Australian Journal of Crop Science AJCS
AJCS 7(12):1927-1934 (2013) ISSN:1835-2707

Nutritional and metabolic profiling of the globe artichoke (*Cynara scolymus* L. *'Capuanella'* heads) in province of Caserta, Italy

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Abstract

Globe artichoke (*Cynara scolymus* L.*)* is a typical vegetable of the countries of the Mediterranean basin. It produces edible immature flower bud and his cultivation has a great economic importance in Italy, first world producer. In the recent years, local cultivars have been gradually replaced with the modern ones, mostly due to the increasing demand of early products for the large-scale retail trade. The valorization of local resources, such as specific cultivars useful in productive processes, may contrast this tendency. In this framework, the aim of this research is the nutritional and metabolic characterization of the *Cynara scolymus* L. *'Capuanella'*, a typical artichoke of the *Campania* Region. The nutritional profile (i.e. moisture content, dietary fiber, ash, total proteins, lipids and carbohydrates, total and free amino acids, fatty acid composition, folic acid, C vitamin, total phenolic compounds) of edible immature flower bud of globe artichoke '*Capuanella'* was determined and compared to the Italian artichoke nutritional profile reported in the National Institute of Research on Food and Nutrition (INRAN) tables. On a fresh weight basis, the Capuanella artichoke total protein content was higher in comparison with the INRAN tables (3.08 vs. 2.70g/100 g, respectively), whereas essential amino acids were lower in Capuanella with respect to the INRAN values (826.3 vs. 884 mg/100 g, respectively). Among the fatty acids of Capuanella artichoke, the most abundant were the essential n-6 linoleic (55.20 mg/100 g) and palmitic (34.80 mg/g) acids, representing about 72% of the total fatty acids. Ascorbic acid was 13.70 mg/100 g, while folic acid represents 17% of Recommended Dietary Allowance (65.00 µg/100 g). Phenolic compounds were found to be abundant in Capuanella artichoke (1878.21 mg/100 g); in particular, the chlorogenic acid (425.46 mg/100 g) represents about 23% of total phenolic compounds. Finally, as confirmed by the AFLP analysis, the Capuanella artichoke belongs to the "Romanesco" group.

Keywords: Amino acids, AFLP analysis, Artichoke 'Capuanella', Chlorogenic acid, *Cynara scolymus* L., Nutritional values, Phenolic content, Traditional cultivars.
Abbreviations: INRAN_Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione; RDA_Recommended Dietary Allowance. For the amino acids the standard three-letter code has been used.

Introduction

Globe artichoke (*Cynara scolymus* L., Family Asteraceae) is an herbaceous perennial species with a diploid genetic set of 34 chromosomes (Bianco, 1990). Originated in the Mediterranean region, where it was known by the ancient Greeks and Romans (Sonnante et al., 2007b; Foury, 1967), globe artichoke is generally propagated via 'ovoli' (underground dormant shoots), offshoots or suckers, stumps or dried shoots from the parent plant. Flower heads consist of a receptacle, bearing broad, fleshy, green violet bracts (the edible part), surrounding a central cluster of violet-blue florets. The globe artichoke is cultivated for the 87% in the Countries of the Mediterranean basin, primarily along the coasts, where it finds the ideal conditions of growth and reproduction (FAOSTAT, 2007). Winter low temperatures, in fact, determine the type of artichoke to be cultivated: in the south and near the coasts, precocious cultivar prevails, while in the inside and northern areas, only spring or late cultivar are present. In Italy, globe artichoke is cultivated on a surface of 43.000 ha, supplying a production of about 50.000 t (43% of the world production, followed by Spain and France with about 15%), for about 600 million of Euros (FAOSTAT, 2007). The artichoke crop is known and appreciated for its pharmacological and nutraceutical qualities due to its content in polyphenolic antioxidants and bioactive substances (Ceccarelli et al., 2010; Englisch et al., 2000; Bundy et al., 2004); therefore, it could play an important role in a balanced diet, specially considering the growing consumer's request for high quality food (Feifer, 2011). Moreover, it could be interesting for the food market economy and for its potential pharmaceutical and industrial application Artichoke is also commonly being used in European cooking for its nutraceutical proprieties, and its international demand is increasing. Global artichoke consumption increased by 44% over the last 20 years (from 0.42 pounds per capita to 0.65 pounds per capita), and. this is particularly significant given the growing world population over that period (Lattanzio et al., 2009). During the centuries, many artichoke cultivar and ecotypes were differentiated in Italy (Basnizki and Zohary, 1994; Dellacecca et al., 1976). Four morpho-productive groups have been recognized and located: Spinoso, Violetto, Catanese and Romanesco (Sonnante et al., 2002; Porceddu et al., 1976). In particular, the Sele Plain, where the IGP "Artichoke of Paestum" (also known as Tondo di Paestum') is cultivated, represents Campania's areas mainly interested

to artichoke cultivation. It represents about 70% of the regional amount (Magnifico et al., 2005). Moreover, in Campania there are a lot of other local cultivar of minor economic importance, but of appreciable qualitative potential, that are essentially destined to the local market. Recognized with D.M. 14.06.2002, Italian legislature the artichoke Capuanella belongs to this local cultivar. Currently, although still cultivated by few farmers, it has been replaced slowly but inexorably by more modern early cultivars. The market, in fact, favours the early vegetables, and the late qualities, such as the artichoke Capuanella, have less economic importance. However, the interest in the local cuisine justified by the attempt to recover the crops belonging to popular tradition and the maintenance of biological diversity that representing a key to any future strategy for sustainable agricultural production. In this framework, availability of nutritional data is important both to understand the possible beneficial effects of globe artichoke 'Capuanella'on human health and to support their possible production and marketing. Thus, this research will provide nutritional values of the artichoke 'Capuanella'. The analyses have been carried out on fresh material [(artichoke heads), the edible portion] for the determination of protein, lipids and carbohydrates content. Then, C vitamin, folic acid and amino acid (free and proteic) content, have been determined. Moreover, we have characterized the polyphenolic components in the collected artichoke. An AFLP analysis has been performed to confirm the existing data about the Capuanella artichoke related group

