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Influence of genotype, harvest time and plant part on polyphenolic composition of globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori]

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ABSTRACT

Globe artichoke is an ancient herbaceous plant native to the Mediterranean Basin. The edible part of the plant (head) is particularly rich in polyphenols, whose therapeutic properties are well documented. A field experiment was conducted in Sicily (south Italy) to examine the influence of genotype and harvest time on the polyphenol content and profile of different head parts. The concentrations of 19 phenolic compounds were determined by HPLC-DAD-ESI/MS^n analysis. It was observed that individual phenolic substances were preferentially accumulated in specific head parts and genotypes. Apigenin 7-*O*-glucuronide was found to be the major flavonoid, with 6298 mg kg^{-1} DM in 'Romanesco clone C3' receptacle, whereas chlorogenic acid represented the main caffeoylquinic acid, reaching 14841 mg kg^{-1} DM in the inner bracts of 'Violetto di Sicilia'. Our findings prove also the influence of climatic conditions on the phenolic profile and thus suggest giving specific consideration to harvest time.

1. Introduction

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is an herbaceous perennial plant native to the Mediterranean Basin. The edible part of the plant is the immature inflorescence (head or capitulum), which is harvested when it is still in rapid growth. It constitutes nearly 35–55% of the fresh weight of the head (Bianco, 1990) and consists of the enlarged receptacle and the tender thickened bases of the bracts. Nowadays, artichoke heads production is widely distributed all over the world (121 kha), even though it is mainly concentrated in Mediterranean regions, which produce 800 kt per year (about 65% of the global production). Italy represents the leading producer (about 474 kt per year), followed by Spain and France (about 215 and 55 kt per year, respectively) (FAO Statistical Database, 2007). Within Italy, this crop is cultivated mainly in the south, particularly in Apulia (17 kha), Sicily (15 kha) and Sardinia (13 kha) (ISTAT, 2007), where it contributes significantly to agricultural economy.

Not only is globe artichoke a tasty food in the Mediterranean diet but also known since ancient times in folk medicine for its choleretic and diuretic effects. In recent years, an increasing demand for functional foods with added value has led to a renewed interest in this crop. Several *in vitro* and *in vivo* experiments have demonstrated the health promoting effects of globe artichoke extracts (Brown & Rice-Evans, 1998; Gebhardt, 1997; Perez-Garcia, Adzet, & Canigueral, 2000; Wang et al., 2003; Zapolska-Downar et al., 2002). The beneficial properties have always been related to the phenolic compounds present in the heads (Lattanzio, Cardinali, Di Venere, Linsalata, & Palmieri, 1994; Lattanzio & Van Sumere, 1987; Schütz, Kammerer, Carle, & Schieber, 2004) and leaves (Llorach, Espin, Tomas-Barberan, & Ferreres, 2002; Wang et al., 2003). The most abundant phenolic substances reported in artichoke heads are caffeoylquinic acid derivatives (Lattanzio et al., 1994), particularly chlorogenic acid (5-*O*-caffeoylquinic acid), 1,5-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid (Schütz et al., 2004), and flavonoids such as apigenin and luteolin (both glycosides and rutinosides) (Lattanzio & Van Sumere, 1987; Schütz et al., 2004) as well as different cyanidin caffeoylglucoside derivatives (Aubert & Foury, 1981). The importance of these compounds seems to be linked to their well established dual role as a protective pool against oxidative damages

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caused by free radicals (Racchi et al., 2002; Rice-Evans & Miller, 1996) and as substrates for oxidative browning reactions by both enzymatic and chemical mechanisms (Lattanzio et al., 1994). The action of phenolics as strong antioxidants is due mainly to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans & Miller, 1996).

Compared to other vegetables, artichoke is a promising source of antioxidant compounds and contains high levels of total polyphenols in its edible part (Brat et al., 2006). In many studies performed until now, artichoke leaves have been used primarily as a raw material (Adzet & Puigmacia, 1985; Brown & Rice-Evans, 1998; Perez-Garcia et al., 2000; Zapolska-Downar et al., 2002), whereas the phenolic composition of other plant parts is not well investigated, notwithstanding that its edible part is used worldwide both as a fresh and a canned taste product and that the utilisation of by-products of artichoke processing, such as stem and outer bracts, has been increasingly taken into consideration (Llorach et al., 2002) as animal feedstuff (Megías, Hernández, Madrid, & Martinez-Teruel, 2002), for fibre production (Femenia, Robertson, Waldron, & Selvendran, 1998), and for the recovery of functional ingredients (Larossa, Llorach, Espin, & Tomas-Barberan, 2002). Few studies (Di Venere et al., 2004; Fratianni, Tucci, De Palma, Pepe, & Nazzaro, 2007; Wang et al., 2003) were carried out to investigate the content of individual phenolic compounds in relation to genotype. Considering that Italy holds the most important germplasm (Mauromicale & Ierna, 2000a), with a great number of commercial and local varieties, the characterisation and quantification of these substances could allow to select artichoke varieties on the basis of their phenolic profile. In fact, this information seems to be useful in order to define the suitability of the different varieties to fresh consumption and/or industrial processing, because polyphenols have an important role from both a nutraceutical and technological point of view. Moreover, only few studies (Mulinacci et al., 2004; Schütz et al., 2004; Sànchez-Rabaneda et al., 2003) have reported the simultaneous determination of all caffeoylquinic acids and flavonoids in artichoke extracts. In this view, the objective of the present study was to evaluate quantitatively and qualitatively the individual phenolic compounds of globe artichoke genotypes in relation to inflorescence parts (leaves, floral stem, receptacle, and inner and outer bracts) and harvest time (winter and spring).

Although previous studies (Lattanzio & Van Sumere, 1987; Schütz et al., 2004) have evaluated the phenolic contents and profile of globe artichoke heads, only few genotypes (Alamanni & Cossu, 2003; Curadi, Ceccarelli, Graifenberg, & Picciarelli, 2004), scarcely representative of the genetic background of the subspecies and often purchased at local supermarket (Brat et al., 2006; Racchi et al., 2002) were considered. Therefore, our intention also was to provide useful information about the suitability of some genotypes widely cultivated and new 'seed' propagated cultivars for a specific use (fresh market, food processing, pharmaceutical applications, etc.) and to orientate specifically the heads production also in relation to harvest time.

