JOURNAL OF FUNCTIONAL FOODS 7 (2014) 599–608

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Evaluation of Latin-American fruits rich in phytochemicals with biological effects

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ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form
18 December 2013
Accepted 20 December 2013
Available online 11 January 2014

Keywords:
Diabesity
Fruits
Berries
Polyphenols
Phytochemicals
Antioxidants

ABSTRACT

This work aimed to provide a thorough description of the polyphenolic composition of five Latin-American fruits of increasing interest, which have certain anti-diabetic effects (açaí, maqui, Cape gooseberry, papaya and noni), and to correlate their antioxidant capacity and anti-diabesity activities (lipase and α-glucosidase inhibition), and examine their potential use by the food industry. The phytochemical profiling of the fruits revealed a wide range of bioactive phenolics. The inhibition of pancreatic lipase was significant for maqui, and maqui and papaya were the best inhibitors of α-glucosidase. Regarding the DPPH, $ABTS^{\cdot+}$ and FRAP assays, maqui berries displayed the highest activity. The ORAC method and the superoxide radical scavenging assays revealed maqui and açaí as the best performers. These Latin-American fruits are of great value regarding nutrition and health benefits, and the development of products for the control of diabetes and obesity.

1. Introduction

In the last decade, numerous publications have dealt with the high content of bioactive compounds, mostly polyphenols, present in certain fruits and their protective effects on human health. Besides their antioxidant properties, it is widely accepted that most of these phenolics have an affinity for proteins, exhibiting inhibitory activity on some functional enzymes (Birari & Bhutani, 2007). In this aspect, the inhibition of α-glucosidase, a key enzyme that catalyzes the final step in the digestive process of carbohydrates, could delay the breakdown of oligosaccharides and disaccharides into monosaccharides, diminishing glucose absorption and consequently reducing postprandial hyperglycaemia (Rubilar et al., 2011). Berry polyphenols have been reported as being inhibitors of α-glucosidase *in vitro* (Boath, Stewart, & McDougall, 2012). The current therapeutic approaches for the treatment of obesity involve the inhibition of dietary triacylglycerol absorption, via inhibition of pancreatic lipase (PL) by orlistat (Birari & Bhutani, 2007). Many polyphenolic extracts are active against this enzyme; for example, polyphenol-rich water extracts from litchi (*Litchi chinensis* Sonn.) show *in vitro* inhibitory effects (Wu et al., 2013), while extracts from certain berries have been described as effective inhibitors of PL *in vivo* (McDougall, Kulkarni, & Stewart, 2009).

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1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.12.025

The increase in human obesity has been accompanied by a growing incidence of diabetes. The close relationship between these two conditions has led to the adoption of the term *diabesity* (Schröder, 2007). In this sense, some fruits of Latin-American origin represent good sources of bioactive compounds with certain anti-diabetic effects and are receiving increasing interest. Açaí (*Euterpe oleracea* L.) is a palm-tree berry from the Amazon area in South America. Potential benefits have been attributed to açaí fruits, extracts and juices: antioxidant, anti-inflammatory (Schauss et al., 2006), hypocholesterolaemic (De Souza et al., 2012) and anti-diabetic activity (Kim, Hong, Jung, Jeong, & Cho, 2012). Maqui (*Aristotelia chilensis* L.) is a common edible berry from central and southern Chile, and it is a source of natural colorant due to the presence of anthocyanins. Various reports have linked the phenolics of maqui berries with their high antioxidant capacity (Rubilar et al., 2011), *in vitro* inhibition of adipogenesis and inflammation (Schreckinger, Wang, Yousef, Lila, & De Mejia, 2010), cardioprotection (Céspedes, El-Hafidi, Pavon, & Alarcon, 2008) and *in vitro* and *in vivo* anti-diabetic effects (Rojo et al., 2011; Rubilar et al., 2011). Cape gooseberry (*Physalis peruviana* L.) is an herbaceous perennial semi-shrub that grows in sub-tropical zones. Its calyx represents an essential source of carbohydrates during the first 20 days of growth and development. Anti-inflamatory, hypocholesteroleamic and antihepatotoxic effects have been attributed to *P. peruviana* (Ramadan, 2012). Papaya (*Carica papaya* L.) fruits grow in tropical and sub-tropical regions and are marketed around the world. Numerous papers have described beneficial effects of this fruit against chronic diseases such as cancer (Nguyen, Shaw, Parat, & Hewavitharana, 2013), diabetes (Juárez-Rojop et al., 2012) and obesity (Athesh, Karthiga, & Brindha, 2012). Noni (*Morinda citrifolia* L.) is a tropical and sub-tropical plant used as a folk medicine in Pacific islands to treat a broad range of diseases. Recently, several health benefits have been attributed to noni fruits, juice or extracts, namely hypolipidemic and anti-oxidative effects (Lin et al., 2012), hepatoprotection (Wang, Nowicki, Anderson, Jensen, & West, 2008), anti-diabetic (Sabitha, Adhikari Prabha, Shetty Rukmini, Anupama, & Asha, 2009) and anti-cancer (Brown, 2012) effects.

In addition to the above-mentioned bioactivities, it has been reported that these five fruits also display significant anti-diabetic activity (Juárez-Rojop et al., 2012; Kim et al., 2012; Lee et al., 2012; Pujiyanto, Lestari, Suwanto, Budiarti, & Darusman, 2012; Rojo et al., 2011). To the best of our knowledge, there are insufficient data in the literature (arising from the same assaying procedure and conditions) to allow a comprehensive comparison of the different antioxidants and enzymatic activities of these polyphenol-rich fruits. Moreover, the polyphenolic composition has been reported for some of these fruits, mainly açaí and maqui, but phenolic characterization studies on Cape gooseberry, noni and papaya are scarce. Hence, the aim of this study was to evaluate the α-glucosidase- and lipase-inhibitory activities and the antioxidant activities of five fruits rich in bioactive compounds (native from different countries in Latin America), together with their phytochemical profiling, making a comparison of the species, their origin and the analytical methods studied.

