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Biochemical characterization of artichoke (*Cynara cardunculus* var. *scolymus* L.) spring genotypes from Marche and Abruzzo regions (Central Italy)

A. Galieni, [1] [(*)] F. Stagnari [2], M. Pisante [2], C. Platani [1], N. Ficcadenti [1]

[1] *Centro di Ricerca Orticoltura e Florovivaismo, CREA, Via Salaria, 1, 63077 Monsampolo del Tronto (AP), Italy.*

[2] *Facoltà di Bioscienze e Tecnologie Agroalimentari e Ambientali, Università degli Studi di Teramo, Via R. Balzarini, 1, 64100 Teramo, Italy.*

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[(*)] Corresponding author:
angelica.galieni@crea.gov.it

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Ten artichoke genotypes from Marche and Abruzzo Regions [Ascolano (As), Castorano (Cs), Clone Monsampolo, Jesino (Je), Mazzaferrata (Mz), Montelupone A, Montelupone B, Urbisaglia1 (Ub_1), Urbisaglia2 and Violetto Tardivo di Pesaro] were characterized for their quality traits and peculiar end-use attitudes, in comparison with the reference Romanesco Clone C3 (Cl_C3). Total polyphenols content (TPC), total flavonoid content (TFC) and antiradical activity were assessed in the receptacle and external bracts of both main and first order capitula. Cl_C3 showed high TPC and TFC values in the receptacle of the main flower heads (7.4 mg gallic acid equivalents, GAE, g^{-1} dry weight, DW, and 3.6 mg rutin equivalents, RUE, g^{-1} DW, respectively), confirming its attitude for fresh consumption. Je and Ub_1 showed great and stable (among main and first order capitula) head quality, highlighting their potential for breeding programs to enhance the content of functional compounds. Conversely, Mz and As could be appreciable for processing or pharmaceutical applications, being characterized by great TPC (external bracts, first order capitula: 2.8 and 2.7 mg GAE g^{-1} DW, respectively) and TFC (external bracts, first order capitula: 2.1 and 2.7 mg GAE g^{-1} DW, respectively) values in the waste parts. High correlations between TPC and TFC with antiradical activity were also observed. Our results suggest the possibility to promote the utilization in genetic breeding programs of the autochthonous artichoke populations, according to their peculiar characteristics, including also their biochemical composition.

1. Introduction

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] belongs to the family of *Asteraceae* (*Compositae*) and it is an herbaceous perennial crop mainly cultivated in the Mediterranean Basin (about 65% of world production) followed by Americas and China (Sihem *et al*., 2015, Lombardo *et al.*, 2017). In Italy it plays an important role in the agro-food

chain with over 43.8 Kha and approximately 366 Kt of floral heads produced (FAO, 2016).

The Italian gene pool of globe artichoke includes hundreds of varieties and ecotypes, grouped into four main types i.e. "Catanesi", "Romaneschi", "Spinosi" and "Violetti". According to the harvesting period, they are classified as early or late distinct clonal varietal groups, with the former having a typical autumn-winter cycle in the southern Regions, while late including spring genotypes mainly grown in central Regions (Ciancolini *et al*., 2013 a, b). The varietal constitution of artichoke is restricted to clonal selection carried out within local populations propagated by agamic way which represent a patrimony of agrobiodiversity and a biological, cultural and economic heritage (Ficcadenti *et al*., 2013). However, several cases of homonymy and synonymy (different varieties are called with the same name in the first case, while the same variety comes call with different names in the second) as well as unsatisfactory, uniformity and identity of accessions occur (Ficcadenti *et al*., 2013). Traditional agricultural and food production must be safeguarded to avoid processes of globalization and homologation and, consequently, identification, collection, characterization and conservation of agrobiodiversity as well as development of genetic improvement strategies, particularly linked to nutritional and organoleptic traits, have been undertaken in these years (Mauromicale and Ierna, 2000; Ficcadenti *et al*., 2010; Ciancolini *et al*., 2012).

Recently, a renewed and growing interest for artichoke cultivation has been observed worldwide mainly due to its potential uses as functional food: its large immature inflorescences, called capitula or heads, represent a rich source of bioactive compounds - including polyphenols with a strong antiradical activity (Schütz *et al*., 2004) - inulin, fibres and minerals (Lattanzio *et al*., 2009; Lombardo *et al*., 2010; Pandino *et al*., 2011). Furthermore, the utilization of by-products of artichoke processing (i.e. external bracts) involves animal feedstuff (Megías *et al*., 2002) or extraction of functional molecules (Larossa *et al*., 2002). It emerges that the types and amount of bioactive substances and their activity (i.e. polyphenols content and the related antioxidant activity) could be used to characterize and select specific genotypes.

