Food Chemistry 141 (2013) 3480–3485

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Influence of taro (*Colocasia esculenta* L. Shott) growth conditions on the phenolic composition and biological properties

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ARTICLE INFO

Article history:
Received 10 April 2013
Received in revised form 31 May 2013
Accepted 3 June 2013
Available online 12 June 2013

Keywords:
Colocasia esculenta (L.) Shott
Wound repair
Oxidative/nitrosative stress
Enzymatic inhibition
Phenolic compounds

ABSTRACT

Colocasia esculenta (L.) Shott, commonly known as taro, is an essential food for millions of people. The leaves are consumed in sauces, purees, stews, and soups, being also used in wound healing treatment. Nowadays, the consumers' demand for bioactive compounds from the diet led to the development of new agricultural strategies for the production of health-promoting constituents in vegetables. In this work, two strategies (variety choice and irrigation conditions) were considered in the cultivation of *C. esculenta*. The effect on the phenolic composition of the leaves was evaluated. Furthermore, a correlation between the biological activity of the different varieties and their chemical composition was established. Qualitative and quantitative differences in the phenolic composition were observed between varieties; furthermore, the irrigation conditions also influenced the composition. *C. esculenta* varieties were able to scavenge several oxidant species and to inhibit hyaluronidase, but data suggest that metabolites other than phenolics are involved. The results show that cultivation strategies can effectively modulate the accumulation of these types of bioactive compounds. Furthermore *C. esculenta* wound healing potential can be attributed, at least in part, to the protection of the wound site against oxidative/nitrosative damage and prevention of hyaluronic acid degradation.

1. Introduction

Nowadays, the consumers' demand for a higher content of bioactive compounds in the diet has increased. Bioactive metabolites, like polyphenols, are not strictly required in the diet, but when present at sufficient levels promote good health and prevent diseases of different origins (Padula et al., 2012; Rouphael et al., 2012). Numerous studies relate the ingestion of phenolic compounds from the diet to a lower risk of cardiovascular diseases and development of cancers. Furthermore, they have long been recognised to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective and antiviral activities (Abad-García, Berrueta, Garmón-Lobato, Gallo, & Vicente, 2009; Padula et al., 2012). Therefore, much attention has now been placed on the agricultural practices which will enhance the nutritional content of horticultural crops being produced today. New investigation has provided the improvement of the vegetables properties and quality through pre-harvest factors (cultivar selection, grafting), optimisation of environmental conditions (light, temperature, humidity), as well as growing conditions (soilless culture, irrigation management, salinity and fertilisation) (Padula et al., 2012; Rouphael et al., 2012).

Colocasia esculenta (L.) Shott is an annual herbaceous plant belonging to the Araceae family, commonly known as taro (Prajapati, Kalariya, Umbarkar, Parmar, & Sheth, 2011). *C. esculenta* is cultivated mainly due to its tuber, an essential food for millions of people, and it is considered the 14th cultivated vegetable/staple around the world (Oscarsson & Savage, 2007). Depending on the varieties and local cultural traditions, other plant tissues, such as leaves, flowers and stems, are also consumed, especially in sauces, purees, stews and soups (Ejoh, Mbiapo, & Fokou, 1996; Ferreres et al., 2012b). Apart from its nutritional value, *C. esculenta* leaves and tubers are also used in the treatment of cutaneous wounds (Agyarea et al., 2009).

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0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.009

Few studies have previously addressed the phenolic composition of *C. esculenta*, anthocyanins (cyanidin and pelargonidin derivatives) and flavones (apigenin and luteolin derivatives) being already described in different varieties (Ferreres et al., 2012b).

Taro crop is one of the important crops in Azores archipelago, mainly in São Jorge, Pico and São Miguel Island, were its production covers 55 hectares and is estimated in c.a. 1000 tons per year (INE, 2012). Therefore, seeking the valorisation of taro crop in the Azores archipelago, this work aimed to study the influence of two pre-harvest factors (variety and irrigation conditions) on the phenolic composition of *C. esculenta* from Azores islands, as well as to relate the phenolic profile of the different varieties with their biological capacity.

