Journal of Food Composition and Analysis 42 (2015) 56–62

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

Pasteurization of blackberry juice preserves polyphenol-dependent inhibition for lipid peroxidation and intracellular radicals

Gabriela Azofeifa [a,*], Silvia Quesada [a], Ana M. Pérez [b], Fabrice Vaillant [c], Alain Michel [d]

[a] Departamento de Bioquímica, Escuela de Medicina, Universidad de Costa Rica, Código Postal 11501-2060, San José, Costa Rica
[b] Centro Nacional de Ciencia y Tecnología de Alimentos (CITA), Universidad de Costa Rica, Código Postal 11501-2060, San José, Costa Rica
[c] Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR 95 QUALISUD, 73 rue Jean-François Breton, 34398 Montpellier Cedex 5, France
[d] Laboratoire de Pharmacologie et Physiopathologie Expérimentales, Faculté de Pharmacie, Université Montpellier 1, UMR 95 QUALISUD, 15 Av. C. Flahault, 34093 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 13 January 2015
Accepted 13 January 2015
Available online 28 March 2015

Keywords:
Blackberry juice
Rubus adenotrichos
Antioxidant activity
Lipid peroxidation
Pasteurization
Nutrient retention
Food processing
Bioactive non-nutrient
Food composition
Food analysis

ABSTRACT

Berries are commonly consumed as juice, and juice-processing conditions could affect their bioactive compounds. This study evaluated the effect of thermal treatments on the antioxidant capacity of blackberry juice polyphenols. Pasteurized blackberry juices were prepared at 75 °C for 15 s (JP75) and 92 °C for 10 s (JP92). Polyphenol analysis showed that for JP75 and JP92, anthocyanin concentrations decreased significantly, compared to non-pasteurized juice (NPJ), whereas ellagitannins were not significantly affected. The evaluation of the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity showed a significant decrease of 26% for JP75 and 27% for JP92, and, for the NO (nitric oxygen) scavenging capacity, the activity was reduced 15% for JP75 and 16% for JP92. There were no significant reductions observed for the peroxidation inhibitory capacity of the pasteurized juices for any of the oxidation substrates tested: liposomes, liver homogenates and erythrocytes. Furthermore, the intracellular antioxidant capacity showed no significant differences due to thermal treatments. The concentration of phenols necessary to scavenge 50% of the radical oxygen species was 204 ± 9 μg/mL for NPJ, 219 ± 10 μg/mL for JP75 and 220 ± 9 μg/mL for JP92. This study revealed that pasteurized blackberry juices maintained their biological properties related to inhibition of peroxidation and their capacity to scavenge intracellular radicals.

1. Introduction

Research over the past few years has supported the health beneficial effects of berries. Different trials have demonstrated that berry consumption contributes to protect against cardiovascular disease (Basu et al., 2010) and metabolic syndrome (Basu and Lyons, 2012). Specifically, berry consumption lowers the oxidation of LDL (low density lipoproteins) and is associated with lowering blood pressure and improving insulin resistance (Basu and Lyons, 2012). *In vitro* and *in vivo* studies revealed that berry compounds have a potential for cancer prevention through the regulation of cancer cell proliferation, apoptosis and tumor angiogenesis signaling pathways (Seeram, 2008). In addition, anti-inflammatory effects have been described for strawberries, mulberries and blackberries, mainly through immuno-modulatory mechanisms by the inhibition of cytokines (Cuevas-Rodríguez et al., 2010; Liu and Lin, 2013).

Blackberries are one of the most consumed tropical berries. This type of berry, which is from the *Rubus* spp. group, has a high level of polyphenolic compounds that contributes to its high antioxidant capacity. The total phenolics in blackberries range from 114 to 1056 mg/100 g FW (fresh weight) and this value is higher than other kinds of berries, such as cranberries (120–315 mg/100 g FW) or strawberries (43–443 mg/100 g FW) (Howard and Hager, 2007; Szajdek and Borowska, 2008). The main polyphenols that are present in the blackberries are anthocyanins and ellagitannins (Kaume et al., 2012).