2. Material and methods

2.1. Chemicals

The compounds 2,2-diphenyl-1-picrylhidracyl radical (DPPH$^{\cdot}$), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)diammonium salt (ABTS$^{\cdot+}$), 2,4,6-tripyridyl-S-triazine (TPTZ), ferric chloride hexahydrate, fluorescein (free acid), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (APPH), monobasic sodium phosphate, dibasic sodium phosphate, Folin Ciocalteu's Reagent, β-nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), nitrotetrazolium blue chloride (NBT), triazine hydrochloride, 4-nitrophenyl α-D-glucopyranoside, α-glucosidase from *Saccharomyces cerevisiae*, acarbose and potassium phosphate were obtained from Sigma–Aldrich (Steinheim, Germany). Meanwhile, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and magnesium chloride hexahydrate were purchased from Fluka Chemika (Neu-Ulm, Switzerland); sodium carbonate (anhydrous), sodium benzoate, and potassium sorbate were bought from Panreac Química S.A. (Barcelona, Spain). LIPASE-PS™ (Kit) was obtained from Trinity Biotech (Jamestown, NY, USA). Ultrapure water was produced using a Millipore water purification system.

2.2. Fruits

Lyophilized maqui^{CHI2}, açaí^{BRZ2}, noni^{ECU} and papaya^{ECU} fruits, provided by Ecuadorian Rainforest LLC. (Belleville, NJ, USA), were obtained from Chile (CHI), Brazil (BRZ) and Ecuador (ECU). Açaí^{COL} was supplied by Corpocampo S.A. (Bogotá, Colombia (COL)). Açaí^{BRZ1} was provided by Amazon Dreams Industria e Comercio S.A. (Belem, Pará, Brazil). Cape gooseberry^{COL} fruits and calyx were provided by Arc. Eurobanan S.L. (Santa Fé de Bogotá, Colombia). Maqui^{CHI1} and maqui^{CHI3} were provided by INTA-UCHILE (Santiago, Chile): maqui^{CHI1} was lyophilized and maqui^{CHI3} was spray-dried and microencapsulated by atomization.

2.3. Extraction

Each sample (100 mg) was mixed with 1 mL of methanol/water (70:30, v/v). For the HPLC analysis samples were acidified with 1% of formic acid. Then, the samples were vortexed and sonicated in an ultrasonic bath for 60 min. The samples were kept at 4 °C overnight and sonicated again for 60 min. A centrifugation (model EBA 21, Hettich Zentrifugen) step (9500 *xg*, 5 min) was used to separate the supernatant from the solid residue. This supernatant was filtered through a 0.45-μm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and stored at 4 °C before the analyses were performed.

2.4. Identification of phenolic compounds by HPLC–DAD–ESI/MS^n and quantification by RP-HPLC–DAD

The chromatographic analyses for the identification were carried out on a Luna C18 column (250 × 4.6 mm, 5 mm particle size; Phenomenex, Macclesfield, UK). Water/formic acid (99:1, v/v) and acetonitrile were used as the mobile phases A

and B, respectively, with a flow rate of 1 mL/min. The linear gradient started with 8% solvent B, reaching 15% solvent B at 25 min, 22% at 55 min, and 40% at 60 min, which was maintained to 70 min. The injection volume was 30 μL. Chromatograms were recorded at 280, 320, and 360 nm. The HPLC–DAD–ESI/MS*n* analyses were carried out using an Agilent HPLC 1100 series model equipped with a photodiode array detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany). The equipment consisted of a binary pump (model G1312A), an autosampler (model G1313A), a degasser (model G1322A), and a photodiode array detector (model G1315B). The HPLC system was controlled by ChemStation software (Agilent, version 08.03). The mass detector was an ion trap spectrometer (model G2445A) equipped with an electrospray ionization interface, and was controlled by LCMSD software (Agilent, version 4.1). The ionization conditions were 350 °C and 4 kV, for capillary temperature and voltage, respectively. The nebulizer pressure and nitrogen flow rate were 65.0 psi and 11 L/min, respectively. The full-scan mass covered the range of *m*/z from 100 to 1200. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. The mass spectrometry data were acquired in the positive ionization mode for anthocyanins and in the negative ionization mode for other flavonoids. The MS*n* was carried out in the automatic mode on the more-abundant fragment ion in MS($n-1$).

For the quantification, all samples were also centrifuged for 5 min at 9500*g*. Each supernatant was filtered through a 0.45-μm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) before injection into the HPLC system, as described by Gironés-Vilaplana, Villaño, Moreno, and García-Viguera (2013). Chromatograms were recorded at 280, 320, 360 and 520 nm. Anthocyanins were quantified as cyanidin 3-*O*-glucoside at 520 nm, flavonols and xanthone derivatives as quercetin 3-*O*-glucoside at 360 nm, ellagic acid derivatives as ellagic acid 3-*O*-glucoside at 360 nm and cinnamic acids as 5-*O*-caffeoylquinic acid at 320 nm.

