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Phenolic composition and antioxidant capacity of cultivated artichoke, Madeira cardoon and artichoke-based dietary supplements

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ABSTRACT

Cynara cardunculus var. *scolymus* (artichoke) and *Cynara cardunculus* var. *ferocissima* (Madeira cardoon) are two Asteraceae plants used in Madeira Archipelago in diet and also for medicinal issues. The present work aimed to compare the phenolic composition and quantify the main compounds of these two plants and two artichoke-based dietary supplements (juice and dragées). The methanolic extract of the endemic plant cardoon was basically composed of caffeoylquinic acid isomers. The same compounds were observed in artichoke extract, where there was a larger number and variety of other phenolic compounds. Variations in qualitative and quantitative composition of the three artichoke based products were extensive, with only 3 components being common to all 3 products. Mono-*O*-diglycosilated flavonoids (luteolin, apigenin and quercetin) were found in the artichoke products but not in Madeira cardoon. 1,3-*O*-dicaffeoylquinic acid (cynarin), usually considered mainly responsible for the biological properties of artichoke, was found in high amounts ranging in most samples but was not detected in the analyzed commercial dragées. The antioxidant assays results reflected the highly diversified composition of the artichoke-based products, showing a high radical scavenger and reducing capacities, while the extract from Madeira cardoon was a poor antioxidant.

1. Introduction

Cardoon (*Cynara cardunculus* L.) is the designation of a group of Mediterranean species, traditionally used in Southwest Europe such as globe artichoke, cultivated cardoon and wild cardoon. Those applications consider the usage of the blanched leaves, fleshy leaf petioles and the receptacles in soups, stews and salads (Fernández, Curt, & Aguado, 2006). Cardoon leaves are used for their cholagogue, choleretic and choliokinetic actions, for treatment of dyspepsia and as anti-diabetics (Koubaa, Damak, McKillop, & Simmonds, 1999). Flowers of cardoon plants are rich in proteases, namely cardoonsins A and B, due to which aqueous extracts of flowers have been used for centuries in the Iberian Peninsula for manufacturing of ovine and/or caprine milk cheeses (Silva & Malcata, 2005).

Few studies concerning the phenolic composition of cardoon are published. Mono-CQA, di-CQAs and some succinyl-diCQAs along with some luteolin and apigenin glucosides were found to be the main components of wild and cultivated cardoons in Sicily (Pinelli et al., 2007). The antioxidant activity of cardoon lyophilized infusion against superoxide radical, hydroxyl radical, and hypochlorous acid was evaluated and the positive results were attributed to the presence of CQA derivatives and flavonoids (Valentão et al., 2002).

Artichoke (*Cynara cardunculus* var. *scolymus*) is known since 4th century B.C. and used as a food and also as an herbal remedy (Schütz, Kammerer, Carle, & Schieber, 2004).

It is cultivated all over the world today and in 2002, the global annual production was of 1.3 million tons (Lattanzio, Kroon, Linsalata, & Cardinali, 2009; Schütz et al., 2004).

Several studies described numerous pharmacological activities associated to artichoke, such as hepatoprotective, antioxidative, anticarcinogenic, hypocholesterolemic, antiobacterial, anti-HIV, bile-expelling and urinative effects (Kukić et al., 2008; Lattanzio et al., 2009; Shen, Dai, & Lu, 2010).

Phenolic compounds including mono- and di-isomers of caffeoylquinic acid (CQA), and flavonoid *O*-glycosides (luteolin and apigenin derivatives) were described as the main responsible compounds for the biological properties of this plant and synergistic effects are often observed (Pandino, Lombardo, Mauromicale, & Williamson, 2011a; Schütz, Muks, Carle, & Schieber, 2006; Schütz et al., 2004).

1,3-*O*-dicaffeoylquinic acid (cynarin) is considered the one with the highest capacity to inhibit cholesterol biosynthesis and LDL oxidation (Lattanzio et al., 2009). High levels of total cholesterol in the plasma are associated to atherosclerosis, which is the primary cause of coronary heart disease (CVD) (Bundy, Walker, Middleton, Wallis, & Simpson,

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2008). The need to lower the incidence of this disease has led to several investigations that revealed that the consumption of plants rich in this type of phytochemicals is associated to lower risk of CVD.

Artichoke is sold as a dietary supplement in formulations such as dragées, capsules, juice and alcoholic/aqueous extracts in most European countries and in the USA; in Germany, artichoke is considered a medicinal plant and such formulations are dispensed under medical prescription (Fritsche, Beindorff, Dachtler, Zhang, & Lammers, 2002; Schütz et al., 2006). In Madeira Archipelago (Macaronesia region), artichoke is widely produced and used as food (leaves and heads) and as infusion/teas of the leaves for liver problems and digestive issues.

Cynara cardunculus var. *ferocíssima* is an endemic plant from Madeira Archipelago, locally known as Madeira cardoon. This plant belongs to the same genus of artichoke and is used locally for similar purposes described for artichoke. It grows in semiarid places of Madeira and Porto Santo islands generally in association with *Carduus tenuiflorus* locally known as blue cardoon, and *Scolymus maculates,* the golden cardoon. These three plants are very easy to tell apart, Madeira cardoon showing the thinner and sharper spines. There is no written information related to the traditional medicinal uses of this plant and the available information is passed verbally through generations.

The aims of this work were to investigate the phenolic profile of artichoke (cultivated) and cardoon (wild) extracts; to quantify the main compounds detected; to assess *in vitro* antioxidant capacity of the plant extracts by three well established colorimetric methods (ABTS, DPPH and FRAP); and to explore the relationship between phenolic composition and antioxidant capacity. In addition, two commercial artichoke-based products were analyzed and compared to the plant extracts in terms of their phenolic profile.

For clarity sake, throughout this work *Cynara cardunculus* var. *scolymus* will be referred as artichoke and *Cynara cardunculus* var. *ferocissima* as Madeira cardoon.

2. Material and methods

2.1. Chemical and standards

The following reagents were obtained from Merck (Darmstadt, Germany): potassium persulfate (99%), sodium chloride (99.5%), disodium phosphate dodecahydrated (99%), glacial acetic acid (100%), sodium carbonate (p.a.) and ferrous sulfate heptahydrate (99%). 2,2-diphenyl-1-picrylhydrazyl (DPPH) (>95%), Trolox (≥99.8%, HPLC), 2,2'azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (≥99%, HPLC), 2,4,6-Tri(2-Pyridyl)-s-triazine (TPTZ) (≥99.0%, TLC) and Folin–Ciocalteu's phenol reagent were purchased from Fluka (Lisbon, Portugal). Potassium chloride (>99.5%), gallic acid (99%, HPLC), potassium acetate (p.a.), rutin (≥98%, HPLC) and ferric chloride hexahydrate (97%–100%) were purchased from Panreac (Barcelona, Spain); potassium dihydrogen phosphate (99.5%), aluminium chloride (98%) and sodium acetate trihydrate (pure) were purchased from Riedel-de Haën (Hanover, Germany).

All solvents used for plant extraction were AR grade, purchased from Fisher. LC-MS grade acetonitrile, 99.9% (LabScan, Gliwice, Poland) and ultra-pure water (Milli-Q water purification system, EUA) were used for HPLC analysis.

Stock solutions of standard compounds (100 μg/mL) were prepared in ethanol for HPLC–DAD–ESI/MS^n identification and stored in a refrigerator at −20 °C until use. Standards used: caffeic acid (>99%), apigenin (>99%), quercetin (>99%) and luteolin (>99%) from Extrasynthese (Lyon, France) and 5-*O*-caffeoylquinic acid (99%) from Acros Organics. 1,3-*O*-dicaffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid and 3,4,5-*O*-tricaffeoylquinic acid (>98% by HPLC for all) were obtained from Chengdo Biopurify Phytochemicals, Ltd China (Sichuan, China).

2.2. Plant material and sample preparation

Fresh leaves of artichoke (100 g of artichoke) were obtained from a certified producer in Camacha (Madeira Island, July 2010) and those from Madeira cardoon (100 g of Madeira cardoon) were collected in the wild in Ponta de São Lourenço (Madeira Island, July 2010) and identified by taxonomist Fátima Rocha and vouchers were deposited in the Madeira Botanical Garden Herbarium collection.

The leaves were dried at room temperature (20 °C), protected from direct sunlight and ground to fine powder in a mechanic grinder to 60 mesh size. The powdered plant material was submitted to ultrasound-assisted solvent extraction: plant material (1 g) was extracted with methanol (25 mL) using a sonicator Bandelin Sonorex (Germany) at 35 kHz and 200 W for 60 min at room temperature. The solutions were filtered and concentrated under reduced pressure in a rotary evaporator (40 °C). Stock solutions with concentrations (m/v) of 5 mg/mL were prepared by dissolving each dried extract in initial HPLC mobile phase (CH_3CN/H_2O (20/80, v/v)). For each sample three independent extractions were done.

Two commercial manufactured artichoke food supplements (juice and dragées) were purchased from local drugstores. The juice label claimed it to be pure artichoke juice made from fresh plants 100% organically grown and that three 15 mL portions per day should be taken, pure or diluted in water or tea. Dragées (700 mg each) claimed to be composed of powdered artichoke plant (250 mg) plus artichoke extract (50 mg), microcrystalline cellulose and magnesium stearate as excipients; the contents in cynarin is claimed to be 0.5–0.8% and the recommended daily intake is 3 dragées.

Dragées (3 units) were ground in a mortar and extracted by the same method. Juice samples were diluted with ultra pure water (2:10, v/v). These solutions were filtered through 0.45 μm micropore membranes prior to use and 10 μL was injected for HPLC–DAD–ESI/MS^n analysis.

2.3. HPLC–DAD–ESI/MS^n analysis of phenolic profile

The HPLC separation was performed on a Dionex ultimate 3000 series instrument (California, EUA) coupled to a binary pump, a diode-array detector (DAD), an autosampler and a column compartment.