2. Materials and methods

2.1. Plant material, experimental design and management practices

Nine globe artichoke genotypes (Table 1) differing in their biological and morphological profiles, were selected. Planting of semi-dormant offshoots ("ovoli") or seeds (achenes) was performed manually in August 2006. The plant material was arranged in a randomized block experimental design with four replications, adopting a planting density of 1.0 plant m^{-2} (plant spaced 0.80 m apart, in rows separated apart by 1.25 m). Each experimental unit consisted of 10 plants.

A fertilisation program, commonly used in the area for globe artichoke crops, was adopted [150 kg N, 80 kg phosphorus pentoxide (P_2O_5) and 100 kg of potassium oxide (K_2O) per ha]. Drip irrigation was carried out when accumulated daily evaporation reached 35 mm, 100% of maximum evapotranspiration (ETP). Pest control followed standard commercial practice. No gibberellic acid was applied to the plants during the crop cycle.

2.2. Plant cultivation

Field experiments were conducted during the 2006–2007 growing season at the experimental station of Catania University, on Catania Plain (10 m a.s.l., 37°25′ N, 15°30′ E) (Sicily, southern Italy), in a typic and/or vertic xerochrepts soil (USDA, Soil Taxonomy). The Catania Plain is a typical area for globe artichoke cultivation in the Mediterranean region. The soil characteristics were: clay 45%, silt 28%, sand 27%, organic matter 1.0%, pH 7.2, total nitrogen 0.1%, available P_2O_5 10 ppm and exchangeable K_2O 110 ppm.

The local climate is semiarid-Mediterranean, with mild winters and hot, rainless summers. The mean 30-year maximum monthly temperature ranges between 14.8 °C (January) and 30.6 °C (July) and the minimum temperature between 7.8 °C (January) and 22.3 °C (August).

2.3. Morphological description of the cultivars

Heads were harvested with the floral stem including 2–3 leaves at marketing stage, regardless of size. At this stage, the length of central floral buds was about 2 mm (Mauromicale & Ierna, 2000b). After harvesting, the stem was removed from the flower by cutting 0.5 cm under the receptacle. All heads were weighed and their maximum diameter and length measured. Head length/diameter ratio, an important index of head shape, was calculated. This ratio is a relatively constant trait of each genotype, varying from 0.9 to 1.1 and $\geqslant$1.2 in spherical/subspherical and in long shaped types, respectively. The colour of outer and inner bracts was recorded.

Table 1
Head characteristics of nine genotypes of globe artichoke.

Genotype	Fresh weight (g)	Length/diameter ratio	Colour of outer bracts	Colour of inner bracts	Bracts
Concerto	201 ± 4	1.25 ± 0.08	Deep purple	Yellowish-green	Spineless
Harmony F_1	225 ± 17	1.15 ± 0.01	Green	Yellow	Spineless (but mucronate)
Madrigal F_1	219 ± 37	1.15 ± 0.03	Green	Yellow	Spineless
Romanesco clone C3	144 ± 5	1.05 ± 0.03	Purple with green shades	Yellow-greenish with purple shades	Spineless
Tema 2000	167 ± 3	1.25 ± 0.03	Deep purple	Yellow with purple shades	Spineless (but mucronate)
Tempo	176 ± 66	1.30 ± 0.03	Purple with green shades	Yellow–purple	Spineless
Tondo di Paestum	210 ± 50	1.00 ± 0.02	Purple with light green shades	Yellow–purple	Spineless
Violet de Provence	125 ± 1	1.45 ± 0.10	Purple with green shades	Yellow–purple	Spineless
Violetto di Sicilia	122 ± 22	1.45 ± 0.05	Green with purple shades	Yellow-greenish	Spineless

2.4. Sample preparation

Five globe artichoke heads per replicate and per harvest were washed with tap water and manually separated into outer bracts (10–15 external bracts), inner bracts (remaining bracts) and receptacle. The floral stem and leaves, previously separated from their head, were also cleaned and dried. Each fraction of the inflorescence including floral stem was sliced into small pieces (<0.5 cm) and immediately blended using a domestic food processor on an ice bath for 2 min. Subsequently, the samples were lyophilised and stored at −20 °C until analysis.

2.5. Solvents and reagents

Reagents and solvents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout. Chromabond, 1000 mg, solid phase extraction cartridges were obtained from Macherey & Nagel (Düren, Germany). Apigenin 7-*O*-glucoside and 1,3-di-*O*-caffeoylquinic acid (cynarin) were from Roth (Karlsruhe, Germany); 5-*O*-caffeoylquinic acid (chlorogenic acid), luteolin 7-*O*-glucoside, and narirutin were obtained from Extrasynthése (Lyon, France); caffeic acid was purchased from Fluka (Buchs, Switzerland).

2.6. HPLC analysis

Polyphenol analyses were carried out as described previously by Schütz et al. (2004) using a series 1100 HPLC (Hewlett-Packard, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detection system. The column used was a 150 × 3.0 mm i.d., 4 μm particle size, C_{18} Hydro-Synergi from Phenomenex (Torrance, CA, USA), with a security guard 4 × 3.0 mm i.d., C_{18} ODS column, operated at 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10–18% B (20 min), 18–24% B (10 min), 24–30% B (15 min), 30% B isocratic (20 min), 30–55% B (5 min), 55–100% B (5 min), 100% B isocratic (8 min) and 100–10% B (2 min). Total run time was 90 min. Simultaneous monitoring was performed at 280 nm (narirutin), 320 nm (hydroxycinnamic acids), 330 nm (apigenin derivatives), and 350 nm (luteolin derivatives) at a flow rate of 0.4 mL/min. Spectra were recorded from 200 to 600 nm.