Nowadays, only few studies have investigated on polyphenols content, discriminating among the different head parts of artichoke (see for example Fratianni *et al*., 2007; Lombardo *et al*., 2010; Soumaya *et al*. 2013; Sihem *et al*., 2015); besides, the simultaneous determinations of total polyphenols and flavonoids content with radical scavenging capability, have not been considered at all. Consequently, in the present work we aimed at investigating such important biological properties in eleven spring accessions of artichoke collected in Central Italy (Marche and Abruzzo regions). The primary objectives were to obtain a preliminary: (i) genotype's characterization of the selected Central-Italy artichoke accessions from a biochemical point of view; (ii) evaluation of the suitability of the selected artichoke genotypes for fresh consumption or by-products production.

2. Materials and Methods

Plant material, management practices and head sampling

The study was carried out in 2014 at the experimental field of the Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics (CREA-OF), located in Monsampolo del Tronto (AP) (latitude 42°52'59.1" N, longitude 13°48'01.9" E), in the coastal area of the Marche Region (Central Italy) a typical area for globe artichoke cultivation.

Ten artichoke accessions from Marche and Abruzzo Regions, named as "Clone Monsampolo" (Cl_MSP), "Ascolano" (As), "Castorano" (Cs), "Jesino" (Je), "Mazzaferrata" (Mz), "Montelupone A" (ML_A), "Montelupone B" (ML_B), "Urbisaglia1" (Ub_1), "Urbisaglia2" (Ub_2) and "Violetto Tardivo di Pesaro" (VT_PS), were collected on the base of their peculiar sensory features and were compared with the reference genotype "Romanesco Clone C3" (Cl_C3), characterized by high market standards of the flower heads (purple with green shades, round shape, regular size and thick consistency). The selected globe artichoke genotypes differ for their biological and morphological profiles, as briefly synthetized in Table 1.

Plant material (shoots, named "carducci") was transplanted in August 2011 in rows spaced 1.00 m apart with row spacing of 1.20 m; each plot (artichoke genotype) consisted of thirty plants. The fertilization program, typical of the area, consisted in: 150 kg ha^{-1} of N, 80 kg ha^{-1} of phosphorus pentoxide (P_2O_5) and 100 kg ha^{-1} of potassium oxide (K_2O), respectively. The experimental field was kept weed-free by mechanical weed control and no pest control was needed.

Table 1 - Head characteristics of the eleven selected genotypes of globe artichoke

Genotype	Acronym	Colour of outer bracts	Colour of inner bracts	Bracts
"Clone C3"	Cl_C3	Green with Purple shades	Yellow	Spineless
"Clone Monsampolo"	Cl_MSP	Green	Yellowish-green	Spineless
"Ascolano"	As	Purple with green shades	Yellow-greenish with purple shades	Spineless
"Castorano"	Cs	Purple with light green shades	Yellow-purple	Spineless (but mucronate)
"Jesino"	Je	Purple with Green shades	Yellow purple	Spineless
"Mazzaferrata"	Mz	Purple with Green shades	Yellow-greenish	Spineless
"Montelupone A"	ML_A	Purple with Green shades	Yellow-purple	Spineless
"Montelupone B"	ML_B	Purple with Green shades	Yellow-purple	Spineless (but mucronate)
"Urbisaglia 1"	Ub_1	Purple	Yellow-purple	Spineless
"Urbisaglia 2"	Ub_2	Purple	Yellow-purple	Spineless
"Violetto tardivo PS"	VT_PS	Purple	Yellow-purple	Spine

At the marketing stage, six capitula per artichoke genotype were harvested, without floral stem, in two subsequently times (10th April and 10th May, considered as early and mid-spring), allowing to compare both main (first sampling data) and first order (second sampling data) capitula. Each flower head was separated into 'external bracts (~15 bracts)' (waste part) and 'receptacle' (edible fraction), freeze-dried, homogenized and stored at -20°C until biochemical characterization.

Chemical analysis

The extraction of polyphenols and flavonoids were carried out as described by Gouveia and Castilho (2012 a).

