Food Chemistry 211 (2016) 185–197

Contents lists available at ScienceDirect

Food Chemistry

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

Characterization of antioxidant polyphenols from *Myrciaria jaboticaba* peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study

Merichel Plaza [a,*], Ângela Giovana Batista [b], Cinthia Baú Betim Cazarin [b], Margareta Sandahl [a], Charlotta Turner [a], Elin Östman [c], Mário Roberto Maróstica Júnior [b]

[a] Lund University, Department of Chemistry, Centre for Analysis and Synthesis, P.O. Box 124, SE-221 00 Lund, Sweden
[b] School of Food Engineering, University of Campinas (UNICAMP), P.O. Box 6121, 13083-862 Campinas-SP, Brazil
[c] Food for Health Science Centre, Lund University, Sweden

ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 1 April 2016
Accepted 27 April 2016
Available online 6 May 2016

Keywords:
Myrciaria jaboticaba
Jaboticaba
Polyphenols
Glycemia
Insulin
Antioxidant capacity
HPLC-qTOF-MS
HPLC-DAD-ECD-CAD

ABSTRACT

Brazilian berries, such as *Myrciaria jaboticaba* (jaboticaba), are good sources of polyphenols with a recognized function in oxidative stress attenuation proved in non-clinical studies. In the present study, the polyphenols profile and their contribution to the antioxidant capacity of the jaboticaba peel were analyzed using high-performance liquid chromatography (HPLC) with photodiode array (DAD), electrochemical (ECD), charged aerosol (CAD), and mass spectrometry (MS) detections. Anthocyanins, ellagitannins and gallotannins, ellagic acid and derivatives, and flavonols were found in jaboticaba. Anthocyanins were the phenolics found in higher concentrations. However, ellagitannins were the main contributors to the total antioxidant capacity. Moreover, the effect of jaboticaba peel intake on antioxidant and glucose parameters in a single-blind placebo-controlled crossover study was investigated. The serum antioxidant capacity was significantly higher when the subjects had consumed the test meal containing jaboticaba. Serum insulin decreased subsequent to the second meal at 4 h after jaboticaba peel consumption.

1. Introduction

Energy imbalance and excess of some nutrients could lead to the overproduction of free radicals and consequently lead to oxidative stress *in vivo*. Oxidative stress is characterized by the insufficient capacity of biological systems to neutralize the overproduction of free radicals and is involved in several chronic diseases, such as insulin resistance, type 2 diabetes (Ghosh & Konishi, 2007; Guo et al., 2012; Matsuzawa-Nagata et al., 2008), cardiovascular diseases (Keaney et al., 2003), high blood pressure (Roberts, Vaziri, Liang, & Barnard, 2001; Roberts, Vaziri, Wang, & Barnard, 2000), and osteoarthritis (Tanaka, Hamanishi, Kikuchi, & Fukuda, 1998). The increasing occurrence of these diseases has become a major challenge for health care professionals, who have sought bioactive compounds as the solution to such problems (Leite et al., 2011; Prior et al., 2010; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003).

Phenolic compounds are the major contributors of antioxidant capacity in the diet (Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007). They represent a large group of secondary metabolites in plants, and could scavenge free radicals due to their capacity for electron donation. However, their antioxidant effect depends on their stability in physiologic conditions, as well as the number and position of hydroxyl groups in the molecule (Plaza et al., 2014b).

In vivo studies have shown that circulating polyphenols and other dietary antioxidant compounds may be widely distributed in tissues and act as exogenous antioxidants, neutralizing free radicals, reducing oxidative stress and supporting the endogenous antioxidant system (Chuang & McIntosh, 2011). The consumption of polyphenol-rich vegetables and especially berries, small globular fruits ranging in colour from red to purple, has been linked to some health benefits (Cao, Russell, Lischner, & Prior, 1998; Mazza, Kay, Cottrell, & Holub, 2002; Prior et al., 2010). Berries are rich in flavonoids and other polyphenols with greater antioxidant value, such as tannins and anthocyanins. The latter are responsible for the fruit's colour and have important functional effects (Prior et al., 2008). There is an increasing interest in

* Corresponding author.
E-mail addresses: Merichel.Plaza@chem.lu.se, merichelpla@gmail.com (M. Plaza).

http://dx.doi.org/10.1016/j.foodchem.2016.04.142
0308-8146/

anthocyanins due to their property of attenuating oxidative stress and, consequently, decreasing biomarkers associated with, for example, type 2 diabetes (Ghosh & Konishi, 2007), insulin resistance (Guo et al., 2012; Jayaprakasam, Olson, Schutzki, Tai, & Nair, 2006), hypercholesterolemia (Kalt et al., 2008; Kim, Bartley, Rimando, & Yokoyama, 2010) and neurodegenerative diseases (Williams & Spencer, 2012).

Jaboticaba is a native and popular plant from South America, known as the Brazilian berry that produces globular fruits with a deep purple peel and white sweet pulp. The main species is *Myrciaria jaboticaba* (Vell.) Berg. or jaboticaba sabará found in the Southeast part of Brazil, but other species exist, such as *Myrciaria cauliflora* (DC). Jaboticaba species has been reported to contain certain anthocyanins, phenolic acids, flavonoids and tannins (Reynertson et al., 2006; Wu, Dastmalchi, Long, & Kennelly, 2012). Although not extensively consumed, the main polyphenols of the fruits are concentrated in the peel, which has indicated high antioxidant capacity (Abe, Lajolo, & Genovese, 2012; Leite-Legatti et al., 2012; Lima, Correa, Saczk, Martins, & Castilho, 2011).

However, so far a full characterization of the phenolic composition and their antioxidant properties of the species *M. jaboticaba* peel have not been carried out. We have developed a method based on high-performance liquid chromatography (HPLC) with photodiode array (DAD), electrochemical (ECD), charged aerosol (CAD) and mass spectrometric (MS) detection. Different detectors were used for identification and structural elucidation (DAD and MS), for quantification (CAD) and for analyzing the antioxidative properties (ECD) of the phenolic compounds present in this fruit. Furthermore, we have examined whether the phenolic compounds from *M. jaboticaba* peel are able to protect healthy subjects from oxidative damage, as well as their influence on postprandial glucose and insulin levels.

2. Materials and methods

2.1. Chemicals and materials

All of the chemicals were of analytical grade. The flavonoid standards were purchased from Extrasynthese (Lyon, France). HPLC grade methanol was supplied by Honeywell Burdick and Jackson (Muskegon, MI, USA), meanwhile formic acid and ammonium formate were acquired from Sigma Aldrich (Steinheim, Germany). Syringe filters (17 mm, PTFE, 0.2 μm) were purchased from VWR International (West Chester, PA, USA), and ultrapure water was obtained using a Milli-Q system (Millipore, Billerica, MA, USA). Methanol, LC-MS grade, was provided by Scharlau (Barcelona, Spain) and ethanol (99.7%) was from Solveco (Rosenberg, Sweden). Deagglomerated alumina (α-Al_2O_3) suspensions with grain sizes 0.1 μm was provided by Struers (Ballerup, Denmark). (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were obtained from Sigma–Aldrich (São Paulo, Brazil).

2.2. Sample pre-treatment

Jaboticaba fruits (*Myrciaria jaboticaba*) were bought at a local market in Campinas, São Paulo State, Brazil. The fruits were washed, manually peeled and frozen at −18 °C. The peels were dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil) with temperature ranging from −30 to 25 °C, 300 μm Hg for 95 h, and stored at −20 °C.

2.3. Extraction and characterization of phenolic compounds from *M. jaboticaba* peel

2.3.1. Pressurized hot water extraction

Pressurized hot water extractions (PHWE) were performed on a Dionex ASE 200 system (Thermo Fisher, Germering, Germany) using a degased (sonication during 40 min) solvent mixture of water/ethanol/formic acid (94/5/1, vol%) at 99 °C for 1 min. The choice of conditions was based on an optimized method used for the extraction of anthocyanins from red cabbage (Arapitsas & Turner, 2008). Prior to each experiment, the extraction cell heat-up was carried out for 6 min. Likewise, all extractions were performed in 11 mL extraction cells, containing 1 g of jaboticaba dry peel. Samples were prepared in triplicate.

The extraction procedure was as follows: (i) the extraction cell was loaded into the oven, (ii) the cell was filled with solvent up to a pressure of 1500 psi; (iii) heat-up time was applied, (iv) static extraction with all system valves closed was performed; (v) the cell was rinsed (with 60% cell volume using extraction solvent); (vi) the solvent was purged from the cell with nitrogen gas and (vii) depressurization took place. Between extractions, the system was completely rinsed in order to prevent any extract carry-over. The extracts obtained were protected from light and stored at −20 °C until analysis. To prepare the sample solutions, the extracts were diluted to 25 mL with MilliQ water. The water extracts were filtered through a 0.2 μm PTFE filters (VWR International, West Chester, PA, USA) prior to HPLC separation without further clean up.

2.3.2. Characterization of phenolic compounds by HPLC-DAD-ECD-CAD

The phenolic compounds of the *M. jaboticaba* peel extracts were analyzed with a HPLC-DAD-ECD-CAD system according to a previous work with some modifications (Plaza, Kariuki, & Turner, 2014a). It consisted of an ultimate-3000® HPLC system from Dionex (Thermo Fisher, Germering, Germany) that was equipped with an online degasser, a dual ternary pump, an auto-sampler with cooler, a column oven, and a DAD with scanning capabilities, all controlled by Chromeleon 6.80 (Thermo Fisher) software. The DAD was set at 280 nm (phenolic compounds), 350 nm (flavonols) and 520 nm (anthocyanins).

