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Hydroxycinnamic acids and flavonols in native edible berries of South Patagonia

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ABSTRACT

Diverse edible berries are native to the Patagonian region of Southern Chile. These berries are underused because their nutritional properties are relatively unknown. In this work, the profiles and concentrations of hydroxycinnamic acid derivatives and flavonols, and the antioxidant capacity of the berry extracts, were studied using HPLC–DAD–ESI–MS/MS and CUPRAC assays, respectively. In total, 46 compounds were identified, including 17 hydroxycinnamic acid derivatives and 26 flavonols. Caffeoylquinic acid isomers were the most abundant compounds, and quercetin and myricetin derivatives were the main flavonols found. The berries from *Ribes* genera showed a high diversity and concentration of these 2 families of compounds and contained 3-caffeoylquinic acid and quercetin-3-rutinoside at the highest concentrations. The Patagonian berries, especially the berries of *Rubus* and *Ribes* genera, had high cupric reducing antioxidant capacity, comparable with that described for berries from the Northern hemisphere. These results contribute to promote the nutritional study of these fruits.

1. Introduction

Hydroxycinnamic acid derivatives (HCADs) are secondary plant metabolites synthesised by the phenylpropanoid pathway (Aksamit-Stachurska, Korobczac-Sosna, Kulma, & Szopa, 2008). HCADs play diverse roles in plant physiology and activities, which are correlated with protein synthesis, allelopathy, and photosynthesis (Robbins, 2003). The biological effects of HCADs in humans are related to their antioxidant functions. HCADs have been reported to inhibit cardiovascular disorders, cancer, and neurological disorders such as Alzheimer's and Parkinson's diseases, and possess antigenotoxic activity (Ferguson, Zhu, & Harris, 2005; Robbins, 2003).

Flavonols are extensively studied phenolic compounds that also produce important biological effects that are related to their antioxidant activity (Tai et al., 2012). Flavonols have been studied for the protection they offer against pathologies such as various types of cancer (Tsimplouli, Demetzos, Hadzopoulou-Cladaras, Pantasis, & Dimas, 2012) and cardiovascular disorders (Perez-Vizcaino & Duarte, 2010) and for their antibacterial properties (Urzua, Echeverria, & Espinoza, 2012).

In Chile, the governmental program "Elige vivir sano" (Choose to live healthy) (http://www.eligevivirsano.cl/) was designed in the same way as the World Health Organization program that promoted eating fruits to prevent and reduce the obesity and overweight problems that affect more than 64% of the world's population (OECD, 2010). Chile cultivates and exports many fruits, being the world leader in producing table grapes, and being an important producer of apples, kiwis, plums, peaches, and blueberries, which are grown especially in the central zone of the country (ODEPA, 2012). In the extreme south of the country, wild edible berries are produced by several vascular plants from Grossulariaceae, Ericaceae, Rosaceae, Myrtaceae, Onagraceae, and other families (Vidal, 2007), but these berries are not used widely because their nutritional properties have not been studied. Specifically, HCAD and flavonol profiles have not been described for Patagonian berries from the *Ribes* genera such as *Ribes magellanicum* ("parra silvestre" or "parrilla", in Spanish) or *Ribes cucullatum* ("parrillita"); however, profiles have been described for *Ribes nigrum*, a European berry, which contains *p*-coumaric, ferulic, and caffeic acid derivatives, the most important of these being caffeoylglucose (Gavrilova, Kajd-Zanoska, Gjamovski, & Stefova, 2011; Zheng

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et al., 2012), as well as for flavonols such as myricetin, quercetin, isorhamnetin, and kaempferol derivatives (Zheng et al., 2012). In *R. nigrum*, 2 HCADs were recently described: *p*-coumaroylglucose and caffeoylglucose, with the latter being more abundant (Gavrilova et al., 2011). Profiles of these compounds have not been described in the fruits of *Rubus geoides* ("frambuesa silvestre", "frutilla silvestre" or "frutilla de Magallanes"), but have reported in other berries from *Rubus* genus members, such as those from *Rubus glaucus* and *Rubus adenotrichus*, in which ellagic acids derivatives and flavonols such as quercetin-glucoronide and quercetin-glucoside are the most abundant compounds (Mertz Chaynier, Gunata, & Brat, 2007). In other berries that have been studied, such as those from *Gaultheria mucronata* ("chaura"), *Gaultheria antarctica* ("mutilla"), *Myrteola nummularia* ("sarapito" or "daudapo"), and *Fuchsia magellanica* ("chilco"), HCAD and flavonol profiles have not been yet described.

Several methodologies are used to analyse phenolic compounds, the most important of which is HPLC using C18 analytical columns and mobile phases containing water acidified with acetic, formic, and phosphoric acid, and methanol or acetonitrile as the organic modifier (Hokkanen, Matilla, Jaakola, Pirtilla, & Tolonen, 2009; Takenaka et al., 2003). UV–vis absorbance with photodiode arrays (PDAs) and mass spectrometry (MS) are used for detection because these techniques are extremely sensitive (Robbins, 2003). MS, which is used widely for analysing complex mixtures of phenolic compounds in plants (Thabti, Elfalleh, Hannachi, Ferchichi, & Campos, 2012), allows the compounds being studied to be identified using information about their fragmentation patterns without having to isolate the compounds, as required for more sophisticated analysis using NMR. In this context, flavonols and phenolic acids in several foods have been detected with high sensitivity using the negative ionisation mode (Ferreres, Taveira, Pereira, Valentao, & Andrade, 2010; Mertz, Cheynier, Gunata, & Brat, 2007; Ruiz et al., 2013b; Takenaka et al, 2003).