The Folin-Ciocalteu reagent method was used to evaluate the total polyphenols content (TPC) of the external bracts and receptacle following the method of Gouveia and Castilho (2011). Plant extracts were dissolved in methanol (10 mg mL^{-1}); aliquots of 50 µL were added to 1.25 mL of Folin-Ciocalteu (dilution, 1:10) and 1.0 mL of a 7.5% Na_2CO_3 solution. Solutions were maintained at room temperature for 30 min and the TPC was determined at 765 nm using a Beckman DU640B spectrophotometer (Beckman Coulter, Brea, California, USA). Gallic acid standard solutions were used to calibrate the method, so results were expressed as mg gallic acid equivalents (GAE) per g^{-1} dry weight (DW).

Total flavonoids content (TFC) was calculated following the procedure described by Gouveia and Castilho (2012 a) and estimated as rutin equivalents (RUE), i.e. expressed as mg RUE g^{-1} DW. Methanolic solutions (500 µL of sample solution) of the plant extracts (2.5 mg mL^{-1}) were mixed with 1.5 mL of methanol, 2.8 mL of water, 100 µL of potassium acetate (1 M) and 100 µL of aluminium chloride (10% in methanol). The absorbance of reaction mixture was read after 30 min at room temperature and at 415 nm using a Beckman DU640B spectrophotometer.

The radical scavenging activity of the extracts was determined using the stable radicals: (i) 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) - TEAC/ABTS assay (ABTS) (Re *et al*., 1999), modified as described by Gouveia and Castilho (2012 a); and (ii) 2,2-diphenyl-1-picrylhydrazyl - DPPH assay (Gouveia and Castilho, 2012 b). In each assay, Trolox was employed as reference standard and results were expressed as µmol Trolox equivalent (TE) g^{-1} DW.

Reagents and solvents were purchased from Sigma Chemicals Co. (St. Louis, MO). All reagents were of analytical grade.

Statistical analysis

In order to test (*F*-test) the effect of genotype on all the investigated variables, a one-way analysis of variance (ANOVA) was performed. The experiment was conducted following a complete randomized design and each sampled capitula represented a single repetition. When significant differences were detected, the means were compared based on the standard error of the difference (SED) between means, with significance being assigned using the least significant difference (LSD) value at the 5% ($p<0.05$) level of significance. Before the ANOVA, the data were analyzed to test for normality and homoschedasticity assumptions, through graphical methods.

To interpret and summarize the association between treatments (artichoke genotypes: Cl_C3, Cl_MSP, As, Cs, Je, Mz, ML_A, ML_B, Ub_1, Ub_2 and VT_PS) and variables (TPC, TFC, ABTS, DPPH) the principal component analysis (PCA) was applied. The PCA was performed separately for main and first order capitula; each principal component (PC) was

calculated as a linear combination of the standardized original variables by using the eigenvectors of the correlation matrix. The results were visually explored in a two-dimensional PCA correlation biplot: standardized PC1 and PC2 scores were plotted as symbols, while the correlations between PCs and standardized variables (factor loadings) were plotted as vectors.

Statistical analyses were performed with the R software (R Core Team, 2017).

3. Results and Discussion

The TPC and TFC in the receptacle and external bracts of main and first order capitula of the eleven artichoke genotypes, are reported in Table 2. TPC ranged from 1.5 to 9.2 mg GAE g^{-1} DW, while TFC ranged from 1.8 to 4.1 mg RUE g^{-1} DW, matching with the literature data or, in some circumstances, resulting slightly higher (Lombardo *et al*., 2010; Pandino *et al*., 2011; Gouveia and Castilho, 2012 a; Pandino *et al*., 2012 a; Sihem *et al*., 2015; Dabbou *et al*., 2017; Marques *et al*., 2017; Petropoulos *et al*., 2017). Both traits were significantly (p<0.05) influenced by genotype: differences are related to both head part (receptacle or external bracts) and their location on plant architecture (main or first order capitula) (Table 2). Clear trends were observed: Je gave the highest TPC content (5.1 mg GAE g^{-1} DW, averaged over head parts and harvest time), followed by Ub_1, Ub_2 and VT_PS (5.0, 5.0 and 4.3 mg GAE g^{-1} DW on average, respectively), while Cl_MSP resulted as one of the worst genotypes in terms of polyphenols concentra-