The separation was performed on a Phenomenex Gemini C_{18} column (5 μm, 250 × 3.0 mm i.d.; Phenomenex) with a sample injection volume of 10 μL. The mobile phase was composed of acetonitrile (A) and water/formic acid (100/0.1, v/v) (B). A gradient program was used as follows: 20% A (0 min), 25% A (10 min), 25% A (20 min), 50% A (40 min), 100% A (42–47 min), 20% A (49–55 min). The mobile phase flow rate was 0.4 mL/min; the chromatogram was recorded at 280 nm and 320 nm and spectral data for all peaks were accumulated in the range of 190–400 nm. Column temperature was controlled at 30 °C.

For HPLC–DAD–ESI/MS^n analysis, a model 6000 ion trap mass spectrometer (Bruker Esquire, Bremen, Germany) fitted with an ESI source was used. Data acquisition and processing were performed using Esquire control software. Negative ion mass spectra of the column eluate were recorded in the range *m/z* 100–1000 at a scan speed of 13,000 Da/s. High purity nitrogen (N_2) was used both as drying gas at a flow of 10.0 mL/min and as a nebulizing gas at a pressure of 50 psi. The nebulizer temperature was set at 365 °C and a potential of +4500 V was used on the capillary. Ultra-high-purity helium (He) was used as collision gas at a pressure of 1×10^{-5} mbar and the collision energy was set at 40 V. The acquisition of MS^n data was made in *auto* MS^n mode, with an isolation width of 4.0 *m/z*. For MS^n analysis, the mass spectrometer was scanned from 10 to 1000 *m/z* with a fragmentation amplitude of 1.0 V (MS^n up to MS^4) and two precursor ions.

Three independent assays were performed for each sample.

2.4. HPLC–DAD quantification of phenolic content

The quantitative analysis was performed with the HPLC system described above using a modified gradient that allowed for the separation of the CQA compounds (Gouveia & Castilho, 2012b). The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B). The gradient program was used as follows: 20% B (0–1 min), 78% B (8–10 min), 76% B (12–14 min), 75% B (16–18 min), 73% B (20 min), 50% B (40 min), 0% B (41–45 min), 80% B (46–50 min). The flow rate was 0.4 mL/min and the injections volume 10 μL. UV detection was performed at 320 nm. Three independent assays were performed for each sample and the content of each compound was expressed in terms of milligrams per 100 g dried plant (mg/100 g).

2.5. Total phenolic content (TPC)

The Folin–Ciocalteu method described by Zheng and Wang (2001) was used with some modifications (Gouveia & Castilho, 2011). Plant extracts were dissolved in methanol to yield a concentration (w/v) of 10 mg/mL. 50 μL aliquots were mixed with 1.25 mL of Folin–Ciocalteu reagent (diluted 1:10 fold) and 1 mL of 7.5% sodium carbonate solution. After 30 min, absorbance was measured at a $\lambda = 765$ nm, at room temperature. The results were expressed milligrams as gallic acid equivalents (GAE) per 100 g dried plant (mg GAE/100 g).

2.6. Total flavonoid content (TFC)

Total flavonoid content was established using an adapted method (Akkol, Göger, Kosar, & Baser, 2008). Methanolic solutions of the extracts with a concentration of 2.5 mg/mL were prepared. In a 10 mL test tube, 0.5 mL of sample solution, 1.5 mL of methanol, 2.8 mL of water, 0.1 mL of potassium acetate (1 M) and 0.1 mL of aluminium chloride (10% in methanol) were added and mixed. After 30 min, at room temperature, decrease in absorbance was measured at a $\lambda = 415$ nm. The total flavonoid content was expressed as milligrams of rutin equivalent (RUE) per 100 g dried plant (mg RUE/100 g).

2.7. ABTS$^{\bullet+}$ radical scavenging activity

The ABTS method used was adapted version of a previously reported method (Re et al., 1999). The ABTS$^{\bullet+}$ radical was prepared by reacting 50 mL of 2 mM ABTS$^{\bullet+}$ solution with 200 μL of 70 mM potassium persulfate solution. This mixture was kept in the dark for 16 h at room temperature. This solution is reported as being stable in this form for two days (Re et al., 1999).

For each analysis, the ABTS$^{\bullet+}$ solution was diluted with pH 7.4 phosphate buffered saline (PBS) solution to an initial absorbance of 0.700 ± 0.021 at 734 nm. This solution was freshly prepared for each analysis. For evaluation of the radical scavenging activity, an aliquot of 100 μL methanolic solution (10 mg/mL) was added to 1.8 mL of ABTS$^{\bullet+}$ solution and the absorbance decrease, at a $\lambda = 734$ nm, was recorded after 6 min. Results were expressed in terms of μmol Trolox equivalent per 100 g dried plant (μmol eq. Trolox/100 g).

2.8. DPPH radical scavenging activity

The DPPH assay was performed as described before by (Gordon, Paiva-Martins, & Almeida, 2001) introducing some modifications (Gouveia & Castilho, 2012a). Briefly, 100 μL of the methanolic solution (10 mg/mL) was added to 3.5 mL of a 0.06 mM methanol DPPH radical solution. The decrease in absorbance was determined at 516 nm until it reached a plateau (after 30 min), in the dark. The DPPH antioxidant capacity was determined using a Trolox standard curve and results were expressed as μmol Trolox equivalent per 100 g dried plant (μmol eq. Trolox/100 g).

2.9. Ferric reducing activity (FRAP assay)

The ferric reducing ability of the samples was established by the FRAP assay (Benzie & Strain, 1996). FRAP reagent was freshly prepared by mixing 2.5 mL of solution $FeCl_3x6H_2O$ (20 mM), 2.5 mL of solution TPTZ (10 mM in 40 mM of HCl) and 25 mL of acetate buffer 0.3 M (pH 3.6) and was incubated at 37 °C.

The extracts were dissolved in methanol to yield a final concentration (w/v) of 1 mg/mL. For each analysis, 30 μL of methanolic solution was added to 180 μL of distilled water and 1.8 mL of FRAP solution. The increase of absorbance was recorded at $\lambda = 593$ nm in 15 second intervals, during 30 min at 37 °C. The FRAP results were expressed as mmol $FeSO_4 \cdot 7H_2O$ per mg dried plant (mmol Fe(II)/mg).

2.10. Statistical analysis

All measurements were performed in triplicate and results are expressed as mean ± SD.

Significant differences in antioxidant activity and total phenolic content of the different extracts were determined using one-way ANOVA. The statistical probability was considered to be significantly different at the level of $p < 0.05$.

3. Results and discussion

In this work, a reversed phase C_{18} stationary phase column was used with a previously reported HPLC–DAD–ESI/MS^n method proved to be "suitable" for the screening of phenolic compounds present in polar extracts of plants from the Asteraceae family (Gouveia & Castilho, 2009, 2010). A slightly modified HPLC method was employed in order to achieve a complete separation of CQA compounds, in particular of 3,4-*O*-dicaffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid isomers. This second HPLC analysis was used in order to quantify the main CQA derivatives, namely 5-*O*-caffeoylquinic acid, five dicaffeoylquinic acid (1,3-*O*-diCQA, 3,4-*O*-diCQA, 3,5-*O*-diCQA, 1,5-*O*-diCQA and 4,5-*O*-diCQA) present in the plants and food supplements under study.

3.1. Identification of phenolic compounds by HPLC–DAD–ESI/MS^n

Similarities and dissimilarities on the phenolic profiles of artichoke and Madeira cardoon were studied. Commercial artichoke-based products were also analyzed and compared to the plant extracts.

The base peak chromatograms (BPC) of the analyzed samples are given in Fig. 1. Most detected compounds were well separated and the retention times were reproducible in the three assays performed for each sample.

In the four analyzed samples, more than 30 compounds were identified and Figs. 2–4 show their chemical structures. Complete characterization was achieved by tandem mass spectrometry experiments and UV absorption data (Table 1).

3.1.1. Identification of hydroxycinnamic acid derivatives (**2**, **3**, **4**, **5**, **6**, **8**, **10**, **15**, **16**, **17** *and* **20**)

The detected mono- and dicaffeoylquinic acids isomers were identified by comparison with standard solutions and with previous reports: 3-*O*-caffeoylquinic acid (compound **2**; $t_R = 4.4$ min), 5-*O*-caffeoylquinic acid (compound **3**; $t_R = 5.0$ min), 1,3-*O*-dicaffeoylquinic acid (compound **5**, $t_R = 6.5$ min), 3,4-*O*-dicaffeoylquinic acid (compound **15**, $t_R = 12.1$ min), 1,5-*O*-dicaffeoylquinic acid (compound **16**, $t_R = 12.4$ min), 3,5-*O*-dicaffeoylquinic acid (compound **17**, $t_R = 13.6$ min) and 4,5-*O*-dicaffeoylquinic acid (compound **20**, $t_R = 14.4$ min). The fragmentation spectrum for isomer 1,5-*O*-diCQA is presented in Fig. 5.

Compound **4** ($t_R = 6.0$ min) gave a $[M-H]^-$ ion at *m/z* 353 and was only found in the artichoke's extract. Its MS^n fragmentation behaviour was identical to those of caffeoylquinic acids.