For calibration, appropriate volumes of the standard stock solutions (1000 mg/L) were diluted with methanol, and nine concentration levels (0.5, 2, 5, 10, 20, 40, 100, 200 and 500 mg/L) were analysed.

For quantification, peak areas were correlated with the concentrations according to the calibration curve. Mono-caffeoylquinic acids were calculated as chlorogenic acid, and di-caffeoylquinic acids were quantified as cynarin. Apigenin and luteolin derivatives were calculated as apigenin 7-*O*-glucoside and luteolin 7-*O*-glucoside, respectively. Naringenin 7-*O*-glucoside was calculated as narirutin. All data presented are mean values ± standard deviation of two independent experiments ($n = 2$).

2.7. LC–MS analysis

Analyses were performed with the HPLC system described above coupled on-line with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. LC–MS parameters have been reported in detail by Schütz et al. (2004).

2.8. Sample preparation for HPLC analysis of phenolic compounds

Amounts of 4 g of freeze-dried artichoke samples were homogenised with a Grindomix GM 200 knife mill (Retsch, Haan, Germany) and extracted by stirring with aqueous methanol (60%, v/v) for 1 h at ambient temperature. After filtration through a filter paper, the extracts were evaporated to dryness *in vacuo* at 30 °C, and the residue was dissolved in water. After the solution had been adjusted to pH 7.0, the volume was made up to 25 mL with deionized water.

Purification and fractionation were carried out using C_{18} reversed-phase cartridges, which were activated with 5 mL of methanol and then rinsed with 10 mL of water. Aliquots of 4 mL of the extracts were applied to the sorbent. Hydroxycinnamic acid derivatives were subsequently eluted with 50 mL of 10% aqueous methanol (fraction I). Rinsing with 50 mL of pure methanol eluted neutral compounds (fraction II). The eluates were evaporated to dryness *in vacuo*, and the residues obtained were dissolved in 4 mL (fraction I) and 1 mL (fraction II) of 50% aqueous methanol, respectively.

3. Results and discussion

3.1. Head characteristics of genotypes

The main characteristic of heads, reported in Table 1, showed a high variability amongst genotypes with respect to size, shape (length/diameter, *L*/*W* ratio) and colour of outer and inner bracts. The average fresh weight of heads varied from 122 ('Violetto di Sicilia') to 225 g ('Harmony F_1') and the *L*/*W* ratio, as an index of the shape, ranged between 1.00 and 1.05 (spherical heads) of 'Tondo di Paestum' and 'Romanesco clone C3' and 1.45 (cylindrical/conical heads) of 'Violetto di Sicilia' and 'Violetto di Provenza'. The colour of outer bracts varied from deep green ('Harmony F_1' and 'Madrigal F_1') to deep purple ('Concerto' and 'Tema 2000') with a wide range of different colour hues. The basis colour of inner bracts was yellow ('Harmony F_1' and 'Madrigal F_1'), yellow with greenish ('Concerto' and 'Violetto di Sicilia') or purple shades (for example, 'Tempo' and 'Tondo di Paestum').

3.2. Evaluation of phenolic profile in artichoke heads

Our study provides a qualitative and quantitative evaluation of the phenolic profile of globe artichoke mature heads of nine genotypes. In total, 19 compounds were identified by their UV/Vis and mass spectrometric data (Table 2). Because these were reported in a previous study (Schütz et al., 2004), their data are not presented in this communication. The total phenolic content, calculated as the sum of individual phenolic contents determined by HPLC, depended significantly ($P \leqslant 0.001$) on genotype and site of the inflorescence (data not shown). Amongst the genotypes investigated, 'Violetto di Sicilia', a typical variety of Sicily, is suggested for use in pharmaceutical preparations because of its highest total amount of phenolics in the floral stem, and outer and inner bracts (about 16903, 1565 and 30994 mg kg^{-1} of DM, respectively). In contrast, 'Romanesco clone C3' was found to possess maximum contents in the receptacle (9382 mg kg^{-1} of DM) and thus appears more suitable for fresh consumption, due to its beneficial antioxidant properties. The cultivars 'Harmony F_1' and 'Tondo di Paestum' displayed the lowest contents of total polyphenols in outer and inner bracts (515 and 862 mg kg^{-1} of DM, respectively) and in receptacles and floral stems (1666 and 732 mg kg^{-1} of DM, respectively). These findings suggest that these varieties are more suitable for food processing, due to their presumed lower propensity to browning during handling and storage (Lattanzio et al., 1994).

Table 2
Phenolic contents (mg kg^{-1} of DM) of globe artichoke outer bracts in relation to genotype.