An ECD was attached just after the DAD. The online amperometric detection setup included a thin layer flow cell (Bioanalytical Systems Inc., West Lafayette, IN) with dual glassy carbon disk electrodes (3 mm diameter) and a working electrode imbedded in a PEEK block (Bioanalytical Systems Inc.). A spacer gasket with a thickness of 16 μm (Bioanalytical Systems Inc.) was placed between the PEEK block comprising of the working electrode and the stainless steel block serving as counter electrode. This resulted in a total cell volume of 1.0–1.2 μL. The reference electrode was fixed in a compartment in the stainless steel block. The ECD redox potential was measured at +0.6 V vs. Ag/AgCl. A battery-powered potentiostat (Palmsens, Palm Instrument BV, Houten, The Netherlands) was connected to the flow cell and controlled by HP iPAQ Pocket PC (HP 2200 series, Hewlett-Packard Company). Palm Time software (vs. 2.3.0.0, Palmsens) was used to collect the data.

A corona CAD instrument from ESA Biosciences Inc. (part of Thermo Fisher, Germering, Germany) was placed in series after the ECD detector. Data processing was carried out with Chromoleon 6.8 software (Thermo Fisher). The nitrogen gas supply for the CAD-device was monitored and regulated automatically to 35 psi, the response full-scale range was set to 20 pA, and a medium filter was applied.

Separation was carried out with porous-shell fused core Ascentis Express C18 analytical column (150 mm × 2.1 mm, 2.7 μm) from Supelco (Bellefonte, PA, USA). The mobile phases consisted of (A) 60 mM ammonium formate buffer (pH 1.5) in water, and

(B) methanol with 5% of formic acid in a gradient elution analysis programmed as follows: 0 min, 5% (B); 0–5 min, 5% (B); 5–35 min, 40% (B); 35–40 min, 40% (B); with 10 min of post-time for column conditioning at a flow rate of 300 μL min^{-1}. It has been shown that the CAD response strongly depends on the amount of organic solvent in the mobile phase (Plaza et al., 2014a). In order to achieve a uniform CAD response and to be able to quantify the phenolic compounds with just one phenolic standard an inverse methanol gradient (make-up gradient) requiring a dual gradient pump system was needed (de Villiers, Gorecki, Lynen, Szucs, & Sandra, 2007; Gorecki, Lynen, Szucs, & Sandra, 2006). The make-up gradient started 0.3 min after the elution gradient. All solvents were purged continuously with nitrogen to remove oxygen. The column temperature was set at 50 °C, the injection volume was 2 μl and the vial tray was held at 4 °C.

Phenolic compounds were quantified with CAD detector. The calibration curve of cyanidin 3-glucoside was selected to quantify all phenolic compounds. The cyanidin 3-glucoside standard solution was injected in triplicate at six concentrations levels (1–100 μg mL^{-1}). The calibration curve of cyanidin 3-glucoside was obtained by plotting peak area as function of concentration (μg mL^{-1}). Responses obtained in the examined ranges were expressed by a linear equation, $y = ax \pm b$ ($y = 3.3017x - 0.0023$), with good r^2 determination coefficient value (0.997).

2.3.3. Identification of phenolic compounds by HPLC-DAD-MS

The structural elucidation of phenolic compounds was carried out by a Waters Acquity UPLC chromatographic system (Waters Corp., Manchester, UK) that was equipped with a DAD and a quadrupole and orthogonal acceleration time-of-flight tandem mass spectrometer Xevo G2 qTOF with electrospray ionization (ESI) (Waters MS Technologies, Manchester, UK). The HPLC instrument was equipped with a binary solvent pump, an auto-sampler, and a column heater compartment. The system was controlled by Waters® Empower™ Chromatography software; while MassLynx™ (V 4.1, SCN 779, Waters Corp., Manchester, UK) was used for MS data acquisition and treatment. The separation conditions were the same as stated in Section 2.3.2 with the following exception. The mobile phases consisted of (A) water with 0.5% of formic acid, and (B) methanol with 0.5% of formic acid. UV–vis spectra were recorded in the range of 200–500 nm. The ESI interface was operated in both positive and negative modes, and full-scan HPLC-qTOF-MS spectra were obtained by scanning from *m/z* 50 to *m/z* 1000. The mass spectrometer was calibrated using a solution of sodium formate. Data were collected in continuum mode and the mass was corrected during acquisition using an external reference (Lock-Spray™ interface) comprising of a 10 μL min^{-1} solution of leucine-enkephalin (2 ng μL^{-1}). The capillary and cone voltage were set at 3 kV and 30 V, respectively. Nitrogen was used as both cone gas (50 l h^{-1}) and desolvation gas (1000 l h^{-1}). The source and desolvation temperatures were set at 120 and 400 °C, respectively. Simultaneous acquisition of exact mass at high and low collision energy, MS^E (where E represents collision energy), was used to obtain full scan accurate mass fragment, precursor ion, and neutral loss information. The collision energy in function 1 (low energy) was off while in function 2 (high energy) the collision energy was ranged from 15 to 60 V.

2.4. Clinical study design

2.4.1. Subjects

Ten healthy volunteers, 5 men and 5 women aged 26.5 ± 3.4 years, with normal body mass index (BMI) (mean ± standard deviation (sd) = 22.9 ± 1.1 kg/m^2) participated in the study. The inclusion criteria were age between 20 and 35 years, BMI between 18 and 25 kg/m^2, non-smoker and no known metabolic disorders or food allergies. Approval of the study was given by the Regional Ethical Review Board in Lund, Sweden (Dnr 2009/587).

2.4.2. Meals

Both breakfast and lunch meals were based on 50 g potentially available starch. The control meal was given to the subjects as 250 mL of water and 121.5 g white wheat bread prepared according to Rosen et al. (2009). The test meal included 27.6 g *M. jaboticaba* peel powder, which was mixed with water into a jam and served with the same bread and water as the control meal. The dose was based on the total phenolic contents which has not indicated any adverse effects in humans. According to our findings 27.6 g of jaboticaba peel offers 1.25 g of total phenolic (Table 1). To standardize both meals in terms of reducing sugar (Leite et al., 2011), 12.0 g of sucrose was added to the control meal. The sucrose was mixed with non-caloric green and red food colourings to resemble the jaboticaba jam.

The lunch consisted of commercial, low fibre, high glycemic index white wheat bread, butter and ham. The sandwiches were served as standardized double-sandwiches either whole or cut diagonally. The lunch meal was served with 300 mL water.

2.4.3. Study design

The design was single-blinded, placebo controlled, randomized, crossover, with one week wash out in between both treatments. The subjects were asked to refrain from all vegetable foods and drink for two days (48 h) prior to the meal studies and they were asked to continue the restricted diet during the follow-up 24 h. The restricted diet consisted of unflavoured dairy products, meat, fish, eggs, refined wheat flour products and rice.

After their evening meals, the subjects were fasting until the breakfast was served the subsequent morning at the research department. The subjects arrived to the department at 7:45 am. An intravenous cannula was inserted into an antecubital vein for blood sampling. Fasting blood samples were collected before the breakfast. The subjects received the control or test meal at 8:00 am and finished within 10 min. Three hours after breakfast, the test subjects were provided a lunch (at 11:00 a.m.), and measurement of physiological test variables continued after this meal. Samples for blood glucose and insulin, as well as antioxidant capacity, were obtained at fasting and at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after commencing the breakfast.

The subjects were encouraged to standardize their meal pattern and to maintain their regular eating habits during the experimental period. They were also instructed to avoid alcohol, excessive physical exercise or food rich in dietary fibres the day prior to the test days. Furthermore, they should not have taken antibiotics or probiotics during the previous 2 weeks period. During the experimental days the subjects were told to maintain a constant, low physical activity, preferably reading or similar.

2.4.4. Blood analysis

First, blood glucose collected from finger-prick was measured repeatedly using microcuvettes and a glucometer (HemoCue® B-glucose, HemoCue AB, Ängelholm, Sweden). Venous blood was withdrawn repeatedly for determination of serum insulin and antioxidant capacity. Insulin was measured using a commercial enzyme immunoassay kit (Mercodia, Uppsala, Sweden). The HOMA-IR ((insulin (mU L^{-1}) × glucose (mmol L^{-1}))/22.5) and HOMA-β ((20 × insulin (mU L^{-1}))/(glucose (mmol L^{-1}) − 3.5)) indexes were calculated using the glucose and insulin fasting values.

The antioxidant capacity of serum was measured according to the hydrophilic oxygen radical absorbance capacity (ORAC) assay, using AAPH as radical, trolox as standard and a Synergy HT, Biotek

Table 1
Identification (UV–vis and MS characteristics, molecular formula (MF) and mass precision (mDa)), quantification (CAD response for cyanidin-3-glucoside standard, mg 100 g^{-1} dry weight sample ± standard deviation (sd)), antioxidant capacity (ECD response, peak area μA*s 100 g^{-1} dry weight sample ± sd) and individual antioxidant capacity (ECD area/concentration, μA*s mg^{-1}) of phenolic compounds in the *M. jaboticaba* peel. All measurements were done in triplicate for each extraction.