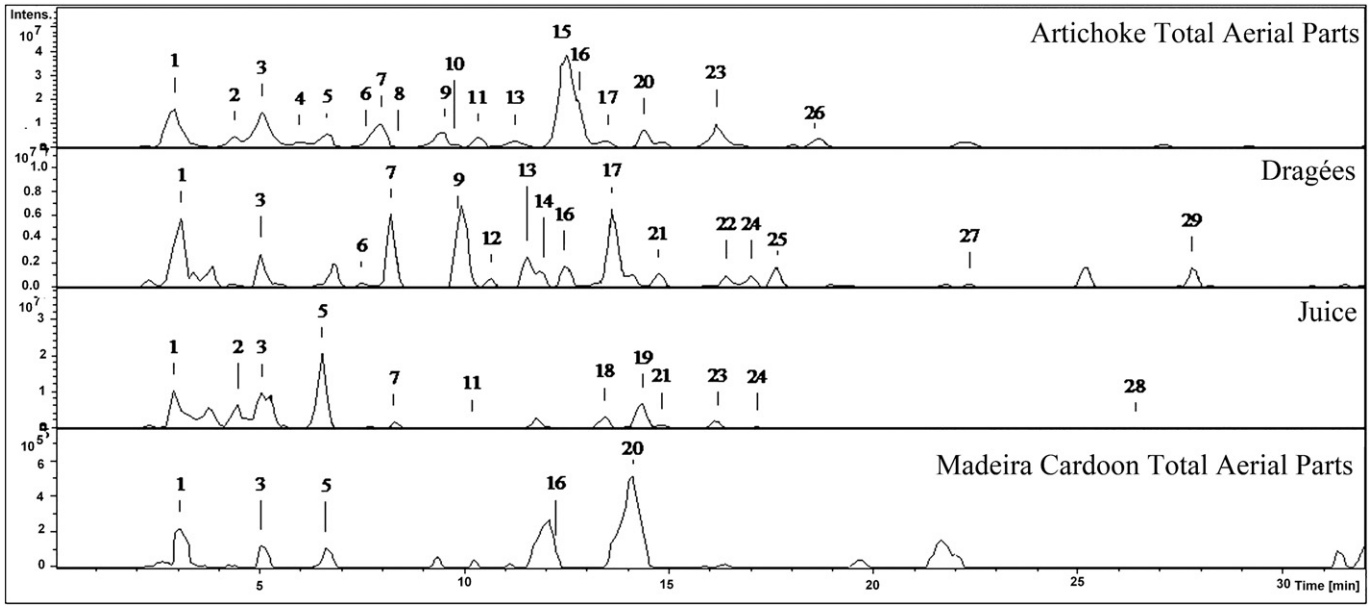

Fig. 1. HPLC–DAD–ESI/MS^n analysis of the methanolic extracts of artichoke and Madeira cardoon total aerial parts and artichoke-based dragées and juice — HPLC–MS negative ion ESI/MS^n base peak chromatogram (BPC).

Unequivocally identification was not possible to establish but compound **4** is possibly a *cis*-isomer or an epimer of 5-*O*-CQA (Jaiswal, Sovdat, Vivan, & Kuhnert, 2010).

Compound **6** (t_R = 7.6 min) gave a [M–H]$^-$ ion at *m/z* 367 and its MS^2 fragmentation gave a fragment ion at *m/z* 191 [quinic acid–H]$^-$ as base peak. This compound was characterized as 5-*O*-feruloylquinic acid (Clifford, Zheng, & Kuhnert, 2006) and it was detected in the artichoke's extract and dragées sample.

Artichoke leaf extract revealed the presence of compounds **2**, **3**, **5**, **15**, **16**, **17** and **20**. In *C. ferocissima* leaf extract only compounds **3**, **5**, **16**, **17** and **20** were detected. For the artichoke-based products, compounds **2** and **5** were found in the juice but not in the dragées; compounds **16** and **17** were observed in the dragées but not in the juice. Compound **3** was present in both juice and dragées.

Compound **8** (t_R = 8.4 min) gave a [M–H]$^-$ ion at *m/z* 677. Its MS^n fragmentation showed sequential loss of 162 Da, forming a MS^2 ion at *m/z* 515. Fragmentation of this MS^2 ion gave a similar fragmentation pattern to that observed from 1,3-*O*-diCQA (compound **5**). Given its low retention time, the hypothesis of this compound to be a triCQA was discarded. In previous works from our group (Gouveia & Castilho, 2009, 2010) triCQA was positively identified (using a reference sample) in other plants analyzed by the same method at t_R = 28.7 min; The 162 Da elimination was thus attributed to a hexoside residue. Therefore, compound **8** was identified as 1,3-*O*-dicaffeoylquinic acid *O*-hexoside and it was only found in the artichoke extract.

Compound **10** (t_R = 9.8 min) exhibited a [M–H]$^-$ ion at *m/z* 631 and in the MS^2 spectrum a fragment ion at *m/z* 353 was observed as base peak. This fragment resulted from the neutral loss of a 278 Da residue which probably corresponds to a diglycoside moiety composed of two sugar units (146 Da + 132 Da) (Jin et al., 2008). The further MS^n fragmentation revealed a CQA part, MS^3 ion at *m/z* 191 and compound **10** was identified as a caffeoylquinic acid diglycoside.

3.1.2. Identification of flavonoids (**7**, **9**, **13**, **14**, **19**, **21**, **23**, **22**, **24**, **27**, **28** and **29**)

MS^n fragmentation of the ion [M–H]$^-$ gave the deprotonated aglycone ion (Y_0^-) by the loss of the sugar residue. The nomenclature

Caffeoyl position	Compound
R_3	3-*O*-caffeoylquinic acid (**2**)
R_5	5-*O*-caffeoylquinic acid (**3**)
-	Caffeoylquinic acid (**4**)
R_1; R_3	1,3-*O*-dicaffeoylquinic acid (**5**)
R_5	5-*O*-feruoylquinic acid (**6**)
R_1; R_3	1,3-*O*-dicaffeoylquinic acid hexoside (**8**)
R_3, R_4	3,4-*O*-dicaffeoylquinic acid (**15**)
R_1, R_5	1,5-*O*-dicaffeoylquinic acid (**16**)
R_3, R_5	3,5-*O*-dicaffeoylquinic acid (**17**)
R_4, R_5	4,5-*O*-dicaffeoylquinic acid (**20**)

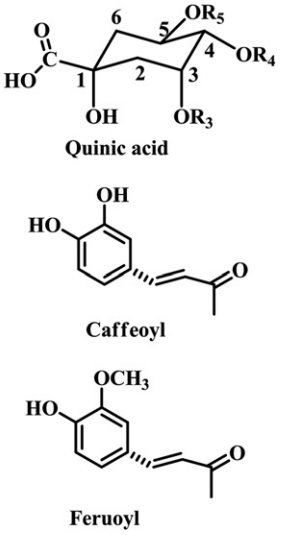

Fig. 2. Chemical structures of caffeoylquinic acid derivatives characterized.

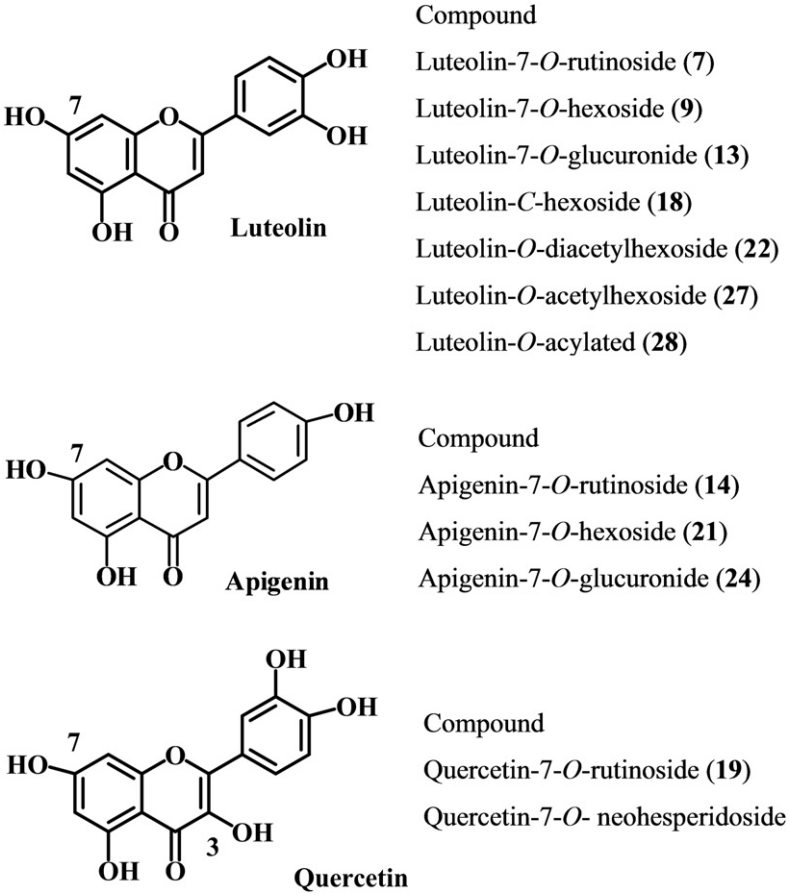

Fig. 3. Chemical structures of flavonoids characterized.

proposed by Ma et al. (Cuyckens & Claeys, 2004) for MS^n fragmentations of flavonoids was adopted in this work. For free aglycones, the $^{i,j}A^-$ and $^{i,j}B^-$ labels correspond to ions containing intact A- and B-rings, respectively, in which I and j indicate the C-ring bonds that have been broken. For conjugated aglycones, Y_0^- is used to refer to the aglycone fragment $[M–H–glycoside]^-$.

Seven compounds presenting luteolin as aglycone were discovered and described. Free luteolin aglycone was only found in the dragées samples and was labelled as compound **29** ($t_R = 27.7$ min), based on the order of elution.

Compound **7** ($t_R = 8.0$ min) displayed a $[M–H]^-$ ion at *m/z* 593. Its MS^2 fragmentation gave a fragment ion at *m/z* 285 due to a neutral loss of 308 Da. This moiety is probably a combined loss of two sugar units (162 Da + 146 Da) (Fig. 6).

Since the aglycone radical ion was not detected in the MS^2 spectrum and based on the rules reported by Ablajan et al. (Ablajan et al., 2006) this compound was characterized as a flavonoid mono-*O*-diglycoside.

The MS^2 spectrum did not revealed fragment ions related to the glycan part, Y_n^-. According to Cuyckens et al. (Cuyckens, Rozenberg, de Hoffmann, & Claeys, 2001) this observed behaviour is typical for diglycosides with 1 → 6 interglycosidic linkage.