Table 2 - Total polyphenols content [TPC, mg gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and total flavonoids content [TFC, mg rutin equivalents (RUE) g^{-1} DW] in the receptacle and in the external bracts of different artichoke genotypes

Genotype§	Main capitula		First order capitula	
	TPC (mg GAE g^{-1} DW)	TFC (mg RUE g^{-1} DW)	TPC (mg GAE g^{-1} DW)	TFC (mg RUE g^{-1} DW)
Receptacle				
Cl_C3	7.4 ± 0.11	3.6 ± 0.23	3.8 ± 0.57	2.5 ± 0.38
Cl_MSP	3.6 ± 0.28	1.9 ± 0.16	3.1 ± 0.09	1.9 ± 0.12
As	5.7 ± 0.42	2.4 ± 0.21	3.9 ± 0.55	2.2 ± 0.19
Cs	6.3 ± 0.91	2.5 ± 0.30	2.6 ± 0.03	1.9 ± 0.08
Je	8.2 ± 1.09	3.3 ± 0.44	5.4 ± 0.47	3.0 ± 0.34
Mz	4.9 ± 0.73	2.8 ± 0.17	6.8 ± 1.35	3.5 ± 0.61
ML_A	5.8 ± 1.34	4.1 ± 1.01	2.1 ± 0.19	1.8 ± 0.19
ML_B	4.3 ± 0.22	2.3 ± 0.31	4.0 ± 0.64	3.1 ± 0.66
Ub_1	8.0 ± 0.58	3.4 ± 0.36	4.7 ± 0.66	2.9 ± 0.69
Ub_2	9.2 ± 1.55	3.9 ± 0.74	4.4 ± 0.15	2.6 ± 0.15
VT_PS	6.8 ± 0.45	3.5 ± 0.42	4.2 ± 0.45	3.2 ± 0.45
F-test	**	*	**	*
SED	1.2	0.7	0.9	0.6
External bracts				
Cl_C3	3.2 ± 0.12	2.6 ± 0.07	2.1 ± 0.06	2.3 ± 0.18
Cl_MSP	2.2 ± 0.15	2.0 ± 0.00	1.7 ± 0.11	2.0 ± 0.10
As	3.0 ± 0.19	2.4 ± 0.08	2.7 ± 0.42	2.7 ± 0.30
Cs	2.9 ± 0.28	2.1 ± 0.17	2.1 ± 0.04	2.6 ± 0.20
Je	3.9 ± 0.53	2.5 ± 0.18	3.0 ± 0.46	2.9 ± 0.56
Mz	2.4 ± 0.14	2.5 ± 0.08	2.8 ± 0.16	2.1 ± 0.04
ML_A	2.1 ± 0.25	2.4 ± 0.25	1.5 ± 0.08	2.3 ± 0.02
ML_B	2.1 ± 0.16	2.0 ± 0.07	2.2 ± 0.28	2.3 ± 0.28
Ub_1	4.8 ± 0.30	2.9 ± 0.17	2.4 ± 0.03	2.3 ± 0.22
Ub_2	4.1 ± 0.61	2.7 ± 0.25	2.2 ± 0.19	2.3 ± 0.02
VT_PS	4.0 ± 0.11	3.2 ± 0.05	2.2 ± 0.05	3.0 ± 0.21
F-test	**	**	**	NS
SED	0.4	0.2	0.3	

Data refer to both main and first order capitula (two different harvest times, at early and mid-spring 2014). Means ± standard errors of n=6 independent replicates are reported.

* p<0.05; ** p<0.01; *** p<0.001; NS = not significant.

SED, standard error of differences between means.

§ The list of the used acronomys is reported in Table 1.

tion in artichoke heads (on average 2.7 mg GAE g^{-1} DW) together with ML_A and ML_B (on average 2.9 and 3.2 mg GAE g^{-1} DW, respectively) (Table 2). These results were quite confirmed by TFC data (Table 2), indicating those genotypes' suitable for fresh consumption rather than food processing. Lower antioxidant compounds (i.e. polyphenols) is, indeed, considered a qualitative trait required by industry, thanks to the scarce propensity to enzymatic browning phenomena after cutting and storage operations (Lattanzio *et al*., 1994; Lombardo *et al*., 2010). The reference genotype (Cl_C3) confirmed its high value for fresh consumption, registering higher TPC and TFC, only in the combination early-spring harvest (main capitula)/receptacle (Table 2), mostly appreciated by consumers and with the highest commercial value.