2.5. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was assessed by modification of a previously-reported procedure (Chan, Sun, Reddy, & Wu, 2010). Briefly, each well contained 100 μL of 2 mM 4-nitrophenyl α-D-glucopyranoside in 10 mM potassium phosphate buffer (pH 7.0) and 20 μl of the sample or acarbose (positive control), also in buffer. The reaction was initiated by the addition of 100 μL of the enzyme solution (56.66 mU/mL). The plates were incubated at 37 °C for 10 min. The absorbance of the 4-nitrophenol released from 4-nitrophenyl α-D-glucopyranoside was measured at 400 nm. The increase in absorbance was compared with that of the control (buffer instead of sample solution), to calculate the inhibitory activity and the IC_{50} (sample concentration which reduced the enzyme concentration by 50%).

2.6. Lipase inhibitory effect

Lipase-PS™ reagents were obtained from Trinity Biotech (Procedure No. 805, Trinity Biotech, Jamestown, NY, USA). The lipase activity was determined in microscale 96-well micro plates (Nunc, Roskilde, Denmark) in an Infinite® M200 micro plate reader (Tecan, Grödig, Austria), as described by Gironés-Vilaplana et al. (2013). The recorded rate of increase in absorbance at 550 nm, due to the formation of quinone diimine dye, was used to determine the pancreatic lipase activity in the samples prepared.

2.7. Antioxidant capacity

The free radical scavenging activities were determined using the DPPH·, $ABTS^{\cdot+}$, and FRAP (ferric reducing antioxidant power) methods adapted to a microscale, according to Mena et al. (2011). The antioxidant activity was evaluated by measuring the variation in absorbance at 515 nm after 50 min of reaction with the radical (for DPPH·), at 414 nm after 50 min ($ABTS^{\cdot+}$), and at 593 nm after 40 min for FRAP. The assays were performed using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite® M200 micro plate reader (Tecan, Grödig, Austria). All the reactions were started by adding 2 μL of the corresponding diluted sample to the well containing the stock solution (250 μL). The final volume of the assay was 252 μL. The antioxidant activity was also determined using the ORAC-FL assay, according to Ou, Hampsch-Woodill, and Prior (2001). The results were expressed as mM Trolox/100 mg dry weight.

The superoxide radical ($O_2^{\cdot-}$) scavenging activity was also determined spectrophotometrically, in a 96-well plate reader, by monitoring the effect of extracts on the $O_2^{\cdot-}$ induced reduction of NBT at 560 nm. Superoxide radicals were generated by the NADH/PMS system, according to a described procedure (Ferreres et al., 2009). The experiments were performed in triplicate and the results expressed as the IC_{50}.

2.8. Statistical analysis

The data are presented are mean values ($n = 3$) ± standard deviation. All the data were subjected to analysis of variance (ANOVA) and a Multiple Range Test (Tukey's test), using IBM SPSS statistics 21 software (SPSS Inc., Chicago, IL, USA). Pearson's correlation analysis was performed to corroborate the relationships between selected parameters.

3. Results and discussion

3.1. Phenolic compounds

The HPLC–DAD–ESI/MS^n analysis of the hydromethanolic extracts of the Latin-American fruits revealed a wide range of different phenolic compounds. Anthocyanins and ellagic acid derivatives were only detected in açai and maqui fruits (Table 1), whilst flavonols, xanthones and hydroxycinnamic acid derivatives were widely distributed in the fruits (Table 2).

The açaí fruit samples (açaí^{BRZ1}, açaí^{BRZ2} and açaí^{COL}) contained diverse anthocyanins (cyanidin-*O*-hexosides and pelargonidin and peonidin rutinosides (**A7**, **A8**, **A10**, **A12** and **A13**)) and flavonols (quercetin, kaempferol and isorhamnetin glycosides (**F6**, **F8**, **F13**, **F14**, **F16**, **F17**, **F18**, **F19**)), in accordance with other reports (Gironés-Vilaplana, Valentão, et al., 2012;

Table 1 – Anthocyanins and ellagic acid derivatives identified and quantified (mg/100 g dried weight) in açaí and maqui fruits.

Compounds	Rt	$[M-H]^+$	MS^n	Fruits					
				Açaí^{BRZ1}	Açaí^{BRZ2}	Açaí^{COL}	Maqui^{CHI1}	Maqui^{CHI2}	Maqui^{CHI3}
Anthocyanins									
A1 Delphinidin 3-O-sambubioside-5-O-glucoside	5.8	759	465, 303	–	–	–	250.25 ± 11.44	125.21 ± 24.65	354.51 ± 27.49
A2 Delphinidin 3,5-O-diglucoside	6.6	627	465, 303	–	–	–	240.35 ± 8.45	251.45 ± 19.31	114.38 ± 1.34
A3 Cyanidin 3,5-O-diglucoside	11.7	611	449, 287	–	–	–	134.65 ± 3.28*	77.07 ± 3.60*	11.47 ± 0.90*
A4 Cyanidin 3-O-sambubioside-5-O-glucoside	12.0	743	581, 287	–	–	–			
A5 Delphinidin 3-O-sambubioside	15.7	597	303	–	–	–	63.22 ± 0.37	18.63 ± 1.87	72.45 ± 6.22
A6 Delphinidin 3-O-glucoside	16.5	465	303	–	–	–	210.90 ± 1.84	110.69 ± 3.41	167.55 ± 12.58
A7 Cyanidin 3-O-galactoside	17.7	449	287	55.60 ± 7.81	10.49 ± 0.22	27.21 ± 1.92	–	–	–
A8 Cyanidin 3-O-glucoside	19.6	449	287	–	3.89 ± 0.36	–	–	–	–
A9 Cyanidin 3-O-sambubioside	20.2	581	287	–	–	–	82.21 ± 0.48	23.44 ± 2.71	56.29 ± 4.61
A10 Cyanidin-3-O-rutinoside	21.6	595	287	81.49 ± 9.49	31.21 ± 1.62	305.21 ± 30.01	–	–	–
A11 Cyanidin 3-O-glucoside-5-O-rhamnoside	22.4	595	449, 287	–	–	–	2.54 ± 1.87	7.59 ± 1.87	1.96 ± 0.75
A12 Pelargonidin 3-rutinoside	25.2	579	433, 271	28.88 ± 1.48	–	1.60 ± 0.32	–	–	–
A13 Peonidin 3-O-rutinoside	26.8	609	463, 301	3.45 ± 0.97	3.44 ± 0.52	13.78 ± 6.90	–	–	–
Total				143.42 ± 16.71	49.02 ± 1.82	347.81 ± 35.86	984.12 ± 7.32	614.08 ± 47.44	881.84 ± 46.07
Ellagic acid derivatives									
EA1 Granatin B	26.4	951	933, 301	–	–	–	0.53 ± 0.11	0.54 ± 0.13	0.39 ± 0.00
EA2 Ellagic acid hexoside	34.3	463	301	–	–	–	2.01 ± 0.15	1.23 ± 0.00	1.17 ± 0.08
EA3 Dehydrogaloyl-hexahydroxydiphenoyl hexoside	40.1	615	463, 301	–	–	–	14.29 ± 0.20	12.42 ± 1.54	5.88 ± 0.38
Total				–	–	–	16.83 ± 0.42	14.20 ± 1.48	7.44 ± 0.43