Compound	Genotype								
	Concerto	Harmony F_1	Madrigal F_1	Romanesco clone C3	Tema 2000	Tempo	Tondo di Paestum	Violet de Provence	Violetto di Sicilia
1 CQ ac	nd[a]	nd	nd	nd	nd	nd	nd	nd	nd
3 CQ ac	nd	nd	21.5 ± 1.3	25.1 ± 4.5	nd	nd	nd	nd	nd
5 CQ ac	20.3 ± 1.5	nd	34.8 ± 0.8	32.2 ± 4.1	47.3 ± 1.1	25.1 ± 1.9	73.1 ± 1.6	158.2 ± 14.7	139.4 ± 12.6
4 CQ ac	17.8 ± 0.6	nd	47.6 ± 1.6	30.8 ± 3.2	nd	28.2 ± 1.4	154.3 ± 3.3	nd	114.2 ± 5.8
Caf ac	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,3 di CQ ac	55.6 ± 0.5	58.5 ± 4.6	22.7 ± 1.0	26.4 ± 1.1	14.1 ± 0.9	17.6 ± 0.03	22.4 ± 0.2	37.7 ± 5.0	28.2 ± 0.3
Lut rut	nd	2.9 ± 0.4	9.8 ± 0.5	7.1 ± 0.4	4.5 ± 0.1	5.3 ± 1.5	12.1 ± 3.0	8.0 ± 0.6	34.8 ± 3.4
Lut glc	16.9 ± 1.5	6.0 ± 1.6	25.5 ± 2.2	12.0 ± 0.1	nd	13.4 ± 4.1	7.2 ± 2.1	6.9 ± 0.8	14.4 ± 1.5
Lut glr	nd	nd	13.1 ± 0.6	12.0 ± 1.0	11.1 ± 0.4	12.8 ± 1.1	15.3 ± 1.0	37.8 ± 0.8	74.1 ± 15.0
Nar	20.5 ± 0.1	15.3 ± 0.6	14.6 ± 0.6	19.1 ± 1.1	nd	55.3 ± 4.9	16.3 ± 0.7	25.7 ± 1.4	53.0 ± 5.0
Nar glc	10.2 ± 1.5	16.0 ± 0.1	29.8 ± 1.8	38.8 ± 1.8	nd	8.7 ± 0.6	nd	34.3 ± 0.01	nd
di CQ	nd	nd	nd	nd	nd	nd	nd	nd	nd
3,4 di CQ ac	nd	nd	nd	nd	nd	nd	nd	nd	nd
3,5 di CQ ac	8.0 ± 0.02	nd	11.4 ± 0.3	nd	nd	10.2 ± 0.8	nd	nd	55.0 ± 7.5
1,5 di CQ ac	7.0 ± 0.5	nd	24.2 ± 1.2	11.6 ± 0.6	nd	21.2 ± 0.04	18.1 ± 0.1	nd	53.6 ± 0.7
Api rut	49.3 ± 3.5	18.7 ± 0.4	38.8 ± 0.3	182.7 ± 2.2	25.6 ± 0.5	30.4 ± 5.4	12.5 ± 0.8	23.1 ± 1.3	nd
Api glc	23.8 ± 1.8	111.2 ± 3.9	22.3 ± 1.2	71.2 ± 1.3	11.0 ± 1.0	72.6 ± 21.1	61.6 ± 8.9	41.0 ± 1.0	13.5 ± 1.3
Api glr	850.4 ± 36.2	286.8 ± 17.3	637.2 ± 2.9	270.3 ± 5.2	762.0 ± 7.9	811.3 ± 38.4	567.3 ± 9.3	567.1 ± 40.8	985.0 ± 89.7
4,5 di CQ ac	nd	nd	nd	nd	nd	nd	nd	nd	nd

Note: 1 CQ ac, 1-*O*-caffeoylquinic acid (Rt = 6.7 min); 3 CQ ac, 3-*O*-caffeoylquinic acid (Rt = 8.9 min); 5 CQ ac, 5-*O*-caffeoylquinic acid (or chlorogenic acid) (Rt = 17.5 min); 4 CQ ac, 4-*O*-caffeoylquinic acid (Rt = 18.8 min); Caf ac, caffeic acid (Rt = 20.3 min); 1,3 di CQ ac, 1,3-di-*O*-caffeoylquinic acid (or cynarin) (Rt = 31.7 min); Lut rut, luteolin 7-*O*-rutinoside (Rt = 49.8 min); Lut glc, luteolin 7-*O*-glucoside (Rt = 51.0 min); Lut glr, luteolin 7-*O*-glucuronide (Rt = 52.8 min); Nar, narirutin (Rt = 54.9 min); Nar glc, naringenin 7-*O*-glucoside (Rt = 58.8 min); di CQ, di-caffeoylquinic acids (Rt = 55.7 min); 3,4 di CQ ac, 3,4-di-*O*-caffeoylquinic acid (Rt = 58.4 min); 3,5 di CQ ac, 3,5-di-*O*-caffeoylquinic acid (Rt = 62.5 min); 1,5 di CQ ac, 1,5-di-*O*-caffeoylquinic acid (Rt = 64.1 min); Api rut, apingenin 7-*O*-rutinoside (Rt = 62.3 min); Api glc, apigenin 7-*O*-glucoside (Rt = 66.7 min); Api glr, apigenin 7-*O*-glucuronide (Rt = 71.6 min); 4,5 di CQ ac, 4,5-di-*O*-caffeoylquinic acid (Rt = 73.0 min). Rt, indicated in parenthesis, means retention time.
[a] nd, Not detected.

In accordance with previous findings (Nichiforesco, 1966), the polyphenols were not distributed uniformly in the four head parts. Regardless of the genotype, the inner bracts and receptacles contained the highest total polyphenols concentrations (on average, 4991 and 4809 mg kg^{-1} of DM respectively), which was observed also by Fratianni et al. (2007). The floral stem exhibited high polyphenol contents of about 4200 mg kg^{-1} DM, whereas the outer bracts were characterised by the lowest concentrations (971 mg kg^{-1} DM).

Concerning the individual phenolic compounds, remarkable differences among genotypes and location within the flower were found, as reflected by a significant ($P \leqslant 0.001$) "genotype × location" interaction (Tables 2–5). As shown in Tables 2 and 3, 1-*O*-caffeoylquinic acid was not detected in outer bracts but only in inner bracts of the cultivars 'Madrigal F_1' and 'Violetto di Sicilia'. This compound showed the highest value (Table 5) in 'Madrigal F_1' floral stem (1111 mg kg^{-1} of DM), followed by 'Violetto di Sicilia' inner bracts (648 mg kg^{-1} DM; Table 3). The latter variety was found to be also the best source of 3-*O*-caffeoylquinic acid, with 226, 138 and 284 mg kg^{-1} DM in the inner bracts, receptacle and floral stem, respectively. The contents of 4-*O*-caffeoylquinic acid ranged from approximately 16 (inner bracts of 'Harmony F_1') to 574 mg kg^{-1} DM ('Violetto di Sicilia' floral stem). High contents (525 mg kg^{-1} DM) of this compound were also found in 'Violetto di Sicilia' inner bracts (Table 3). Chlorogenic acid was present mainly in receptacle and stem, even though the highest value was found in 'Violetto di Sicilia' inner bracts (14841 mg kg^{-1} of DM; Table 3). Caffeic acid was present in traces only in one sample ('Romanesco clone C3' receptacle; Table 4). The absence of the latter compound in fresh tissues is in agreement with previous findings (Schütz et al., 2004), and its presence may result from hydrolysis of mono- and di-caffeoylquinic acids during food processing. Cynarin is a phenolic compound typical of globe artichoke and well known for its choleretic activity (Gebhardt, 1997). Its presence was dependent on genotype and location within the inflorescence. The highest concentration was found in floral stem, particularly in 'Tema 2000' (90 mg kg^{-1} DM), 'Madrigal F_1' (85 mg kg^{-1} DM) and 'Violet de Provence' (83 mg kg^{-1} DM). The floral stem was also the best source of 3,4-di-*O*-caffeoylquinic acid, where it ranged from approximately 53 ('Tondo di Paestum') to about 598 mg kg^{-1} DM ('Violetto di Sicilia').

