuela Politécnica Superior de Orihuela, Crta. Beniel km. 3,2, E 03312 Orihuela, Alicante, Spain. Tel.: +34 966749737; fax: +34 966749677.
E-mail address: mviuda@umh.es (M. Viuda-Martos).

http://dx.doi.org/10.1016/j.indcrop.2014.10.047
0926-6690/

et al., 2011). In a study conducted by Toledo-Romaneiko (2009) on six different borojo clones, it has been evidenced that the nutritional composition of borojo depends on the type of clone studied; in fact, values of Fe ranged between 22 and 70 mg/kg, vitamin A between 764 and 3015 UI/100 g and vitamin C between 14 and 22 mg/100 g; also total phenols content ranged between 39.6 and 112 mg of GAEs/100 g.

Besides the healthy promoter characteristics, borojo has showed antimicrobial activity towards *Escherichia coli* and *Staphylococcus aureus*, and this activity has been attributed to the presence of polyphenols (Sotelo et al., 2010). Actually in Colombia there is an interest to generate high value-added products from borojo which maintain nutritional properties of the pulp or seed. Thus, the recovery of valuable compounds from natural resources is nowadays conducted using the so called "5-stage universal recovery processing" (Galanakis, 2012).

The aims of the present work were to determine (i) the organic acid and sugar contents, (ii) the volatile profile and (iii) to evaluate the antioxidant and antibacterial properties of borojo (*B. patinoi* Cuatrecasas) fruit from the rainforests of Colombia.

2. Materials and methods

2.1. Plant material

A total of twelve Borojo (*B. patinoi*) fruits with no visible external cuts or spoilage were purchased from a local market in Cali (Colombia).

2.2. Sample preparation

Borojo fruits were cleaned with tap water and then separated into peel and pulp (the edible part) and seeds (the non-edible part). Immediately, the edible portion was chopped and homogenized for 10 s whilst the non-edible portion was discarded. The time between chopping the fruit and beginning the extraction was 5 min.

To obtain the extracts, three different methodologies were used. In the first procedure 10 g of the edible part of borojo were placed in a capped centrifuge tube and 30 mL of methanol–water (80–20, v/v) were added, after which the mixture was homogenized in an Ultra-Turrax (IKA, T25D, Staufen, Germany) during 3 min at 18,000 rpm. The tube was then centrifuged at 2739 × *g* for 20 min at 4 °C and the supernatant was transferred to a round-bottomed flask and evaporated to dryness using a rotary evaporator R-205 (Büchi, Flawil, Switzerland) under reduced pressure (<100 mbar) at 40 °C. Five millilitres of methanol were added to the residue, and the mixture was well shaken in a Vortex for 2 min.

In the second procedure, ten grams of borojo samples were mixed with 30 mL of ethanol, vortexed for 1 min and homogenized in an Ultra-Turrax during 3 min at 18,000 rpm. The tube was then centrifuged at 2739 × *g* for 20 min at 4 °C and the supernatant was recovered. Thirty millilitres of acetone–water (70:30, v/v) were added to the residue, followed by shaking, homogenizing and centrifugation. Supernatants were combined and evaporated to dryness using a rotary evaporator R-205 under reduced pressure at 40 °C. Five millilitres of methanol were added to the residue, and the mixture was well shaken in a Vortex for 2 min.

In the third procedure, ten grams of borojo fruit were extracted with 10 mL of water using an ultrasonic water bath (Selecta S.A. Barcelona, Spain) without temperature control, during 3 h. Then, the mixtures were centrifuged at 2739 × *g* for 20 min at 4 °C. After centrifugation supernatants were filtered through a 0.45 μm Millipore filter (Millipore Corporation, Bedford, USA).

The extracts obtained were stored at −20 °C and measured before 24 h. The three fractions obtained were: borojo extracted with methanol (BE_M), borojo extracted with ethanol:acetone (BE_{EA}) and borojo extracted with water (BE_w).

2.3. Total phenol content

To determine the total phenol content (TPC) of borojo extracts the Folin-Ciocalteu's reagent (Singleton and Rossi, 1965) was used. The results were expressed as mg Gallic acid equivalents (GAE)/100 g sample (fresh weight (fw)). Each assay was carried out in triplicate.

2.4. Total flavonoid content

For the total flavonoid content (TFC), the method described by Blasa et al. (2005) was used. The results were expressed in mg rutin equivalents (RE)/100 g of sample (fw) as mean of three replicates.

2.5. Determination of polyphenolic compounds

Twenty microliters of the different extracts (BE_M, BE_{EA} and BE_w) were injected into a Hewlett-Packard HPLC series 1100 instrument (Woldbronn, Germany) equipped with UV-Vis Diode Array Detector. Separations were realized on a C_{18} Teknokroma column (Mediterranea sea_{18}, 25 cm × 0.4 cm, 5 μm particle size, Teknokroma, Barcelona, Spain). Spectral data from all peaks were accumulated in the range 200–400 nm, and the chromatograms were recorded at 280, 320 or 360 nm. Phenolic compounds were analyzed, in standard and sample solutions, using a gradient elution at 1 mL/min with the following gradient programme, started with 95% A, 75% A at 20 min, 50% A at 40 min, 20% A at 50 min and 20% A at 60 min. The mobile phase was composed by formic acid:water (4.5:95.5) (A) and acetonitrile as solvent B according to a described procedure (López-Vargas et al., 2013). The quantitative analysis of the components was achieved with reference to authentic standards (phenolic acid standards: catechin, epicatechin, caffeic, ferulic, synapic, *p*-coumaric, gallic, chlorogenic acids; flavonoids standards: rutin, quercetin, luteolin, apigenin and luteolin-7-*O*-glucoside) (Extrasynthese, Genay, France). Compound identification was carried out by comparing UV absorption spectra and retention times of each compound with those of pure standards injected in the same conditions. The compounds were quantified through calibration curves of standard compounds as mean of three replicates.

2.6. Antioxidant activity

2.6.1. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the different borojo extracts was determined by using the potassium ferricyanide–ferric chloride method, as described by Oyaizu (1986). The ferric reducing activity of a sample was estimated in μM Trolox equivalent (TE)/100 g of sample (fw). Each assay was carried out in triplicate.

2.6.2. Ferrous ion-chelating ability assay

Ferrous ions (Fe^{2+}) chelating activity was measured by inhibiting the formation of Fe^{2+}–ferrozine complex after treatment of test material with Fe^{2+}, following the method of Carter (1971). Results were expressed in μM EDTA equivalent/100 g sample (fw) as mean of three replicates.

2.6.3. DPPH radical scavenging ability assay

The radical scavenging ability of the different extracts obtained from borojo was measured using the stable radical DPPH