In previous studies, HCAD extraction and chromatographic analysis were optimised (Ruiz et al., 2013b), and the anthocyanin profiles of Patagonian berries were determined (Ruiz et al., 2013a). The aim of this study was to examine the unreported profiles of phenolic compounds such as flavonols and HCADs in methanolic extracts of Patagonian berries, using HPLC–DAD–ESI–MS/MS to identify the compounds based on their fragmentation patterns. The results obtained are complemented with the determination of cupric reducing antioxidant capacity (CUPRAC) of the berry extracts, in order to promote the interest for the nutritional study of these fruits.

2. Materials and methods

2.1. Reagents and standards

The commercial standards of 3-caffeoylquinic (98.87%), 4-caffeoylquinic, 5-caffeoylquinic (98.28%), 1,5-dicaffeoylquinic (99.49%), 3,5-dicaffeoylquinic (98.07%), 3,4-dicaffeoylquinic (96.92%), and 4,5-dicaffeoylquinic (92.57%) acids were obtained from Phytolab (Vestenbergsgreuth, Germany). The standards quercetin-3-galactoside (>97%), quercetin-3-glucoside (>90%), quercetin-3-rhamnoside (85%), rutin (quercetin-3-rutinoside), miricetin (85%), quercetin, kaempferol (90%), and TROLOX (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were obtained from Sigma–Aldrich (Steimheim, Germany). Formic acid, acetonitrile, methanol, water (HPLC grade), $CuCl_2 \times 2H_2O$ (extra pure), and ammonium acetate (ACS grade) were provided by Merck (Darmstadt, Germany). Neocuproine hemihydrate (2-9-Dimethyl-1,10-phenanthroline) (⩾99.0% pure) was obtained from Fluka. Solid phase extractions were conducted using Oasis MCX mixed phase cartridges (Waters, Mildford, MA).

2.2. Berry samples

Mature fruits of mutilla (*G. antarctica*), chaura (*G. mucronata*), parra silvestre (*R. magellanicum*), parrillita (*R. cucullatum*), sarapito (*M. nummularia*), frutilla silvestre (*R. geoides*), and chilco (*Fuchsia magellanica*) were collected in Region of Magallanes in Chilean Patagonia in February 2010 and kept frozen at −20 °C until analysis. Taxonomic identification was performed by Professor Roberto Rodriguez, Director of the Herbario CONC of the Department of Botany, University of Concepcion.

2.3. Extraction of HCADs and flavonols

An ultrasonic bar homogenizer (Cole Palmer Series 4710), a mechanical shaker (Edyman KL2) and a centrifuge (Heraeus-Christ GMbH, Osterode, Germany) were used for preparing samples. Samples were extracted using a methanol/water 93/7 (v/v) mixture, and then cleaned using a modified SPE method, as described previously (Ruiz et al., 2013b), with Oasis MCX cartridges that combine cation exchange and reversed phases. Crude extracts (1 mL) were dried in a rotary evaporator, reconstituted in 1.0 mL of 0.1 N of hydrochloric acid, and then loaded onto an MCX cartridge equilibrated previously with 5.0 mL of methanol and 5.0 mL of water. Sugars were removed by washing with 5.0 mL of 0.1 N hydrochloric acid and 5.0 ml of water, and 3 × 5.0 mL of methanol was used to elute flavonols and HCADs that, being anthocyanins, were retained in the solid phase during this process. Lastly, HCAD extracts were dried in a rotary evaporator and reconstituted in 1 mL of the mobile phase.

2.4. HPLC–DAD–ESI–MS/MS conditions

HPLC–DAD–MS/MS analyses of HCADs and flavonols were carried out using a Shimadzu HPLC system (Tokyo, Japan) equipped with a quaternary LC-10ADVP pump with an FCV-10ALVP elution unit, a DGU-14A degasser unit, a CTO-10AVP oven, and a UV–vis diode array spectrophotometer (model SPD-M10AVP) coupled in tandem with a QTrap LC/MS/MS 3200 Applied Biosystems MDS Sciex system (Foster city, CA, USA). The instrument was controlled and data were collected using CLASS-VP DAD Shimadzu Chromatography Data System and Analyst software (version 1.5.2) for MS/MS analysis.

Flavonols and HCADs were analysed by HPLC as described (Ruiz et al., 2013b) using a C18 column (Kromasil; 250 mm × 4.6 mm, 5 μm) with a C18 precolumn (Nova-Pak, Waters; 22 mm × 3.9 mm, 4 μm) at 30 °C. For chromatography, a mobile phase gradient of 0.1% formic acid in water and acetonitrile was used at a flow rate of 0.5 mL min^{-1}. The acetonitrile gradient was from 15% to 25% in 14 min, from 25% to 35% in 11 min, from 35% to 100% in 1 min, and from 100% to 15% in 1 min, followed by stabilization for 10 min. Hydroxycinnamic acid and flavonol identities were assigned using optimised ESI–MS/MS conditions: negative ionisation mode, −5 V collision energy, −4000 V ionisation voltage, capillary temperature at 450 °C, and an auxiliary flow rate of 15 arbitrary units.

HCADs were quantified at 320 nm and flavonols at 360 nm by external calibration using 3-caffeoylquinic acid for HCADs, and myricetin, quercetin, or kaempferol for flavonols. Results were expressed as μmol/g fresh weight.

2.5. CUPRAC assay

It was carried out as described (Ribeiro, Magalhaes, Reis, Lima, & Segundo, 2011). CUPRAC (cupric reducing antioxidant capacity) is an in vitro assay used to determine antioxidant capacity of hydrophilic and lipophilic dietary polyphenols; the assay is simple,