Results

Nutritional values

Nutritional values of the globe artichoke 'Capuanella' heads (hereafter Capuanella artichoke) of Caserta province are reported in Table 1. They have been compared with values reported by INRAN for the related Italian artichoke heads (hereafter INRAN artichoke). Our results showed that nutritional values of Capuanella artichoke were similar to those of INRAN artichoke harvested in Italy. The protein content of Capuanella artichoke (3.08 ± 0.09 g/100 g) was higher while lipids (0.18 ± 0.01 g/100 g) were slightly lower than that of INRAN artichoke. The total dietary fiber was 6.01 ± 0.29, a little more than that of INRAN artichoke. Capuanella artichoke had a significant folic acid content (65.00 ± 4.15 μg/100 g; Table 2). The content of vitamin B-complex was quite interesting and similar to that reported for other Italian artichoke (68 μg/100 g) (VV. IEO, 2011). In the Capuanella artichoke, the folic acid content represented the 17% of RDA (Recommended Dietary Allowance) uptake for adults. Ascorbic acid content (13.70 ± 0.68 mg/100 g) was similar to the INRAN artichoke (12 mg/100 g).

Amino acid content

The free and total (free plus protein-derived) amino acids are reported in Table 3 and compared with those available for INRAN artichoke. Two essential amino acids, leucine (Leu) and lysine (Lys) were the most abundant (187.89 ± 4.84 and 155.95 ± 5.30 g/100 g, respectively) when determined as total amino acids; among the non-essential amino acids, Asx (aspartic acid plus asparagine) and Glx (glutamic acid plus glutamine) were the most representative (617.71 ± 25.51 and 328.37 ± 13.89 g/100 g, respectively).

Sulphur amino acids (methionine and cysteine, 25.37 ± 0.73 and 6.03 ± 1.96 g/100 g, respectively) resulted 2.5-fold lower than those of the INRAN artichoke (methionine and cysteine, 43 and 38 g/100 g, respectively). Total free amino acid content of Capuanella artichoke was 0.342 mg/100 g. Among the essential amino acids, the most abundant free amino acids were asparagine (Asp) and glutammine (Glu) (305.23 ± 12.91 and 10.272 ± 0.275 mg/100 g, respectively). Free proline (Pro), glutamine (Gln) and arginine (Arg) (6.014 ± 0.248, 3.121 ± 0.080 and 2.955 ± 0.109 mg/100 g, respectively) were the main non-essential amino acids. This analysis also evidenced the presence of non-protein amino acids whose total amount was 3.294 mg/100 g; in particular, o-phospho-L-serine (Phser) and taurine (Taur) were the most abundant (1.133 ± 0.029 and 0.423 ± 0.009 mg/100 g, respectively) whereas the remaining non-protein amino acids did not exceed 1.738 mg/100 g of product.

Fatty acid content

The fatty acid composition showed that saturated fatty acid content was lower (43.27 mg/100 g; 34.65%) than that of unsaturated ones (81.60 mg/100 g; 65.35%; Table 4). The most abundant saturated fatty acid was palmitic acid (C16:0), representing the 27.87% of total fatty acids. Linoleic acid (n-6; C18:2), α-linolenic acid (n-3; C18:3), and γ-linolenic acid (n-6; C18:3) were the main unsaturated fatty acid components (Table 4). In particular, linoleic acid (n-6; 18:2) was the main component of the unsaturated fatty acid fraction (55.20 ± 2.70 mg/100 g), representing 44.20% of total fatty acids. The amounts of unsaturated fatty acids was about 2 fold more than saturated fatty acids; this finding confirms the beneficial use of artichokes for human heart (Siri-Tarino et al., 2010). No data was found in INRAN database.

Total phenolic content

The content of total phenols evidenced a relative abundance of phenolic phytochemicals in Capuanella artichoke; in fact, all the tested samples yielded average value of 1878.21 ± 105.18 mg/100 g, expressed as gallic acid equivalents (GAE; see Methods). Generally, the quantitative amount of phenols in different artichoke heads depends on their germplasm, as well as on their harvest time and on growth conditions (Lattanzio et al., 2005; Ceccarelli et al., 2010; Guida et al., 2013). Furthermore, the chlorogenic acid was identified and quantified by using RP-HPLC analysis and a commercial standard (Table 2). The chlorogenic acid content was 625.46 ± 23.25 mg/100 g. The result was in agreement with that of the globe artichoke 'Romanesco' from the central region of Italy (300 to 530 mg/100 g) (Di Venere et al., 2007).