Consecutive fragmentation of the ion at *m/z* 285 gave, as main fragments, ions at *m/z* 241 $[M–H–CO_2]^-$, 175 $[M–H–C_3O_2–C_2H_2O]^-$ and 151 ($^{1,3}A^-$) indicating that the aglycone is luteolin with 7-OH as favoured glycosilation position (Cuyckens & Claeys, 2004). Thus, compound **7** was characterized as luteolin-7-*O*-rhamnosyl(1 → 6) hexoside (luteolin-7-*O*-rutinoside) (Fig. 7) and was found in all artichoke-based samples, including the plant extract, but it was not found in cardoon extract.

Compound **9** ($t_R = 9.4$ min) showed a $[M–H]^-$ ion at *m/z* 447 and was identified as luteolin-7-*O*-hexoside. The MS^2 spectrum base peak

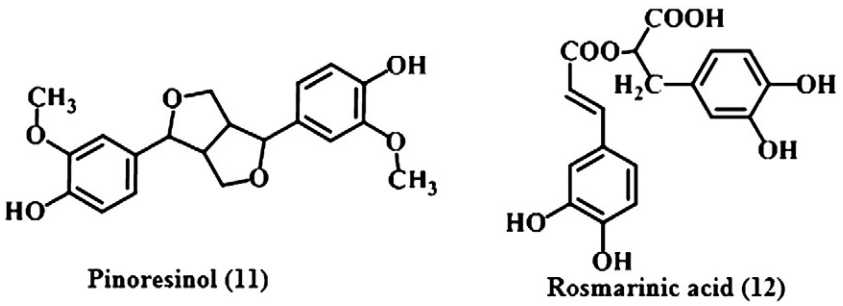

Fig. 4. Chemical structures of pinoresinol and rosmaniric acid.

Table 1
Characterization of phenolic compounds of Madeira cardoon and artichoke extracts, and artichoke-based dietary supplements (juice and dragées) by HPLC–DAD–ESI/MS^n.

No.	Assigned identity	t_R (min)	UV λ_{max} (nm)	$[M-H]^-$ *m/z*	HPLC–DAD–ESI/MS^n *m/z* (% base peak)	Sample
1	Caffeic acid-*O*-hexoside	2.9	225, 278	683	MS^2 [683]: 341 (100) MS^3 [683→341]: 179 (100), 161 (16.0), 143 (17.1), 113 (17.9) MS^4 [683→341→179]: 143 (100), 131 (51.5), 119 (50.7), 113 (33.6), 89 (64.1)	Artichoke Cardoon Dragées Juice
2	3-*O*-Caffeoylquinic acid	4.4	240, 300,324	353	MS^2 [353]: 191 (100), 179 (16.0) MS^3 [353→191]: 173 (49.3), 127 (100), 111 (40.5), 109 (28.4) MS^4 [353→191→127]: 109 (100), 85 (48.3)	Artichoke Juice
3[a]	5-*O*-Caffeoylquinic acid	5.0	242, 300, 326	707	MS^2 [707]: 354 (11.6), 353 (100) MS^3 [707→353]: 191 (100) MS^4 [707→353→191]: 173 (100), 127 (99.5), 111 (59.1), 93 (68.6)	Artichoke Cardoon Dragées Juice
4	5-*O*-Caffeoylquinic acid isomer	6.0	240, 299, 320	353	MS^2 [353]: 191 (100) MS^3 [353→191]: 173 (40.3), 127 (100), 111 (37.0), 93 (48.1), 85 (55.5) MS^4 [353→191→127]: 109 (100)	Artichoke
5[a]	1,3-*O*-Dicaffeoylquinic acid	6.5	242, 300, 321	515	MS^2 [515]: 353 (100), 335 (27.6), 179 (28.3), 191 (17.4) MS^3 [515→353]: 191 (100), 179 (37.2), 135 (11.1) MS^4 [515→353→191]: 173 (79.9), 127 (100), 85 (71.7), 111 (61.1)	Artichoke Cardoon Juice
6	5-*O*-Feruoylquinic acid	7.6	327	367	MS^2 [367]: 191 (100) MS^3 [367→191]: 127 (100), 111 (21.0), 93 (35.7)	Artichoke Dragées
7	Luteolin-7-*O*-rutinoside	8.0	255, 267, 359	593	MS^2 [593]: 285 (100) MS^3 [593→285]: 285 (100), 241 (15.1), 199 (12.6), 175 (23.0), 151 (16.2) MS^4 [593→285→241]: 241 (23.7), 215 (31.4), 199 (100)	Artichoke Dragées Juice
8	1,3-*O*-Dicaffeoylquinic acid hexoside	8.4	–	677	MS^2 [677]: 516 (24.1), 515 (100) MS^3 [677→515]: 353 (100), 323 (30.2), 179 (11.7), 161 (14.1) MS^4 [677→515→353]: 191 (100), 179 (19.3)	Artichoke
9	Luteolin-7-*O*-hexoside	9.4	256, 268, 350	447	MS^2 [447]: 286 (16.3), 285 (100) MS^3 [447→285]: 285 (100), 259 (25.0), 241 (33.7), 199 (41.9), 175 (50.3)	Artichoke Dragées
10	Caffeoylquinic acid diglycoside	9.8	240, 299, 320	631	MS^2 [631]: 354 (11.3), 353 (100), 191 (39.5) MS^3 [631→353]: 191 (100) MS^4 [631→353→191]: 173 (100), 155 (42.6), 127 (42.2), 111 (68.2), 93 (45.4), 85 (46.9)	Artichoke
11	Pinoresinol-4-*O*-hexoside	10.3	–	519	MS^2 [519]: 358 (18.7), 357 (100) MS^3 [519→357]: 342 (13.6), 151 (100), 136 (50.7) MS^4 [519→357→151]: 136 (100)	Artichoke Juice
12	Rosmarinic acid-*O*-hexoside	10.7	–	521	MS^2 [521]: 359 (100) MS^3 [521→359]: 223 (19.8), 197 (13.7), 179 (13.7), 161 (100) MS^4 [521→359→161]: 133 (100)	Dragées
13	Luteolin-7-*O*-glucuronide	11.3	256, 267, 349	461	MS^2 [461]: 285 (100), 286 (16.5) MS^3 [461→285]: 199 (76.2), 217 (82.3), 243 (100)	Artichoke Dragées
14	Apigenin-7-*O*-rutinoside	11.9	268, 338	577	MS^2 [577]: 270 (13.9), 269 (100) MS^3 [577→269]: 228 (100), 225 (77.0), 169 (16.0), 151 (54.4)	Dragées
15	3,4-*O*-Dicaffeoylquinic acid	12.1	242, 300, 325	515	MS^2 [515]: 353 (100), 335 (17.5), 191 (33.2), 179 (21.9), 173 (34.0) MS^3 [515→353]: 191 (51.0), 179 (74.0), 173 (100), 135 (20.9) MS^4 [515→353→173]: 155 (77.0), 111 (100	Artichoke
16	1,5-*O*-dicaffeoylquinic acid	12.4	242, 300, 328	515	MS^2 [515]: 354 (20.9), 353 (100), 191 (30.4) MS^3 [515→353]: 191 (100) MS^4 [515→353→191]: 173 (33.5), 127 (100), 93 (29.1), 85 (76.0)	Artichoke Cardoon Dragées
17	3,5-*O*-Dicaffeoylquinic acid	13.3	242, 300, 327	515	MS^2 [515]: 353 (100), 191 (12.9), 179 (2.2) MS^3 [515→353]: 191 (100), 179 (32.2), 173 (2.5), 135 (13.3) MS^4 [515→353→191]: 173 (63.7), 127 (100), 111 (15.9)	Artichoke Cardoon Dragées
18	Luteolin-*C*-hexoside	13.4	255, 268, 350	579	MS^2 [579]: 459 (100), 313 (19.4), 271 (58.1), 235 (15.5) MS^3 [579→459]: 441 (46.9), 357 (100), 235 (46.3), 205 (27.0), 151 (41.3) MS^4 [579→459→357]: 339 (100), 169 (14.7), 125 (37.5)	Juice
19	Quercetin-7-*O*-rutinoside	14.3	255, 265, 355	609	MS^2 [609]: 302 (18.5), 301 (100) MS^3 [609→301]: 286 (100), 283 (68.1), 199 (14.1), 125 (57.1) MS^4 [609→301→286]: 258 (100), 199 (92.1)	Juice
20	4,5-*O*-Dicaffeoylquinic acid	14.4	242, 300, 326	515	MS^2 [515]: 353 (100), 299 (10.5), 203 (23.4), 179 (29.9), 173 (27.8) MS^3 [515→353]: 191 (18.5), 179 (57.0), 173 (100), 135 (12.6) MS^4 [515→353→191]: 127 (16.0), 109 (18.2), 93 (100)	Artichoke
21	Apigenin-7-*O*-hexoside	14.7	266, 338	431	MS^2 [431]: 270 (27.2), 269 (100) MS^3 [431→269]: 268 (62.8), 225 (39.7), 169 (50.1), 149 (100)	Dragées Juice
22	Luteolin-*O*-diacetylhexoside	16.4	255, 266, 350	533	MS^2 [533]: 490 (32.3), 489 (100) MS^3 [533→489]: 286 (14.9), 285 (100) MS^4 [533→489→285]: 241 (13.5), 213 (43.6), 199 (48.4), 175 (67.4), 151 (35.0), 133 (80.6), 121 (100)	Dragées
23	Quercetin-7-*O*-neohesperidoside	16.1	255, 266, 355	609	MS^2 [609]: 463 (16.9), 447 (4.34), 302 (18.5), 301 (100) MS^3 [609→301]: 286 (100), 283 (47.4), 199 (55.6), 125 (62.4) MS^4 [609→301→286]: 242 (24.8), 201 (55.1), 199 (100)	Juice
24	Apigenin-7-*O*-glucuronide	17.1	265, 338	445	MS^2 [445]: 270 (15.0), 269 (100), 175 (11.0) MS^3 [445→269]: 225 (42.6), 182 (64.1), 151 (100), 107 (92.0)	Dragées Juice
25	Dimer of rosmaniric acid	17.5	–	359	MS^2 [359]: 197 (25.8), 179 (20.6), 162 (10.6), 161 (100) MS^3 [359→161]: 133 (100)	Dragées
26	Unknown	18.7	–	433	MS^2 [433]: 399 (30.7), 397 (100), 259 (15.5), 160 (26.2) MS^3 [433→397]: 317 (100)	Artichoke
27		22.3		489		Dragées