* **A3** and **A4** coeluted and were quantified together in maqui^{CHI1}, maqui^{CHI2} and maqui^{CHI3}. Rt: retention time of.

Table 2 – Non-red polyphenols and hydroxycinnamic acids identified and quantified (mg/100 g dried weight) in Latin-American fruits: açaí, maqui, Cape gooseberry, papaya, and noni.

Non-red polyphenols				Açaí	Maqui				Cape goseberry		Papaya	Noni
	Rt	$[M-H]^-$	MS^n	BRZ1	BRZ2	COL	CHI1	CHI2	CHI3	Fruit	Calyx	ECU	ECU
F1	19.8	771	609, 301	–	–	–	–	–	–	–	34.56 ± 1.39	–	–
F2	25.1	755	593, 285	–	–	–	–	–	–	–	5.00 ± 0.13	–	–
F3	25.6	421	366, 241	–	–	–	–	–	–	–	–	0.84 ± 0.13	–
F4	27.0	631	479, 317	–	–	–	3.20 ± 0.24	1.95 ± 0.31	1.71 ± 0.17	–	–	–	–
F5	30.3	479	317	–	–	–	2.47 ± 0.34	2.01 ± 0.30	2.07 ± 0.18	–	–	–	–
F6	32.5	447	285	8.05 ± 0.67	–	5.18 ± 0.50	–	–	–	–	–	–	–
F7	32.8	479	317	–	–	–	1.92 ± 0.38	2.58 ± 0.58	2.46 ± 0.18	–	–	–	–
F8	33.4	447	285	9.98 ± 0.71	–	7.96 ± 1.04	–	–	–	–	–	–	–
F9	33.8	573	366, 241	–	–	–	–	–	–	–	–	4.90 ± 1.12	–
F10	34.7	269	-	–	–	–	–	–	–	–	–	–	11.37 ± 1.03
F11	35.3	573	366, 241	–	–	–	–	–	–	–	–	0.78 ± 0.20	–
F12	37.2	741	609, 301	–	–	–	–	–	–	–	–	–	0.86 ± 0.37
F13	40.6	609	301	3.53 ± 0.32		6.17 ± 0.86	5.13 ± 0.87	7.18 ± 1.65	2.46 ± 0.58	1.74 ± 0.54	147.57 ± 2.55	0.72 ± 0.24	4.04 ± 1.14
F14	42.5	463	301	–	5.34 ± 1.12	–	2.17 ± 0.60	1.28 ± 0.49	2.42 ± 0.71	–	–	1.30 ± 0.56	–
F15	44.0	463	301	–	–	–	–	1.70 ± 0.70	–	–	–	1.65 ± 0.21	–
F16	47.8	433	301	–	1.46 ± 0.21	–	1.55 ± 0.09	3.71 ± 0.60	1.16 ± 0.19	–	–	–	–
F17	50.2	433	301	–	2.76 ± 0.03	–	2.24 ± 0.09	3.74 ± 0.29	2.74 ± 0.58	–	–	–	–
F18	51.9	593	285	1.10 ± 0.10	–	1.57 ± 0.08	0.74 ± 0.29	0.53 ± 0.17	0.55 ± 0.06	0.44 ± 0.17	8.31 ± 0.01	–	2.47 ± 0.05
F19	54.4	623	315	1.42 ± 0.33	–	0.68 ± 0.11	–	–	–	–	–	–	–
F20	55.3	447	301	–	0.82 ± 0.27	–	–	–	–	–	–	–	–
			TOTAL	24.07 ± 3.37	17.75 ± 0.91	21.56 ± 2.18	19.42 ± 2.40	24.41 ± 1.47	15.58 ± 1.82	2.18 ± 0.71	195.44 ± 3.88	10.20 ± 2.08	18.74 ± 2.82
Hydroxycinnamic acids													
Q1	7.9	515	191	–	6.68 ± 0.17	–	–	–	–	–	–	–	–
Q2	11.6	353	191, 179	–	18.84 ± 0.61	–	–	–	–	–	71.54 ± 2.62	–	–
Q3	14.8	431	-	–	–	–	–	–	–	–	–	–	1.06 ± 0.14
Q4	18.9	353	191	–	–	–	–	8.84 ± 0.86	–	–	51.72 ± 2.48	–	–
Q5	30.1	337	191	–	7.33 ± 0.77	–	–	–	–	–	–	–	–
			TOTAL	–	32.86 ± 1.17	–	–	8.84 ± 0.86	–	–	123.26 ± 5.10	–	1.06 ± 0.14