Genetic study using AFLP markers

To confirm that the globe artichoke 'Capuanella' belongs to the group of artichokes 'Romanesco' the Amplified Fragment Length Polymorphism (AFLP) analysis was performed (Vos et al., 1995). Thirty cultivated plants of globe artichoke 'Capuanella'(in Capua and Maddaloni towns, named Cap-C and Cap-M clones, respectively, Fig. 1) and five genomic DNA obtained from four globe artichoke cultivars belonging to *C. scolyum* 'Violetto' di Toscana, *C. scolymus* 'Catanese', *C. scolymus* 'Spinoso Sardo' and two *C. scolymus* 'Romanesco' were used. A total of 236 fragments ranging in size from 62 to 578 bp was scored using five different primer combinations selected whereas the number of total polymorphic fragments was 139 (Table 5 and Fig. 2). Genetic similarity matrix among the 35 globe artichoke cultivars were calculated based on the similarity

Table. 1. Nutritional value of artichoke 'Capuanella' heads from Caserta district. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on fresh weight basis.

Artichoke heads	Moisture (%)	Dietary fiber (g/100g)	Ash (g/100g)	Proteins (g/100g)	Lipids (g/100g)	Carbohydrates (%)
C. scolymus 'Capuanella'	79.60	6.01±0.29	0.96±0.33	3.08±0.09	0.18 ±0.01	10.17
C. scolymus INRAN	91.30	5.50	n.r.	2.70	0.20	n.r.

n.r., not reported

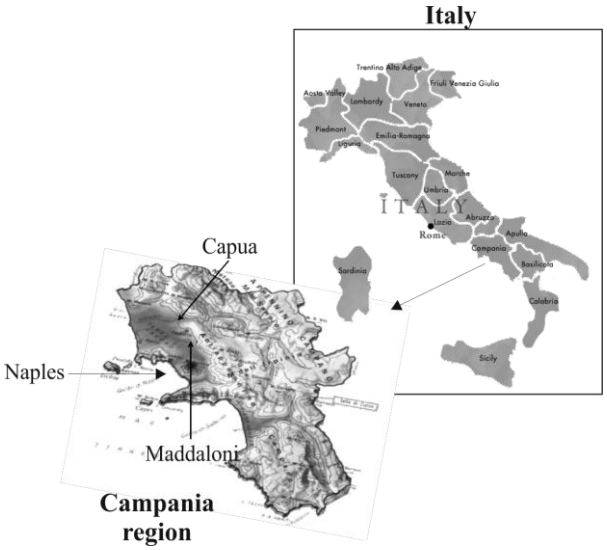

Fig 1. The bold arrows indicate Capua and Maddaloni artichoke 'Capuanella' sampling area.

Table 2. Vitamins and other metabolites content of artichoke 'Capuanella'heads from Caserta district (100 g). Values are means (±SD) of triplicate analyses (n = 3) and are expressed on fresh weight basis.

Component	Content
Folic acid	65.00±4.15 µg
Vitamin C	13.70 ±0.68 mg
Phenolic compounds	1878.21±105.18 mg
Chlorogenic acid	425.46±0.23 mg

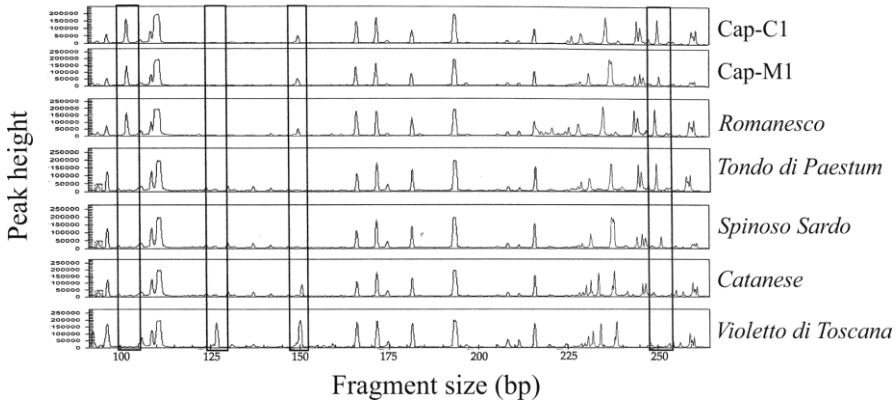

Fig 2. AFLP electropherogram profiles of two artichoke Capuanella samples (Cap-C1 and Cap-M1) compared to reference samples generated using number 3 primer combination (see Table 5).

coefficient of Jaccard (Jaccard, 1908). Similarity values among individual samples ranged from 0.624 to 1.000. In particular, Cap-M15 genotype of globe artichoke 'Capuanella' and *C. scolyum* 'Violetto di Toscana' were the most distantly related (0.624). The dendrogram using UPGMA shows relationships among 35 globe artichoke cultivars analysed (Fig. 3). The analysis shows the presence of two main groups, hereafter named Group I and Group II). Group I contains all globe artichoke 'Capuanella' genotypes (Cap-C1-15 and Cap-M1-15) and *C. scolymus* 'Romanesco'. Group II contains *C. scolymus* 'Violetto di Toscana' *C. scolymus* 'Catanese' and *C. scolymus* cv 'Spinoso Sardo'. Bootstrap value for Group I and Group II supported 92 % of the clustering. Thus, the genetic relationship obtained by using AFLP analysis confirms an association between the globe artichoke 'Capuanella' and *C. scolymus* 'Romanesco', as previously reported (Reg Campania, 2005). *C. scolymus* cv. Tondo di Paestum is clustered separately.

Table 3. Total and free amino acid composition of artichoke 'Capuanella' heads. Protein amino acids are reported in bold. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on fresh weight basis.