ID	Rt (min)	Compounds identified	UV–vis maxima (nm)	$[M-H]^-$ (*m*/*z*), MF, mDa	Main fragments detected (*m*/*z*), MF, mDa	Concentration (mg 100 g^{-1} dry weight of sample)	ECD peak area 100 g^{-1} dry weight sample ((μA*s) 100 g^{-1})	Individual antioxidant capacity (μA*s mg^{-1})
1	2.1	HHDP-galloylglucose	216.8, 279.8	633.0737, $C_{27}H_{21}O_{18}$, 0.9	481.0507, $C_{20}H_{17}O_{14}$, 2.2 331.0627, $C_{13}H_{15}O_{10}$, −3.8 300.9982, $C_{14}H_5O_8$, −0.2 275.0190, $C_{13}H_7O_7$, −0.2 249.0433, $C_{12}H_9O_6$, 3.4 169.0123, $C_7H_5O_5$, 3.4	77.6 ± 5.7	19.0 ± 1.3	0.24
2	2.8	Non-identified phenolic (1)	242.5, 271.2			35.5 ± 1.5	22.3 ± 2.0	0.63
3	3.2	Bis-HHDP-glucose (Casuariin)	195.8, 216.8, 231.0, 260.5	783.0704, $C_{34}H_{24}O_{22}$, 2.3	481.0596, $C_{20}H_{17}O_{14}$, −2.2 300.9982, $C_{14}H_5O_8$, −0.2 275.0190, $C_{13}H_7O_7$, −0.2 257.0065, $C_{13}H_5O_6$, −2.1 229.0117, $C_{12}H_5O_5$, −2.0	75.1 ± 5.1	12.8 ± 0.8	0.17
4	3.6	Non-identified phenolic (2)	241.8, 269.5			100.9 ± 5.1	14.8 ± 1.0	0.15
5	5.7	Bis-HHDP-glucose isomer (Pedunculagin)	195.8, 231.8, 258.0	783.0646, $C_{34}H_{24}O_{22}$, 2.4	481.0596, $C_{20}H_{17}O_{14}$, −2.2 300.9982, $C_{14}H_5O_8$, −0.2 275.0190, $C_{13}H_7O_7$, −0.2 257.0095, $C_{13}H_5O_6$, 1.2 229.0148, $C_{12}H_5O_5$, 1.1	111.3 ± 8.1	23.0 ± 1.3	0.21
6	11.1	Galloyl-bis-HHDP-glucose (Casuarinin)	208.8, 280.0	935.0803, $C_{41}H_{27}O_{26}$, 1.2	783.0417, $C_{34}H_{23}O_{22}$, −3.5 765.0485, $C_{34}H_{21}O_{21}$, −3.4 633.0737, $C_{27}H_{21}O_{18}$, 0.9 481.0641, $C_{20}H_{17}O_{14}$, 2.3 300.9982, $C_{14}H_5O_8$, −0.2 275.0190, $C_{13}H_7O_7$, −0.2 169.0150, $C_7H_5O_5$, −1.4	185.5 ± 13.8	17.3 ± 0.2	0.09
7	11.5	Galloyl-bis-HHDP-glucose isomer (Casuarictin)	207.8, 280.0	935.0803, $C_{41}H_{27}O_{26}$, 1.2	783.0589, $C_{34}H_{23}O_{22}$, 2.3 765.0485, $C_{34}H_{21}O_{21}$, −3.4 633.0634, $C_{27}H_{21}O_{18}$, 0.9 481.0596, $C_{20}H_{17}O_{14}$, −2.2 300.9982, $C_{14}H_5O_8$, −0.2 275.0190, $C_{13}H_7O_7$, −0.2 169.0123, $C_7H_5O_5$, 1.3	212.6 ± 16.8	40.8 ± 0.7	0.19

Table 1 (*continued*)

ID	Rt (min)	Compounds identified	UV–vis maxima (nm)	$[M-H]^-$ (*m/z*), MF, mDa	Main fragments detected (*m/z*), MF, mDa	Concentration (mg 100 g^{-1} dry weight of sample)	ECD peak area 100 g^{-1} dry weight sample ((μA*s) 100 g^{-1})	Individual antioxidant capacity (μA*s mg^{-1})
8	12.8	Delphinidin-3-glucoside (Myrtillin)*	275.3, 520.6	465.1032 $[M]^+$, $C_{21}H_{21}O_{12}$, −0.1	303.0485, $C_{15}H_{11}O_7$, −2.0	356.3 ± 1.0	41.1 ± 1.2	0.12
9	15.1	Cyanidin-3-glucoside (Kuromarin)*	278.6, 513.2	449.1098 $[M]^+$, $C_{21}H_{21}O_{11}$, 1.4	287.0554, $C_{15}H_{11}O_6$, −0.2	2866.3 ± 40.1	78.8 ± 0.7	0.03
10	15.7	HHDP-digalloylglucose (Tellemagrandin I)	265.8	785.0845, $C_{34}H_{25}O_{22}$, 0.8	633.0737, $C_{27}H_{21}O_{18}$, 0.9 483.0775, $C_{20}H_{19}O_{14}$, −2.2 300.9982, $C_{14}H_5O_8$, −0.2 275.0156, $C_{17}H_7O_4$, −3.5 169.0150, $C_7H_5O_5$, −1.4	51.3 ± 2.9	7.4 ± 0.5	0.14
11	17.5	HHDP-trigalloylglucose (Tellemagrandin II)	218.8, 274.8	937.0929, $C_{41}H_{29}O_{26}$, 4.5	785.0787, $C_{34}H_{25}O_{22}$, −2.8 767.0733, $C_{34}H_{23}O_{21}$, 0.1 765.0541, $C_{34}H_{21}O_{21}$, −3.4 633.0840, $C_{27}H_{21}O_{18}$, −4.3 613.0433, $C_{27}H_{17}O_{17}$, 1.8 483.0799, $C_{20}H_{19}O_{14}$, 2.4 481.0641, $C_{20}H_{17}O_{14}$, −2.2 465.0636, $C_{20}H_{17}O_{13}$, 1.1 300.9982, $C_{14}H_5O_8$, −0.2 275.0156, $C_{17}H_7O_4$, −3.5 229.0148, $C_{12}H_5O_5$, 1.1 169.0150, $C_7H_5O_5$, −1.4	74.8 ± 1.7	11.0 ± 0.3	0.15
12	17.7	Ellagic acid pentoside	252.8, 359.8	433.0424, $C_{19}H_{13}O_{12}$, 1.7	300.9982, $C_{14}H_5O_8$, −0.2 275.0122, $C_{13}H_7O_7$, −3.6 229.0117, $C_{12}H_5O_5$, −2.0	55.1 ± 5.1	4.0 ± 0.4	0.07
13	19.8	Ellagic acid*	253.8, 367.8	300.9982, $C_{14}H_5O_8$, −0.2	275.0190, $C_{13}H_7O_7$, −0.2 229.0117, $C_{12}H_5O_5$, −2.0	142.8 ± 11.7	15.0 ± 1.3	0.11
14	20.3	Pentagalloyl hexose	218.8, 279.8	939.1077, $C_{41}H_{31}O_{26}$, −2.7	787.0782, $C_{34}H_{27}O_{22}$, −4.0 769.0838, $C_{34}H_{25}O_{21}$, 0.7 617.0837, $C_{27}H_{21}O_{17}$, −4.4 601.0721, $C_{27}H_{21}O_{16}$, −2.9 465.0680, $C_{20}H_{17}O_{13}$, 1.1 447.0517, $C_{20}H_{15}O_{12}$, −0.4 431.0574, $C_{20}H_{15}O_{11}$, 0.3 313.0553, $C_{13}H_{13}O_9$, −0.7 295.0443, $C_{13}H_{11}O_8$, −1.1	32.8 ± 3.0	2.8 ± 0.2	0.09

(*continued on next page*)

Table 1 (*continued*)

ID	Rt (min)	Compounds identified	UV–vis maxima (nm)	$[M-H]^-$ (*m*/*z*), MF, mDa	Main fragments detected (*m*/*z*), MF, mDa	Concentration (mg 100 g^{-1} dry weight of sample)	ECD peak area 100 g^{-1} dry weight sample ((μA*s) 100 g^{-1})	Individual antioxidant capacity (μA*s mg^{-1})
					279.0506, $C_{13}H_{11}O_7$, 0.1 169.0123, $C_7H_5O_5$, −1.4			
15	20.4	Non-identified phenolic (3)	262.8, 352.8			82.5 ± 1.9	–	–
16	24.2	Quercetin-3-rhamnoside (Quercitrin)*	255.8, 348.8	447.0863, $C_{21}H_{19}O_{11}$, −2.0	301.0248, $C_{15}H_9O_7$, −1.5	60.7 ± 2.1	2.6 ± 0.2	0.04
					Total	4521.0 ± 59.8	312.6 ± 3.1	

* Identified confirmed using commercial standards

microplate reader (Winooski, USA) set with fluorescent filters (excitation: 485 nm and emission: 520 nm), as described in a previous study (Batista et al., 2014).

2.4.5. Statistical analysis

Data from each person and test day were normalized for baseline measurements to examine changes in blood glucose, serum insulin and ORAC for each person over time and total AUC (area under the curve) were used for statistics. The statistical analyses of all data were based on two-way ANOVA followed by Bonferroni tests and paired Student's *t* test for AUC data. The limit of significance was set at $P < 0.05$. The statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software.

