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Chemical and Morphological Characteristics of New Clones and Commercial Varieties of Globe Artichoke (*Cynara cardunculus* var. *scolymus*)

Gaetano Pandino · Sara Lombardo · Giovanni Mauromicale

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Abstract The globe artichoke is a widely consumed vegetable in the Mediterranean Basin, with Italy being the leading producer. In southern Italy, its cultivation contributes to local economic stability and social development. The producers are increasingly choosing to replace autochthonous varieties, such as 'Violetto di Sicilia', with cultivars bred or selected outside of the region, putting pressure on the maintenance of traditional varieties. Here, we have undertaken a detailed morphological and chemical analysis of a group of clones selected from a population of 'Violetto di Sicilia'. All the traits measured displayed genetic variation, particularly the total content of phenolics and minerals. The capitula of the 'Violetto di Sicilia' clones contained, on average, 6.3 g kg^{-1} of fresh weight total phenolics, compared with 4.5 g kg^{-1} in the two commercial varieties. The clones also had more inulin than commercial varieties (254 *vs*. 225 g kg^{-1} of dry matter), as well as a good mineral content. The set of clones is of interest in the context of the proposed improvement of the crop through breeding and selection of genotypes with high nutritional quality and a specific end-use (industrial processing or fresh consumption).

Keywords Globe artichoke · Inulin · Minerals · New clones · Total phenolics · Total protein

Introduction

Globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] is an herbaceous perennial plant native to the Mediterranean Basin, belonging to the family of Asteraceae (ex Compositae). The edible part of the plant is the immature inflorescence, commonly called capitulum or head, which consists of a receptacle protected by fleshy leaves (bracts). The receptacle is usually considered fully edible, consumed throughout the world as raw, boiled, steamed, fried, and as component of many recipes, because it combines good sensory properties with a healthy image. In particular, it is characterized by high content of minerals [1–3], polyphenols [4–6] and inulin [7]. Mediterranean people appreciate this vegetable and they have introduced it in other parts of the world. Nowadays, the annual global production of capitula is widely distributed all over the world (~1,320 Kt) and especially in the South Europe (Italy, Spain, France, Greece), Middle East (Turkey, Syria, Israel), North Africa (Egypt, Morocco, Algeria, Tunisia), South America (Peru, Argentina, Chile), United States, and recently also in China [8]. Italy is the leading producer in the world (about 475 Kt per year) [8] and holds the greatest artichoke biodiversity, characterized by a large number of local clonal varietal groups with high level of heterozygosis, since the genetic instability of early genotypes and the unavoidable hidden heterogeneity of the propagation organs [9, 10]. At present, the germplasm of globe artichoke includes about 100–120 genotypes grouped in relation to harvest time ('early' or 'late') and to morphological parameters (such as the colour of the external bracts and presence/absence of spines) [11, 12]. Porceddu et al. [13] proposed to divide the germplasm of globe artichoke in four groups: 'Spinosi' ('Spinoso di Palermo', 'Spinoso Sardo', etc.), 'Violetti' ('Violetto di Toscana', 'Nostrano', etc.), 'Romaneschi' ('Castellamare', 'Tondo di Paestum', 'Blanc Hyerois', etc.) and 'Catanesi' ('Violetto di Sicilia', 'Violet de Provence', etc.). However, the genotypes with major commercial importance are only 11–12 [14]. In Italy,

G. Pandino · S. Lombardo · G. Mauromicale (✉)
Dipartimento di Scienze delle Produzioni Agrarie e Alimentari,
Università degli Studi di Catania,
via Valdisavoia 5,
95123 Catania, Italy
e-mail: g.mauromicale@unict.it

the genotypes mainly cultivated are 'Romanesco', 'Violet de Provence' and 'Violetto di Sicilia'. The latter is an autochthonous genotype, vegetatively propagated, cultivated in the South Italy, including Sicily, where it represents an important component of regional economic stability and social development and provides employment almost the whole year due to its long growth cycle. In addition, recent studies highlighted that southern Italy and especially Sicily are implicated as the origin of globe artichoke domestication [11, 15]. Nowadays, many farmers have introduced in Sicily either several genotypes selected abroad and well adapted to local climate and soil, such as 'Violet de Provence' (South France), or some best fitting marked demand, such as 'Romanesco'. This has allowed a market expansion in Italy and abroad, but it is also leading to a progressive erosion of local germplasm. In the latter view, a recent study via AFLP fingerprinting on 'Violetto di Sicilia' varietal type indicated a significant genetic variation within populations, presumably as a consequence of the multiclonal composition [10]. This highlights the requirement of implementing "on farm" germplasm preservation strategies, and the need for clonal selection programmes in order to provide more uniform plant material and to offer well-defined clones to growers.

The objective of this study was to characterize variation with respect to both the morphology and chemical constitution of the capitulum among a set of clones selected from a population of the autochthonous globe artichoke variety 'Violetto di Sicilia', with a view to germplasm conservation and defining parents in the context of proposed improvement through breeding. The data were expected to be relevant for obtaining protected designation of origin (PDO) or protected geographical indication (PGI) status, to select materials in relation to their end use (fresh consumption or food processing), to evaluate the effectiveness of clonal selection procedures, and to improve the crop's content of health-promoting compounds.