	Artichoke 'Capuanella'		Artichoke INRAN
Amino acid[a]	Total amino acids [b] (mg/100g)	Free amino acid content [c] (mg/100g)	Total amino acids [b] (mg/100g)
		Essential amino acids	
His*	37.29±1.39	0.060±0.001	39
Ile	95.01±3.97	0.140±0.005	121
Leu	187.89±4.84	0.180±0.006	196
Lys	155.95±5.30	0.092±0.002	81
Met	25.37±0.73	0.193±0.008	43
Phe	109.92±4.29	0.274±0.010	115
Thr	98.24±3.83	0.937±0.004	122
Trp	n.r.		17
Val	116.64±3.68	0.511±0.022	150
		Non-essential amino acids	
AAAA	-	0.154±0.005	-
Ala	114.71±3.90	1.126±0.046	144
Arg	148.07±4.59	2.955±0.109	169
Asn	-	305.23±12.91	-
Asp		3.121±0.080	
Asx	617.71±25.51		413
□-ala		0.027±0.001	-
Cys	6.03±1.96	-	38
Ethan	-	0.473±0.015	-
GABA	-	1.040±0.029	-
Gln	-	3.994±0.168	-
Glu		10.272±0.275	
Glx	328.37±13.89		300
Gly	111.03±3.37	0.266±0.001	127
Orn	-	0.044±0.001	-
Phser	-	1.133±0.029	-
Pro	517.85±13.93	6.014±0.248	136
Ser	109.49±4.50	2.891±0.098	139
Taur	-	0.423±0.009	-
Tyr	52.29±2.04	0.167±0.007	69
Total (g)	2.832	0.342	2.419

[a] free and protein amino acids. Three letter code has been used: AAAA: L-α-aminoadipic acid; Ala: L-alanine; Asn: L-asparagine; Asx, L-asparagine + L-aspartic acid; Arg: L-arginine; Asp: L-aspartic acid; Cys: L-half cystine; Ethan: ethanolamine; GABA: γ-amino-n-butyric acid; Gln: L-glutamine; Glu: L-glutamic acid; Glx, L-glutamine + L-glutamic acid; Gly: glycine; His: L-Histidine; Ile: L-isoleucine; Leu: L-leucine; Lys: L-lysine; Met: L-methionine; Orn: L-ornithine; Phe: L- phenylalanine; Phser: o-phospho-L-serine; Pro: L-proline; Ser: L-serine; Taur: taurine; Thr: L-threonine; Trp: tryptophan; Tyr: L-tyrosine; Val: L-valine; β-ala: β-alanine. n.d., not determined; (-), not detected; n.r, not determined.

[b] free plus protein-derived amino acids (see text). [c] free amino acids content for *C. scolymus* L. INRAN and other cultivars is not previously published.

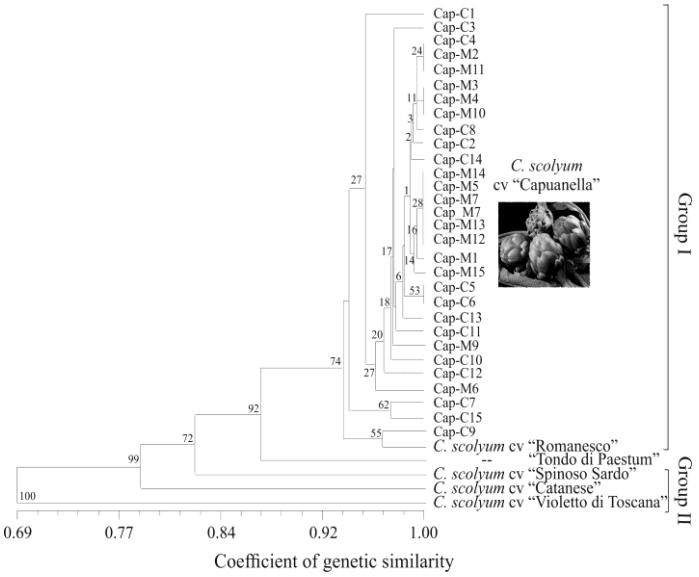

Fig 3. Dendrogram for artichoke cultivars generated by UPGMA analysis based on AFLP data using Jaccard coefficient of genetic similarity estimates. The analysis shows high similarity between the globe artichoke 'Capuanella' and the Romanesco group artichoke (group I cluster).

Discussion

Globe artichoke 'Capuanella' is a medium size dark green variety, ready to harvest between the end of March and the beginning of April. Its heads ("*capolini"* in Italian) are commonly being used in the local diets between the cities of Naples and Caserta. In this study, we have evaluated the nutritional values of this cultivar. The results suggest that globe artichoke 'Capuanella' is an important source of many essential nutrients. The content of total proteins (3.08 g/100 g) was similar to that previously reported for both INRAN artichoke (2.70 g/100 g in INRAN database) and other artichoke varieties (Ceccarelli et al., 2010; Alamanni et al., 2001; VV. IEO, 2011). The total amino acid content (2.83 g/100 g) was higher than that of INRAN artichoke (2.42 g/100 g). Asparagine was the main free amino acid in line with its role as *N*-mobilizer in plant species (Brouquisse et al., 1998; Lea et al., 2007). The lipid content was similar to that reported for INRAN artichoke. A qualitative analysis of fatty acid composition showed that, among the unsaturated fatty acids, the Capuanella artichoke amount of the essential linoleic acid (n-6; C18:2; 44.20% of total fatty acids), α-linolenic acid (n-3; C18:3), and γ-linolenic acid (n-6; C18:3) were the most abundant. Total phenol content (425.46 ± 0.23) was similar to that reported for different artichoke cultivars (Lombardo et al., 2009) whereas there were no data in the INRAN database. In particular, the chlorogenic acid content determined because of its important ability to regulate the glucose levels in blood through the inhibition of the glucose-6-phosphatase (Karthikesan et al., 2010). It resulted to be similar to that of the globe artichoke 'Romanesco'. The presence of phenol phytochemicals justifies the therapeutic properties observed and addresses the extension of the artichoke cultivation as novel source of natural nutraceutical for human nutrition (Lattanzio et al., 2009). The antioxidant properties of Capuanella artichoke resulted also from the presence of ascorbic acid. Moreover, a good amount of folic acid detected and resulted to be about 17% of the RDA uptake for adults. Folic acid plays an important role in the nucleic acid and protein synthesis and in erythropoiesis. It is particularly important for tissues that undergo proliferation and differentiation. For these reasons, it is essential in preventing birth defects (Wilton et al., 2010). Finally, the AFLP analysis confirmed that the Capuanella artichokes is strictly related to the Romanesco group, as previously reported (Reg Campania, 2005).

