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Quantification of phytoprostanes – bioactive oxylipins – and phenolic compounds of *Passiflora edulis* Sims shell using UHPLC-QqQ-MS/MS and LC-IT-DAD-MS/MS

Sonia Medina [a,b], Jacinta Collado-González [a], Federico Ferreres [a], Julián Londoño-Londoño [b,*], Claudio Jiménez-Cartagena [b], Alexandre Guy [c], Thierry Durand [c], Jean-Marie Galano [c], Angel Gil-Izquierdo [a,*]

[a] Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), Espinardo, Murcia, Spain
[b] Faculty of Engineering, Food Engineering Program, Corporación Universitaria Lasallista, Caldas-Antioquia, Colombia
[c] Institut des Biomolécules Max Mousseron, UMR 5247 CNRS-University of Montpellier – ENSCM, Montpellier, France

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ABSTRACT

The genus *Passiflora*, comprising about 500 species, is the largest in the Passion flower family. *Passiflora edulis* Sims f. *edulis* (gulupa) is one of the most important fruits cultivated in Colombia. In recent years and due to its organoleptic and bioactive properties, its exports have significantly increased. In this work, six new bioactive oxylipins –phytoprostanes – were detected in gulupa shell by a UHPLC-QqQ-MS/MS method: F_{1t}-phytoprostanes and D_{1t}-phytoprostanes were the predominant and minor classes, respectively. Moreover, the polyphenol profile of the shell was investigated and we were able to detect and quantify phenolic compounds that have not been described previously, like luteolin-8-*C*-(2-*O*-rhamnosyl)hexoside and quercetin-3-*O*-(6″-acetyl)glucosyl-2″-sinapic acid. Consequently, this study provides new insights into the importance of gulupa shell as a valuable option in the design of new beverages rich in antioxidant phytochemicals, as part of a well-balanced diet, and in the process and quality control of such products.

1. Introduction

Colombia is one of the most important countries with respect to the production of tropical fruits. Among these fruits, the production of gulupa (*Passiflora edulis* Sims f. *edulis*) has been increased in recent years by growth of the Colombian horticultural sector. So, in 2014 the area of gulupa cultivated in Colombia had reached 608 ha and its production was more than 8000 tonnes. All this has allowed the opening up of very important international markets like Europe and Japan. The pleasant organoleptic properties of gulupa might explain its increasing popularity in these countries.

Gulupa is a native species of the southern Andes, growing at altitudes between 1600 and 2600 m above sea level (a.s.l.) and at temperatures between 16 and 22 °C (Jiménez et al., 2011). *Passiflora edulis* has sedative, diuretic, anthelmintic, anti-diarrheal and other uses in South America (Dhawan, Dhawan, & Sharma, 2004). Its pulp is used to prepare juices and smoothies and it is considered a source of vitamins, minerals and ascorbic acid. In addition, gulupa shell has been reported to contain carotenoids with antioxidant activity (Franco, Cartagena, Guillermo Correa, Rojano, & Piedrahita, 2014), aromatic volatile compounds like ethyl acetate, ethyl butyrate, and ethyl hexanoate, among others and phytoconstituents like glycosides, phenols, alkaloids, and amino acids (Dhawan et al., 2004).

Color, texture, and flavor are important attributes in food quality, but newly described compounds like phenolic compounds and

Abbreviations: ALA, α-linolenic acid; BHA, *tert*-butylhydroxyanisole; Bis-Tris, Bis-(2-hydroxyethyl)-amino-*tris*(hydroxymethyl)-methane; *cis*-OPDA, *cis*-(+)-12-oxo-phytodienoic acid; DW, dry weight; HPLC, high performance liquid chromatography; HPLC-DAD-ESI-MSn, high-performance liquid chromatography-diode array detection-electrospray ionization-tandem mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; MRM, multiple reaction monitoring; Nfr2, nuclear factor erythroid-2; OS, oxidative stress; PUFA, polyunsaturated fatty acids; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SPE, solid phase extraction; UHPLC-QqQ-MS/MS, ultra-high performance liquid chromatography-triple quadrupole-tandem mass spectrometry.