Tropical highland blackberry (*Rubus adenotrichos*) is commonly processed as a juice or juice concentrate. In major producing countries (Colombia, Ecuador and Costa Rica), blackberry-based beverages are regularly produced by local industries (Gancel et al., 2011). The juice-processing conditions, such as enzymatic

* Corresponding author at: Departamento de Bioquímica, Facultad de Medicina, Universidad de Costa Rica, 2060 San José, Costa Rica. Tel.: +506 2511 4483; fax: +506 2511 4450.
E-mail address: gabriela.azofeifacordero@ucr.ac.cr (G. Azofeifa).

http://dx.doi.org/10.1016/j.jfca.2015.01.015
0889-1575/

reactions, thermal treatments or microfiltration steps, can affect the bioactive compounds and their subsequent antioxidant activity. Thermal decomposition is one of the main causes of bioactive compound loss (D'Archivio et al., 2010; Rawson et al., 2011). Studies evaluating the effect of thermal treatments on fruit phenolic compounds have been reported for mango (Santhirasegaram et al., 2013), grapefruit (Igual et al., 2010), peach (Oliveira et al., 2012), orange (Lo Scalzo et al., 2004) and berries (Aracibia-Avila et al., 2012; Gancel et al., 2011; Piasek et al., 2011; Zhang et al., 2012). However, these studies focused on changes in the phenolic composition of the fruits after thermal treatments rather than on the changes in their potential beneficial activity. The impact on biological activities have also been evaluated for other industrial process, for example, a study on blackberries demonstrated that despite a reduction of polyphenol concentration by the microfiltration process, the capacity to inhibit lipid peroxidation was not affected (Azofeifa et al., 2011).

The main objective of this study was to evaluate the effect of thermal treatments on the antioxidant capacity of blackberry polyphenols. We evaluated the antioxidant capacities for free radical-scavenging and inhibiting lipid peroxidation and intracellular reactive oxygen species (ROS). We believe that these capacities are close indicators of biological properties.

2. Materials and methods

2.1. Chemicals

All organic solvents were obtained from JT Baker (Griesheim, Germany). The HPLC standard ellagic acid was obtained from Fluka (Buchs, Switzerland), and cyanidin-3-glucoside was obtained from Extrasynthese (Lyon, France). Amberlite XAD-7, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azobis-2-methyl-propion-amidine-dihydro-chloride (AAPH), *tert*-butyl hydroperoxide (TBHP), thiobarbituric acid (TBA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,7-dichlorodihydrofluorescein diacetate (DCFDA), quercetin, gallic acid and fluorescein were acquired from Sigma Aldrich (St. Louis, MO, USA). For cell culture, EMEM medium, containing L-glutamine, fetal bovine serum, streptomycin, penicillin and trypsin, were purchased from Sigma–Aldrich (St. Louis, MO, USA). For the Griess reagent, sodium nitroprusside (SNP), sulfanilamide, naphthylethylenediamine dihydrochloride and sodium nitrite were acquired from Merck (Darmstadt, Germany).

2.2. Blackberry juice preparation and pasteurization

Full-ripe blackberries (*R. adenotrichos* Schltdl. cv. 'vino') were collected from different farms in the province of Cartago, Costa Rica (altitude 1864–2517 m, latitude 09°39′57.1″N–09°44′40.3″N, longitude 83°53′32.1″W–84°00′06.3″W). A microfiltrated juice was prepared as previously indicated (Vaillant et al., 2008). Blackberries were pressed, and the juice was treated with a commercial enzymatic preparation of polygalacturonase (Ultrazym – Novozymes, Bagsvaerd, Denmark), for 1 h at 35 °C with constant agitation to reduce viscosity and facilitate microfiltration. Cross-flow ultrafiltration was performed in a tubular ceramic membrane (Membralox® 1 P19-40, Pall Exekia, Bazet, France) with an average pore size diameter of 0.2 μm. Microfiltration allowed for the removal of suspended solids that could lead to bias.