(continued on next page)

Table 1 (*continued*)

No.	Assigned identity	t_R (min)	UV λ_{max} (nm)	$[M-H]^-$ *m/z*	HPLC–DAD–ESI/MS^n *m/z* (% base peak)	Sample
	Luteolin-*O*-acetylhexoside		256, 265, 350		MS^2 [489]: 285 (100) MS^3 [489 → 285]: 285 (22.1), 241 (33.3), 239 (100), 199 (13.6), 151 (55.6)	
28	Luteolin-*O*-acylated	26.4	256, 266, 350	593	MS^2 [593]: 285 (100) MS^3 [593 → 285]: 164 (100), 243 (87.7), 241 (72.3), 245 (10.7) MS^4 [593 → 285 → 164]: 242 (100), 164 (94.6), 151 (41.8)	Juice
29	Luteolin	27.7	–	285	MS^2 [285]: 285 (100), 243 (86.0), 217 (76.4), 175 (59.8), 171 (40.6), 241 (33.2) MS^3 [285 → 241]: 241 (56.8), 199 (100)	Dragées

[a] Comparison with a reference standard; – their UV spectra have not been properly observed due to low intensity.

was the $[aglycone–H]^-$ ion at *m/z* 285 and its MS^n fragmentation gave luteolin characteristic fragments (described above).

Compound **13** ($t_R = 11.3$ min) exhibited a $[M–H]^-$ ion at *m/z* 461. Under MS^n fragmentation, a glucuronic acid group was eliminated (neutral loss of 176 Da) to form the $[aglycone–H]^-$ ion at *m/z* 285. The aglycone fragmentation behaviour was identical to that described above for luteolin. Thus, **13** was identified as luteolin-7-*O*-glucuronide and it was found in the artichoke extract and dragées.

In the dragées sample, at a retention time of 16.4 min, a compound with $[M–H]^-$ ion at *m/z* 533 was detected. This ion easily lost a neutral 44 Da group (CO_2) to form a MS^2 base peak at *m/z* 489. Fragmentation of this ion gave $[luteolin–H]^-$ ion at *m/z* 285 and its characteristic ions. The MS^3 neutral loss of 204 Da can be related to an acetylhexoside moiety (162 + 42 Da). Therefore, **22** was identified as luteolin-*O*-diacetylhexoside.

Compound **27** ($t_R = 22.3$ min) exhibited a $[M–H]^-$ ion with *m/z* 489. Its MS^n fragmentation led to the same fragmentation pattern described for compound **22**, but with only an acetyl residue. So, **27** was identified as luteolin-*O*-acetylhexoside.

Another compound with $[M–H]^-$ ion at *m/z* 593 was observed in the artichoke juice but, as opposed to compound **7**, compound **28** occurred at a very high retention time ($t_R = 26.4$ min). The same fragmentation pathway found for compound **7** was also found in this case. Attending to the high retention time, the neutral loss of 308 Da is more likely due to an acyl group such as caffeoyl (162 Da) + malonyl (146 Da) that to sugar moieties. The UV data have a very low intensity and no useful information could be obtained from them. Therefore, compound **28** was characterized as luteolin-*O*-acylated.

Compound **14** ($t_R = 11.9$ min) gave a $[M–H]^-$ ion at *m/z* 577 and its MS^2 spectrum revealed a neutral loss of 308 Da rising a fragment ion at *m/z* 269. This loss, as mentioned above, was associated to a mono-diglycoside (162 + 146 Da). As for compound **7**, the absence of MS^2 fragment ions related to the glycan part and a base peak correspondent to the deprotonated aglycone ion, (Y_0^-) suggest a 1 → 6 interglycosidic linkage. The fragmentation of the ion at *m/z* 269 resulted in fragments consistent with apigenin at *m/z* 225 $[M–H–CO_2]^-$ and 151 ($^{1,3}A^-$). So, **14** was identified as apigenin-7-*O*-rhamnosyl(1 → 6)hexoside (apigenin-7-*O*-rutinoside).

Two other apigenin derivatives were found in the dragées and juice samples. Compound **21** ($t_R = 14.7$ min) displayed a $[M–H]^-$ ion at *m/z* 431 and its MS^2 spectrum gave a fragment ion at *m/z* 269 indicating the loss of 162 Da. The further MS^n fragmentation gave the characteristic fragments of apigenin and **21** was characterized as apigenin-7-*O*-hexoside.

Compound **24** ($t_R = 17.1$ min) gave a $[M–H]^-$ ion at *m/z* 445 and was identified as apigenin-7-*O*-glucuronide. The presence of glucuronic acid moiety was observed by the neutral loss of a 176 Da residue. The apigenin common fragments were obtained in the MS^n experiments.

Other than luteolin and apigenin, another flavonoid aglycone was found in the juice–quercetin.

Compounds **19** ($t_R = 14.3$ min) and **23** ($t_R = 16.1$ min) showed a $[M–H]^-$ ion at *m/z* 609 and its MS^2 fragmentation revealed a loss of

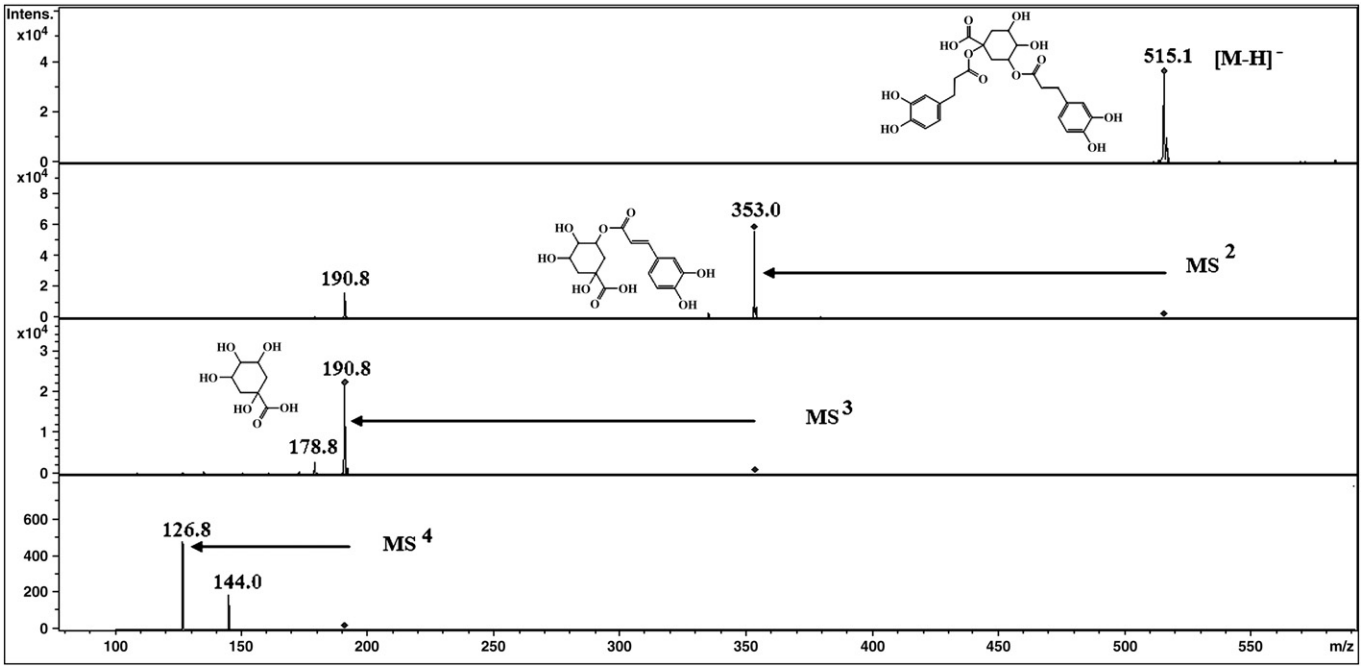

Fig. 5. ESI/MS^n negative mode of compound **16**. Sequential fragmentation, MS^1 to MS^4 of the ion at *m/z* 515.