2. Materials and methods

2.1. Plant material

All *C. esculenta* leaves were collected in São Miguel island (Azores archipelago) in June 2010, at the same stage of development (Table 1): sample WD was collected in Ajuda da Bretanha region, while the remaining ones were from Furnas region, situated 53 km away from the first location. The island has a typical climate, so all samples were subjected to the same environmental conditions: temperate rainy climate with dry summer, with temperatures ranging from 17.5 °C in February (minimum values) and 22 °C in August (maximum values) and with a monthly average 80% of humidity. The soil in this island is terrigenous and pumice.

The leaves from the different varieties come from local farmers' traditional cultures, without fertilisers, differing just in the natural soil watering, because they have distinct hydric conditions: inundation with cold water without drip irrigation, inundation with hot water without drip irrigation or soil without irrigation (Table 1).

The plant material was immediately transferred to the laboratory and dried in an oven at 30 °C for two weeks. The dried material was powdered (mean particle size lower than 910 μm) and stored in a desiccator. The analysed samples correspond to a mixture of the leaves of three different individuals from the same variety. Voucher specimens were deposited at the Laboratory of Pharmacognosy of the Faculty of Pharmacy of Porto University, under the identification CEGWC-L-062010, CERC-L-062010, CEWC-L-062010, CEWD-L-062010 and CEWH-L-062010 (leaves from "giant white", "red" and the "white" varieties samples from three different culture conditions, respectively).

2.2. Standards and reagents

Standards of luteolin-3′,7-di-*O*-glucoside, apigenin-8-*C*-glucoside, apigenin-6-*C*-glucoside, luteolin-8-*C*-glucoside, luteolin-6-*C*-glucoside and apigenin-6-*C*-glucoside-7-*O*-glucoside were purchased from Extrasynthese (Genay, France). HPLC-grade methanol, acetic acid, ethanol, potassium di-hydrogen phosphate, di-sodium tetraborate, iron(II) sulphate ($FeSO_4{\cdot}7H_2O$), 4-dimethylaminobenzaldehyde (DMAB) and *N*-(1-naphthyl) ethylenediamine dihydrochloride were obtained from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH•), butylated hydroxytoluene [BHT, 2,6-bis(1,1-dimethylethyl)-4-methylphenol], sodium cromoglycate, β-nicotinamide adenine dinucleotide reduced form (NADH), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), sodium nitroprusside dehydrate (SNP), linoleic acid, hyaluronidase (HAase) from bovine testes (type IV-S), L-ascorbic acid and sulphanilamide were purchased from Sigma (St. Louis, MO, USA). Sodium formate, trizma hydrochloride (Tris–HCl) and bovine serum albumin (BSA) were from Sigma–Aldrich (Steinheim, Germany). Hyaluronic acid (HA) sodium salt from *Streptococus equi* was from Sigma–Aldrich (Prague, Czech Republic). Water was deionised using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Extraction

Extraction was performed by mixing dried powdered leaves of each sample with water (0.2 g/50 mL), as follows: 0.5 h of sonication followed by 1 h of stirring (200 rpm) at room temperature. The extract obtained was filtered under vacuum and concentrated to dryness, under reduced pressure (40 °C).

2.4. HPLC-DAD qualitative and quantitative analyses

Analysis was performed following the procedure of our previous work (Ferreres et al., 2012b). Redissolved aqueous extract (ca. 50 mg/mL in water) was analysed on an analytical HPLC-DAD unit (Gilson) using a Luna C18 column (250 × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, UK). The mobile phase consisted of two solvents: water–acetic acid (1%) (A) and methanol (B), starting with 20% B and using a gradient to obtain 35% B at 30 min, 50% B at 40 min, 90% B at 42 min and 100% B at 50 min. The flow rate was 1 mL/min. Spectral data from all peaks were collected in the range of 200–400 nm and chromatograms were recorded at 340 nm for flavonoids and at 320 nm for phenolic acids. The data were processed on Unipoint System software.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external calibration standards. Since there is no commercially available standard, di-*C*-glycosides, mono-*C*-(*O*-glycosyl)glycosides, di-*C*-(*O*-glycosyl)glycosides of luteolin and apigenin were quantified as luteolin-3′,7-di-*O*-glucoside and apigenin-6-*C*-glucoside-7-*O*-glucoside, respectively. Chrysoeriol-8-*C*-hexoside was quantified as chrysoeriol. The other compounds were quantified as themselves.