'Violetto di Sicilia' revealed the highest contents of 3,5- and 1,5-di-*O*-caffeoylquinic acids in all head parts analysed. In accordance with Schütz et al. (2004), a higher amount of these di-caffeoylquinic acids could be attributed to isomerization of 1,3-di-*O*-caffeoylquinic acid, whereas 4,5-di-*O*-caffeoylquinic acid was detected only in trace amounts and reached the maximum value in 'Violet de Provence' receptacle (about 57 mg kg^{-1} DM).

Among the flavonoids, apigenin 7-*O*-glucuronide was the predominant compound in receptacle and outer and inner bracts, showing the highest concentrations in the receptacle of 'Romanesco clone C3' (6298 mg kg^{-1} DM) and 'Concerto' (3648 mg kg^{-1} DM). Apigenin 7-*O*-glucoside and -rutinoside were detected mainly in the receptacle, particularly in 'Harmony F_1' (981 and 738 mg kg^{-1} of DM, respectively).

In the floral stem the prevalent flavonoids were luteolin 7-*O*-rutinoside and glucoside (Table 5). The first ranged from 5.7 ('Concerto') to 200 mg kg^{-1} DM ('Madrigal F_1'), whereas the second ranged from 22.6 ('Tondo di Paestum') to 298 mg kg^{-1} DM ('Violetto di Sicilia'). The inner bracts of 'Violetto di Sicilia' (150 mg kg^{-1} DM) and receptacle of 'Concerto' (249 mg kg^{-1} DM) are worth being mentioned for their high contents of luteolin 7-*O*-rutinoside and -glucoside, respectively. Luteolin 7-*O*-glucuronide revealed higher values in the receptacle, ranging from 60 ('Violet de Provence') to 1683 mg kg^{-1}DM ('Romanesco clone C_3'). Narirutin and naringenin 7-*O*-glucoside were minor compounds in all parts of the plant analysed in this study.

The cultivar 'Violetto di Sicilia' was additionally analysed also for its phenolic contents of the leaves attached to the floral stem (data not shown). The leaves are characterised by high total levels of polyphenolics, which justifies their wide use in phyto- pharmaceutical applications (Gebhardt, 1997). Among the hydroxycinnamic acids,

Table 3
Phenolic contents (mg kg^{-1} of DM) of globe artichoke inner bracts in relation to genotype.

Compound	Genotype								
	Concerto	Harmony F_1	Madrigal F_1	Romanesco clone C3	Tema 2000	Tempo	Tondo di Paestum	Violet de Provence	Violetto di Sicilia
1 CQ ac[a]	nd[b]	nd	95.8 ± 3.6	nd	nd	nd	nd	nd	647.7 ± 42.7
3 CQ ac	nd	nd	101.4 ± 5.3	nd	nd	nd	nd	nd	225.6 ± 19.1
5 CQ ac	18.6 ± 0.2	53.2 ± 4.1	370.4 ± 83.0	73.1 ± 7.6	33.8 ± 0.7	161.9 ± 29.8	61.2 ± 2.2	300.8 ± 121.8	14840.8 ± 249.9
4 CQ ac	nd	16.3 ± 1.3	123.2 ± 4.0	64.3 ± 7.0	nd	79.6 ± 2.4	nd	nd	524.7 ± 17.4
Caf ac	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,3 di CQ ac	19.4 ± 0.7	31.6 ± 2.4	60.5 ± 0.2	36.4 ± 3.5	11.8 ± 0.5	40.9 ± 4.9	11.5 ± 0.7	42.1 ± 4.2	73.4 ± 15.2
Lut rut	nd	2.8 ± 0.2	4.7 ± 0.2	4.9 ± 0.1	2.0 ± 0.1	22.9 ± 2.1	5.4 ± 0.3	3.9 ± 0.2	149.5 ± 23.2
Lut glc	6.3 ± 0.5	3.9 ± 0.1	nd	6.4 ± 0.1	nd	2.7 ± 0.3	4.2 ± 0.2	4.4 ± 0.2	24.7 ± 7.2
Lut glr	nd	nd	51.3 ± 6.1	15.0 ± 0.1	9.7 ± 0.2	82.4 ± 21.0	17.3 ± 0.2	41.5 ± 0.7	289.7 ± 16.9
Nar	5.6 ± 2.9	nd	nd	15.4 ± 5.5	nd	16.2 ± 0.7	nd	15.8 ± 2.0	49.2 ± 9.6
Nar glc	nd	nd	nd	nd	nd	38.4 ± 2.5	nd	nd	30.6 ± 4.1
di CQ	nd	nd	nd	nd	nd	nd	nd	nd	210.5 ± 16.3
3,4 di CQ ac	nd	14.9 ± 6.1	nd	nd	nd	36.8 ± 2.7	nd	38.8 ± 20.4	342.0 ± 80.4
3,5 di CQ ac	nd	43.7 ± 19.0	311.9 ± 284.3	26.7 ± 5.6	nd	132.8 ± 22.3	nd	136.6 ± 109.5	3871.9 ± 878.1
1,5 di CQ ac	nd	25.4 ± 4.2	677.5 ± 561.4	26.1 ± 5.2	nd	215.2 ± 48.5	nd	207.5 ± 151.5	7497.7 ± 1170.3
Api rut	31.8 ± 0.2	126.0 ± 3.4	15.0 ± 0.8	55.9 ± 1.1	19.2 ± 0.7	69.8 ± 14.6	35.8 ± 3.1	60.2 ± 2.4	205.9 ± 42.2
Api glc	38.9 ± 5.2	20.1 ± 1.5	65.2 ± 1.6	9.3 ± 0.1	8.5 ± 0.5	23.9 ± 1.8	5.0 ± 0.2	25.8 ± 2.4	281.3 ± 72.6
Api glr	963.7 ± 0.1	524.7 ± 43.7	2041.0 ± 427.4	1228.2 ± 14.9	1188.7 ± 28.3	1184.5 ± 421.8	972.7 ± 51.5	1124.4 ± 56.3	1723.3 ± 19.5
4,5 di CQ ac	nd	nd	nd	nd	nd	nd	nd	nd	nd

[a] See note to Table 2 for the list of abbreviations of the identified compounds with their relative retention time values.
[b] nd, Not detected.