Quantified at 360 nm: **F1**: Quercetin 3-*O*-rutinoside 7-*O*-hexoside, **F2**: Kaempferol 3-*O*-rutinoside 7-*O*-hexoside, **F3**: Mangiferin, **F4**: Myricetin 3-*O*-galoylglucoside, **F5**: Myricetin 3-*O*-galactoside, **F6**: Kaempferol 3-*O*-galactoside, **F7**: Myricetin 3-*O*-glucoside, **F8**: Kaempferol 3-*O*-glucoside, **F9**: Mangiferin gallate, **F10**: Lucidin, **F11**: Isomangiferin gallate, **F12**: Quercetin 3-*O*-rutinoside-7-*O*-pentoside, **F13**: Quercetin 3-*O*-rutinoside, **F14**: Quercetin 3-*O*-galactoside, **F15**: Quercetin 3-*O*-glucoside, **F16**: Quercetin 3-*O*-xyloside, **F17**: Quercetin 3-*O*-arabinoside, **F18**: Kaempferol 3-*O*-rutinoside, **F19**: Isorhamnetin 3-*O*-rutinoside, **F20**: Quercetin 3-*O*-rhamnoside. Quantified at 320 nm: **Q1**: 3,5-*O*-Dicaffeoylquinic acid, **Q2**: 3-*O*-Caffeoylquinic acid, **Q3**: Asperulosidic acid, **Q4**: 5-*O*-Caffeoylquinic acid, **Q5**: 5-*O*-*p*-Coumaroylquinic acid. Samples were labelled as follows: açaí[1]: açai^{BRZ1}, açaí[2]: açaí^{BRZ2}, açaí[3]: açaí^{COL}, maqui[1]: maqui^{CHI1}, maqui[2]: maqui^{CHI2}, maqui[3]: maqui^{CHI3}.

Table 3 – α-glucosidase inhibition and pancreatic lipase activity (U/L) of all the fruits.

Fruit		α-Glucosidase IC_{50}*	Lipase U/L
Açaí			
BRZ1		–	120.91 ± 10.95 ef
BRZ2		2.14 ± 0.18 a	51.03 ± 12.70 bc
COL		–	137.46 ± 16.27 f
Maqui			
CHI1		0.33 ± 0.02 a	19.62 ± 3.39 a
CHI2		1.10 ± 0.17 a	26.82 ± 5.48 ab
CHI3		0.81 ± 0.08 a	21.30 ± 2.92 a
Cape gooseberry			
Fruit (COL)		56.03 ± 0.32 c	180.83 ± 14.98 g
Calyx (COL)		–	100.22 ± 9.57 de
Papaya			
ECU		1.58 ± 0.26 a	107.58 ± 11.16 e
Noni			
ECU		27.32 ± 2.79 b	74.63 ± 4.37 cd
LSD	$P < 0.05$	0.988	8.388

Means ($n = 3$) in the same columns followed by different letters are significantly different at $P < 0.05$ according to Tukey's test.
* Samples without data did not inhibit 50% of enzyme.

Gordon et al., 2012). Considering the origin of the fruits, only açaí^{BRZ2} had hydroxycinnamic acid derivatives (3,5-*O*-dicaffeoylquinic (**Q1**), 3-*O*-caffeoylquinic (**Q2**) and 5-*O*-*p*-coumaroylquinic acids (**Q5**)), but displayed significantly-smaller amounts of anthocyanins (49.02 ± 1.82 mg/100 g dry weight (dw) total anthocyanins content (TAC)) compared to açaí^{BRZ1} (TAC: 143.42 ± 16.71 mg/100 g dw) and açaí^{COL} (TAC: 347.81 ± 35.86 mg/100 g dw). Cyanidin 3-*O*-rutinoside (**A10**) was the major anthocyanin quantified in açaí fruits (56.8, 63.7, and 87.7% of the total anthocyanins in açaí^{BRZ1}, açaí^{BRZ2} and açaí^{COL}, respectively), followed by cyanidin 3-*O*-galactoside (**A7**) (Table 1). Regarding flavonols, the levels were similar among the three studied açaís, but the phytochemical profile of açaí^{BRZ2} was completely different from those of açaí^{BRZ1} or açaí^{COL} (Table 2). This was probably due to differences in the ripening stage or growth conditions, which are directly related to the nutritional profile of these fruits (Gordon et al., 2012).

With respect to the maqui berry powders, different glycosides and di-glycosides of delphinidin and cyanidin were found (**A1**, **A2**, **A3**, **A4**, **A5**, **A6**, **A9**, **A11**) (Table 1), in accordance with previous reports (Gironés-Vilaplana, Mena, García-Viguera, & Moreno, 2012; Gironés-Vilaplana, Valentão, et al., 2012). Flavonols (quercetin and myricetin derivatives (**F4**, **F5**, **F7**, **F13**, **F14**, **F15**, **F16**, **F17**, **F18**)), one ellagic acid hexoside (**EA2**), and two ellagitannins (granatin B (**EA1**) and dehydrogaloyl-hexahydroxydiphenoyl hexoside (**EA3**), the latter identified for the first time in maqui fruits) were also identified at quantifiable levels (Table 1). It is important to note the higher anthocyanin concentrations in maqui with respect to açaí, particularly in maqui^{CHI1} (TAC: 984.12 ± 7.32 mg/100 g dw). Moreover, maqui^{CHI2} exhibited a slightly-higher level of flavonols than the other two maquis, but also a lower content of anthocyanins (Table 2). Furthermore, maqui^{CHI2} was the only maqui fruit containing 5-*O*-caffeoylquinic acid (**Q4**), while maqui^{CHI3} had the lowest level of flavonols.