Materials and methods

Chemicals and reagents

nor-leucine (*nor*-Leu), Folin-Ciocalteau reagent, gallic acid, hydrogen peroxide, solvents (hexane, acetonitrile and chloroform, analytical grade) and salts were obtained from Sigma-Aldrich S.r.l. (Milan, Italy). Fatty acid methyl esters (Supelco^{TM} 37 component FAME mix) were obtained from Supelco (Park-Bellefonte, PA, USA). Chemicals and solvents for the Kjeldahl method and for the automated amino acid analysis were provided by Carlo Erba Reagents (Milan, Italy) and Biochrom (Cambridge, U.K.), respectively.

Plant material and sampling

Globe Artichokes (*Cynara scolymus* L.) 'Capuanella' were grown under typical conditions of soil, irrigation, and illumination in Capua and Maddaloni (Fig. 1). Thirty mature artichokes were manually and randomly picked across the field in the spring of 2012 (see "Genetic study using AFLP markers" paragraph). They were placed in polyethylene bags and transported at 4 °C to the laboratory. Samples were then selected to eliminate damaged and poor quality units and to obtain uniformity. Sample preparation: artichoke heads [medium size, dark green colour, closely gathered leaves and an almost spherical (about 5 cm in diameter, 6 cm in height) and scaled head] were washed under cold running water. Petals were pulled off and stems were cu to one cm or less. The top quarter of each artichoke was cut and sharp tips were snipped off. Samples were, and then powdered with porcelain mortar and pestle (i.d. 260 mm, 640 mL) in liquid N_2 until particles of homogeneous size were obtained. Finally, samples were then transferred into 50 mL polypropylene bottles (Falcon, Becton Drive, Franklin Lakes, NJ, USA), covered with silver paper and stored at -80°C until use. Aliquots from each of the frozen powered samples were used for all the following analyses (except moisture content).

Dietary fiber, ash and moisture content

Dietary fiber was determined as previously reported (Lee et al., 1992). Ash content was determined according to the AOAC Official Method 942.05 (AOAC, 1997). For moisture level, sample (2.0 g) was dried in a thermostatically controlled oven, at uniform temperature of 550°C and 105 °C, respectively, until the constant weight was obtained.

Macronutrient content

Total protein content: Nitrogen concentration was obtained by using the Kjeldahl method [AOAC 920.54 (AOAC, 1997)] and total protein content was estimated using a nitrogen factor of 6.25. Samples (about 1.0 g) were analyzed using a Mineral Six digester and an Auto Disteam semi-automatic distilling unit (International PBI). *Total lipid content:* Aliquots (5.0 g) of frozen globe artichoke 'Capuanella'sample were lyophilized using an FTS-System Flex-Dry™ instrument (SP Scientific, Stone Ridge, NY USA). The materials obtained were extracted by the Soxhlet apparatus with $CHCl_3$ for 4 h and then dried using a rotary evaporator to obtain the lipid extracts, which were weighed giving the amount of extracted fat. *Total carbohydrate content:* Total carbohydrates were obtained by subtracting (moisture + crude protein + crude fat + ash + dietary fiber) from 100 (Sabah El-Kheir and Murwa, 2010).

Amino acid composition

For the analysis of free amino acid composition, aliquots of about 200 mg of globe artichoke 'Capuanella' were precipitated first with ethanol and then with sulfosalicylic acid in the presence of *nor*-Leu as internal standard as previously reported (Di Maro et al., 2011). For the analysis of total (free and protein) amino acids, about 10 mg of globe artichoke 'Capuanella' were hydrolysed with HCl containing 0.02% phenol and *nor*-Leu as internal standard as previously reported (Iriti et al., 2009).
Aliquots of both samples were directly analysed on a Biochrom 20 amino acid analyser (Biochrom, Cambridge, U.K.), equipped with a post-column ninhydrin derivatization system, adapting the procedure previously reported (Stein and Moore, 1963).

Gas chromatographic analysis of fatty acid methyl esters

Fatty acid methyl esters content were performed by a gas

Table 4. Fatty acid composition before saponification from total lipids of artichoke 'Capuanella' heads. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on fresh weight basis.

Fatty acid	C:D (lipid numbers)	Fatty acid content (mg/100g)	Types of omega fatty acids
Saturated			
Lauric	C12:0	2.41±0.07	-
Myristic	C14:0	2.46±0.09	-
Palmitic	C16:0	34.80±1.76	-
Stearic	C18:0	3.60±0.17	-
Unsaturated			
Oleic	C18:1 c	6.00±0.25	n-9
Linoleic	C18:2	55.20±2.70	n-6
α-linolenic	C18:3	20.40±0.78	n-3
and	+		and
γ-linolenic	C18:3		n-6
Total fatty acids (mg)		124.87	

Fatty acid composition for C. scolymus L. INRAN is not previously published.