Table 4
Phenolic contents (mg kg^{-1} of DM) of globe artichoke receptacle in relation to genotype.

Compound	Genotype								
	Concerto	Harmony F_1	Madrigal F_1	Romanesco clone C3	Tema 2000	Tempo	Tondo di Paestum	Violet de Provence	Violetto di Sicilia
1 CQ ac[a]	84.0 ± 6.7	nd[b]	72.6 ± 0.4	58.7 ± 0.4	nd	nd	nd	nd	130.6 ± 0.4
3 CQ ac	75.3 ± 4.6	nd	64.2 ± 0.5	61.1 ± 3.1	nd	45.5 ± 2.3	nd	nd	138.4 ± 7.6
5 CQ ac	888.9 ± 104.6	82.5 ± 26.4	268.2 ± 1.8	288.3 ± 22.8	20.3 ± 1.4	104.8 ± 7.2	166.1 ± 12.4	492.0 ± 130.5	4717.0 ± 257.2
4 CQ ac	88.2 ± 8.1	17.4 ± 2.8	69.5 ± 0.6	60.2 ± 5.4	21.2 ± 0.9	46.0 ± 2.8	60.2 ± 2.2	90.1 ± 1.7	157.1 ± 7.2
Caf ac	nd	nd	nd	1.3 ± 0.1	nd	nd	nd	nd	nd
1,3 di CQ ac	nd	nd	28.4 ± 0.5	33.6 ± 0.1	14.4 ± 0.6	26.3 ± 2.9	30.0 ± 0.03	30.4 ± 1.0	45.3 ± 1.8
Lut rut	33.9 ± 12.4	37.8 ± 8.6	12.3 ± 2.1	17.9 ± 1.6	nd	9.8 ± 0.2	18.2 ± 2.0	11.4 ± 0.7	5.0 ± 0.4
Lut glc	248.7 ± 60.3	109.9 ± 21.0	51.3 ± 9.8	45.9 ± 3.9	nd	29.8 ± 2.7	54.2 ± 6.8	13.6 ± 2.5	3.0 ± 0.3
Lut glr	72.0 ± 1.3	352.0 ± 160.4	318.2 ± 8.1	1683.1 ± 35.6	110.2 ± 7.8	187.9 ± 22.7	175.4 ± 3.6	60.2 ± 17.0	144.9 ± 3.9
Nar	66.8 ± 26.6	8.1 ± 1.6	32.9 ± 5.5	36.8 ± 3.3	nd	32.4 ± 1.8	nd	47.6 ± 2.8	nd
Nar glc	106.8 ± 41.1	44.0 ± 6.0	26.3 ± 4.5	16.2 ± 1.1	24.7 ± 1.4	21.9 ± 1.9	nd	20.3 ± 0.1	nd
di CQ	nd	nd	nd	nd	nd	nd	nd	nd	nd
3,4 di CQ ac	51.0 ± 4.9	23.8 ± 11.8	nd	nd	15.4 ± 1.3	22.4 ± 2.1	46.2 ± 2.0	nd	114.9 ± 3.3
3,5 di CQ ac	146.8 ± 14.6	75.2 ± 55.4	121.5 ± 6.4	204.1 ± 41.7	12.0 ± 0.1	58.1 ± 1.9	132.2 ± 1.3	232.8 ± 122.4	1172.3 ± 156.7
1,5 di CQ ac	359.7 ± 52.7	50.4 ± 37.5	181.4 ± 4.1	241.4 ± 48.0	12.5 ± 2.6	52.5 ± 0.2	86.8 ± 6.1	181.5 ± 79.9	1102.0 ± 144.8
Api rut	368.4 ± 130.1	737.8 ± 128.0	332.7 ± 88.3	234.2 ± 22.6	nd	465.6 ± 48.4	nd	273.9 ± 10.6	26.8 ± 0.8
Api glc	946.0 ± 89.2	981.4 ± 137.8	342.1 ± 98.8	100.8 ± 9.1	19.5 ± 3.3	404.7 ± 65.8	184.6 ± 17.2	94.9 ± 4.4	7.9 ± 0.5
Api glr	3647.8 ± 319.6	2423.7 ± 132.7	1793.5 ± 17.9	6298.5 ± 206.3	1776.9 ± 17.8	2134.4 ± 263.7	711.8 ± 21.6	831.6 ± 95.2	511.7 ± 15
4,5 di CQ ac	nd	nd	9.6 ± 0.1	nd	nd	nd	nd	56.8 ± 11.8	nd

[a] See note to Table 2 for the list of abbreviations of the identified compounds with their relative retention time values.
[b] nd, Not detected.

chlorogenic acid was the predominant compound with approximately 25155 mg kg^{-1} DM. Considerable amounts of 3,5- and 1,5-di-*O*-caffeoylquinic acids were also found (1680 and 1391 mg kg^{-1} DM, respectively). In contrast, modest quantities were observed for the mono- and di-caffeoylquinic acid derivatives not mentioned above. 1-,3- and 4-*O*-caffeoylquinic acids reached 189 mg kg^{-1} DM on average; cynarin (1,3-di-*O*-caffeoylquinic acid), together with 3,4- and 4,5-di-*O*-caffeoylquinic acids, were found at levels of 237 mg kg^{-1} DM on average. Caffeic acid was also present in appreciable amounts (about 75 mg kg^{-1} DM).