2.5. Antioxidant activity

2.5.1. DPPH• scavenging activity

DPPH• scavenging was monitored spectrophotometrically at 515 nm on a Multiskan Ascent plate reader (Thermo, Electron Corporation), following the procedure described by Ferreres et al. (2012a). For each extract, a dilution series (five concentrations) was prepared: the concentration in each well ranged from 0.041 to 2.622 mg/mL. IC_{50} was calculated from three independent

Table 1
Characterisation of *C. esculenta* samples.

Sample	Variety	Soil watering
GWC	Giant white	Inundation of the plots with cold water without drip irrigation
RC	Red	Inundation of the plots with cold water without drip irrigation
WD	White	Dry land
WH	White	Inundation of the plots with hot water without drip irrigation
WC	White	Inundation of the plots with cold water without drip irrigation

assays, performed in triplicate. Ascorbic acid was used as positive control.

2.5.2. Superoxide radical ($O_2^{\bullet -}$) scavenging activity

The effect of the extract on the superoxide radical-induced reduction of NBT was monitored spectrophotometrically in a Multiskan Ascent plate reader (Thermo, electron corporation), in kinetic function, at 562 nm. Superoxide radicals were generated by the NADH/PMS system, as previously reported (Ferreres et al., 2012a). For each extract, five different concentrations were tested (0.031–4.917 mg/mL final concentration). IC_{50} was calculated from three independent assays, performed in triplicate. Ascorbic acid was used as positive control.

2.5.3. Nitric oxide ($^{\bullet}NO$) radical scavenging activity

The anti-radical activity was determined spectrophotometrically in a Multiskan Ascent plate reader (Thermo, electron corporation), according to a previously described procedure (Ferreres et al., 2012a), with final extracts concentrations between 0.061 and 1.052 mg/mL. IC_{50} was calculated from three independent assays, performed in triplicate. Ascorbic acid was used as positive control.

2.5.4. Lipid peroxidation inhibition assay

Peroxidation of fatty acyl groups was determined following the methodology proposed by Ferreres et al. (2012c), with slight modifications. The reaction mixture contained 250 μL of linoleic acid 4 mM, 150 μL of Tris–HCl (100 mM, pH 7.5), 50 μL of $FeSO_4{\cdot}7H_2O$ 4 mM and 50 μL of serial dilutions of the five different extracts prepared in distiled H_2O (final concentration: 0.036–2.273 mg/mL). Linoleic acid peroxidation was initiated by the addition of 50 μL of ascorbic acid 5 mM, and the mixture was immediately incubated for 1 h at 37 °C. After the incubation period, 3 mL of ethanol was added to each test tube. The mixtures were vortexed and the absorbance was immediately measured at 233 nm in a Helios α (Unicam) spectrophotometer, at room temperature. Three independent assays were performed in triplicate. BHT was used as positive control.

2.6. Hyaluronidase (HAase) inhibition assay

HAase inhibition assay was performed as proposed by Ferreres et al. (2012c). The reaction mixture contained 50 μL of HA stock solution, 100 μL of buffer (0.2 M sodium formate, 0.1 M NaCl and 0.2 mg/mL BSA, pH 3.68), 200 μL of water and 50 μL of serial dilutions of the five different extracts prepared in distiled H_2O (0.021–1.052 mg/mL on well). The reaction was started by the addition of 50 μL of HAase (600 U/mL) prepared in NaCl 0.9%.