* Corresponding authors at: Faculty of Engineering, Food Engineering Program, Corporación Universitaria Lasallista, Carrera 51, 118 Sur, 57 Caldas-Antioquia, Colombia (J. Londoño Londoño). Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain (A. Gil-Izquierdo).

E-mail addresses: julondono@lasallistadocentes.edu.co (J. Londoño-Londoño), angelgil@cebas.csic.es (A. Gil-Izquierdo).

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phytoprostanes (PhytoPs) – not described to date in gulupa – can increase our knowledge of the intrinsic benefit of gulupa consumption. It has been suggested that phenolic compounds might be associated with beneficial effects on conditions such as cardiovascular disease, diabetes, obesity, and skin disorders, among others (Tomás-Barberán & Gil, 2008). The pathogenesis of these diseases could be associated with oxidative stress (OS) and with high levels of reactive oxygen species (ROS). Regarding OS in plants, PhytoPs are formed from α-linolenic acid (ALA) oxidation, by a non-enzymatic pathway (Collado-González, Durand et al., 2015). Recently, it has been reported that PhytoPs have a wide range of biological activities. These bioactive compounds can protect plants from oxidative damage, by participating in plant defense and the detoxification response (Loeffler et al., 2005). In humans, they can be considered as nutraceuticals, since they possess anti-inflammatory and apoptosis-inducing activities (Durand et al., 2011) and can regulate immune function (Gilles et al., 2009). Also, a promising neuroprotective effect of B_1-PhytoPs was described by Minghetti and colleagues, since they protected undifferentiated neuronal cells (SH-SY5Y cells) against OS induced by hydrogen peroxide and promoted myelination (Minghetti et al., 2014).

There are several recent studies on the PhytoPs profiles in different plant matrices – like olive oil (Collado-Gonzalez et al., 2015), almond (Carrasco del Amor et al., 2015), red wine and must (Marhuenda et al., 2015), and macroalgae (Barbosa et al., 2015). However, no previous reports exist concerning the PhytoPs content in gulupa fruit. In this context, the main goals of the current research were to perform, for the first time, the fingerprinting of PhytoPs in gulupa shell and to evaluate the phenolics profile in the same tissue, to obtain a qualitative and quantitative profile of these bioactive compounds in gulupa.

2. Materials and methods

2.1. Standards and reagents

Ten PhytoP standards (9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, 9-D_{1t}-PhytoP, 9-*epi*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, *ent*-16-B_1-PhytoP, 9-L_1-PhytoP, and *ent*-9-L_1-PhytoP) were synthesized according to previous reports (Durand et al., 2004; El Fangour et al., 2004; El Fangour, Guy, Vidal, Rossi, & Durand, 2005; Guy, Flanagan, Durand, Oger, & Galano, 2015). The PhytoP chemical structures are shown in Fig. 1A. Quercetin-3-*O*-rutinoside (rutin), 5-*O*-caffeoylquinic acid, cyanidin-3-*O*-glucoside and Bis-Tris (bis (2-hydroxyethyl)amino-*tris*(hydroxymethyl)methane) and BHA (*tert*-butylhydroxyanisole) were purchased from Sigma Aldrich (St. Louis, MO, USA).

Milli-Q® ultrapure water was used in this experiment (Millipore, Bedford, MA, USA). All solvents used were LC-MS grade like hexane was obtained from Panreac (Castellar del Valles, Barcelona, Spain) and methanol and acetonitrile were purchased from Baker (Phillipsburg, New Jersey, USA). For solid phase extraction (SPE), the selected cartridges were Strata X-AW (100 mg (3 mL)$^{-1}$) from Phenomenex, (Torrance, CA, USA). The internal standard d_4-15-F_{2t}-isoprostane (8-*iso*-$PGF_{2\alpha}$-d_4) was acquired from Cayman Chemicals (Ann Arbor, MI, USA).