3. Results and discussion

3.1. Characterization of the M. jaboticaba peel

In order to carry out the extraction of the phenolic compounds from *M. jaboticaba*, PHWE was used. PHWE is an environmentally sustainable technique that presents important advantages over traditional solvent extraction techniques, offering safe, green and rapid extractions. Increasing the temperature decreases the dielectric constant of water, resulting in the possibility of tuning its solvent properties, produces an enhancement on the solubility of the analytes, an improvement of water diffusivity and a decrease in water viscosity, which allows better penetration into the matrix and faster mass transfer of the compounds of interest (Plaza & Turner, 2015). *M. jaboticaba* presents high amount of anthocyanins that for chemical stability reasons require acidic conditions and lower extraction temperature. Thus, the use of additives in the extraction solvent (i.e. formic acid) to lower the pH was needed. The extraction conditions used in the present work to extract phenolic compounds from *M. jaboticaba* were based on a previous optimized study to extract anthocyanins from red cabbage (Arapitsas & Turner, 2008). After the PHWE of the polyphenols from *M. jaboticaba*, the extract was ready for further analysis.

A method based on HPLC-DAD-ECD-CAD-MS was adapted to characterize the phenolic compounds of *M. jaboticaba* PHWE extract. The HPLC-DAD-ECD-CAD method used in this study was based on an optimized method in a previous work, with some modification (Plaza et al., 2014b). In the present work, higher amounts of formic acid were needed in the mobile phase (5% instead 0.5%, v/v). As it is commented above, the pH needs to be very low to stabilize the flavylium species of the anthocyanins and to reduce the presence of non-charged forms guaranteeing a better resolution and the typical absorbance is in the range of 520–540 nm (Mulinacci & Innocenti, 2012, Chap. 22). However, 5% (v/v) of formic acid in the mobile phase is not recommended to use when the HPLC is connected to the MS detector. Therefore, 0.5% (v/v) of formic acid was used when the samples were analyzed by HPLC-DAD-MS.

The beauty of using the hyphenated system HPLC-DAD-ECD-CAD is that information about light absorbing properties (DAD), quantitative (CAD) and oxidisability properties (ECD) of the phenolic compounds can be obtained in just one run. The phenolic compounds were detected by a DAD, which was set to record chromatograms between 200 and 600 nm, with selected wavelengths of 280 nm (for identification of phenolic compounds), 350 nm (for flavonols) and 520 nm (for anthocyanins). The DAD allowed a preliminary characterization of the compounds based on their retention time and UV–vis spectra by comparison with standards, when available. The ECD was used to estimate the antioxidant capacity of each compound and its contribution to the total antioxidant capacity. The oxidation potential (0.6 V) employed was the optimized one in previous study of phenolic compounds from apples (Plaza et al., 2014a).

The quantification of phenolic compounds was carried out with a CAD detector using the calibration curve of cyanidin 3-glucoside. This detector allowed the quantification of all the separated phenolic compounds without their respective standards, because the signal of the compounds is proportional to the weight of sample present independent of chemical structure. This results in an equal response for equal mass amounts (de Villiers et al., 2007; Gorecki et al., 2006). Cyanidin 3-glucoside was selected to quantify all phenolic compounds because it is normally found in high concentrations in *Myrciaria* species (Reynertson, Yang, Jiang, Basile, & Kennelly, 2008).

Fig. 1 shows the chromatograms at 280, 350 and 520 nm, CAD chromatogram and amperogram obtained by HPLC-DAD-ECD-CAD from *M. jaboticaba* extract. The data clearly demonstrates that sixteen most likely phenolic compounds could be separated in an analysis time of around 25 min. As shown in Fig. 1, the peak profile of the HPLC-ECD amperogram aligned well with the chromatographic peaks in the DAD and CAD profiles.

A HPLC-DAD-MS^E method was set up, with the aim to identify the phenolic compounds present in *M. jaboticaba* extracts. The careful analysis of the separated compounds, using the information provided by the DAD detector as well as the MS detector installed in series, together with the information that could be found in the literature and from commercial standards (when available) allowed the identification of 13 phenolic compounds in *M. jaboticaba* extract. Table 1 lists the compounds tentatively identified. In fact, combining the information provided by the UV–vis spectra

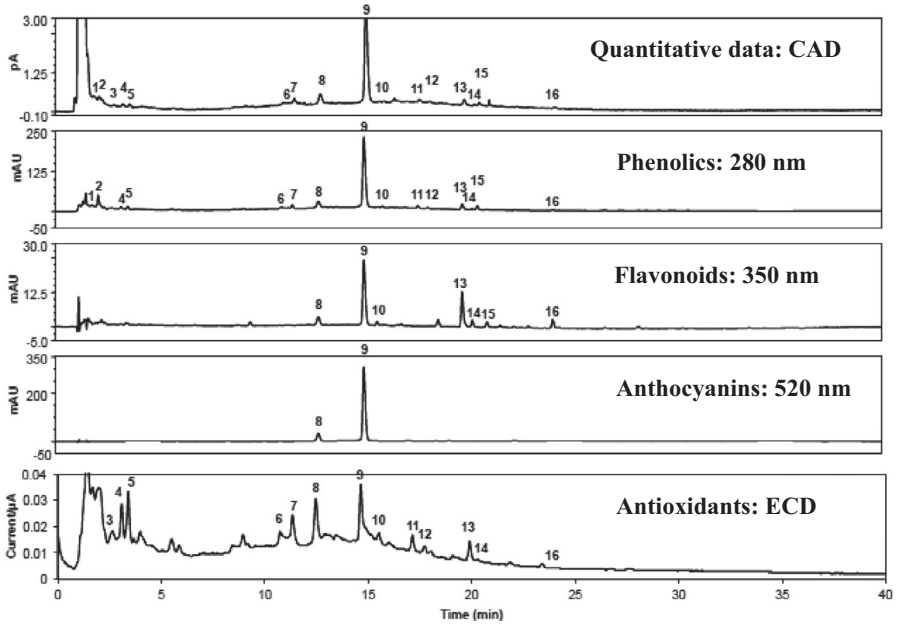

Fig. 1. Chromatograms and amperograms corresponding to the LC-DAD-ECD-CAD analysis of *M. jaboticaba* peel extract at 280 nm, 350 nm and 520 nm and amperogram.

of the separated compounds as well as the information of their MS spectra and the MS^E fragmentation patterns, it was possible to significantly increase the certainty of the tentative assignments. This data is also shown in Table 1.

3.1.1. Identification of phenolic compounds by HPLC-DAD-MS

As it can be observed in Fig. 1 and in Table 1, almost all the main peaks separated in HPLC analyses could be identified. The extract was characterized by the presence of anthocyanins, mainly cyanidin 3-glucoside (kuromanin) and delphinidin 3-glucoside (myrtillin), different ellagitannins and gallotannins, flavanols, as well as ellagic acid and its related compounds.

3.1.1.1. Anthocyanins. The two predominant peaks in the chromatogram obtained at 520 nm (peaks 8 and 9) are anthocyanins (see Fig. 1). These well-known compounds present in *Myrciaria* species and *M. jaboticaba* have been described to possess interesting functional properties, including antioxidant capacity (Einbond, Reynertson, Luo, Basile, & Kennelly, 2004; Leite et al., 2011; Lenquiste, Batista, Marineli, Dragano, & Marostica Junior, 2012; Reynertson et al., 2006). Nevertheless, the negative ESI ionization employed for the detection of phenolic compounds did not allow identification of the anthocyanins (peaks 8 and 9). With the aim to identify these components, positive ESI ionization analysis was carried out. When presented under positive ESI conditions peak 9 revealed the existence of a molecule ion $[M]^+$ at m/z 449.1098 that produced a main fragment at m/z 287.0554. The combination of this information with its UV–vis spectrum (maximum absorbance at 513.2 nm and co-chromatography (commercial standard)) provided the identification of this peak as cyanidin 3-glucoside (kuromanin) (Fig. 2a). The other anthocyanin (peak 8) found at a lower concentration was delphinidin 3-glucoside (myrtillin) which presented at m/z 465.1032 $[M]^+$ with a main fragment at m/z 303.0485 and maximum absorbance at 520.6. The identification of this peak was also confirmed using mass spectra data and co-chromatography (commercial standard).

3.1.1.2. Ellagitannins and gallotannins (hydrolysable tannins). The hydrolysable tannins are polyesters of a sugar moiety and organic acids (Serrano, Puupponen-Pimia, Dauer, Aura, & Saura-Calixto, 2009). If the acid component is gallic acid, the compounds are called gallotannins. However, if the ester is hexahydroxydiphenic acid (HHDP) (forming ellagic acid when hydrolysed through elimination of water), the hydrolysable tannins are called ellagitannins. Most ellagitannins are mixed esters with both HHDP and gallic acid (example of chemical structure in Fig. 3). These compounds present in *Myrciaria* species have been described to possess interesting functional properties, including antioxidant, antimicrobial, anti-inflammatory, anti-atherogenic and anticancer activities (Lipinska, Klewicka, & Sojka, 2014).