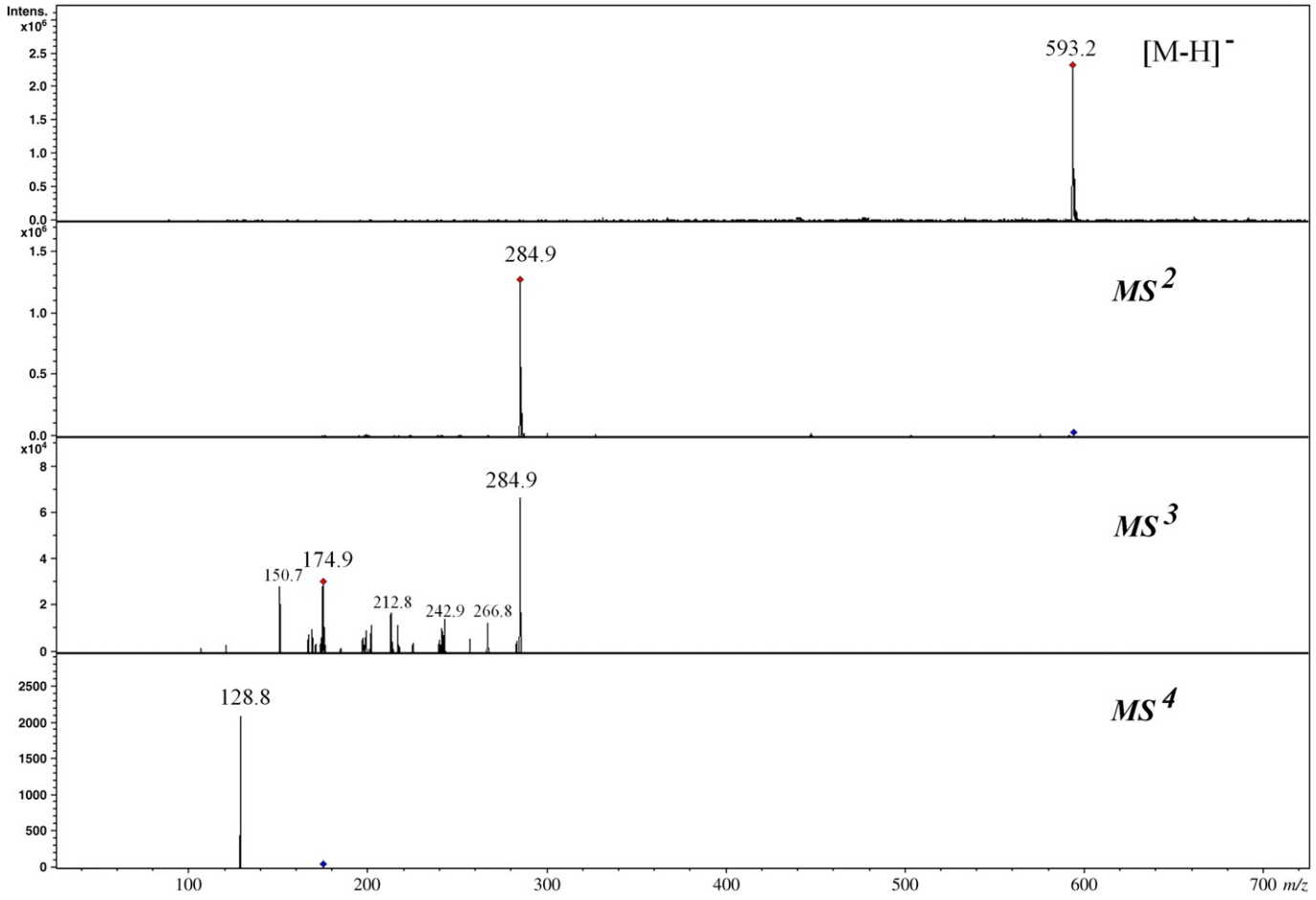

Fig. 6. ESI/MS^n negative mode of compound **7**. Sequential fragmentation, MS^1 and MS^2 of the ion at *m/z* 593.

308 Da forming a MS^2 base peak ion at *m/z* 301 that corresponds to the deprotonated aglycone ion, Y_0^-. Further fragmentation of this ion revealed the characteristic fragment ions of quercetin, for example at *m/z* 151 ($^{1,2}A^-$–CO), 179 ([$^{1,2}A^-$–H]$^-$) and 271 ([M–H–CH_2O]$^-$).

As mentioned before, the loss of 308 Da can be associated to a mono-diglycoside moiety (162 Da + 146 Da). Further analysis of each MS^n spectra allowed to distinguish the interglycosidic linkage position of these two moieties.

For flavonoids *O*-neohesperidosides ((1→2) interglycosidic linkage), the formation of fragment ions related to the glycane part shows a higher intensity than that of their *O*-rutinoside analogues ((1→6) interglycosidic linkage).

In the MS^2 spectrum of **19** it was not possible to observe those glycan fragments, therefore the 308 Da moiety should correspond to a *O*-rutinoside residue. The exact opposite was found for compound **23**, where fragments such as Y_1^- at m/z 463 and Y_0^- at m/z 447 were observed. So, this flavonoid is probably a *O*-neohesperidoside.

In conclusion, **19** was identified as quercetin-7-*O*-rhamnosyl(1→6) hexoside (quercetin-7-*O*-rutinoside) and **23** as quercetin-*O*-rhamnosyl (1→2)hexoside (quercetin-*O*-neohesperidoside). For **19**, the possibility of substitution on position 3-OH was further excluded by comparison with a standard.

3.1.3. Other compounds (**11, 12**, **18** *and* **25**)

Compound **11** (t_R = 10.3 min) showed a [M–H]$^-$ ion at *m/z* 519 and was detected in the leaf extract and juice from artichoke. This compound was identified as pinoresinol-4-*O*-hexoside and was previous described by us for *Helichrysum melaleucum* (Gouveia & Castilho, 2010).

Compound **12** (t_R = 10.7 min) displayed a [M–H]$^-$ ion at *m/z* 521. Its MS^2 fragmentation showed the loss of a 162 Da group, forming a fragment ion at *m/z* 359 (Fig. 8). The consequent MS^n fragmentation gave, as most important fragments, ions at *m/z* 161, 223, 197 and 133. This behaviour was described before for salviaflaside (Chen, Zhang, Wang, Yang, & Wang, 2011), a hexoside of rosmarinic acid commonly found in *Salvia* species (Li, 1998). This compound was only found in the dragées of artichoke.

Compound **25** (t_R = 17.5 min) exhibited a [M–H]$^-$ ion at *m/z* 359 and was also only present in the dragées. Its fragmentation gave fragment ions at *m/z* 161 (base peak), *m/z* 197 (*ca.* 26% of base peak), *m/z* 179 (*ca.* 20% of base peak). According to Liu et al. (2007) this fragmentation pattern corresponds do the dimer of rosmarinic acid.

The UV spectra of compound **18** (t_R = 13.4 min) showed maximum absorption bands at 255, 268 and 350 nm, the typical absorptions bands of flavones.

Nevertheless, the MS^n fragmentation behaviour revealed a distinct fragmentation pathway from luteolin *O*-glycosylated derivatives. It was also possible to detect a MS^2 fragment ion $^{0,2}X^-$ [M–H–120]$^-$ at *m/z* 459. This ion can suggest the presence of a flavonoid *C*-glycosylated. However, it was not possible to observe any more fragment ions indicative of this type of compounds.

3.2. HPLC–DAD quantification of phenolic content

The quantification method used for assessment of caffeoylquinic acid isomers was developed and validated before (Gouveia & Castilho, 2012b) and the artichoke and cardoon samples were analyzed in the same conditions. The method was linear in the range of 0.1–700 μg/mL with correlation coefficients (R^2) higher than 0.999; the LOD values

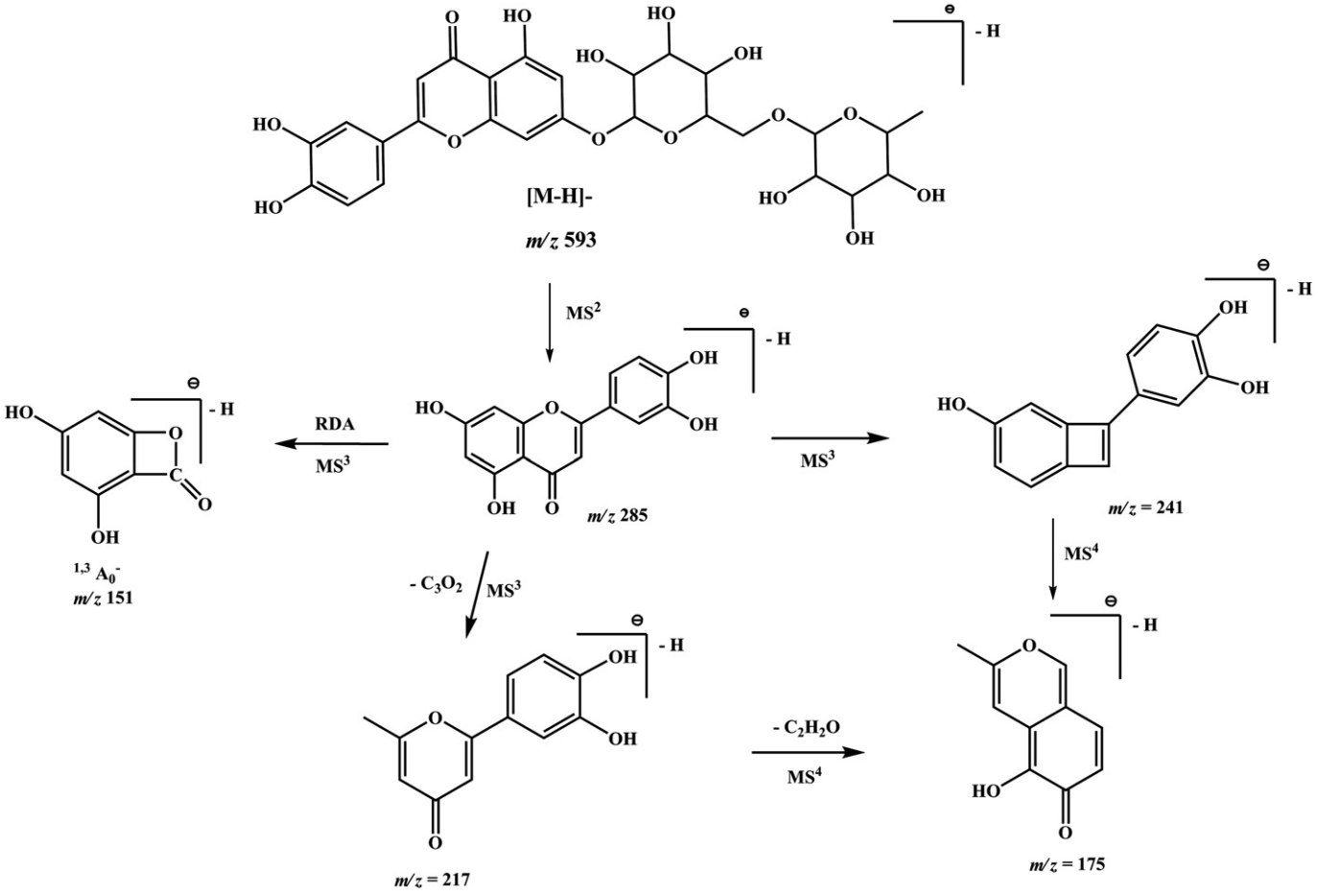

Fig. 7. Proposed fragmentation pathway for compound 7, luteolin-7-*O*-rutinoside.

ranged from 0.760 to 5.95 μg/mL and LOQ values from 2.29 to 18.0 μg/mL. The % RSD for the intraday and interday precision was very good with values lower than 1.5%.