As previously observed (Fratianni *et al*., 2007; Lombardo *et al*., 2010; Pandino *et al*., 2011; Pandino *et al*., 2012 b; Pandino *et al*., 2013 a; Sihem *et al*., 2015), polyphenols were not uniformly distributed in the different floral head parts (Fig. 1): regardless of the harvest time, higher TPC values were observed in the receptacle (5.2 mg GAE g^{-1} DW, averaged over genotypes) while lower in the external bracts (2.7 mg GAE g^{-1} DW, averaged over genotypes). Besides, no differences emerged in terms of TFC (2.5 *vs*. 2.8 mg GAE g^{-1} DW in external bracts and receptacle respectively, averaged over genotypes). The different amount of antioxidant compounds in the various head parts is of interest to identify genotypes rich in these molecules in the by-products (external bracts) and hence interesting for the industrial processes (i.e. animal feedstuff, fiber production, recovery of functional ingredients) (Femenia *et al*., 1998; Larossa *et al*., 2002; Megías *et al*., 2002; Lattanzio *et al*., 2009). Nonetheless, we observed some differences in terms of relative TPC among artichoke accessions, with particular regards in terms of relative TFC (Fig. 1): the genotypes Cl_MSP, As and Cs were characterized by the highest relative TFC in the waste products (Fig. 1), suggesting useful utilization for the by-products processes, with external bracts representing a potential innovative source for flavonoid extraction.

Lastly, TPC values lowered in both receptacle and external bracts shifting from main to first order capitula (Fig. 2A); this trend was confirmed by all the accessions with the exception of Mz (Fig. 2A), which gave the highest TPC values in the first order flower heads (Table 2), so maintaining a high content of functional compounds during the growing cycle. A similar behavior was recorded for some of the selected artichoke accessions in terms of TFC (see for example Cl_MSP, As and ML_B) (Fig. 2B). This was probably attributable to the environmental conditions recorded during the harvest season. Despite the solar radiation levels show the stronger effect on polyphenols accumulation in the artichoke' receptacle (Pandino *et al*., 2013 b), in our study the lower temperatures observed in April (-10% on average with respect to May - considering the mean air temperatures recorded during the first 10 days of each month) could have affected TPC and TFC, as previously observed in other crops (Klimov *et al.*, 2008; Hykkerud *et al*., 2018).

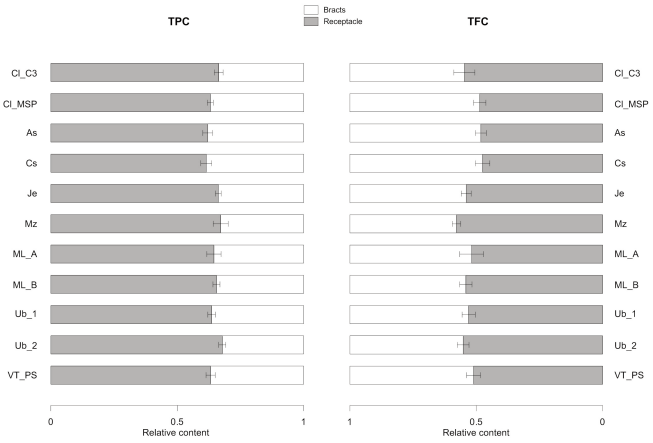

Fig. 1 - Relative proportion (as percentage) of total polyphenols content [TPC, gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and total flavonoids content [TFC, mg rutin equivalents (RUE) g^{-1} DW] in the receptacle and external bracts averaged over both main and first order capitula of different globe artichoke genotypes (see Table 1 for the list of acronyms). Data represent means ± standard errors, n=12 independent replicates.

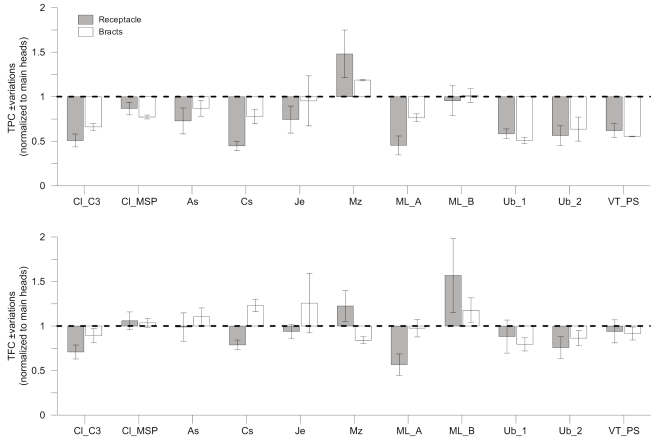

Fig. 2 - Variations normalized to main capitula values (dashed line) of (A) total polyphenols content [TPC, mg gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and (B) total flavonoids content [TFC, mg rutin equivalents (RUE) g^{-1} DW] in the receptacle and external bracts of different globe artichoke genotypes (see Table 1 for the list of acronyms). Data represent means ± standard errors, n=6 independent replicates.

