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Original Article

Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. *chiloensis* form *chiloensis*) using HPLC-DAD–ESI-MS and free radical quenching techniques

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ABSTRACT

A comparative analysis of methanol extracts from fruits, rhizomes and leaves of the Chilean white strawberry (*Fragaria chiloensis* spp. *chiloensis* var *chiloensis*) was performed by means of reversed phase high-performance liquid chromatography coupled to diode array detection and electrospray ionization mass spectrometry (HPLC–DAD and HPLC–ESI-MS). The total phenolic, total flavonoid and total anthocyanin content of the extracts was measured and compared. For the first time, some 18 phenolic compounds were tentatively identified in rhizomes and 18 in leaves of the Chilean strawberry. The products were mainly procyanidins, ellagitannins, ellagic acid and flavonol derivatives. The different extracts of the native strawberry presented antioxidant activity, which was close to that exhibited by the white fruits. The rhizomes and leaves proved to be a good source of phenolic antioxidants. The obtained information can be used to characterize the local cultivars by metabolite profiling and provide a reference HPLC fingerprint for future comparison of chemical changes associated to the plant response towards environmental factors and pathogens.

1. Introduction

In the southern part of South America, native berries were relevant as a food source for hunter-gatherers during the growing season. *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis* is a wild species of *Fragaria* endemic to southern Chile which produces light red or "white" strawberries. It is also one of the progenitors of the worldwide known commercial red strawberry (*Fragaria* × *ananassa* Duch.). The leaves and fruits of the Chilean strawberry have been used as food and medicine by the Mapuche aborigines in the Andean region in Chile (Retamales et al., 2005) and Argentina (Ladio et al., 2007), and were also gathered by the extinct Kawashkar culture in the channels and islands of Chilean Patagonia and Tierra del Fuego.

Berries are appreciated all over the world as a pleasant-tasting food often associated with medicinal or health-improving effects (Hancock et al., 2007). Berries such as strawberries (*Fragaria* × *ananassa*), cranberries (*Vaccinium macrocarpon*), raspberries (*Rubus idaeus*), blueberries (*Vaccinium corimbosum*) and cloudberries (*Rubus chamaemorus*) can be consumed raw or after processing as jams, ice creams, liquors and juices, and are a good source of phenolic antioxidants (Chen and Zuo, 2007; Määtä-Riihinen et al., 2003, 2004; Zuo et al., 2002b).

Several reports describe the chemical composition of different berries, mainly focusing on the identity of phenolic and flavonoid compounds and the antioxidant/free radical-scavenging effect of the extracts and isolated/identified compounds. Comparatively little is known about the chemistry of the native Chilean strawberry, and studies are urgently needed to support the efforts to develop this species as a crop (Retamales et al., 2005). The characterization of small molecules such as secondary compounds in crop plants can be used for metabolomic studies with high potential in food chemistry, food component analysis (Wishart, 2008) and to assess selected metabolites in the progeny from breeding programs (McDougall et al., 2008). The impact and

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perspectives of the metabolomic approach in plant science have been discussed by Sumner et al. (2003) as well as by Weckwerth and Fiehn (2002). An approach for the safety assessment of novel plant food (such as the native strawberry) has been proposed by Knudsen et al. (2008).

Previous studies of the fruits of *F. chiloensis* ssp. *chiloensis* f. *chiloensis* have shown the presence of 1-*O*-*E*-cinnamoyl-β-D-rhamnopyranoside, 1-*O*-*E*-cinnamoyl-α-xylofuranosyl-(1 → 6)-β-D-glucopyranose and 1-*O*-*E*-cinnamoyl-β-D-xylopyranoside, the aminoacid tryptophan, ellagic acid and cyanidin-3-*O*-glucoside (Cheel et al., 2005). The antioxidant properties of several cultivars of red strawberries are well known and were described by Asami et al. (2003) and Skupien and Oszmianski (2004), and we have reported a comparison of the antioxidant activity between the red strawberry cultivar Chandler and the white strawberry growing in the same location in Chile (Simirgiotis et al., 2009b).

Several analytical techniques such as high-performance liquid chromatography with UV–vis photodiode array detection (HPLC–DAD) (Chen et al., 2001; Zuo et al., 2002a), gas–liquid chromatography with flame ionization (GC–FID) (Zuo et al., 2008), gas–liquid chromatography coupled with mass spectrometry (GC–MS) (Chen and Zuo, 2007; Zuo et al., 2008, 2002b), capillary electrophoresis with electrochemical detection (CE–ED) (Peng et al., 2008) and capillary electrophoresis coupled to electrospray ionization time-of-flight mass spectrometry (CE–ESI-TOF-MS) (Gómez-Caravaca et al., 2008) have been used to detect, characterize and quantify phenolic compounds present in different food and medicinal plants.