Among the ellagitannins detected galloyl-bis-HHDP-glucose (casuarinin) (peak 6) and galloyl-bis-HHDP-glucose isomer (casuarictin) (peak 7) were the main compounds (Fig. 1). Peak numbers 6 and 7 were isomers. The presence of different isomers of galloyl-bis-HHDP-glucose in *M. cauliflora* has been described previously (Wu et al., 2012). Casuarinin has higher polarity than casuarictin, causing casuarinin to elute first because it has an open glucose ring structure and therefore an additional hydroxyl group (casuarinin is 16 OH groups vs. casuarictin is 15 OH groups). Therefore, peak 6 would correspond to casuarinin and peak 7 to casuarictin. These peaks presented a maximum absorbance at 280 nm and a clear molecular ion with m/z 935.0803 $[M-H]^-$. This ion produced fragments with m/z 783.0589, 633.0634, 481.0507, 300.9982, 275.0190, and 169.0123 (Table 1 and Fig. 2b). The fragmentation pattern of these compounds is well described, presenting fragments as a result of the loss of a galloyl group (m/z 783.0761), a HDDP group (m/z 633.0737) and both (m/z 481.0596) (Wu et al., 2012). Both the molecular ion as well as the fragments confirmed the presence of casuarinin and casuarictin (Table 1).

Other ellagitannins found in high concentrations were bis-HHDP-glucose (pedunculagin) (peak 5) and casuariin (peak 3). Both ellagitannins demonstrated the same molecular ion m/z 783.0646 and 783.0704 $[M-H]^-$ and the fragmentation patterns were identical (Table 1 and Fig. 1). These ions showed fragments at m/z 481.0596 that could most probably be derived from the loss of a HHDP unit together with other fragments at m/z 300.9982, 275.0190, 257.0095, 229.0148 corresponding to those typically from the HHDP unit $[HHDP]^-$ and its fragmentation (Table 1). Pedunculagin and casuariin are isomers and could be distinguished by their retention times. As well as the isomers of ellagitannins above, casuariin has higher polarity than pedunculagin, thus

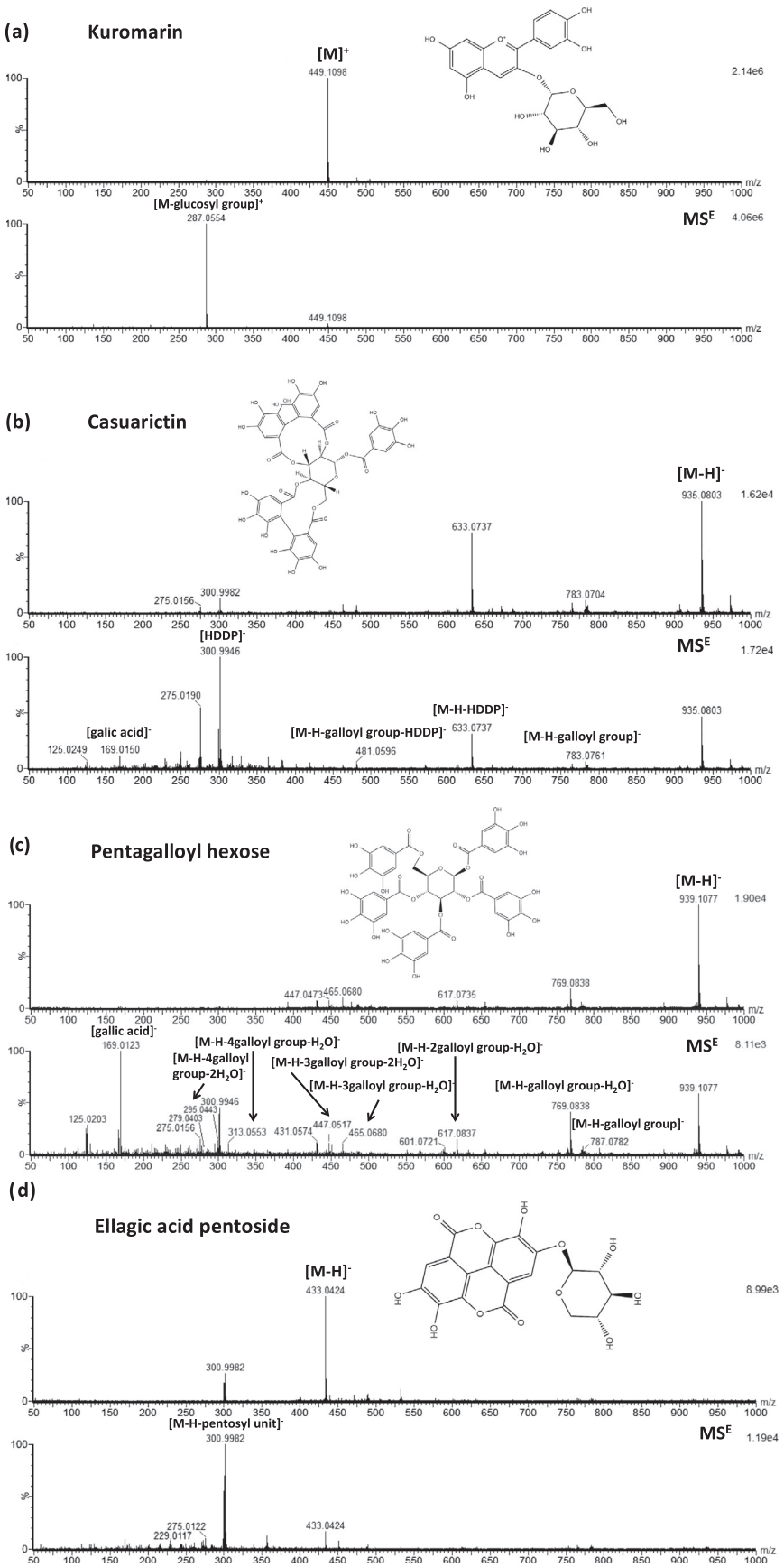

Fig. 2. MS spectra and MS^E fragmentation pattern of the phenolic compounds found in *M. jaboticaba* peel' PLE extract: a) cyanidin-3-glucoside (peak 9); b) galloyl-bis-HHDP-glucose isomer (peak 7); c) pentagalloyl hexose (peak 14); and d) ellagic acid pentoside (peak 12).

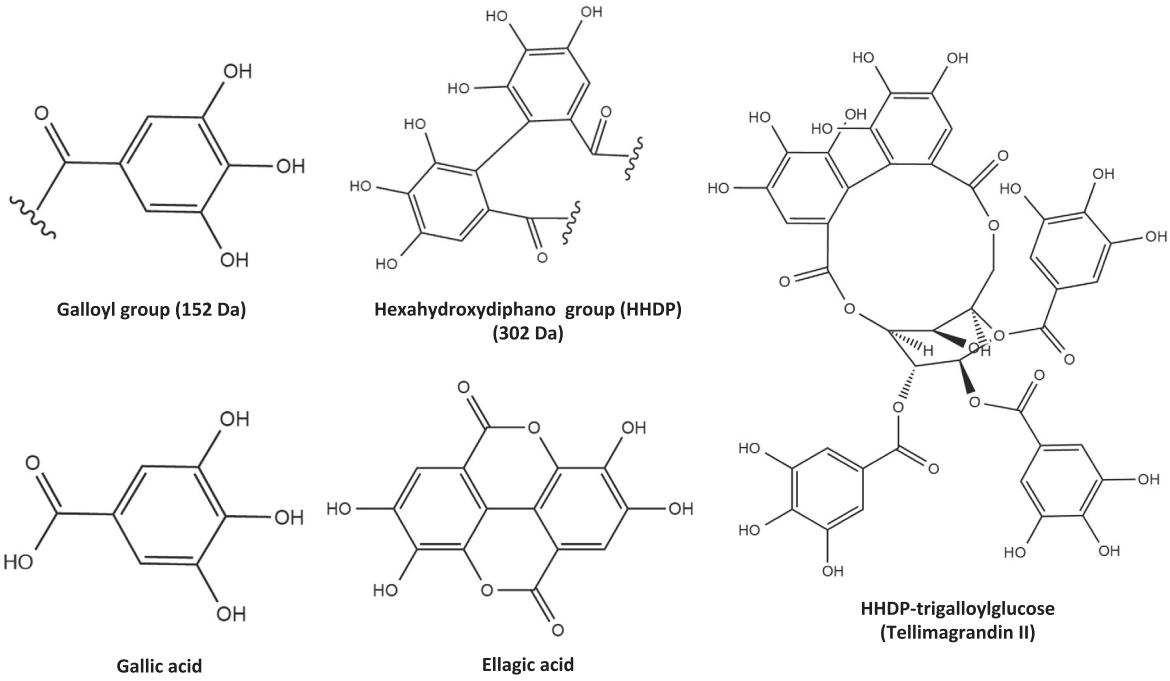

Fig. 3. Example of structures of the hydrolysable tannins and their main ester substituents found in *M. jaboticaba* peel.

eluting casuariin first because it has an open glucose ring structure and therefore an additional hydroxyl group (casuariin 14 OH groups vs. pedunculagin 13 OH groups).

The first eluted compound (peak 1) was tentatively identified as HHDP-galloylglucose with a maximum absorbance at 279.8 nm and molecular ion *m*/*z* at 633.0737 $[M-H]^-$. The fragmentation pattern of this compound presented a fragment at *m*/*z* 481.0507 as a result of the loss of a galloyl group and at *m*/*z* 331.0627 as a result of the loss of a HHDP unit (see Table 1).

The ellagitannin HHDP-digalloylglucose (tellimagrandin I) (peak 10) has previously been described in *Myrciaria* species (Wu et al., 2012). This peak presented a molecular ion at *m*/*z* 785.0845 $[M-H]^-$. The MS^E fragmentation produced ions at *m*/*z* 633.0737 and 483.0775 corresponding to the loss of a galloyl group and HHDP unit, respectively.