Two pasteurized juices were prepared from the microfiltrated juice using a pilot-scale, UHT processing system, model FT74X (Armfield Inc., Ringwood, UK). One of the juices was pasteurized at 75 °C for 15 s, and the other was processed at 92 °C for 10 s. As a result, three samples were obtained: one non-pasteurized juice and two pasteurized. The selected temperatures for pasteurization represent mid (70–80 °C) and high (>90 °C) pasteurization conditions commonly used for fruit juices in previous publications (Hager et al., 2010; Mena et al., 2013; Santhirasegaram et al., 2013; Zhang et al., 2012).

2.3. Polyphenol purification

Each of the three blackberry juice samples were subjected to polyphenol purification using an Amberlite XAD-7 column (150 mm × 20 mm) packed in water. Five hundred milliliters of each juice was loaded onto the Amberlite column and washed with 2 L water to remove sugars. Finally, the phenolic compounds were eluted with 200 mL methanol/water (80:20), concentrated under a vacuum (40 °C) and freeze-dried (−40 °C, 133 × 10^{-3} mBar for 48 h). The three lyophilized polyphenol extracts: non-pasteurized juice (NPJ), juice pasteurized at 75 °C (JP75) and juice pasteurized at 92 °C (JP92), were stored at −30 °C until further chemical and antioxidant analysis. Only one batch of each extract (NPJ, JP75, JP92) was prepared, however to characterize the extracts, each assay described next was performed in three independent experiments.

2.4. Analytical characterization of the blackberry juices

2.4.1. Total polyphenol content

The total phenolic content of the polyphenol extracts was determined using the Folin–Ciocalteu assay that was modified by George et al. (2005). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of polyphenol extract. Samples were analyzed in triplicate.

2.4.2. Phenolic compounds by HPLC

The polyphenol extracts were analyzed by HPLC for anthocyanins and ellagitannins following the protocol described by Mertz et al. (2007) and Gancel et al. (2011). Briefly, the HPLC quantitative analysis was performed using a Dionex liquid chromatograph system that was equipped with a UVD 340 U photodiode array detector (Dionex Corporation, Sunnyvale, CA, USA) and an endcapped, reversed-phase Lichrospher ODS-2 column (250 mm × 4.6 mm i.d., 5 μm) (Interchim, Montluçon, France). HPLC solvents consisted of 2% aqueous formic acid (solvent A) and acetonitrile/water/formic acid (80:18:2, v/v/v, solvent B). The chromatographic conditions were: 30 °C, 0.3 mL/min flow rate, 20 μL injection volume and the gradient used for separation was: 0 min, 5% B; 0–20 min linear gradient from 5% to 25% B; 20–25 min linear gradient from 25% to 100% B; 25–30 min linear gradient from 100% to 5% B and 30 to 35 min, 5% B.

Previously, Mertz et al. (2007) identified sanguiin H6, lambertianin C and cyanidin glucosides following the same HPLC procedure by including an additional hyphenation of the diode array detector (DAD) to an electrospray Ion Trap Mass spectrometry detector (ESI-TRAP-MS/MS). As the same method was followed in this paper and the retention times coincide, a tentative identification of the phenolic compounds was performed based on the work of Mertz et al. (2007). The quantification of polyphenols in the extract was performed using calibration curves with five concentrations that were established with standards of ellagic acid for ellagitannins and cyanidin-3-glucoside for anthocyanins. Samples were analyzed in triplicate.

2.5. Antioxidant assays

2.5.1. DPPH radical-scavenging activity (RSA)

The radical-scavenging activities of the polyphenol extracts were evaluated by assessing the direct DPPH-scavenging activity in the samples (Azofeifa et al., 2013). DPPH (0.25 mM) was prepared