Radical scavenging activity (ABTS and DPPH assays) registered differences among thesis similar to those observed for TPC and TFC data (Table 3). ABTS values ranged from 21.2 to 146.8 µmol TE g^{-1} DW and DPPH ranged from 12.8 to 204.3 µmol TE g^{-1} DW, showing the same order of activity previously found in other antioxidant capacity assays on artichoke (Gouveia and Castilho, 2012 b; Rouphael *et al.*, 2017). The highest activity was concentrated in the receptacle (85.7 and 103.8 µmol TE g^{-1} DW for ABTS and DPPH, respectively *vs.* 41.9 and 38.3 µmol TE g^{-1} DW for ABTS and DPPH, respectively in the external bracts) regardless of genotype and harvesting time (Table 3) (Sihem *et al*., 2015). Also for these biochemical traits, Je, Ub_1 and Ub_2 ranged at the first positions while Cl_MSP as the genotype with the lowest ABTS and DPPH values (Table 3). Moreover, we found significant (p<0.001) linear relationships between these variables, confirming previous results (Alghazeer *et al*., 2012; Lombardo *et al*., 2013). Follows the higher measured Pearson's correlation coefficients: TPC *vs.* ABTS, r=0.84; TPC *vs.* DPPH, r=0.91; TFC *vs.* ABTS, r=0.76; TFC *vs.* DPPH, r=0.81. Indeed, phenolic compounds are known to have the ability to block the chain reaction of reactive oxygen and nitrogen species through different pathways involving (i) direct reaction with free radicals, (ii) sequester metal ions able to spread the chain reaction, (iii) synergic action with other antioxidants (Khasawneh *et al*., 2014).

The relationships between TPC, TFC, ABTS and DPPH, classified based on the analysed capitula parts

Table 3 - Radical scavenging activity (µmol trolox equivalents (TE) g^{-1} DW) obtained from two different assays: trolox equivalent antioxidant capacity with 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the receptacle and in the bracts of different artichoke genotypes

Genotype§	Main capitula		First order capitula	
	ABTS (µmol TE g^{-1} DW)	DPPH (µmol TE g^{-1} DW)	ABTS (µmol TE g^{-1} DW)	DPPH (µmol TE g^{-1} DW)
Receptacle				
Cl_C3	146.8 ± 6.91	165.5 ± 7.75	61.1 ± 14.29	61.3 ± 26.77
Cl_MSP	71.3 ± 8.85	47.3 ± 12.82	42.3 ± 7.39	31.7 ± 2.95
As	87.2 ± 14.10	109.9 ± 14.97	73.4 ± 7.23	78.2 ± 14.87
Cs	97.4 ± 28.90	127.2 ± 31.56	34.4 ± 5.83	33.5 ± 7.20
Je	119.5 ± 19.14	185.6 ± 34.58	76.0 ± 12.66	56.9 ± 11.52
Mz	86.6 ± 19.63	108.4 ± 28.80	95.0 ± 12.93	178.2 ± 42.51
ML_A	104.2 ± 32.54	144.8 ± 57.25	31.4 ± 3.08	26.2 ± 9.39
ML_B	68.2 ± 8.77	80.0 ± 12.90	82.3 ± 19.76	47.2 ± 12.23
Ub_1	140.3 ± 16.58	171.5 ± 19.28	67.5 ± 12.89	111.0 ± 26.09
Ub_2	128.2 ± 27.06	204.3 ± 49.60	95.2 ± 5.22	91.7 ± 9.76
VT_PS	107.4 ± 7.40	138.2 ± 12.15	70.2 ± 16.40	84.3 ± 22.80
F-test	NS	*	**	**
SED		42.4	16.7	28.5
External bracts				
Cl_C3	57.3 ± 5.56	42.9 ± 4.49	37.6 ± 8.53	18.2 ± 2.77
Cl_MSP	27.6 ± 0.77	12.8 ± 3.78	21.2 ± 3.39	16.1 ± 2.57
As	45.5 ± 4.93	37.0 ± 4.82	40.8 ± 3.63	42.6 ± 10.25
Cs	34.1 ± 8.87	38.1 ± 6.55	32.1 ± 2.04	24.2 ± 3.38
Je	64.7 ± 12.85	80.5 ± 13.40	54.4 ± 10.96	53.7 ± 16.83
Mz	27.5 ± 2.79	21.7 ± 5.90	48.1 ± 5.17	34.0 ± 8.95
ML_A	31.9 ± 6.81	36.6 ± 1.91	27.4 ± 3.67	17.9 ± 5.71
ML_B	27.5 ± 1.78	27.2 ± 5.04	31.5 ± 10.47	20.9 ± 7.52
Ub_1	80.9 ± 8.72	93.9 ± 10.09	35.8 ± 4.19	38.4 ± 2.52
Ub_2	58.8 ± 16.82	71.4 ±20.49	39.1 ± 6.21	19.1 ± 6.07
VT_PS	57.6 ± 2.45	59.4 ± 4.90	39.2 ± 6.46	36.7 ± 3.85
F-test	**	**	NS	**
SED	11.5	12.7		10.8