Peak 11 was tentatively identified as HHDP-trigalloylglucose (tellimagrandin II) with a maximum absorbance at 274.8 nm and molecular ion *m*/*z* at 937.0929 $[M-H]^-$ (Fig. 3). In the fragmentation pattern, the loss of one, two and three galloyl group was observed (*m*/*z* at 785.0787, 633.0840, and 481.0696, respectively), as well as the loss of a galloyl group and HHDP at *m*/*z* 483.0753.

The compound 14 was tentatively identified as pentagalloyl hexose, considering its molecular ion at *m*/*z* 939.1077 and the presence of fragments with loss of a H_2O molecule and 1, 2, 3 and 5 galloyl groups (*m*/*z* 769.0838, 617.0837, 465.0680 and 313.0552, respectively) (Table 1 and Fig. 2c). Pentagalloyl hexose was the only gallotannin found in *M. jaboticaba*.

3.1.1.3. Ellagic acid and derivatives. Ellagic acid was found in high concentrations (peak 13). This peak showed a maximum absorbance at 367.8 nm and a clear molecular ion *m*/*z* 300.9982 $[M-H]^-$. The identification of this peak was confirmed using mass spectra data and co-chromatography (commercial standard). This compound has previously been found in *M. jaboticaba* (Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013a).

Ellagic acid pentoside (peak 12) could be identified since it showed a $[M-H]^-$ at *m*/*z* 433.0424 and MS^E yielding an ion at *m*/*z* 300.9982 [M−H-pentosyl unit]$^-$ and *m*/*z* 275.0122 and 229.0117, which matches the fragmentation pattern of ellagic acid. On the basis of the mass spectra data and the maximum absorbance at 359.8 nm, this compound was identified (Table 1 and Fig. 2d).

3.1.1.4. Flavonols. One flavonol could be identified in the *M. jaboticaba* peel. Peak 16 was identified as a quercetin-3-rhamnoside (quercitrin). This flavonol was determined by its UV–visible spectrum, mass spectra data, retention time and comparison with the commercial standard. Peak 16 showed the molecular ion at *m*/*z* 447.0863 $[M-H]^-$ and it produced a clear fragment corresponding to its aglycone (*m*/*z* 301.0248) (Table 1). The maximum of absorbance was at 348.8. This compound has been described previously in *Myrciaria* species (Borges, Conceicao, & Silveira, 2014).

All of the above compounds, except one, have been previously detected from *Myrciaria* species (Leite et al., 2011; Reynertson et al., 2006; Wu et al., 2012), but are being reported for the first time in the *Myrciaria jaboticaba* species, except for kuromanin, myrtillin and ellagic acid, which have been already described in this fruit (Alezandro et al., 2013a; Leite et al., 2011). Further, this is the first time gallotannin pentagalloyl hexose has been identified in any *Myrciaria* species.

3.1.2. Quantification and individual antioxidant capacity of phenolic compounds

The online HPLC-DAD-ECD-CAD method was used to carry out the quantification (CAD) of the different phenolic compounds found in the PHWE extract of *M. jaboticaba*, as well as to determine their individual antioxidant capacity (ECD).

As shown in Table 1, kuromanin was the compound found in the highest concentration followed by myrtillin, casuarictin, casuarinin, ellagic acid, pedunculagin and a non-identified phenolic compound (phenolic 2 in Table 1). These seven compounds, summarized to 88% of the total mass of detected phenolic compounds based on CAD, and their contribution to the total antioxidant capacity was 74% based on ECD. The kuromanin represented around 63% (2866.2 ± 40.1 mg 100 g^{-1} dry weight of sample) of total phenolic compounds. However the individual contribution

of kuromanin to the total antioxidant capacity was around 25% (78.8 ± 0.7 ECD peak area 100 g^{-1} dry weight). While the contribution of the other six compounds, which represented the 25% of total phenolics, the total antioxidant capacity was 49%. This result means that the latter compounds together had more antioxidant power than kuromanin because even at lower concentrations their contribution to the total antioxidant capacity was higher.

The compound found in the lowest concentration was pentagalloyl hexose, followed by a non-identified phenolic (1), tellimagrandin I, ellagic acid pentoside, quercitrin, tellimagrandin II, casuariin, HHDP-galloyl glucose and non-identified phenolic (3). These compounds only represented 12% of the total phenolic compounds, however their contribution to the total antioxidant capacity was considerable (26%).

Ellagitannins and gallotannins (hydrolysable tannins) were the phenolic class that contributed most to the total antioxidant capacity of *M. jaboticaba* (43%), followed by anthocyanins (38%), other non-identified phenolic (12%), ellagic acid and derivatives (6%), and flavonols (1%). On the other hand, the phenolic group found in higher concentration was anthocyanins (71%) followed by ellagitannins and gallotannins (18%), others non-identified phenolic (5%), ellagic acid and derivatives (5%), and flavonols (1%).

Considering the individual antioxidant capacity (individual antioxidant capacity = ECD area (μA*s 100 g^{-1})/concentration (mg 100 g^{-1})) of each phenolic compound (see Table 1), the non-identified phenolic (1) showed the highest antioxidant capacity, followed by HHDP-galloylglucose, pedunculagin, casuarictin, casuariin, tellimagrandin II, non-identified phenolic (2), tellimagrandin I, myrtillin, ellagic acid, casuarinin, pentagalloyl hexose, ellagic acid pentoside, quercitrin and kuromanin. The non-identified phenolic (3) did not present any detectable antioxidant capacity. The antioxidant capacity of the flavonoids is affected by the substituents on the molecule (Plaza et al., 2014b). The anthocyanin myrtillin presented higher antioxidant capacity most likely because it has one more hydroxyl group (in total three OH groups) on the B-ring of the molecule than kuromanin, which has two OH groups. Also, myrtillin have the hydroxyl groups at the 3′-, 4′-, and 5′-position on the B-ring which enhances the antioxidant capacity (Plaza et al., 2014b). Quercitrin demonstrated a very similar antioxidant capacity to kuromanin. Both flavonoids have the same number of OH groups in the same position. Ellagic acid has higher antioxidant power than ellagic acid pentoside because the glycosylation of the latter decreases its antioxidant capacity (Plaza et al., 2014b). Between the isomers of ellagitannins, casuariin vs. pedunculagin and casuarinin vs. casuarictin, the ones with open glucose rings and one more OH group in the glucose (casuariin and casuarinin) showed lower antioxidant capacity than their respective isomers (pedunculagin and casuarictin). Tellimagrandin II possessed higher antioxidant capacity than tellimagrandin I, where the first one has one more galloyl group than the latter.

3.2. Clinical study

Previous reports have shown that *M. jaboticaba* peel in the diet could have important *in vivo* effects due to its polyphenol composition. Among them, improvement in antioxidant capacity of different tissues (Alezandro, Granato, & Genovese, 2013b; Leite et al., 2011), increment in antioxidant enzymes activity (Alezandro et al., 2013b; Batista et al., 2014); serum HDL-cholesterol enhancement, lower insulin resistance (Lenquiste et al., 2012), and anti-inflammatory effects in the liver and adipose tissue (Dragano et al., 2013) has been shown. However, to the best of our knowledge, this is the first clinical study of the effect of the antioxidant compounds found in *M. jaboticaba* peel on postprandial glucose metabolism and antioxidant bioavailability.

3.2.1. Postprandial antioxidant capacity (ORAC assay)

The results above showed that *M. jaboticaba* peel contains polyphenols of interest that may offer *in vivo* protection against oxidative stress, DNA damage and cancer (Leite-Legatti et al., 2012). Phenolic compounds are the major contributor to dietary antioxidant capacity (Pantelidis et al., 2007), and the administration of these compounds in clinical studies seems to be also effective against metabolic diseases, such as obesity-induced oxidative stress.

A single-blinded crossover study in men ($n = 8$) receiving a high-fat diet showed that the intake of freeze-dried wild blueberry powder (100 g in 500 mL water) promoted an increase in postprandial serum antioxidant capacity: 8.5% at 1 h and 15% at 4 h (Kay & Holub, 2002). Another single-blinded crossover study in men ($n = 5$) fed a high-fat diet showed that the intake of blueberry powder (0.2 g mL^{-1} of water) promoted the bioavailability of 19 anthocyanins in serum and increased serum antioxidant capacity as measured by ORAC (Mazza et al., 2002). In addition, a pilot, randomized, double-blinded, placebo-controlled crossover study in women and men ($n = 12$) verified that the intake of 120 mL of a juice blend (0.47 μg mL^{-1} anthocyanins) was capable of increasing the serum antioxidant capacity (2 h postprandial) and reducing serum lipid peroxidation levels (Jensen et al., 2008). However, berries are also good sources of ellagitannins, which in the present study showed to have greater antioxidant power than anthocyanins (Table 1).