Within this scenario, improved atmospheric pressure ionization (API) methods, especially electrospray ionization mass spectrometry (ESI-MS) coupled to HPLC, have enormously increased the range of natural polar and ionic species in solution amenable to mass spectrometry. Indeed, HPLC using tandem spectrometry (MS/MS) has provided important structural information in the analysis of natural products. In HPLC–DAD and electron spray ionization (ESI) analysis of a complex chemical mixture from a plant extract (e.g. containing flavonol glycosides, phenolic acids and tannins), the polar molecules are separated and detected either in their protonated $[M+H]^+$ or deprotonated $[M-H]^-$ forms, and the structures are characterized by co-chromatography with standard compounds. When the samples of natural products for HPLC analysis are unavailable and when the compounds detected have similar UV spectra, similar molecular ions and similar retention times, the structures of the compounds are characterized by MS–MS analysis. Although definitive structures of all compounds and chemotaxonomic markers in a plant extract forming the protonated or deprotonated molecules detected in the ESI-MS will require more refined analysis by extensive isolation of all the compounds combined with spectroscopy data, ESI tandem mass spectra provide clues to information about the nature of the compounds. The phenolic composition of fruit extracts from the forms *chiloensis* and *patagonica* of *F. chiloensis* was previously compared with that of the commercial strawberry *Fragaria* × *ananassa* cv. Chandler by HPLC–UV detection and mass spectrometry. The phenolic constituents in the three species were mainly proanthocyanidins, hydrolysable tannins, anthocyanins and flavonol glycosides (Simirgiotis et al., 2009b). However, the chemical composition of rhizomes and leaves of this native species has not been previously reported.

Following our studies on the chemistry of South American food plants (Simirgiotis et al., 2009a,b), we have analyzed extracts obtained from Chilean strawberry fruits, rhizomes and leaves by high-performance liquid chromatography with diode array detector coupled with electrospray ion-trap tandem mass spectrometry. In this study we report the phenolic compound composition of the different plant parts as well as the phenolic content, antioxidant properties and chromatographic fingerprints of the methanolic extracts of this native berry.

2. Materials and methods

2.1. Plant material

Rhizomes and leaves of wild growing *F. chiloensis* ssp. *chiloensis* f. *chiloensis* were collected at Las Trancas, Termas de Chillán, VIII Región, Chile, in March 2006. The ripe fruits were harvested in January 2006 in a commercial plantation located in Contulmo, Province of Arauco, VIII Region, Chile. Voucher herbarium specimens were deposited with the number 2865 at the Herbarium of the Universidad de Talca.

2.2. Chemicals

Methanol was obtained from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile and formic acid from Merck (Darmstadt, Germany) were used. Ellagic acid was purchased from ChromaDex (Santa Ana, CA, USA). HCl, KCl, Folin-Ciocalteu phenol reagent, sodium acetate, aluminum chloride hexahydrate and sodium carbonate were from Merck (Darmstadt, Germany). Sephadex LH-20 (Pharmacia), thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany), Amberlite XAD-7 (20–60 mesh), diphenylborinic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH•), quercetin, gallic acid, (+) catequin, nitrobluetetrazolium (NBT), xanthine oxidase and hypoxanthine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents used for chromatography were HPLC grade.

2.3. Instrumentation

The HPLC system used for DAD analysis of extracts was Merck-Hitachi (LaChrom, Tokyo, Japan) equipment consisting of a L-7100 pump, a L-7455 UV diode array detector, and a D-7000 chromatointegrator. Mass spectra were recorded using an Agilent 1100 LC system connected through a split to an Esquire 4000 Ion Trap LC/MS system (Bruker Daltoniks, Germany). The extracts were dissolved in MeOH-formic acid (99:1) (approximately 3 mg/mL), and submitted to LC–MS. The volume injected was 20 μL. Full scan mass spectra were measured between m/z 150 and 2000 u in positive ion mode for anthocyanins and negative ion mode for other compounds. Nitrogen was used as nebulizer gas at 27.5 psi, 350 °C and at a flow rate of 8 L/min. The mass spectrometric conditions for negative ion mode were as follows: electrospray needle, 4000 V; end plate offset, −500 V; skimmer 1, −56.0 V; skimmer 2, −6.0 V; capillary exit offset, −84.6 V; and the operating conditions for positive ion mode were: electrospray needle, 4000 V; end plate offset, −500 V; skimmer 1, 56.0 V; skimmer 2, 6.0 V; capillary exit offset, 84.6 V; capillary exit, 140.6 V. Collision induced dissociation (CID) spectra were obtained with a fragmentation amplitude of 1.00 V (MS/MS) using helium as the collision gas.

2.4. Extraction and sample preparation

The sample solutions for HPLC comparison purposes were prepared as previously reported (Simirgiotis et al., 2009b), with some modifications. Approximately 5 g of each freeze-dried plant part was homogenized in a blender with 50 mL MeOH-formic acid (99:1, v/v) and extracted for 1 h three times at room temperature. The fractions were collected; the solution was filtered and concentrated under reduced pressure to evaporate the solvent. Distilled water was added to ca. 10 mL and the solution loaded