The Cape gooseberry fruit is protected by an accrescent calyx; to the best of our knowledge, there are no reports on its phenolic content. For this reason, the calyx and fruit were analyzed separately; this enabled us to show that there were more compounds and a much-higher level of phenolics in the calyx^{COL} (195.44 ± 3.88 mg/100 g dw) than in the fruit^{COL} (2.18 ± 0.71 mg/100 g dw). Two different quercetin (**F1**, **F13**) and kaempferol glycosides (**F2**, **F18**) were identified and quantified in the calyx, but only trace amounts of quercetin 3-*O*-rutinoside (**F13**) and kaempferol 3-*O*-rutinoside (**F18**) were found in the fruit. The 3-*O*-caffeoylquinic (**Q2**) and 5-*O*-caffeoylquinic (**Q4**) acids were also identified in the calyx, but not in the fruit. A point worth mentioning is that the Cape gooseberry calyx^{COL} had the highest concentrations of flavonols and hydroxycinnamic acid derivatives among all the Latin-American fruits analyzed.

In papaya^{ECU} powder, mangiferin (**F3**) and the galloylated forms of mangiferin (**F9**) and isomangiferin (**F11**) (Table 2), xanthone glycosides, were identified. These were described as potent antidiabetic agents by Sellamuthu, Muniappan, Perumal, and Kandasamy (2009). Mangiferin gallate (**F9**) was the major flavonoid present in papaya^{ECU} (4.90 ± 1.12 mg/100 g dw). Quercetin 3-*O*-rutinoside (**F13**), previously reported for this fruit (Andarwulan et al., 2012; Rivera-Pastrana, Yahia, & González-Aguilar, 2010), was also found, as were both quercetin hexosides (**F14**, **F15**) – at lower levels. Hydroxycinnamic acid derivatives were absent or below the detection threshold.

The noni^{ECU} fruit contained low levels of some flavonol glycosides: quercetin 3-*O*-rutinoside-7-*O*-pentoside (**F12**), quercetin 3-*O*-rutinoside (**F13**), and kaempferol 3-*O*-rutinoside (**F18**) (Table 2). Previous work identified the rutinosides of quercetin and kaempferol (Andarwulan et al., 2012; Dussossoy et al., 2011), but not quercetin 3-*O*-rutinoside-7-*O*-pentoside (**F12**), identified and quantified for the first time in this work. Moreover, lucidin (**F10**) (an anthraquinone characteristic of noni (Deng, West, Jensen, Basar, & Westendorf,

2009)) was also detected and quantified in significant amounts (11.37 ± 1.03 mg/g dw), as well as asperulosidic acid (**Q4**), previously reported by Dussossoy et al. (2011).

3.2. α-glucosidase inhibition

The enzyme α-glucosidase catalyzes the final step in the digestion and breakdown of carbohydrates, so its inhibition can be effective for the regulation of Type II diabetes, by controlling glucose absorption (Rubilar et al., 2011). The IC_{50} values were calculated in order to compare the different Latin-American fruits, as shown in Table 3: açaí^{BRZ1}, açaí^{COL} and Cape gooseberry calyx^{COL} did not reach 50% inhibition of the enzyme activity, while Cape gooseberry fruit^{COL} and noni^{ECU} caused slight inhibition and açaí^{BRZ2}, papaya^{ECU}, maqui^{CHI1}, maqui^{CHI2} and maqui^{CHI3} were exceptionally effective, with lower IC_{50} values than the acarbose positive control (IC_{50} = 3.89 ± 0.79). The *in vitro* anti-diabetic effect of maqui has been previously reported (Rubilar et al., 2011), as have the *in vitro* and *in vivo* effects of its anthocyanins (Rojo et al., 2011). Moreover, myricetin and delphinidin, present in maqui berries, have been reported as the best α-glucosidase inhibitors among the flavonoids (Tadera, Minami, Takamatsu, & Matsuoka, 2006). Papaya fruit was also particularly effective against α-glucosidase, probably due to mangiferin (**F3**) and its derivatives (**F9**, **F11**), which are associated with a strong anti-diabetic effect (Sellamuthu et al., 2009). Several studies have also reported that some parts of the *Carica papaya* plant, such as the leaves, exert significant hypoglycaemic effects (Juárez-Rojop et al., 2012). As described above, açaí^{BRZ2} possessed lower amounts of phenolic compounds than other açaís but exhibited a different flavonoid profile, especially with regard to flavonols (Tables 1 and 2), which probably explains its higher activity with respect to açaí^{BRZ1} and açaí^{COL}. The α-glucosidase inhibition results are consistent with the idea that the differences among fruits in their phytochemical profiles, and the interactions between compounds in the fruit matrix, can contribute to their distinct activities; myricetin, quercetin, delphinidin and mangiferin derivatives being the most relevant (Sellamuthu et al., 2009; Tadera et al., 2006). To support this, the α-glucosidase results were correlated with the TAC ($R^2 = -0.527^{*}$, $P < 0.05$) and with the total non-red polyphenols ($R^2 = -0.652^{**}$, $P < 0.01$). Previous research showed that adding crowberry – containing different types of polyphenols – to blackcurrant juice improved the postprandial glycaemic control in healthy subjects (Törrönen et al., 2012). In this sense, maqui, papaya, and açaí fruits may offer dietary coadjuvants to control hyperglycaemia in diabetic patients; however, further evaluation of their *in vivo* anti-diabetic activity is necessary to verify these beneficial effects.