2.2. Plant material

Gulupa (1860 plants) grown at El Peñol, Antioquia, Colombia (6°15′47.8″ N, 75°15′16.5″ W, Magna Sircas World Geodetic System 84) was harvested at commercial maturity. This region of Antioquia is characterized by an average annual rainfall of 3433 mm and is located at 2140 m a.s.l. The whole fruit was washed and sanitized with hyperacetic acid (2 mg L^{-1}). The shells were freeze-dried in a Labconco 1 L system (Labconco, Kansas City, MO, USA).

2.3. Phytoprostane and phenolic compounds extraction

For the extraction of PhytoPs from gulupa shell samples, 1 g was crushed in a mortar with 15 mL of a solution of MeOH and BHA (1 g of *tert*-butylhydroxyanisole per L of MeOH) and transferred to a polypropylene tube. After that, the samples were vortexed for 5 min and centrifuged for 10 min, at 2000*g* and 4 °C. The supernatant was extracted and filtered through a Sep-Pack C_{18} cartridge (Waters, Milford, MA). The PhytoPs present in the samples were isolated, after dilution, by the SPE method (using a Strata X-AW cartridge) developed by Collado-González, Medina et al. (2015), slightly modified for the gulupa matrix.

After SPE, the target compounds were eluted with 1 mL of MeOH and dried using a SpeedVac concentrator (Savant SPD121P, Thermo Scientific, MA, USA). Then, the dry extract was reconstituted with 200 μL of elution phases A (water/acetic acid (99.99:0.01, *v/v*)) and B (methanol/acetic acid (99.99:0.01, *v/v*)) (90:10, *v/v*). Then, the samples were sonicated for 10 min and centrifuged (10000*g* for 10 min) before being filtered through 0.45-μm PVDF filters (Millipore, MA, USA). Of each sample, 20 μL were injected and analyzed in a UHPLC-QqQ-MS/MS. Three injections per sample were performed.

For the extraction of phenolic compounds, 0.6 g of each freeze-dried sample (shell) was chopped to obtain a mixture as homogenous as possible. Then, it was extracted with 2 mL of methanol/water/formic acid (25:24:1, *v/v/v*), according to the method of Moreno and coworkers (Moreno, Pérez-Balibrea, Ferreres, Gil-Izquierdo, & García-Viguera, 2010), with slight modifications. Briefly, each sample was extracted in an ultrasonic bath at room temperature until homogenization was achieved, followed by centrifugation for 10 min at 10,000*g* (model EBA 21, Hettich Zentrifugen). The supernatant was recovered and filtered through a 0.22-μm PVDF filter (Millex GV, Millipore, Bedford, MA, USA), before being analyzed by HPLC for identification and quantification. This procedure was performed thrice.

2.4. UHPLC-QqQ-MS/MS analyses of phytoprostanes

Separation of the PhytoPs present in the gulupa samples was analyzed with the methodology explain in a previous published report (Collado-González, Durand et al., 2015; Collado-González, Medina et al., 2015) using liquid chromatography (UHPLC) coupled to a 6460 mass spectrometry (triple quadrupole technology) from Agilent Technologies, (Waldbronn, Germany). A column BEH 2.1 × 50 mm, 1.7 μm, C_{18} (Waters, Milford, MA, USA) was used with column temperatures of 6 °C (both sides). The mobile phases employed were solvent A (H_2O/acetic acid; 99.99:0.01, *v/v*) and solvent B (MeOH/acetic acid; 99.99:0.01, *v/v*), using the following gradient profile: 60% B at 0 min, 62% B at 2 min, 62.5% B at 4 min, reaching 65% B at 8 min, and returning to the initial conditions at 8.01 min with a flow rate of 0.2 mL min^{-1}. The MS analysis was applied in the multiple reaction monitoring (MRM) negative ESI mode. The ESI conditions and ion optics were as previously described (Collado-González, Durand et al., 2015; Collado-González, Medina et al., 2015). Data acquisition was performed using MassHunter software, version B.04.00 (Agilent Technologies). The quantification of PhytoPs was performed using authentic standards (described in section 2.1) and d_4-15-F_{2t}-IsoP was used as the internal standard.