There are few studies concerning the phenolic composition of artichoke and its derivate bioproducts (Pandino, Lombardo, Mauromicale, & Williamson, 2011b; Pandino et al., 2011a; Schütz et al., 2004, 2006). However, the amounts/types of compounds detected are affected by several aspects such as part of plant under analysis, experimental parameters (sample extraction, cleaning procedures and also the method of analysis) and the origin/growth conditions of the plant material.

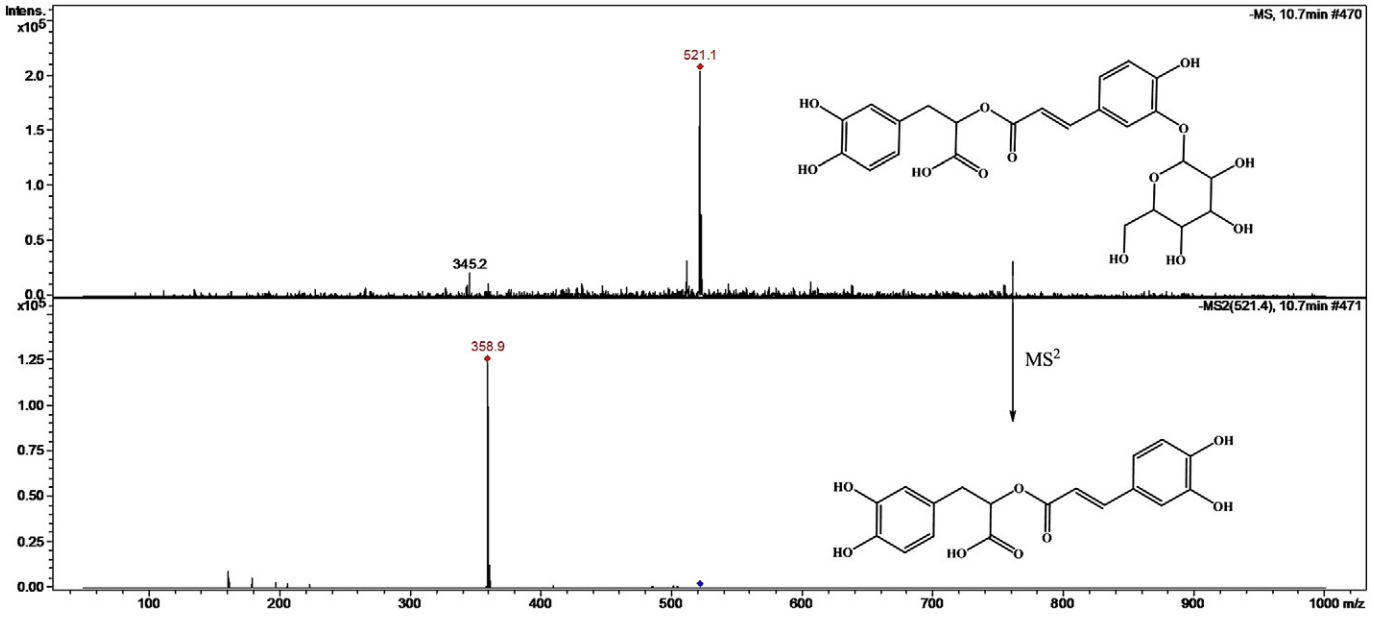

Fig. 8. ESI/MS^n negative mode of compound **12**. Sequential fragmentation, MS^1 and MS^2 of the ion at *m/z* 521.

Groups of plants commonly used in the diet and for medicinal purposes in a specific region should be scientifically studied in terms of their suitable composition. This was one of the mains goals of this study with artichoke and cardoon.

The artichoke leaves, in general, were rich in luteolin and apigenin derivatives while the main source of CQA isomers is the floral stem (Pandino et al., 2011a).

The authors concluded that the production of CQA isomers and flavonoids is stimulated by different biosynthetic enzymes and that the exposition to sunlight increases the production of flavonoid compounds rather than CQA isomers (Pandino et al., 2011a).

Our findings over the leaves of Madeira cardoon showed a lesser content in CQA derivatives than artichoke even though the former grows in arid very much exposed zones of Madeira and Porto Santo islands, while the later is cultivated in more shady areas so other factor may be more relevant. No flavonoids were detected in Madeira cardoon leaves and the flower heads extracts (results not shown) presented no positive results in the antioxidant assays.

The HPLC–DAD–ESI/MS^n screening (Fig. 1) allowed for the identification of several caffeoylquinic acid isomers which are the main compounds in each extract.

However, the 3,4-*O*-diCQA and 1,5-*O*-diCQA isomers were not separated with the required resolution to allow for their quantification by HPLC. Consequently, a modified HPLC gradient was used to overcome this issue.

The obtained separation was good enough to quantify the five CQA isomers (Fig. 9).

This HPLC method has been validated before (Gouveia & Castilho, 2012b) while working also with *Helichrysum* species rich in this class of compounds.

Schütz et al. (2004) also reported a HPLC–DAD method for the quantification of caffeoylquinic acids from artichoke. However, to obtain a good resolution of separation of the dicaffeoylquinic acid isomers more than 70 min are needed, while in the present study these compounds eluted between 10 and 14 min.

As it can be seen from Table 2, the comparison of the *Cynara* subspecies with the bioproducts derived from artichoke showed that the amounts of CQA isomers differ substantially between them. For example, for artichoke extract, the 1,5-*O*-diCQA was the compound present in highest amounts ($5.84 \times 10^2 \pm 0.55$ mg/100 g plant). 5-*O*-CQA was also very abundant ($2.36 \times 10^2 \pm 0.95$ mg/100 g plant), in good agreement to revised literature (Lattanzio et al., 2009). Schütz et al. (2004) also found similar results for lyophilized artichoke heads.

The 5-*O*-CQA was the main CQA derivative in artichoke-based dietary supplements (Table 3): juice (7.31 mg/100 mL, *ca.* 76% of total amount of CQA) and dragées (32.4 mg/100 g of dragées, *ca.* 66% of total amount of CQA).

For Madeira cardoon the 4,5-*O*-diCQA isomer gave the highest content with a value of 89.6 ± 0.44 mg/100 g of plant followed by the 1,5-*O*-diCQA and 5-*O*-CQA.

Cynarin (1,3-diCQA), usually considered mainly responsible for the pharmacological properties of artichoke (Lattanzio et al., 2009), was found in the extract with a value of 23.5 ± 0.064 mg/100 g plant (*ca.* 2.5% of the total amount of CQA compounds) and in the juice at 2.21 ± 0.011 mg/100 mL (*ca.* 23% of the total amount of CQA compounds.

Contrarily to expected, this compound was not found in the dragées samples. Several experiments with samples with increasing concentration were performed and no evidence of this compound was obtained. The possibility of the amount of this compound to be under the limit of detection of the HPLC–DAD method was considered. To overcome this possibility, HPLC–MS^n data were processed with the filter for Extracted Ion Chromatogram (DataAnalysis® Software) with a target mass settled at 515 and no peak with the characteristics (HPLC retention time, mass and UV spectra) of 1,3-*O*-diCQA was ever detected.

The amount of cynarin in Madeira cardoon was quite low (1.17 ± 0.0044 mg/100 g of plant), *ca.* 0.46% of the total CQA compounds measured.

Some care should be taken when considering direct cynarin quantification and comparative evaluation with other studies. As mentioned, this name is attributed to the 1,3-*O*-diCQA isomer according to the new IUPAC nomenclature for cyclitols. In fact, since 1954, when it was first isolated by Panizzi and Scarpati, to 1973 when the new IUPAC rules were established, "cynarin" was the 1,5-*O*-diCQA isomer (Lattanzio et al., 2009). Some confusion still remains, moreover since cynarin is not the most abundant isomer. However, since caffeoylquinic acids can undergo isomerisation or hydrolysis in aqueous solutions, 1,5-*O*-diCQA can form 1,3-*O*-diCQA, and some authors even claim that cynarin is not at all genuine to artichoke but an artefact resulting from that isomerisation both isomers being highly dependent on the physiological state of the vegetal tissues, decreasing

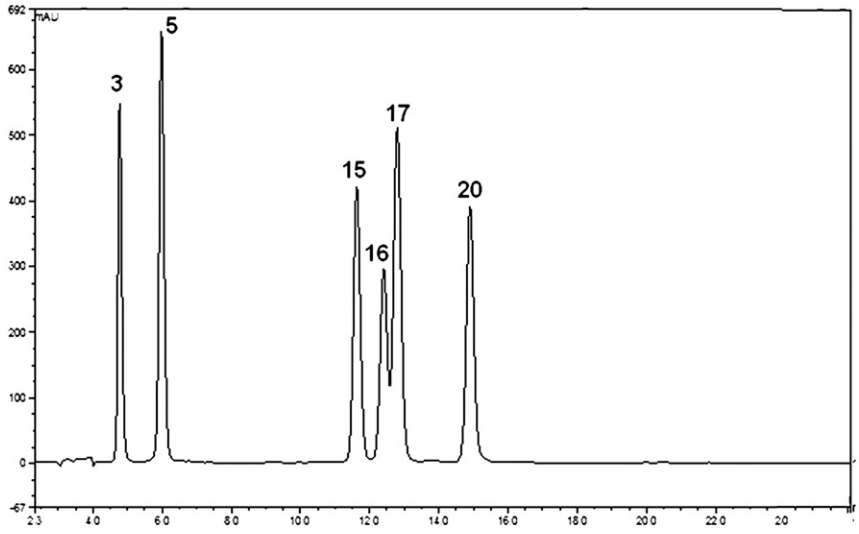

Fig. 9. HPLC separation of standard caffeoylquinic acid isomers with detection at $\lambda = 320$ nm. **3**, 5-CQA; **5**, 1,3-diCQA; **15**, 3,4-diCQA; **16**, 1,5-diCQA , **17**, 3,5-diCQA and **20**, 4,5-diCQA.