Table 5. Primer combination sequences and amplified fragments obtained used for genetic characterization of globe artichoke 'Capuanella' by using AFRP analysis.

N.	Primer combinations	Sequence (5'-3')	Band number	Monomorphic band numbers	Polimorphic band numbers	Polymorphic bands (%)	Range (bp)
1	EcoR I/AGG	GACTGCGTACCAATTCACG	83	27	56	67.5	70-578
	Mse I/CTT	GACGATGAGTCCTGAGTAACTT					
2	EcoR I/ACT	GACTGCGTACCAATTCACT	29	5	24	82.7	71-447
	Mse I/CTC	GACGATGAGTCCTGAGTAACTC					
3	EcoR I/ACG	GACTGCGTACCAATTCACG	51	30	21	41.2	94-452
	Mse I/CAT	GACGATGAGTCCTGAGTAACAT					
4	EcoR I/ACG	GACTGCGTACCAATTCACG	45	26	19	42.2	62-465
	Mse I/CTC	GACGATGAGTCCTGAGTAACTC					
5	EcoR I/ACT	GACTGCGTACCAATTCACT	28	9	19	67.8	83-354
	Mse I/CAA	GACGATGAGTCCTGAGTAACAA					
Total			236	97	139	58.9	

chromatographic analysis as previously reported (Ferrara et al., 2011), using $CHCl_3$ crude extract (1 mg; see "Macronutrient content" section).

Folic acid and ascorbic acid content

Folic acid and ascorbic acid content were detected using the ELISA kit 010060 (EuroKit Srl, Gorizia, Italy) and the HA 3850 kit (HANNA Instruments, Ronchi di Villafranca, Padova, Italy), respectively, as previously reported (Ferrara et al., 2011). This titration method only determines ascorbic acid and not dehydroascrobic acid (DHA).

Determination of total phenol content

In order to define total phenol content, aliquots (5 g) of frozen globe artichoke 'Capuanella' sample were lyophilized extracted with ethanol and then defatted with *n*-hexane as previously reported (Romani et al., 2006). The obtained ethanolic extracts were used for the determination of total phenol content which was determined according to the Folin-Ciocalteau procedure (Kähkönen et al., 1999). Total phenol content was expressed as gallic acid equivalents (GAE, mg/g of fresh weight globe artichoke 'Capuanella').

Determination of chlorogenic acid

The chromatographic analysis of the polyphenols recovered in the ethanolic extract of fresh was performed by RP-HPLC (Romani et al., 2006), using a Waters 1525 (Waters S.p.A., Manchester, UK) instrument equipped with a UV 277 detector (Dosi et al., 2012; Di Maro et al., 2009). A Beckman Coulter C18 column (250 x 4.6 mm; 5 µm particle size) at room temperature was used for separation. The mobile phase included HPLC-grade water containing 0.1% (v/v) formic acid, pH 3.2 (solvent A) and acetonitrile (solvent B). The polyphenols elution was obtained using a four-step linear solvent gradient system starting from 0% up to 100% of solvent B over 40 min at a flow rate of 0.8 mL/min. The percentage of B reached 11% from 0 to 5 min, then 20% from 10 to 15 min, and finally 100% from 25 to 33 min. Three hold-steps were present in the gradient (11% B for 5 min, 20% B for 10 min and 100% B for 7 min). The detection wavelengths were set at 254 and 330 nm. A standard calibration curve was obtained with chlorogenic acid (Sigma-Aldrich, St. Louis, MO, USA) by injecting 5, 10 and 25 µL of a standard solution 1 mg/mL. The chlorogenic acid content of each sample was evaluated comparing the peak areas to the corresponding values of the external calibration curve. The results were expressed as chlorogenic acid concentration (in mg/100g of fresh weight globe artichoke 'Capuanella').

AFLP analysis

Plant materials: Thirty cultivated plants of globe artichoke 'Capuanella' (in Capua and Maddaloni towns, named Cap-C