The enzymatic reaction was stopped by adding 25 μL of di-sodium tetraborate (0.8 M in water), and subsequent heating for 3 min in a boiling water bath. The test tubes were cooled at room temperature and 750 μL of DMAB solution was added. The tubes were incubated at 37 °C for 20 min. The absorbance of the coloured product was measured at 560 nm in a Multiskan Ascent plate reader. Three independent assays were performed in triplicate. Sodium cromoglycate was used as positive control.

3. Results and discussion

3.1. Phenolic composition

The phenolic composition of the leaves from "giant white" and "red" varieties (samples GWC and RC, respectively) was described before by our group (Ferreres et al., 2012b). In the present work, three samples from another variety (samples WC, WH and WD) were analysed, revealing a distinct qualitative profile: "white" variety samples presented mono-*C*-glycosylated, di-*C*-glycosylated and mono-*C*-(*O*-glycosyl)-glycosylated derivatives of luteolin, apigenin and chrysoeriol (Fig. 1 and Table 2), while in "giant white" and "red" varieties (samples GWC and RC, respectively) phenolic acids, flavones di-*C*-(*O*-glycosyl)glycosides and flavones *O*-glycosides were also found (Ferreres et al., 2012b). The three "white" samples exhibit distinct flavones mono-*C*-glycosides diversity (Table 2): samples WD and WC presented five compounds from this class (compounds **7**, **9**, **10**, **13** and **14**), while WH only contained two of them (compounds **7** and **10**).

The amount of phenolic compounds in the three "white" varieties ranged from ca. 3 to 7 g/kg (Table 2), being lower than that found before in the other two varieties (Ferreres et al., 2012b).

Flavones di-*C*-glycosides were the main compounds, representing from 84% to 97% of the determined phenolics (Table 2). In addition, the relative content of flavones mono-*C*-glycosides in WD and WC samples was similar (ca. 15% and 14%, respectively) (Table 2). Apigenin derivatives represent ca. 53% of the phenolic profile of WD, ca. 50% of the phenolic profile of WH and ca. 45% of WC. As for luteolin derivatives, they are found in greater amounts in WH and WC samples (ca. 50% and 52% respectively), being ca. 45% of the phenolic profile of WD. Chrysoeriol-8-*C*-hexoside (**14**), found only in WD and WC samples, represented ca. 3% in both samples (Table 2).

The three major phenolic compounds are, in decreasing order, luteolin-6-*C*-pentoside-8-*C*-hexoside (**6**), apigenin-6-*C*-pentoside-8-*C*-hexoside (**5**) and apigenin-6-*C*-hexoside-8-*C*-hexoside (**8**). These compounds represent more than half of the phenolics determined (ca. 80% in WH, ca. 70% in WD and ca. 65% in WC).

The differences observed among the three samples from the "white" variety could be attributed to the distinct growth conditions. WD is the sample that contains the highest amounts of phenolic compounds. This sample is grown without irrigation, so the higher production of such metabolites, compared with the samples WH and WC, would be expected, since a reduction in water supply usually increases the content of phytochemicals like phenolic compounds (Sánchez-Rodríguez, Moreno, Ferreres, Rubio-Wilhelmi, & Ruiz, 2011).

Regarding sample WH, data suggest that the hot water treatment not only resulted in lower biosynthesis of phenolic compounds, being also noticed lower diversity of compounds (Table 2).

Data from literature obtained with different *C. esculenta* varieties reveal distinct phenolic composition (Ferreres et al., 2012b). Furthermore, sample WC studied herein shares the same geographical origin and similar watering conditions of the "giant white" and "red" varieties samples previously analysed by our group (Ferreres et al., 2012b), but exhibited a distinct phenolic profile. Taken together, the results obtained in this study reinforce the possibility of the occurrence of infraspecific chemical variability in *C. esculenta* pointed before (Ferreres et al., 2012b).