3.3. Pancreatic lipase inhibition

The inhibition of pancreatic lipase, which splits triacylglycerols into absorbable monoacylglycerol and fatty acids, is the main prescribed treatment for obesity in developed countries (McDougall et al., 2009). In order to find alternative sources for obesity prevention and treatment, we searched for inhibitory action of the Latin-American fruits on lipase activity. The results are given in Table 3, expressed in U/L (lipase activity). The activity of the lipase standard was 254 U/L. The maqui fruits exhibited the lowest values and, therefore, the greatest inhibitory effect on pancreatic lipase (26.82, 19.62 and 21.30 U/L for maqui^{CHI1}, maqui^{CHI2} and maqui^{CHI3}, respectively). This result is in line with the strong effect of maqui on lipid metabolism demonstrated previously, specifically the ability of maqui phenolic extracts to reduce adipogenesis and lipid accumulation in 3T3-L1 adipocytes (Schreckinger et al., 2010). The lipase inhibition was correlated strongly with the TAC ($R^2 = -0.682^{***}$, $P < 0.001$), maqui fruits exhibiting the highest anthocyanin content among the fruits examined. A result similar to the

Table 4 – Antioxidant activity of all the fruits.

Fruit	DPPH· mmol Trolox/100 g	ORAC mmol Trolox/100 g	$ABTS^{+}$ mmol Trolox/100 g	FRAP mmol Trolox/100 g	$O_2^{\cdot -}$ IC_{50} (mg/mL)
Açaí					
BRZ1	7.06 ± 0.13 d	28.30 ± 3.88 ef	21.10 ± 1.41 cd	15.99 ± 0.55 c	0.69 ± 0.03 ab
BRZ2	4.80 ± 0.09 b	12.21 ± 0.36 bc	9.17 ± 0.45 b	7.23 ± 0.89 a	0.80 ± 0.11 ab
COL	6.38 ± 0.15 cd	30.36 ± 1.45 ef	20.87 ± 0.75 c	15.79 ± 0.87 c	0.75 ± 0.05 ab
Maqui					
CHI1	13.93 ± 0.65 f	29.90 ± 0.98 ef	25.48 ± 0.82 d	25.42 ± 2.64 d	0.67 ± 0.02 a
CHI2	10.81 ± 1.37 e	26.80 ± 1.64 ef	22.12 ± 3.71 cd	18.12 ± 2.36 c	0.76 ± 0.10 ab
CHI3	6.76 ± 0.25 d	18.18 ± 0.29 d	18.30 ± 1.91 c	16.13 ± 0.96 c	1.83 ± 0.11 d
Cape gooseberry					
Fruit (COL)	1.60 ± 0.06 a	3.29 ± 0.58 a	3.11 ± 0.61 a	4.92 ± 1.24 a	13.91 ± 0.52 e
Calyx (COL)	4.94 ± 0.05 bc	24.29 ± 3.11 e	8.58 ± 1.26 b	11.42 ± 1.59 b	1.19 ± 0.08 bc
Papaya					
ECU	4.41 ± 0.28 b	8.71 ± 0.32 ab	7.09 ± 0.76 ab	6.39 ± 0.66 a	36.43 ± 0.30 f
Noni					
ECU	3.71 ± 0.17 b	15.08 ± 0.22 cd	9.04 ± 0.99 b	6.92 ± 0.85 a	1.37 ± 0.08 cd
LSD $P < 0.05$	0.409	1.707	1.274	1.171	0.155

Means ($n = 3$) in the same columns followed by different letters are significantly different at $P < 0.05$ according to Tukey's test.

α-glucosidase inhibition was also found in açaí fruits regarding lipase inhibition, açaí^{BRZ2} being very active, in contrast to the other two açaís, owing to its differing phytochemical profile. Furthermore, the α-glucosidase and lipase results were directly correlated ($R^2 = 0.828^{***}$, $P < 0.001$). Certain hypocholesterolaemic activity in açaí pulp has been found (De Souza et al., 2012). Some hypolipidaemic effects and an improvement in the serum lipid profile have also been described for noni (Lin et al., 2012). Consequently, it has been demonstrated that maqui, açaí and noni fruits are potent inhibitors of pancreatic lipase *in vitro*, so they may be developed – individually or in synergistic formulations – as natural alternatives for obesity treatment through dietary intervention, even though further *in vivo* research is needed.

3.4. *Antioxidant capacity*

With respect to the DPPH· assays, maqui fruits showed the highest activity against this radical, maqui^{CHI1} and maqui^{CHI2} being the most reactive, followed by açaí samples, due to their high anthocyanin content (Céspedes et al., 2008; Del Pozo-Insfran, Brenes, & Talcott, 2004), as demonstrated by the positive and direct correlation between DPPH· and TAC ($R^2 = 0.816^{***}$, $P < 0.001$). To a lesser degree, Cape gooseberry calyx^{COL}, papaya^{ECU} and noni^{ECU}, exhibited DPPH· scavenging activity. It is important to emphasize that the Cape gooseberry calyx^{COL} was more active than the berry, with regard to this activity (Table 4).