and Cap-M clones, respectively) and five genomic DNA obtained from four globe artichoke cultivars belonging to Violetto di Toscana (CynViTP1 clone), Catanese (CynCatP1 clone), Spinoso Sardo (CynSPS clone) and Romanesco (CynRomP1 and TopMarina9 clones) were used in this study (Sonnante et al., 2004; Sonnante et al., 2007a). The clone TopMarina9 is artichoke "Tondo di Paestum" that belongs to the Romanesco (RegCE_465/04, 2004). *DNA extraction*: Genomic DNA was extracted from globe artichoke 'Capuanella' small leaves as previously report (Doyle and Doyle, 1987). DNA concentration was measured using a Cary 1E spectrophotometer (Varian Inc., Australia) and quality was checked by electrophoresis in 0.8% agarose gel in TBE buffer. *AFLP analysis:* Briefly, the AFLP procedure was performed with minor modifications according to the protocol previously reported (Vos et al., 1995) with some modifications (Sonnante et al., 2004). Approximately 500 ng DNA were simultaneously digested with *Eco*R I and *Mse* I and ligated with specific adapter of same enzymes at 37°C for 3 h. This was done to generate template DNA for pre- and amplification. Pre-amplification was carried out in a final volume of 20 µL with complementary adapter primers (*Eco*R I+and *Mse* I+C). PCR was carried out in a PTC-100 thermal cycler (MJ Research, Waltman, MA, USA) programmed for 72°C for 2 min followed by 20 cycles of 94°C for 20 sec, 56°C for 30 sec, and 72°C for 2 min, and a final incubation of 72°C for 2 min and 60°C for 30 min. For selective amplification, five primer pair combinations were employed in this study: *Eco*R I/AGG-*Mse* I/CTT, *Eco*R I/ACT-*Mse* I/CTC, *Eco*R I/ACG-*Mse* I/CAT, *Eco*R I/ACG-*Mse* I/CTC and *Eco*R I/ACT-*Mse* I/CAA. Each *Eco*R I primer was fluorescently tagged for visualization with Proligo kit (Sigma-Aldrich, Milan, Italia). The PCR amplification was carried out with an initial denaturation step of 94°C for 2 min, followed by the first cycle of 94°C for 20 sec, 66°C for 30 sec, 72°C for 2 min and one degree decrease in annealing temperature in each of the next nine cycles. This was followed by 25 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 3 min. The reactions were incubated at 60°C for 30 min before electrophoresis. PCR products were diluted with sample loading solution (Beckman-Coulter, Fullerton, CA, USA) and diluted reaction products were added to sample loading solution (Beckman-Coulter). DNA size standard kit-600 (Beckman-Coulter) was added to each sample. The samples were electrophoresed and detected using a Beckman-Coulter CEQ 8800 Genetic Analysis System (Beckman-Coulter). The Frag-4 module of CEQ was used to size all the fragments using internal DNA size standard.

Data analysis

All AFLP fragments were scored as binary data (1, peak present; 0, peak absent) along with their sizes. The binary scores were manually compared with the electropherograms to re-confirm presence or absence of peaks. The NTSYS-pc software (Rohlf, 2005) was used to calculate the genetic distance by using Jaccard similarity index (Jaccard, 1908) between all samples. A cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as previously reported (Sneath and Sokal, 1973). Bootstrap values (based on 1000 re-samplings) were used to estimate the reliability of the clustering pattern.

Statistical analysis

Analyses were repeated three times for each sample; mean and standard deviation (SD) of experimental values are reported. Data analysis was carried out with Excel Office 2003 (Microsoft, USA).

Conclusions

C. scolymus 'Capuanella' is cultivated and consumed in the Southern Italian regions (between Naples and Caserta cities). The interest in this vegetable is recently grown, but no nutritional information is available for this vegetable. In the present work, the nutritional values of globe artichoke 'Capuanella', have been defined and compared to data reported in INRAN database for Italian artichokes. The results confirm previous findings on the dry matter, proteins and lipids contents. In addition, they provide information on free amino acids, fatty acids, vitamin C, fibers and total phenols contents. The characterization of globe artichoke 'Capuanella' is very important toimprove its cultivation and future marketing.

Acknowledgments

This study was supported by funds from the Second University of Naples and from AgriGeNet project (Campania region).

References

Alamanni MC, Cossu M, Mura M (2001) Valutazione della composizione chimica e valore nutrizionale del *Cynara scolymus* var Spinoso sardo. Riv Sci Aliment 4: 345-351.

AOAC (1997) Official Methods of Analysis. 17 Edn., Arlington, VA.

Basnizki J, Zohary D (1994) Breeding of seed planted artichoke. Plant Breed Rev 12: 253-269

Bianco VV (1990) Carciofo (*Cynara scolymus* L.), in Italian. Orticoltura. Patron Editore, Bologna

Brouquisse R, Gaudillère JP, Raymond P (1998) Induction of a carbon-starvation-related proteolysis in whole maize plants submitted to light/dark cycles and to extended darkness. Plant Physiol 117: 1281-1291.

Bundy R, Walker AF, Middleton RW, Marakis G, Booth JC (2004) Artichoke leaf extract reduces symptoms of irritable bowel syndrome and improves quality of life in otherwise healthy volunteers suffering from concomitant dyspepsia: a subset analysis. J Altern Complement Med 10: 667-669.

Ceccarelli N, Curadi M, Picciarelli P, Martelloni L, Sbrana C, Giovannetti M (2010) Globe artichoke as a functional food. Mediterr J Nutr Metab 3: 197-201.

Dellacecca VV, Magnifico V, Marzi V, Porceddu E, Scarascia Mugnozza GT Contributo alla conoscenza delle varietà di carciofo coltivate nel mondo. In: Procede. 2nd Int Congress on Artichoke, Bari, 1976. Minerva Med.

Di Maro A, Chambery A, Carafa V, Costantini S, Colonna G, Parente A (2009) Structural characterization and comparative modeling of PD-Ls 1-3, type 1 ribosome-inactivating proteins from summer leaves of Phytolacca dioica L.. Biochimie 91: 352-363.

Di Maro A, Dosi R, Ferrara L, Rocco M, Sepe J, Ferrari G, Parente A (2011) Free amino acid profile of *Malus domestica* Borkh cv. Annurca from the Campania Region and other Italian vegetables. Aust J Crop Sci 5: 154-161.

Di Venere D, Linsalata V, Pieralice M, Cardinali A, Sergio L, Crinò P (2007) Biochemical characterization of clones from two "Romanesco" artichoke landraces. Acta Hortic 730: 443-448.

Dosi R, Carusone A, Chambery A, Severino V, Parente A, Di Maro A (2012) Rapid primary structure determination of myoglobins by a complementary approach based on mass spectrometry and Edman degradation Food Chem 133: 1646-1652.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytocheml Bull 19: 11-15.