3.2. Biological activity

Wound healing consists of an orderly progression of events in order to re-establish the integrity of a damaged tissue. The healing process involves three phases that overlap in time and space: inflammation, tissue formation and tissue remodelling (Gupta et al., 2006). During inflammation highly reactive species, such as superoxide radical ($O_2^{\bullet -}$), peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxyl radicals ($ROO^{\bullet}$) and nitric oxide radical ($^{\bullet}NO$) are produced. Therefore, the wound site is an oxidative/nitrosative environment, rich in reactive oxygen and nitrogen species (ROS and RNS, respectively), protecting the wound against pathogens growth (Khanna et al., 2002). However, when at high concentrations, ROS/RNS cause severe tissue damage, leading to neoplastic transformations that undermine the wound healing process (Lundvig, Immenschuh, & Wagener, 2012).

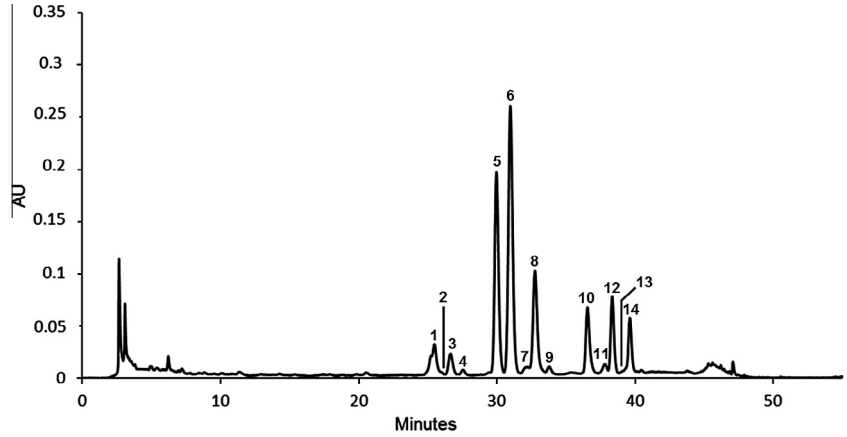

Fig. 1. HPLC-DAD (340 nm) phenolic profile of the aqueous extract from *C. esculenta* "white" variety (sample WD). (1) Luteolin-6-*C*-hexoside-8-*C*-pentoside; (2) apigenin-6,8-di-*C*-hexoside; (3) luteolin-6-*C*-hexoside-8-*C*-pentoside; (4) luteolin-6-*C*-pentoside-8-*C*-hexoside; (5) apigenin-6-*C*-pentoside-8-*C*-hexoside; (6) luteolin-6-*C*-pentoside-8-*C*-hexoside; (7) luteolin-8-*C*-glucoside (orientin); (8) apigenin-6-*C*-hexoside-8-*C*-pentoside; (9) luteolin-6-*C*-glucoside (isoorientin); (10) apigenin-8-*C*-glucoside (vitexin); (11) apigenin-8-*C*-(2-*O*-pentosyl)hexoside; (12) apigenin-6-*C*-hexoside-8-*C*-pentoside; (13) apigenin-6-*C*-glucoside (isovitexin); (14) chrysoeriol-8-*C*-hexoside.

Table 2
Phenolic composition of *C. esculenta* samples (mg/kg dry basis).[a]