Unlike in other floral parts of the artichoke plant, in leaves of 'Violetto di Sicilia' narirutin was the most abundant flavonoid (586 mg kg^{-1} DM), whereas apigenin 7-*O*-glucuronide was detected in low amounts (about 82 mg kg^{-1} DM). Considerable levels were found for luteolin 7-*O*-glucuronide (199 mg kg^{-1} DM), apigenin 7-*O*-rutinoside and -glucoside (166 mg kg^{-1} DM on average). Moreover, it is worth mentioning that two compounds, luteolin 7-*O*-rutinoside and -glucoside, were found only in the heads and not in the leaves, whereas also for leaves naringenin 7-*O*-glucoside was confirmed as a minor compound (18 mg kg^{-1} DM).

The variation of phenolic profiles in relation to harvest time (winter and spring) was evaluated in 'Romanesco clone C3' (Table 6). 'Romanesco clone C3' is a typical spring season genotype, but in the Sicilian areas characterised by high precocity, and thus reaching harvest ripeness from January to February until April. Our results demonstrate that the phenolic content increased by

Table 5
Phenolic contents (mg kg^{-1} of DM) of globe artichoke floral stem in relation to genotype.

Compound	Genotype								
	Concerto	Harmony F_1	Madrigal F_1	Romanesco clone C3	Tema 2000	Tempo	Tondo di Paestum	Violet de Provence	Violetto di Sicilia
1 CQ ac[a]	48.9 ± 0.2	53.4 ± 4.9	1111.1 ± 10.0	nd[b]	101.1 ± 7.0	nd	nd	83.2 ± 21.0	146.2 ± 8.5
3 CQ ac	52.3 ± 0.6	57.4 ± 4.4	88.4 ± 4.5	60.5 ± 4.0	134.5 ± 0.1	66.9 ± 2.3	nd	89.6 ± 15.6	284.0 ± 21.0
5 CQ ac	430.5 ± 15.4	566.8 ± 55.7	1132.8 ± 21.7	333.5 ± 5.4	1341.1 ± 5.5	640.6 ± 10.2	157.4 ± 0.2	395.5 ± 37.8	3792.6 ± 773.9
4 CQ ac	54.8 ± 1.8	53.7 ± 11.4	154.7 ± 3.6	69.5 ± 1.3	125.7 ± 3.1	71.4 ± 1.3	67.7 ± 3.4	126.0 ± 10.2	573.6 ± 6.2
Caf ac	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,3 di CQ ac	21.5 ± 0.1	35.8 ± 4.9	84.8 ± 11.2	38.0 ± 1.0	90.2 ± 3.3	41.7 ± 0.1	31.9 ± 1.8	83.4 ± 24.2	71.6 ± 1.5
Lut rut	5.7 ± 0.7	38.5 ± 1.9	200.0 ± 1.6	80.9 ± 2.0	143.8 ± 0.2	68.0 ± 0.8	73.9 ± 3.4	111.0 ± 0.2	198.4 ± 5.0
Lut glc	83.1 ± 15.8	30.6 ± 1.1	175.9 ± 5.5	49.8 ± 1.4	117.2 ± 9.0	48.9 ± 1.4	22.6 ± 0.5	87.3 ± 0.7	297.6 ± 6.0
Lut glr	12.0 ± 0.7	nd	nd	nd	nd	25.4 ± 2.1	nd	nd	nd
Nar	16.5 ± 1.5	29.9 ± 1.7	28.8 ± 3.7	7.7 ± 0.7	11.0 ± 0.9	nd	15.4 ± 0.4	15.3 ± 2.5	54.8 ± 2.6
Nar glc	nd	6.7 ± 0.4	nd	nd	14.4 ± 2.5	14.6 ± 0.3	nd	21.3 ± 2.0	nd
di CQ	nd	nd	247.1 ± 23.0	nd	nd	nd	nd	nd	nd
3,4 di CQ ac	73.9 ± 2.3	121.3 ± 41.6	92.8 ± 8.0	63.6 ± 3.8	484.6 ± 128.5	83.3 ± 4.6	53.4 ± 2.0	239.0 ± 33.6	597.6 ± 203.0
3,5 di CQ ac	27.6 ± 2.7	529.6 ± 8.1	101.4 ± 55.6	239.2 ± 9.4	180.0 ± 5.5	846.5 ± 75.2	148.5 ± 10.0	361.1 ± 54.9	4441.5 ± 861.7
1,5 di CQ ac	214.6 ± 10.9	1391.1 ± 126.1	3145.1 ± 62.7	683.6 ± 10.0	151.8 ± 10.4	798.7 ± 47.3	136.1 ± 11.4	887.0 ± 53.4	6373.3 ± 3470.6
Api rut	546.8 ± 30.2	14.5 ± 1.4	65.9 ± 0.2	10.8 ± 0.1	77.1 ± 7.8	7.8 ± 0.04	nd	26.6 ± 1.2	34.4 ± 0.6
Api glc	nd	9.9 ± 0.8	18.8 ± 0.5	10.8 ± 0.5	48.0 ± 3.2	5.8 ± 0.4	25.1 ± 0.9	11.9 ± 1.1	25.2 ± 1.0
Api glr	14.9 ± 2.1	nd	nd	41.1 ± 2.1	nd	nd	nd	nd	12.0 ± 0.1
4,5 di CQ ac	nd	nd	28.0 ± 0.7	nd	nd	nd	nd	nd	nd

[a] See note to Table 2 for the list of abbreviations of the identified compounds with their relative retention time values.
[b] nd, Not detected.

Table 6
Phenolic contents (mg kg^{-1} of DM) of globe artichoke cv. Romanesco clone C3 in relation to harvest time and plant part.