Data refer to both main and first order capitula (two different harvest times, at early and mid-spring 2014). Means ± standard errors of n=6 independent replicates are reported.

* p<0.05; ** p<0.01; *** p<0.001; NS = not significant.

SED, standard error of differences between means.

§ The list of the used acronomys is reported in Table 1.

(i.e. receptacle - TPC_Rec, TFC_Rec, ABTS_Rec and DPPH_Rec - and external bracts - TPC_Bra, TFC_Bra, ABTS_Bra and DPPH_Bra), and the eleven artichoke accessions, were summarized by PCA. The results, on the basis of harvest time, are graphically displayed in two correlation bi-plots (Figs. 3A and 3B, respectively); in Table 4 are reported the factor loadings, the eigenvalues and the percentage of the explained variance.

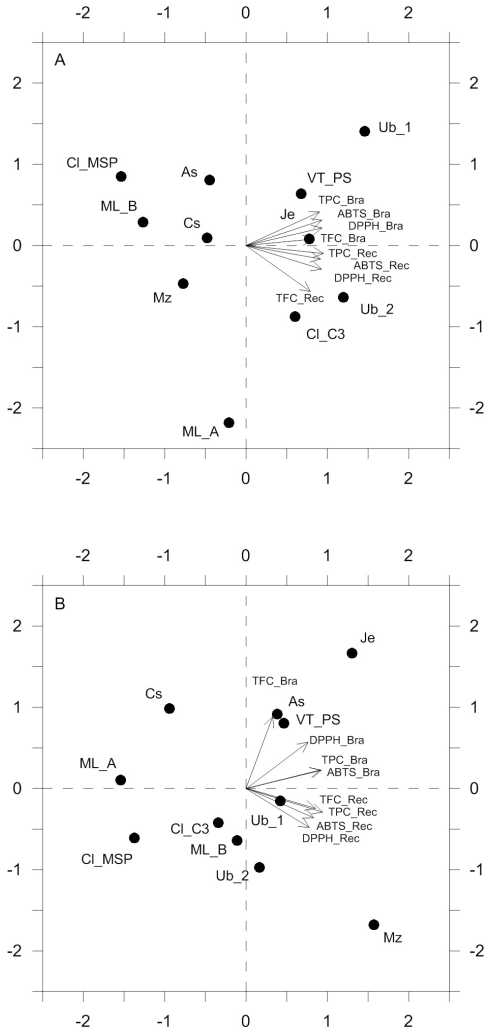

Fig. 3 - Two dimensional principal component analysis (PCA) correlation bi-plot [main capitula/first harvest time (A) and first order capitula/second harvest time (B)]: symbols show the standardized scores on PC1 (x-axis) and PC2 (y-axis) for the eleven artichoke genotypes (see Table 1 for the list of acronyms); vectors coordinates represent the correlations between standardized variables [total polyphenols content in receptacle and external bracts (TPC_Rec and TPC_Bra, respectively), total flavonoids content in receptacle and external bracts (TFC_Rec and TFC_Bra, respectively), radical scavenging activity obtained from two different assays in the receptacle and external bracts (ABTS_Rec and ABTS_Bra, respectively; DPPH_Rec and DPPH_Bra, respectively)] and PCs.