Compound[b]		Sample		
		WD	WH	WC
		Flavones mono-*C*-glycosides		
7	Lut 8-*C*-Gluc (orientin)	89.04 ± 4.79	46.31 ± 3.41	45.98 ± 1.04
9	Lut 6-*C*-Gluc (isoorientin)	76.61 ± 2.21	n.d.	103.66 ± 0.90
10	Api 8-*C*-Gluc (vitexin)	584.48 ± 15.31	20.89 ± 2.82	273.93 ± 12.70
13	Api 6-*C*-Gluc (isovitexin)	31.43 ± 9.27	n.d.	13.88 ± 1.02
14	Chrys 8-*C*-Hex	168.58 ± 11.09	n.d.	105.19 ± 11.68
		Flavones di-*C*-glycosides		
1	Lut 6-*C*-Hex-8-*C*-Pent	287.27 ± 25.24	83.58 ± 1.39	311.39 ± 29.07
2	Api 6,8-di-*C*-Hex	n.q.	n.q.	n.q.
3	Lut 6-*C*-Hex-8-*C*-Pent	197.43 ± 4.19	70.41 ± 8.43	199.18 ± 11.03
4	Lut 6-*C*-Pent-8-*C*-Hex	49.95 ± 4.26	24.86 ± 0.14	69.15 ± 5.69
5	Api 6-*C*-Pent-8-*C*-Hex	1433.39 ± 22.64	666.66 ± 46.31	769.11 ± 41.73
6	Lut 6-*C*-Pent-8-*C*-Hex	2384.15 ± 207.01	1251.22 ± 71.39	1415.39 ± 81.65
8	Api 6-*C*-Hex-8-*C*-Pent	801.01 ± 36.14	420.99 ± 28.24	521.98 ± 9.75
12	Api 6-*C*-Hex-8-*C*-Pent	487.69 ± 5.41	257.48 ± 17.21	303.58 ± 20.12
		Flavones mono-*C*-(*O*-glycosyl)glycosides		
11	Api 8-*C*-(2-*O*-pent)Hex	84.69 ± 6.32	29.72 ± 1.44	16.90 ± 0.75
Total phenolic content (mg/kg)		6675.72	2938.45	4149.36
Flavones mono-*C*-glycosides (%)		14.63 ± 0.57	2.24 ± 0.18	13.84 ± 0.44
Flavones di-*C*-glycosides (%)		84.07 ± 0.57	96.74 ± 0.19	85.87 ± 0.51
Flavones mono-*C*-(*O*-glycosyl)glycosides (%)		1.30 ± 0.03	1.02 ± 0.01	0.44 ± 0.03
Luteolin derivatives (%)		44.55 ± 1.57	50.07 ± 0.38	52.27 ± 3.63
Apigenin derivatives (%)		52.71 ± 1.41	49.92 ± 0.38	44.80 ± 3.78
Chrysoeriol derivatives (%)		2.63 ± 0.18	n.d.	2.93 ± 0.20

[a] Identity of samples as in Table 1. Results are expressed as mean ± standard deviation of three determinations; n.d.: not detected; n.q.: not quantified.
[b] Lut: luteolin; Api: apigenin; Chrys: chrysoeriol; Diosmt: diosmetin; Gluc: glucoside; Hex: hexoside; Pent: pentoside; Rhmn: rhamnoside; Der: derivatives.

In order to evaluate the influence of the phenolic composition on *C. esculenta* wound healing potential we analysed the "giant white" (sample GWC) and "red" (sample RC) varieties which phenolic composition was already described by our group (Ferreres et al., 2012b) and the three different samples from the "white" variety for which phenolic profile is provided in this study (samples WC, WH and WD). The antiradical capacity of the samples was assessed against DPPH$^{\bullet}$, $O_2^{\bullet-}$, $^{\bullet}NO$ and lipid peroxidation (LOO$^{\bullet}$) (Table 3). Ascorbic acid was used as positive control of the first three assays and BHT in the last.

The "giant white" (GWC) variety demonstrated the highest DPPH$^{\bullet}$ scavenging capacity. The "white" varieties WC and WD have a similar activity, while WH displayed the lowest activity (Table 3). GWC and RC samples contained the highest phenolic contents, the last being the richest. However, GWC contains a higher amount of flavones mono-*C*-glycosylated, namely luteolin derivatives [luteolin-8-*C*-glucoside (orientin) and luteolin-6-*C*-glucoside (isoorientin)] (Table 2). These two compounds have great DPPH$^{\bullet}$ scavenging ability, since they are dihydroxylated at positions 3′ and 4′ of the B ring, forming a highly reactive catechol group (Zhang, 2005). Their corresponding apigenin derivatives, also identified in these varieties [apigenin-8-*C*-glucoside (vitexin) and apinenin-6-*C*-glucoside (isovitexin)] (Table 2), only have one hydroxyl substituent at 4′, thus having lower activity (Zielińska & Zieliński, 2011).