Table 2
Contents of individual phenolic compounds in artichoke (extract, juice and dragées) and Madeira cardoon extract.

Sample		5-CQA (**3**)	1,3-diCQA (**8**)	3,4-diCQA (**15**)	1,5-diCQA (**16**)	3,5-diCQA (**17**)	4,5-diCQA (**20**)	Total amount
Artichoke	Extract[1]	$2.36\times10^{2}\pm0.95^{d}$	23.5 ± 0.064^{c}	18.6 ± 0.36^{a}	$5.84\times10^{2}\pm0.55^{c}$	20.4 ± 0.34^{b}	64.9 ± 0.11^{a}	$9.48\times10^{2}\pm2.4^{d}$
	Juice[2]	7.31 ± 0.022^{a}	2.21 ± 0.011^{b}	ND	ND	ND	ND	9.52 ± 0.032^{a}
	Dragées[3]	32.5 ± 0.55^{b}	ND	ND	16.4 ± 0.082^{a}	NQ	ND	48.8 ± 0.63^{b}
Cardoon[1]		74.3 ± 0.23^{c}	1.17 ± 0.0044^{a}	ND	82.7 ± 0.15^{b}	5.75 ± 0.12^{a}	89.6 ± 0.44^{b}	$2.53\times10^{2}\pm0.94^{c}$

Values represented as means ± SD ($n=3$); [1]mg/100 g of plant; [2]mg/100 mL juice; [3]mg/100 g of dragées.
Means in the same row not sharing the same letter are significantly different at $p<0.05$ probability level.
ND — not detected; NQ — not quantified; LOD values from 0.760 to 5.95 μg/mL; LOQ values from 2.29 to 18.0 μg/mL.

sharply as the plant ages. Juice and hydroalcoholic extracts are usually richer in cynarin, since isomerisation occurs during extraction or manufacture.

The brand of juice under analysis was a good example of this assumption, with high levels of 1,3-*O*-diCQA and no 1,5-*O*-diCQA detected. In dragées, allegedly composed of plant leaves and extract, no 1,3-*O*-diCQA isomer was observed, as opposed to the claimed 0.5–0.8%, and the amounts of 1,5-*O*-diCQA and other phenolics were also low. This can be a result of the use of old leaves instead of young ones or of poor manufacture.

For Madeira cardoon extract the total amount of CQA isomers is lower than those obtained for artichoke extracted and processed by the same analytical methods. However, the amount of 4,5-*O*-diCQA isomer is higher in Madeira cardoon rather than it is in artichoke extract.

3.3. Colorimetric assays

As mentioned, the antioxidant capacity is one of the biological properties more extensively studied in artichoke extracts, but for the Madeira cardoon variety there is no scientific study. In the present work we aimed to compare this property in locally produced artichoke and the endemic cardoon variety, collected at the same time of the year and extracted by the same methods. The results obtained for each of the antioxidant methods tested are shown in Table 3.

3.3.1. Total phenolic content (TPC) and total flavonoid content (TFC)

The difference between the TPC values of the artichoke extract (233.64 ± 1.4 mg GAE/100 g) and Madeira cardoon extract (184.49 ± 3.1 mg GAE/100 g) was *ca.* 21%. These results were not expected given the HPLC–DAD results, where taking into account only the caffeoylquinic acids derivatives quantified and their total amount (Table 3), the extract from artichoke presented a difference of 73% of the value obtained for cardoon's extract. This can be explained by the fact that the Folin–Ciocalteu reagent, widely used, is non-specific to phenolic compounds because other non-phenolic compounds with reducing properties (such as organic acids) can also react (Magalhães, Segundo, Reis, & Lima, 2008). However, a high correlation between the results obtained by this method and those obtained by reducing assays (FRAP, DPPH, ABTS, *etc.*) have been reported and consists on the main advantage for routinely use of the Folin–Ciocalteu method.

Despite that the results obtained by the Folin-Ciocalteu method have a lower sensibility and accuracy compared to those obtained by HPLC–DAD methods, in this particular case the same tendency is observed for both methods.

As shown mentioned before, in the HPLC–DAD–ESI/MS^n analysis of the phenolic composition of the extract of artichoke, some flavonoid compounds were present; in the extract from Madeira cardoon no flavonoid type compounds were detected. This was in good agreement with the TFC values of the *Cynara* subspecies which were remarkably different: artichoke extract gave a 97.0 ± 0.074 mg RUE/100 g, *ca.* 97% higher than the extract from Madeira cardoon at 2.86 ± 0.0010 mg RUE/100 g (Table 3).

The quantification of a minor amount of flavonoids in the Madeira cardoon sample and the fact that no compound of this type was detected in the HPLC–DAD analysis can be due either to their concentration limit being under the HPLC–DAD–ESI/MS^n method limit of detection for such compounds, or, more likely, to a false positive result in the colorimetric method.

Table 3
Experimental determinations of total phenolic and flavonoid content and antioxidant capacity against ABTS, DPPH and FRAP for the samples under study.

Sample	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg RUE/100 g)	DPPH	ABTS	FRAP
			μmol eq. Trolox/100 g		mmol Fe(II)/mg
Artichoke	233.6 ± 1.4	97.0 ± 0.074	$3.77\times10^{3}\pm11.8$	$6.94\times10^{3}\pm17.3$	$6.75\times10^{3}\pm58.3$
Madeira cardoon	184.5 ± 3.1	2.86 ± 0.0010	176.8 ± 0.41	419.9 ± 0.78	$1.25\times10^{3}\pm6.4$

Values represented as mean ± SD ($n=3$); GAE — gallic acid equivalents; RUE — rutin equivalent.

3.3.2. Antioxidant capacity assays

There is a large variety of trivial and accurate methods that allow for the measurement of the total antioxidant capacity of plant extracts and/or pure components. In this work, the free radical scavenging capacity and the reducing capacity were measured using the ABTS, DPPH and FRAP method.

The ABTS assay was based in the consumption of the cation radical ABTS•$^+$ by reaction with the antioxidant compounds present in the samples under study. Both extracts showed the capacity to neutralize the radical cation but at different levels (significantly different at $p<0.05$). The ABTS values found for artichoke and Madeira cardoon samples were $6.94\times10^{3}\pm17.3$ and $4.19\times10^{2}\pm0.78$ μmol eq. Trolox/100 g, respectively.

The same order of activity was found in the DPPH assay but the absolute values were lower than those found in the ABTS assay. For instance, the artichoke's extract showed a DPPH value of $3.77\times10^{3}\pm11.8$ μmol eq. Trolox/100 g and Madeira cardoon gave $1.76\times10^{2}\pm0.41$ μmol eq. Trolox/100 g.

The fact that the values obtained by the DPHH were lower than those found by ABTS assay is related to the highest sensibility of the ABTS assay (Arnao, 2000).

The ABTS assay has the advantage of the working solution being soluble in aqueous and organic solvents, at a large range of pH values, and the reaction time is lower than the DPPH assay. The benefit of employing the DPPH assay is due to the higher stability of the DPPH radical and its commercial form being ready to use.

The reducing properties of the samples were measured by the FRAP assay which is based on colour change where the yellow ferric–tripyridyltriazine complex is reduced to the blue ferrous complex, the reaction being accompanied at a wavelength of 593 nm. This reaction is pH-dependent (ideal pH 3.6).

The FRAP results are expressed as mmol Fe(II)/mg. The obtained results indicated artichoke extract as having the highest reducing capacity when compared to cardoon's ($6.75 \times 10^3 \pm 58.3$ and $1.25 \times 10^3 \pm 6.4$ mmol Fe(II)/mg, respectively).

The antioxidant activity of artichoke extracts was reported (Kukić et al., 2008) and different artichoke genotypes were recently studied in terms of their antioxidant capacity (Pandino et al., 2011a).

Both studies gave FRAP values remarkably lower than those found in *Cynara* from Madeira Archipelago reported in this paper. However, comparison of results obtained in independent studies must be considered qualitative since small variations in experimental conditions can largely affect the results.

The antioxidant assays herein described were also used to measure the antioxidant capacity of other Asteraceae plants in the same experimental conditions. The FRAP assays showed a very good correlation to TFC but poor to TPC, while the ABTS assay manifested the opposite trend (Gouveia & Castilho, 2012a).

For instance, *Artemisia argentea* extracts (Gouveia & Castilho, 2011) revealed a highest antioxidant capacity compared to Madeira cardoon but the opposite was found for artichoke.

It is known that CQA isomers and flavonoids such as luteolin and apigenin and their derivatives, detected in artichoke extract, are powerful antioxidants (Kukić et al., 2008). Therefore, the highest antioxidant capacity of artichoke when compared with Madeira cardoon must be related to the wide variety of phenolic compounds present on its composition, as found in the HPLC–ESI/MS^n screening and with the high levels of CQA isomers quantified.

4. Conclusion

Two *Cynara* plant species (one endemic — Madeira cardoon and one introduced — artichoke) used in the traditional folk medicine in Madeira were characterized in terms of their phenolic composition. For Madeira cardoon it was the first time, to our best knowledge, that this plant was analyzed in a scientific manner.

The phenolic profile of the artichoke was found to be much more diversified than that for Madeira cardoon. Caffeoylquinic acids isomers were the main compounds, although flavonoid derivatives, namely mono *O*-diglycosilated compounds, were observed.

The endemism only revealed the presence of caffeoylquinic acids (one mono- and four diCQA) and its antioxidant and reducing capacity are thus diminished when compared to the extract of artichoke.

The HPLC–DAD–ESI/MS^n screening was applied also to food supplements derived from artichoke and the majority of the compounds were common to the artichoke extract. This method showed that the dragées did not present any 1,3-*O*-diCQA, described as the active principle of this food supplement. The freshly prepared extract of artichoke dry leaves has, by far, more phenolic compounds than the over-the-counter preparations with a cynarin contents not much lower than the juice.

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