The consumption of *M. jaboticaba* peel by the subjects increased serum antioxidant capacity against peroxyl radicals as demonstrated by the AUC values (ORAC assay), probably due to the presence of polyphenols, such as anthocyanins and ellagitannins compounds and its metabolites in the blood of the test subjects, as suggested by the aforementioned works (Jensen et al., 2008;

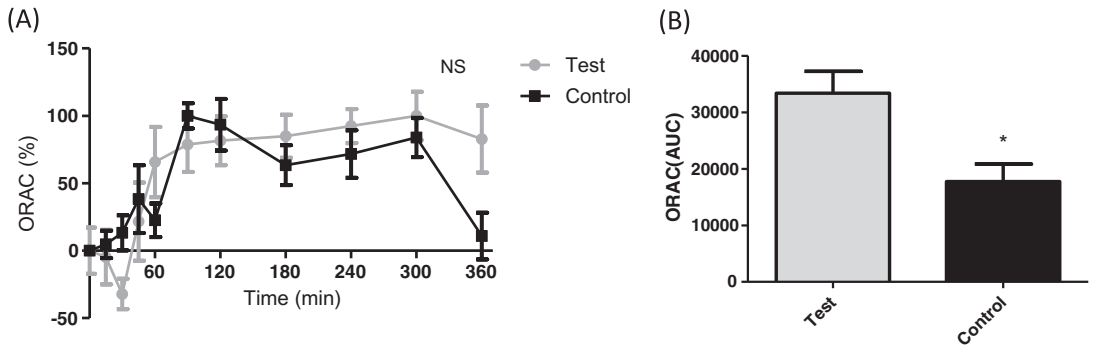

Fig. 4. a) Postprandial ORAC and b) total ORAC values in serum after consumption of *M. jaboticaba* peel or control by healthy subjects. NS = Non-significant; AUC = Area Under the Curve. * Indicates statistical differences when analyzed by two-way ANOVA and Bonferroni *post hoc* (a) or Student's *t* test (b) considering $P < 0.05$. Data expressed as mean ± standard error.

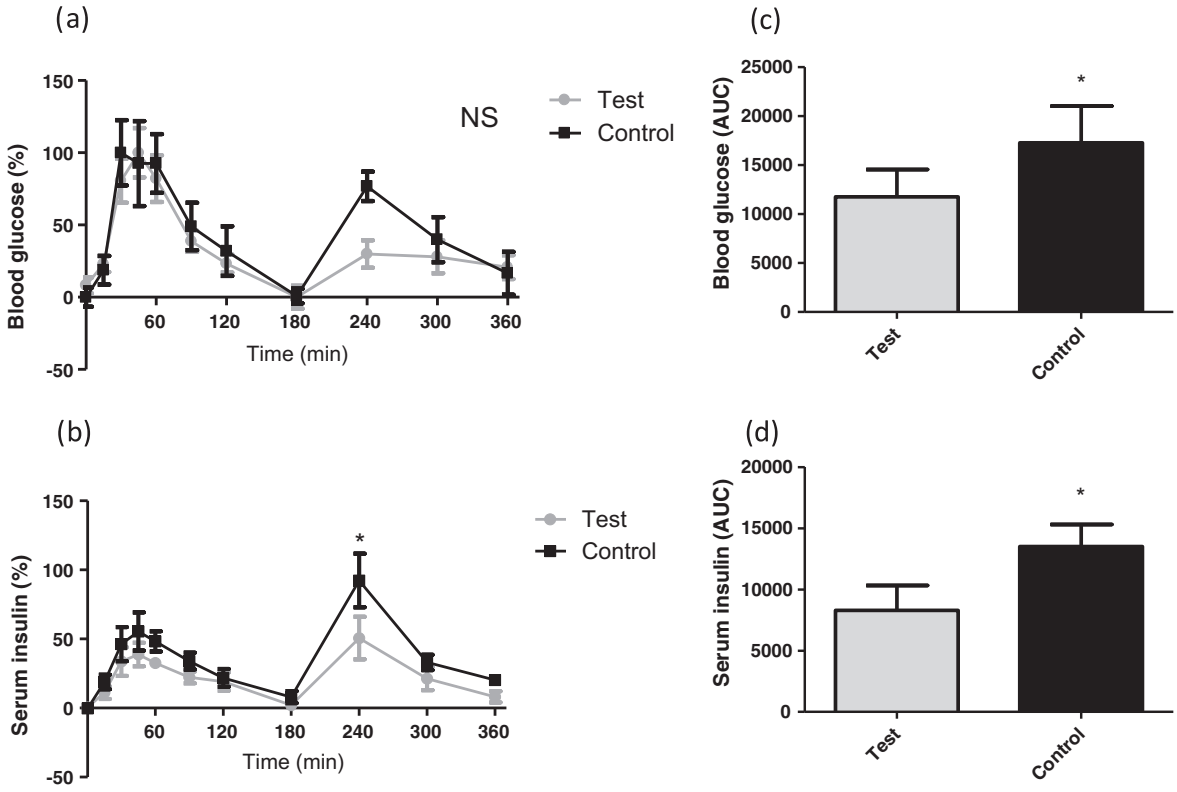

Fig. 5. Postprandial a) glucose and c) insulin and total AUC b) serum glucose and d) insulin after consumption of *M. jaboticaba* peel or control by healthy subjects. NS = Non-significant; AUC = Area Under the Curve. * Indicates statistical differences when analyzed by two-way ANOVA and Bonferroni *post hoc* (a, c) or Student's *t* test (b, d) considering $P < 0.05$. Data expressed as mean ± standard error.

Kay & Holub, 2002; Mazza et al., 2002). The AUC values from the postprandial antioxidant capacity measurements in the serum of the test subjects was 37% higher after intake of the meal containing *M. jaboticaba* peel when compared to the control meal (Fig. 4).

3.2.2. Postprandial glucose and insulin responses

Blood glucose showed a slight, but not significant, decrease at 240 min after the test meal consumption with *M. jaboticaba* peel and insulin levels were significantly decreased at the same time point when compared to the intake of the placebo. These changes corresponded to the lower area under the curve (AUC) for both glucose and insulin postprandial response (Fig. 5b and d). There were no statistical differences between the two meals for HOMA-IR or HOMA-β data.

The lower postprandial glucose and insulin responses after the *M. jaboticaba* peel ingestion could be explained by the ability of phenolic compounds to inhibit digestive enzymes, mainly glucosidase enzymes activities (Alezandro et al., 2013a, 2013b), which could explain the second meal effect (Wolever, Jenkins, Ocana, Rao, & Collier, 1988), as shown in Fig. 5c. Furthermore, phenolic compounds can inhibit the action of two membrane transporters in the intestine (SGLT1 and GLUT2) decreasing the glucose absorption and, consequently, the postprandial response (Manzano & Williamson, 2010). A previous study had shown that the mRNA expressions of SGLT1 and GLUT2 and glucose uptake were decreased in Caco-2 TC7 cells treated with berry extract (Alzaid, Cheung, Preedy, & Sharp, 2013).

In addition, parts of these effects could be explained by the capacity of dietary fibres from jaboticaba peel to result in a second meal effect by quenching glucose and starch to excretion (Batista et al., 2013). The berry polyphenols is also probably released slowly in the gut due to its association with the dietary fibre complex which could influence the insulin signaling pathway and glucose uptake (Chuang & McIntosh, 2011), and in turn explain the results associated with the second meal effect.

The present data confirms previous results of non-clinical studies in which jaboticaba peel intake has been demonstrated to reduce blood glucose levels and insulin resistance in both healthy and obese/insulin resistant rodents (Dragano et al., 2013; Lenquiste et al., 2012). These compounds play a role activating insulin receptor substrates and AKT, which are responsible for the Fox01 phosphorylation that inhibits gluconeogenesis (Dragano et al., 2013; Guo et al., 2012). This interference indicates possible prevention potential of *M. jaboticaba* towards metabolic diseases, such as insulin resistance, diabetes and obesity.

4. Conclusions

The complete phenolic compounds profile of the jaboticaba species, named *Myrciaria jaboticaba*, was studied. Tannins, such as casuarinin, casuarictin, pedunculagin, tellimagrandin I, tellimagrandin II, ellagic acid pentoside, in addition to flavonol as quercetin-3-rhamnoside, were identified and reported for the first time. Although anthocyanins were the major phenolic compounds found in *M. jaboticaba* peel, ellagitannins and gallotannins were the class with higher contribution to the antioxidant capacity. Moreover, the intake of *M. jaboticaba* peel by healthy adults increased their serum antioxidant status and decreased glucose and insulin levels after the second meal, indicating important clinical effects, such as improvement of insulin sensitivity.

Acknowledgements

The presented work has been supported by the Antidiabetic Food Centre, a VINNOVA VINN Excellence Centre at Lund University, Sweden. MRMJ thanks CNPq (Grant 300533/2013-6) and Eras-

mus Mundus for the scholarships. AGB and CBBC are grateful to CNPq (Science without Borders #206092/2014-8) and PNPD/CAPES for scholarships. CT acknowledges the Swedish Research Council for funding VR (2010-333). The authors want to thank Sofia Essén, Centre for Analysis and Synthesis (Lund University), for helpful advice regarding the MS data.

References

Abe, L. T., Lajolo, F. M., & Genovese, M. I. (2012). Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg). *Journal of the Science of Food and Agriculture, 92*, 1679–1687.

Alezandro, M. R., Dubé, P., Desjardins, Y., Lajolo, F. M., & Genovese, M. I. (2013a). Comparative study of chemical and phenolic compositions of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg. *Food Research International, 54*, 468–477.

Alezandro, M. R., Granato, D., & Genovese, M. I. (2013b). Jaboticaba (*Myrciaria cauliflora* (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. *Food Research International, 54*, 650–659.

Alzaid, F., Cheung, H. M., Preedy, V. R., & Sharp, P. A. (2013). Regulation of glucose transporter expression in human intestinal Caco-2 cells following exposure to an anthocyanin-rich berry extract. *PLoS ONE, 8* e78932.

Arapitsas, P., & Turner, C. (2008). Pressurized solvent extraction and monolithic column-HPLC/DAD analysis of anthocyanins in red cabbage. *Talanta, 74*, 1218–1223.