The values obtained for the fruits in the ORAC-Fl assay ranged from to 30.36 3.29 mM Trolox/100 mg dw: açaí^{COL}, maqui^{CHI1} and açaí^{BRZ1} – in decreasing order – showed the highest activities (Table 4). All fruits exhibited great scavenging activity except Cape gooseberry fruit^{COL}, which again showed much lower activity than the calyx. The açaí, maqui, noni and papaya fruits had higher ORAC values than over 100 different kinds of foods, including fruits, vegetables, nuts, dried fruits, spices and cereals from the United States (Wu et al., 2004). Hence, they represent a promising source of antioxidant compounds.

Regarding the ABTS·$^+$ assay, the maqui and açaí berries were expected to be the most-active fruits according to the results of DPPH·, but açaí^{BRZ2} gave a low value, while maqui, with the highest TAC, were the most active. Indeed, the TAC and ABTS·$^+$ values were highly correlated ($R^2 = 0.781^{***}$, $P < 0.001$). Noni^{ECU} and Cape gooseberry calyx^{COL} exhibited good antioxidant capacities, probably owing to their contents of flavonol (kaempferol) derivatives (Table 2).

Fruits rich in anthocyanins were very active in FRAP assay, where maqui^{CHI1} had the highest value, followed by maqui^{CHI2}, maqui^{CHI3}, açaí^{BRZ1} and açaí^{COL} (Table 4), also supported by a strong correlation between the FRAP and TAC values ($R^2 = 0.840^{***}$, $P < 0.001$). Moreover, the FRAP assay values were strongly correlated with DPPH· ($R^2 = 0.928^{***}$, $P < 0.001$), ORAC ($R^2 = 0.811^{***}$, $P < 0.001$) and ABTS·$^+$ ($R^2 = 0.917^{***}$, $P < 0.001$). As in the rest of the antioxidant methods, Cape gooseberry calyx^{COL} showed better activity than the fruit.

The superoxide radical anion plays an important role in the formation of other ROS, such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (Gülçin, 2006). Low IC_{50} values were obtained for the Latin-American fruits (Table 4), suggesting high activities against this ROS, except for Cape gooseberry fruit^{COL} and papaya^{ECU}. The values ranged between 0.67 and 36.43 mg/mL dw, for maqui^{CHI1} and papaya^{ECU}, respectively (Table 4).

As seen from these results, the Latin-American fruits tested can effectively scavenge different types of ROS or free radicals under *in vitro* conditions. The broad range of activities of the fruits indicates that multiple mechanisms may be responsible for the antioxidant activity, linked to their characteristic phenolic compounds (Gironés-Vilaplana, Valentão, et al., 2012). In fact, the fruits with high quantities of anthocyanins (açaí and maqui) exhibited higher activities in all the assays, suggesting that flavonoid glycosides, namely anthocyanins (Del Pozo-Insfran et al., 2004), kaempferol derivatives (Han et al., 2004) and flavonols (Cos et al., 1998; Pulido, Bravo, & Saura-Calixto, 2000), in the natural complex matrix of the fruits may be involved in these actions. These high antioxidant effects of maqui and açaí have also previously been reported (Araya, Clavijo, & Herrera, 2006; Céspedes et al., 2008; Gironés-Vilaplana, Mena, et al., 2012; Gironés-Vilaplana, Valentão, et al., 2012; Schauss et al., 2006). Noni fruit also displayed good activity against the superoxide radical, as previously reported (Calzuola, Luigi Gianfranceschi, & Marsili, 2006), although it was below that of maqui and açaí. Moreover, the bioactivity of these fruits was influenced by extraction procedure, concentration, variety and part of the fruit, origin, genotype, ripening and industrial processing (Speisky, López-Alarcón, Gómez, Fuentes, & Sandoval-Acuña, 2012). The results of certain antioxidant assays and the anti-diabesity effects were somewhat poorly correlated (for example, $R^2 = -0.665$, $P < 0.001$ between lipase and DPPH·, and $R^2 = -0.683$, $P < 0.001$ and $R^2 = -0.640$, $P < 0.001$ between α-glucosidase and ORAC and ABTS·$^+$, respectively). This suggests that some flavonoids can react distinctly against oxidative radicals with respect to their action at the active site of α-glucosidase and pancreatic lipase, but others, such as anthocyanins, may display simultaneous antioxidant and anti-diabesity effects.

4. Conclusions

The phytochemical profiling of fruits presented in this study revealed a diverse range of bioactive phenolics and biological activities. Regarding their potential biological activity, maqui was the best-performing fruit in terms of α-glucosidase and lipase inhibition. Papaya showed high α-glucosidase inhibition and noni fruits also exhibited significant lipase inhibition. The maqui and açaí berries were the most-interesting fruits in terms of antioxidant capacity, due to their high anthocyanin (cyanidin and delphinidin derivatives) contents. Noni fruits and Cape gooseberry calyx also exhibited good antioxidant capacities. The value of these Latin-American fruits as valuable sources of phytochemicals for food product development is clear, regarding nutrition and new dietary options for the treatment of diseases such as obesity and diabetes. Further, *in vivo* research will be conducted, to allow scientifically-backed statements and recommendations for dietary intake to be made.

Acknowledgments

The Authors express their gratitude to the Spanish Ministry of Economy and Competitivity for the funding through the CICYT project AGL2011-23690 and the CONSOLIDER-INGENIO 2010 Research Project FUN-C-FOOD (CSD2007-00063). Part of this work was carried out in international research collaboration within the CYTED Program (Ref. 112RT0460) CORNUCOPIA Thematic Network (URL: redcornucopia.org). AGV also thanks the CSIC and the European Social Funds for the JAE Predoctoral Grant. NB was funded by the FPU Fellowship Program of the Spanish Ministry of Education. The authors also thank Dr. David Walker for corrections of the English language and style.

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