Regarding flavones di-*C*-glycosides, the qualitative analysis has shown that these compounds represent the major constituents of the "white" variety phenolic composition. The *C*-glycosylation, in

Table 3
IC_{50} (mg/mL) found in the antioxidant and hyaluronidase inhibitory potential assays with the extracts from *C. esculenta* samples and with reference compounds.[a]

Samples	IC_{50} (mg/mL)			LOO·	HAase
	DPPH·	·NO	$O_2^{\cdot -}$		
GWC	0.226 ± 0.006	1.247 ± 0.098	0.123 ± 0.002	0.491 ± 0.033	5.219 ± 0.017
RC	0.535 ± 0.032	0.521 ± 0.018	0.145 ± 0.005	0.570 ± 0.048	4.658 ± 0.005
WD	0.746 ± 0.040	1.181 ± 0.107	0.123 ± 0.006	0.788 ± 0.080	2.642 ± 0.106
WH	1.140 ± 0.051[b]	1.145 ± 0.038	0.106 ± 0.011	0.650 ± 0.087	0.333 ± 0.016
WC	0.695 ± 0.054	1.485 ± 0.102	0.076 ± 0.004	0.958 ± 0.043	4.002 ± 0.078
Ascorbic acid	7.100 ± 0.197[c]	0.250 ± 0.015	0.425 ± 0.053	–	–
BHT	–	–	–	4.000 ± 0.171[c]	–
Sodium cromoglycate	–	–	–	–	1.890 ± 0.038

[a] Identity of samples as in Table 1. Values represent mean ± SD of three assays.
[b] Value corresponds to IC_{25}.
[c] Value expressed in μg/mL.

particular at position 8 of the A ring, decreases the activity of the phenolic compounds, as it induces a molecule distortion, affecting the co-planarity and making it difficult to stabilise the phenoxyl radical (Zielińska & Zieliński, 2011). All flavones di-*C*-glycosides identified in this work are substituted at C-8.

In this way, it is possible to conjecture that the highest activity of the "giant white" variety may be due to the higher concentration of flavones mono-*C*-glycosides derived from luteolin. The absence or low amount of these compounds in "white" taro can also explain the lower activity of WD, WC and WH samples, with particular relevance to WH. Nevertheless, comparing with a known antioxidant compound, ascorbic acid, the DPPH· scavenging activity of all taro samples is lower.

The different varieties exhibit considerable $O_2^{\cdot -}$ scavenging activity, being even more effective than ascorbic acid (Table 3). However, unlike the results obtained in the DPPH· assay, it is not possible to speculate a relationship with the phenolic profile of the samples. Even though this activity may probably be due to other compounds present in the analysed extracts, phenolic compounds may have a synergistic effect, probably recycling radicals of other antioxidant metabolites (Kang, 2007).

The "red" variety showed a considerably higher antioxidant capacity against nitric oxide radical than the other two varieties, which have a similar capacity (Table 3). Again, it is not possible to relate the content of phenolic compounds to the activity of the different varieties. For example, WH is the sample with the second strongest activity against this radical, though its phenolic profile is qualitatively the poorest and quantitatively the lowest. Hence, other unidentified compounds may probably be involved in the antioxidant activity of the different analysed varieties. As for DPPH·, ·NO scavenging ability of these samples is significantly lower than that of ascorbic acid.

Oxygen radicals cause tissue damage by lipid peroxidation of structural lipids and proteins from the skin. These agents not only damage the lipids, but also produce lipid hydroperoxides, secondary intermediates that can lead to a lipid peroxidation chain reaction (Altavilla et al., 2001). Lipid peroxidation is considered responsible for the impairment of endothelial cells, keratinocyte capillary permeability, and fibroblast and collagen metabolism, thus affecting the skin homeostasis and leading to several skin pathologies (Altavilla et al., 2001; Briganti & Picardo, 2003).