Compound	Winter harvest				Spring harvest			
	Outer bracts	Inner bracts	Receptacle	Floral stem	Outer bracts	Inner bracts	Receptacle	Floral stem
1 CQ ac[a]	nd[b]	nd	nd	nd	nd	nd	58.7 ± 0.4	nd
3 CQ ac	nd	nd	nd	nd	25.1 ± 4.5	nd	61.1 ± 3.1	60.5 ± 4.0
5 CQ ac	12.3 ± 0.3	26.0 ± 2.5	157.1 ± 7.0	21.5 ± 2.0	32.2 ± 4.1	73.1 ± 7.6	288.3 ± 22.8	333.5 ± 5.4
4 CQ ac	15.4 ± 0.3	18.8 ± 1.0	39.3 ± 3.2	nd	30.8 ± 3.2	64.3 ± 7.0	60.2 ± 5.4	69.5 ± 1.3
Caf ac	nd	nd	nd	nd	nd	nd	1.3 ± 0.1	nd
1,3 di CQ ac	25.4 ± 0.1	15.0 ± 1.3	79.8 ± 0.4	12.2 ± 1.2	26.4 ± 1.1	36.4 ± 3.5	33.6 ± 0.1	38.0 ± 1.0
Lut rut	0.8 ± 0.05	nd	4.7 ± 0.1	13.9 ± 0.8	7.1 ± 0.4	4.9 ± 0.1	17.9 ± 1.6	80.9 ± 2.0
Lut glc	0.6 ± 0.1	nd	2.6 ± 0.2	6.3 ± 0.3	12.0 ± 0.1	6.4 ± 0.1	45.9 ± 3.9	49.8 ± 1.4
Lut glr	nd	nd	857.1 ± 107.6	nd	12.0 ± 1.0	15.0 ± 0.1	1683.1 ± 35.6	nd
Nar	nd	nd	nd	15.2 ± 1.4	19.1 ± 1.1	15.4 ± 5.5	36.8 ± 3.3	7.7 ± 0.7
Nar glc	4.7 ± 0.3	8.8 ± 0.7	nd	4.2 ± 0.6	38.8 ± 1.8	nd	16.2 ± 1.1	nd
di CQ	nd	nd	nd	nd	nd	nd	nd	nd
3,4 di CQ ac	nd	nd	41.1 ± 1.8	nd	nd	nd	nd	63.6 ± 3.8
3,5 di CQ ac	nd	nd	234.2 ± 19.7	nd	nd	26.7 ± 5.6	204.1 ± 41.7	239.2 ± 9.4
1,5 di CQ ac	nd	nd	198.4 ± 33.4	nd	nd	26.1 ± 5.2	241.4 ± 48.0	683.6 ± 10.0
Api rut	27.9 ± 0.8	16.3 ± 1.4	32.1 ± 0.5	6.1 ± 0.4	182.7 ± 2.2	55.9 ± 1.1	234.2 ± 22.6	10.8 ± 0.1
Api glc	19.9 ± 0.1	7.9 ± 1.4	41.9 ± 1.0	2.9 ± 0.2	71.2 ± 1.3	9.3 ± 0.1	100.8 ± 9.1	10.8 ± 0.5
Api glr	576.9 ± 14.0	825.4 ± 17.2	2658.0 ± 227.0	16.9 ± 1.1	270.3 ± 5.2	1228.2 ± 14.9	6298.5 ± 206.3	41.1 ± 2.1
4,5 di CQ ac	nd	nd	nd	nd	nd	nd	nd	nd
Total	684.0	918.2	4346.5	99.1	739.2	1561.6	9382.0	1689.0

[a] See note to Table 2 for the list of abbreviations of the identified compounds with their relative retention time values.
[b] nd, Not detected.

about 16 times from winter to spring harvest, particularly in the floral stem.

As regards the four different parts of the inflorescence, the individual phenolics analysed displayed higher concentrations in spring than in winter harvest. Both in winter and spring harvest, the most abundant component in the inner bracts as well as in the outer bracts and receptacle was apigenin 7-*O*-glucuronide (825 *vs.* 1228 mg kg^{-1} DM), where, regardless of harvest time, this flavonoid contributed to 60% and 64% of the total phenolic content, respectively. The receptacle proved to be the richest head part as revealed by its phenolic profile, particularly in the spring harvest. Several polyphenols, such as 1- and 3-*O*-caffeoylquinic acids, caffeic acid, narirutin and naringenin 7-*O*-glucoside, which were not detected in the winter harvest were present in spring harvest. A similar trend was found in the floral stem, since 1,5- and 3,5-*O*-caffeoylquinic acids absent in winter time, achieved considerable concentrations (684 and 239 mg kg^{-1} of DM, respectively) in spring time.

4. Conclusions

In conclusion, our results demonstrate that the edible part (receptacle) of globe artichoke head represents a good source of bioactive substances. The data also indicate that artichoke by-products (outer bracts and stems) are rich in functional constituents and could thus be an alternative to the traditional application of leaf extracts. Moreover, we have demonstrated that individual phenolic compounds were preferentially accumulated in specific head parts and in specific genotypes. In this view, the head parts, with the exception of the floral stem, represents a promising source of chlorogenic acid and apigenin 7-*O*-glucuronide for industrial extraction. Our data also confirmed the importance of considering the genetic background when determining artichoke polyphenols. In this respect, our intention was to promote genotypes with high polyphenol concentration of the receptacle for fresh consumption and to consider those with higher phenolic content in the waste (leaves, floral stem and bracts) for the recovery of phenolics (Lattanzio et al., 2001). On the other side, genotypes characterised by lower levels of polyphenols would be predestined for minimally processed foods. Our findings, although limited to 'Romanesco clone C3', also indicate the influence of climatic conditions (temperature, light, etc.) on the phenolic profile and thus suggest particular consideration of the harvest time. Further studies investigating the phenolic profile of other artichoke genotypes in relation to harvest time or other stress factors are still required. Finally, screening of local types could be useful to ensure biodiversity and to identify germplasm with a high biosynthetic potential of bioactive substances, for both encouraging a wider cultivation and setting a platform for further breeding programmes aimed to improve the polyphenol content of globe artichoke inflorescence.

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