Englisch W, Beckers C, Unkauf M, Ruepp M, Zinserling V (2000) Efficacy of Artichoke dry extract in patients with hyperlipoproteinemia. Arzneimittelforschung 50: 260-265.

FAOSTAT (2007) http://www.faostat.fao.org.

Feifer J (2011) A Matter of Taste. Men's Health 26: 140.

Ferrara L, Dosi R, Di Maro A, Guida V, Cefarelli G, Pacifico S, Mastellone C, Fiorentino A, Rosati A, Parente A (2011) Nutritional values, metabolic profile and radical scavenging capacities of Wild Asparagus (*A. acutifolius* L.). J Food Compos Anal 24: 326-333.

Foury C (1967) Étude de la biologie florale de l'artichaut (*Cynara scolymus* L.); application à la sélection, 1ère partie: données sur la biologie florale. Ann Amélior Plantes 17: 357-373.

Guida V, Ferrari G, Pataro G, Chambery A, Di Maro A, Parente A (2013) The effects of ohmic and conventional blanching on the nutritional, bioactive compounds and quality parameters of artichoke heads. LWT-Food Sci Technol 53: 569-579

Iriti M, Di Maro A, Bernasconi S, Burlini N, Simonetti P, Picchi V, Panigada C, Gerosa G, Parente A, Faoro F (2009) Nutritional traits of bean (*Phaseolus vulgaris*) seeds from plants chronically exposed to ozone pollution. J Agr Food Chem 57: 201-208.

Jaccard P (1908) Nouvelles rescherches sur la distribution floreale. Bull Soc Vaudoise Sci Nat 44: 223-270.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen MJ (1999) Antioxidant activity of plant extracts containing phenolic compounds. J Agr Food Chem 47: 3954-3962.

Karthikesan K, Pari L, Menon VP (2010) Combined treatment of tetrahydrocurcumin and chlorogenic acid exerts potential antihyperglycemic effect on streptozotocin-nicotinamide-induced diabetic rats. Gen Physiol Biophys 29: 23-30.

Lattanzio V, Cicco N, Linsalata V (2005) Antioxidant activities of artichoke phenolics. Acta Hortic 681: 421-428.

Lattanzio V, Kroon PA, Linsalata V, Cardinali A (2009) Globe artichoke: A functional food and source of nutraceutical ingredients. J Funct Foods 1: 131–144.

Lea PJ, Sodek L, Parry MAJ, Shewry PR, Halford NG (2007) Asparagine in plants. Ann Appl Biol 150: 1-26.

Lee S, Proxy L, De Vries J (1992) Determination of total, soluble and insoluble fiber in foods-enzymatic-gravimetric method, MES–TRIS buffer. Collaborative study. J AOAC Int 75: 395-416.

Lombardo S, Pandino G, Mauro R, Mauromicale G (2009) Variation of phenolic content in globe artichoke in relation to biological, technical and environmental factors. Ital J Agron 4: 181-189.

Magnifico V, Pepe R, Rosati A, Palombo AD, Santonicola L, Donato R (2005) The globe artichocke Bianco di Pertosa. L'informatore Agrario 61: 61-64.

Porceddu E, Dellacecca V, Bianco VV Classificazione numerica di cultivar di carciofo. In: Atti 2° Cong Int sul Carciofo, Bari, 1976. Minerva Med, 1105-1119.

Reg Campania (2005) Il carciofo Capuanella. http://www.agricoltura.regione.campania.it/tipici/tradizionali/carciofo-capuanella.htm.

RegCE_465/04 (2004) Carciofo di Paestum (IGP).

Rohlf FJ (2005) NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Ver. 2.2. Exeter software, New York.

Romani A, Pinelli P, Cantini C, Cimato A, Heimler D (2006) Characterization of Violetto di Toscana a typical Italian variety of artichoke (*Cynara scolymus* L.). Food Chem 95: 221-225.

Sabah El-Kheir MK, Murwa AM (2010) Chemical composition, minerals, protein fractionation, and anti-nutrition factors in leaf of hargel plant (*Solenostemma argel*). Eur J Scient Res 43: 430-434.

Siri-Tarino PW, Sun Q, Hu FB, Krauss RM (2010) Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. Curr Atheroscler Rep 12: 384-390.

Sneath PHA, Sokal N (1973) Numerical Taxonomy. Freeman, W. H., San Francisco.

Sonnante G, Carluccio AV, Vilatersana R, D. P (2007a) On the origin of artichoke and cardoon from the Cynara gene pool as revealed by rDNA sequence variation. Genet Resour Crop Evol 54: 483-495.

Sonnante G, De Paolis A, Lattanzio V, Perrino P (2002) Genetic variation in wild and cultivated artichoke revealed by RAPD markers. Genet Resour Crop Evol 49: 247-252.

Sonnante G, De Paolis A, Pignone D (2004) Relationships among artichoke cultivars and some related wild taxa based on AFLP markers. Plant Genetic Resources: Characterization and Utilization 1: 125-133.

Sonnante G, Pignone D, Hammer K (2007b) The domestication of artichoke and cardoon: from Roman times to the genomic age. Ann Bot 100: 1095-1100.

Stein WH, Moore S (1963) Chomatographic determination of amino acids by the use of automatic recording equipment. Method Enzymol 6: 819-831.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, et al. (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407-4414.

VV. IEO (2011) Food Composition Database for Epidemiological Studies in Italy European Institute of Oncology. http://www.ieo.it/bda2008/uk/index.aspx.

Wilton DC, Foureur MJ (2010) A survey of folic acid use in primigravid women. Women Birth 2: 67-73.