Batista, Â. G., Lenquiste, S. A., Cazarin, C. B. B., da Silva, J. K., Luiz-Ferreira, A., Bogusz, S., Jr., ... Maróstica, M. R. Jr., (2014). Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *Journal of Functional Foods, 6*, 450–461.

Batista, A. G., Lenquiste, S. A., Moldenhauer, C., Godoy, J. T., Reis, S. M. P. M., & Maróstica Júnior, M. R. (2013). Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats. *Brazilian Journal of Nutrition, 26*, 571–581.

Borges, L. L., Conceicao, E. C., & Silveira, D. (2014). Active compounds and medicinal properties of Myrciaria genus. *Food Chemistry, 153*, 224–233.

Cao, G. H., Russell, R. M., Lischner, N., & Prior, R. L. (1998). Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *Journal of Nutrition, 128*, 2383–2390.

Chuang, C., & McIntosh, M. K. (2011). Potential mechanisms by which polyphenol-rich grapes prevent obesity- mediated inflammation and metabolic diseases. *Annual Reviews of Nutrition, 31*, 155–176.

de Villiers, A., Gorecki, T., Lynen, F., Szucs, R., & Sandra, P. (2007). Improving the universal response of evaporative light scattering detection by mobile phase compensation. *Journal of Chromatography A, 1161*, 183–191.

Dragano, N. R. V., Marques, A. C., Cintra, D. E. C., Solon, C., Morari, J., Leite-Legatti, A. V., ... Maróstica Júnior, M. R. (2013). Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. *British Journal of Nutrition, 110*, 447–455.

Einbond, L. S., Reynertson, K. A., Luo, X.-D., Basile, M. J., & Kennelly, E. J. (2004). Anthocyanin antioxidants from edible fruits. *Food Chemistry, 84*, 23–28.

Ghosh, D., & Konishi, T. (2007). Anthocyanins and anthocyanin-rich extracts: Role in diabetes and eye function. *Asia Pacific Journal of Clinical Nutrition, 16*, 200–208.

Gorecki, T., Lynen, F., Szucs, R., & Sandra, P. (2006). Universal response in liquid chromatography using charged aerosol detection. *Analytical Chemistry, 78*, 3186–3192.

Guo, H. H., Xia, M., Zou, T., Ling, W., Liu, G. L., Zhong, R. M., & Zhang, W. (2012). Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. *Journal of Nutritional Biochemistry, 23*, 349–360.

Jayaprakasam, B., Olson, L. K., Schutzki, R. E., Tai, M. H., & Nair, M. G. (2006). Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). *Journal of Agricultural and Food Chemistry, 54*, 243–248.

Jensen, G. S., Wu, X. L., Patterson, K. M., Barnes, J., Carter, S. G., Scherwitz, L., ... Schauss, A. G. (2008). In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-blinded, placebo-controlled, crossover study. *Journal of Agricultural and Food Chemistry, 56*, 8326–8333.

Kalt, W., Foote, K., Fillmore, S. A. E., Lyon, M., Van Lunen, T. A., & McRae, K. B. (2008). Effect of blueberry feeding on plasma lipids in pigs. *British Journal of Nutrition, 100*, 70–78.

Kay, C. D., & Holub, B. J. (2002). The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects. *British Journal of Nutrition, 88*, 389–397.

Keaney, J. F., Larson, M. G., Vasan, R. S., Wilson, P. W. F., Lipinska, I., Corey, D., ... Benjamin, E. J. (2003). Obesity and systemic oxidative stress – Clinical correlates of oxidative stress in the Framingham Study. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*, 434–439.

Kim, H., Bartley, G. E., Rimando, A. M., & Yokoyama, W. (2010). Hepatic gene expression related to lower plasma cholesterol in hamsters fed high-fat diets supplemented with blueberry peels and peel extract. *Journal of Agricultural and Food Chemistry, 58*, 3984–3991.

Leite, A. V., Malta, L. G., Riccio, M. F., Eberlin, M. N., Pastore, G. M., & Marostica, M. R. (2011). Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticaba* Vell Berg). *Journal of Agricultural and Food Chemistry, 59*, 2277–2283.

Leite-Legatti, A. V., Batista, A. G., Dragano, N. R. V., Marques, A. C., Malta, L. G., Riccio, M. F., ... Maróstica Júnior, M. R. (2012). Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. *Food Research International, 49*, 596–603.

Lenquiste, S. A., Batista, A. G., Marineli, R. S., Dragano, N. R. V., & Maróstica Junior, M. R. (2012). Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. *Food Research International, 49*, 153–160.

Lima, A. D. B., Correa, A. D., Saczk, A. A., Martins, M. P., & Castilho, R. O. (2011). Anthocyanins, pigment stability and antioxidant activity in jabuticaba [*Myrciaria Cauliflora* (Mart.) O. Berg]. *Revista Brasileira De Fruticultura, 33*, 877–887.

Lipinska, L., Klewicka, E., & Sojka, M. (2014). The structure, occurrence and biological activity of ellagitannins: A general review. *Acta Scientiarum Polonorum, Technologia Alimentaria, 13*, 289–299.

Manzano, S., & Williamson, G. (2010). Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Molecular Nutrition & Food Research, 54*, 1773–1780.

Matsuzawa-Nagata, N., Takamura, T., Ando, H., Nakamura, S., Kurita, S., Misu, H., ... Kaneko, S. (2008). Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. *Metabolism-Clinical and Experimental, 57*, 1071–1077.

Mazza, G., Kay, C. D., Cottrell, T., & Holub, B. J. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agricultural and Food Chemistry, 50*, 7731–7737.

Mulinacci, N., & Innocenti, M. (2012). Food analysis by HPLC. In *Anthocyanins and Betalains* (pp. 757–776). NW, USA: CRC Press, Taylor & Francis Group.

Pantelidis, G. E., Vasilakakis, M., Manganaris, G. A., & Diamantidis, G. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chemistry, 102*, 777–783.

Plaza, M., Kariuki, J., & Turner, C. (2014a). Quantification of individual phenolic compounds' contribution to antioxidant capacity in apple: A novel analytical tool based on liquid chromatography with diode array, electrochemical, and charged aerosol detection. *Journal of Agricultural and Food Chemistry, 62*, 409–418.

Plaza, M., Pozzo, T., Liu, J. Y., Ara, K. Z. G., Turner, C., & Karlsson, E. N. (2014b). Substituent effects on in vitro antioxidizing properties, stability, and solubility in flavonoids. *Journal of Agricultural and Food Chemistry, 62*, 3321–3333.

Plaza, M., & Turner, C. (2015). Pressurized hot water extraction of bioactives. *TrAC, Trends in Analytical Chemistry, 71*, 39–54.

Prior, R. L., Wilkes, S. E., Rogers, T. R., Khanal, R. C., Wu, X. L., & Howard, L. R. (2010). Purified blueberry anthocyanins and blueberry juice alter development of obesity in mice fed an obesogenic high-fat diet. *Journal of Agricultural and Food Chemistry, 58*, 3970–3976.

Prior, R. L., Wu, X. L., Gu, L. W., Hager, T. J., Hager, A., & Howard, L. R. (2008). Whole berries versus berry anthocyanins: Interactions with dietary fat levels in the C57BL/6J mouse model of obesity. *Journal of Agricultural and Food Chemistry, 56*, 647–653.

Reynertson, K. A., Wallace, A. M., Adachi, S., Gil, R. R., Yang, H., Basile, M. J., ... Kennelly, E. J. (2006). Bioactive depsides and anthocyanins from jaboticaba (*Myrciaria cauliflora*). *Journal of Natural Products, 69*, 1228–1230.

Reynertson, K. A., Yang, H., Jiang, B., Basile, M. J., & Kennelly, E. J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry, 109*, 883–890.

Roberts, C. K., Vaziri, N. D., Liang, K. H., & Barnard, R. J. (2001). Reversibility of chronic experimental syndrome X by diet modification. *Hypertension, 37*, 1323–1328.

Roberts, C. K., Vaziri, N. D., Wang, X. Q., & Barnard, R. J. (2000). Enhanced NO inactivation and hypertension induced by a high-fat, refined-carbohydrate diet. *Hypertension, 36*, 423–429.

Rosen, L. A. H., Silva, L. O. B., Andersson, U. K., Holm, C., Ostman, E. M., & Bjorck, I. M. E. (2009). Endosperm and whole grain rye breads are characterised by low post-prandial insulin response and a beneficial blood glucose profile. *Nutrition Journal, 8*, 42.

Serrano, J., Puupponen-Pimia, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition & Food Research, 53*, S310–S329.

Tanaka, S., Hamanishi, C., Kikuchi, H., & Fukuda, K. (1998). Factors related to degradation of articular cartilage in osteoarthritis: A review. *Seminars in Arthritis and Rheumatism, 27*, 392–399.

Tsuda, T., Horio, F., Uchida, K., Aoki, H., & Osawa, T. (2003). Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition, 133*, 2125–2130.

Williams, R. J., & Spencer, J. P. E. (2012). Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radical Biology & Medicine, 52*, 35–45.

Wolever, T. M., Jenkins, D. J., Ocana, A. M., Rao, V. A., & Collier, G. R. (1988). Second-meal effect: Low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. *The American Journal of Clinical Nutrition, 48*, 1041–1047.

Wu, S.-B., Dastmalchi, K., Long, C., & Kennelly, E. J. (2012). Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. *Journal of Agricultural and Food Chemistry, 60*, 7513–7525.