In early-spring harvesting time (i.e. referring to main capitula data), the first and second principal components explained 90.2% of the total data variability (80.7 and 9.5% for PC1 and PC2, respectively) (Table 4). The variables were grouped into two distinct clusters, separated by PC2: in the upper right quadrant, we found all the chemical data related to the external bracts samples (TPC_Bra, TFC_Bra, ABTS_Bra and DPPH_Bra) while in the bottom right section, those related to the receptacle ones (TPC_Rec, TFC_Rec, ABTS_Rec and DPPH_Rec); all the variables reached high PC1 scores (scores from 0.952 to 0.788 for TPC_Rec and TFC_Rec, respectively) (Table 4). Regarding genotypes, Cl_C3, Ub_2, Je, VT_PS and Ub_1 clustered separately on the right along PC1 and were positively associated with all the investigated variables; conversely, Cl_MSP exhibited the highest negative PC1 score (-1.533) followed by ML_B, Mz, Cs, As and ML_A (Fig. 3A).

With respect to the mid-spring harvesting time (i.e. referring to first order capitula data), the eigenvalues for PC1 and PC2 were 5.28 and 1.71, respectively, thus capturing 87.4% of the total data variability (Table 4). Again, variables were clearly separated by PC2 while genotypes by PC1 (Fig. 3B). In particular, all the variables reached high PC1 scores with the exception of TFC_Bra, which was mainly correlated with PC2 (scores: 0.328 and 0.886 for PC1 and PC2, respectively; Table 4). Je and Mz showed the higher PC1 scores (1.302 and 1.568, respectively) despite they performed very differently with respect to PC2 (scores: 1.668 and -1.679 for Je and Mz, respectively). As a consequence, Je was related to higher TPC, ABTS and DPPH values in the waste fractions (external bracts) while Mz in the edible parts (receptacle).

PCA proved to be a useful tool to summarize the biochemical characteristics of the different investi-

Table 4 - Principal component analysis (PCA): factor loadings, eigenvalues and percentage of the explained variance

Variables	Main capitula		First order capitula	
	PC1	PC2	PC1	PC2
TPC_core	0.952	-0.093	0.941	-0.288
TFC_core	0.788	-0.561	0.853	-0.242
DPPH_core	0.927	-0.286	0.777	-0.477
ABTS_core	0.916	-0.163	0.831	-0.356
TPC_bratee	0.903	0.418	0.926	0.228
TFC_bratee	0.823	0.084	0.328	0.886
DPPH_bratee	0.933	0.220	0.762	0.572
ABTS_bratee	0.930	0.315	0.912	0.226
Eigenvalue	6.455	0.761	5.284	1.711
Explained variance (%)	80.692	9.515	66.056	21.382

gated artichoke genotypes, and clear conclusions could be obtained, confirming the results in terms of single investigated biochemical parameters. In particular, the reference genotype Cl_C3 and the accessions Ub_1, Ub_2, Je and VT_PS confirmed higher TPC, TFC and, consequently, antiradical activity in the main capitula, highlighting their important attitude for fresh consumption. This greater head quality was maintained during all the growing season (i.e. as quality traits of the first order capitula) only for Ub_1, Je and VT_PS. Other genotypes, such as As and, principally, Mz, were clearly characterized by higher bioactive compounds in the first order flower heads and by smaller capitula. These accessions could represent a promising potential as germplasm for future breeding programs to select elite cultivars, characterized by: (i) higher and stable quality traits suitable for fresh consumption (i.e. Je); (ii) high concentrations of biochemical compounds, especially in the waste products, to be used for processing or pharmaceutical applications although further investigations on smaller and waste flower heads are needed.

4. Conclusions

In conclusion, our results confirm that the capitula of globe artichoke could be considered a functional food thanks to its relevant content of bioactive compounds accumulated in both receptacle and external bracts. The properties of the external bracts could be usefully exploited for other end-use purposes, although they are still edible fractions (Fratianni *et al*., 2007; Pandino *et al*., 2011). A great and appreciable variation among genotypes in terms of chemical composition and nutritional value exists. Such biodiversity of the accessions of Abruzzo and Marche Regions should be exploited and utilized, taking into account the peculiarity of each genotype (in terms of both yield and quality) as well as the actual end-use which can be reached.

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