The analysed *C. esculenta* extracts presented a moderate lipid peroxide scavenging potential, that from GWC being the one with greater scavenging potential (IC_{50} = 0.491 mg/mL) and WC the variety with lower activity (IC_{50} = 0.958 mg/mL) (Table 3). The lipid peroxide scavenging potential of WH is similar to that of RC (Table 3), although their phenolic composition is quite different (Table 2 and Ferreres et al., 2012b). On the other hand, the WD and WC samples from the "white" variety present a quite different lipid peroxidation preventing capacity in spite of their similar phenolic composition (Tables 2 and 3, Fig. 1). These results lead us to suggest again that other compounds besides phenolics are involved in the lipid peroxidation scavenging potential of *C. esculenta*.

Although oxidative stress is a key factor in the inflammatory process, hyaluronic acid (HA) also plays a significant role. This non-sulfated, linear glycosaminoglycan (GAG), is present in most living tissues as a high molecular mass polymer (>106 Da) and in significant quantities in the skin (dermis and epidermis), brain, and central nervous system. HA plays a crucial role in tissue repair, including wound healing, as it is involved in dynamic cellular processes, such as cell migration and cell–cell recognition, during wound healing and inflammation (David-Raoudi et al., 2008).

For normal tissue organisation and function, a balance between HA synthesis and degradation is important. High molecular weight tissue HA is degraded extracellularlly by HAase. Non-enzymatically, HA is degraded by reactive oxygen species (ROS). Hyaluronidases are endoglucosaminidases, whereas ROS degrade HA randomly at internal glycosidic linkages (Girish & Kemparaju, 2007). In an unbalanced healing process, where the inflammatory condition is increased by the accumulation of HA fragments, hyaluronidase inhibitors can be important to prevent the accumulation of these low molecular weight hyaluronic acid (LMWHA) fragments and, therefore, avoid the prolonged inflammatory condition (Mio & Stern, 2002).

The WH sample exhibited the strongest potential to inhibit hyaluronidase (Table 3), followed by the other two samples from this variety (WD and WC). GWC and RC displayed the lower capacity. Previous studies speculated that the inhibition of hyaluronidase by flavonoids is higher with the increasing of hydroxyl groups in the flavonoid skeleton; furthermore glycosylation decreases the activity (Hertel, Peschel, Ozegowski, & Müller, 2006). This structure–activity relationship is the same as for ROS/RNS scavenging. In this way, the samples with higher activity should be GWC and RC as referred above for DPPH· scavenging activity. This leads us to infer that the phenolic compounds present in the studied samples are not the main responsible for the HAase inhibition, as the samples from the white variety showed the qualitatively poorest and lowest phenolics content (Table 2). Finally, it is noteworthy that WH sample has higher potential to inhibit hyaluronidase than a commercialised HAase inhibitor, sodium cromoglycate (Yingprasertchai, Bunyasrisawat, & Ratanabanangkoon, 2003).

4. Conclusions

In this study, we demonstrated the impact of the irrigation conditions in the phenolic composition of *C. esculenta* leaves. Dry land culture leads to the accumulation of higher contents of phenolic compounds. Additionally, the speculation of a qualitative and

quantitative infraspecific chemical variability in *C. esculenta* can be reinforced by the results obtained in this study, "giant white" and "red" varieties providing higher diversity and quantity of phenolic compounds.

The wound healing property attributed to *C. esculenta* can be related to its antioxidant activity, namely to its superoxide radical scavenging potential, and to the inhibition of hyaluronidase, thus protecting skin cells from oxidative damage and accelerating the recovery of the wound in the inflammatory state. Phenolic compounds may contribute to these properties.

The present study increased the knowledge about the variation in the composition of *C. esculenta* leaves caused by abiotic and genetic factors, providing useful information about the best cultivation procedure to obtain health-promoting antioxidants and hyaluronidase inhibitors.

Acknowledgements

The authors thank Fundação para a Ciência e a Tecnologia (FCT) for Grant No. PEst-C/EQB/LA0006/2011, to "Programa PROCONVERGÊNCIA" from Secretaria Regional da Economia da Região Autónoma dos Açores, to "TERMAZ" Project promoted by INOVA (Instituto Inovação tecnológica dos Açores), to CYTED Programme (Ref. 112RT0460) CORNUCOPIA Thematic Network and project AGL2011-23690 (CICYT).

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