Materials and Methods

Plant Material, Management Practices and Capitula Sampling

Field experiment was conducted at the experimental station of Catania University, on Catania Plain, a typical area for globe artichoke cultivation in Italy. Five clones of globe artichoke, selected from the varietal type 'Violetto di Sicilia' and labelled 'B_3', 'C_1', 'C_2', 'C_3' and 'S_1', were compared with two commercial varieties ('Violetto di Sicilia' and 'Violet de Provence'). We considered 'Violet de Provence' as a second control genotype because it is biologically and morphologically close to 'Violetto di Sicilia' and is also widely cultivated in the Mediterranean Basin, as well as in other parts of the world. The five clones derived from a clonal programme carried out in four locations of Sicily (Caltagirone, Niscemi, Ramacca and Rosolini), for which a previous molecular study demonstrated a significant genetic variability both within and among populations of the genotype 'Violetto di Sicilia' [10]. The clonal programme was able to identify 35 clones, which well represented the genetic diversity of the genotype 'Violetto di Sicilia'. Among them, we selected these five clones for their earliness, higher total yield and capitulum quality (colour of the external bracts, shape, size and consistency). The plant material (semi-dormant offshoots, named 'ovoli') was planted in August 2008 and arranged in a randomized block experimental design with four replicates. Each field plot consisted of ten plants, spaced 0.80 m apart with row spacing of 1.25 m. Crop management (fertilization, irrigation, weed and pest control) was performed according to the standard local commercial practice. In February–March, at least five disease-free capitula per replicate were harvested at the usual marketing stage and regardless of their size. At this stage, the length of the floral buds was $\leq$2 mm. The capitula were combined, washed with tap water and then subjected to the morphological and chemical characterization. The latter was performed on the edible fraction (receptacle), after the bracts had been manually removed. The receptacles were immediately blended using a domestic food processor at 0 °C (Kenwood multipro, Milan, Italy). Finally, an amount of the resulting slurry was freeze–dried and stored at −20 °C until analysis, another one was used as fresh and the remaining portion was oven-dried.

Reagents and Solvents

Analytical grade chlorogenic acid, methanol, Folin-Ciocalteu reagent, sodium bicarbonate, hydrochloric acid, bovine serum albumin (BSA) and Bradford reagent were purchased from Sigma Aldrich (Milan, Italy). Sucrase, fructanase and kit for enzymatic, spectrophotometric determination of D-glucose and D-fructose were obtained from Megazyme International Ireland Ltd. (Wicklow, Ireland). K, Na, Ca, Mg, Fe, Cu, Mn and Zn standards were obtained from Perkin Elmer (Norwalk, CT). All solutions were prepared using bidistilled water.

Morphological Characteristics

All capitula were weighed and their maximum diameter and length were measured. The ratio length/diameter of the capitulum, as index of capitulum shape, was calculated. After the bracts had been removed, the receptacle was

weighed in order to calculate the percentage of edible fraction of the total capitulum weight.

Chemical Characteristics

Dry Matter Content

An amount (100 g) of homogenized fresh sample was oven-dried at 65 °C (Binder, Milan, Italy), until a constant weight was reached, to determine the dry matter (DM) content. All data presented were expressed as g 100 g^{-1}.

Total Protein Content

The Bradford assay [16] was used to quantify the total protein content. An amount (20 mg) of powdered freeze–dried material was diluted with NaOH (0.5 N), vortexed and centrifuged (3,000 *g*×10 min) at 25 °C, as described by Snyder & Desborough [17]. Then, an aliquot was transferred into plastic cuvettes and Bradford reagent was added (1:1). The solution was thoroughly mixed by inversion, and after being held for 10 min at room temperature, the absorbance was measured at 595 nm using a Shimadzu 1601 UV-visible spectrometer (Shimadzu Corp., Tokyo, Japan). The total protein content was determined on the basis of a standard calibration curve generated with known amounts of BSA standard and expressed as g kg^{-1} of DM.

Total Inulin Content

The total inulin content was performed following the method of Steegmans et al. [18], properly modified. This method is based on an enzymatic hydrolysis of inulin/oligofructonase by fructanase, followed by spectrophotometric determination according to the Megazyme protocol. Briefly, 1 g of freeze–dried sample was added with 40 ml of boiling water and the pH was adjusted to 7.0 with 50 mM of KOH. Then, the solution was kept at 85±2 °C for 15 min. After cooling at room temperature, the volume was made up to 100 ml with deionized water. An aliquot was used for direct analysis with Megazyme kit, one incubated for 30 min at 40±2 °C with sucrase and the other for 60 min at 60±2 °C with fructanase. In every case, the absorbance was measured at 340 nm. The total inulin content was calculated according to Steegmans et al. [18]. All data presented were expressed as g kg^{-1} of DM.

Total Phenolic Content

The Folin-Ciocalteu assay was used to quantify the total phenolic content (TPC) [19]. An amount (5 g) of blended fresh sample was treated with 50 ml of methanol containing 1% hydrochloric acid, homogenized for 2 min in an Ultra-Turrax T18 (Janke & Kunkel Ika-Labortechnik, Staufen, Germany) and stirred at room temperature for 1 h. Then, the solution was filtered, through a Whatman no. 4 filter paper, and an aliquot, properly diluted, was mixed with Folin-Ciocalteu reagent. After 5 min, sodium bicarbonate (10%, w/v) was added and the volume was made up to 20 ml with bidistilled water. The solution was allowed to stand at room temperature for 90 min and the absorbance was measured at 760 nm. The TPC was determined on the basis of a standard calibration curve generated with known amounts of chlorogenic acid and expressed as g kg^{-1} of fresh weight (FW).

Mineral Content

For the determination of mineral content, 1 g of the oven-dried sample was put in a muffle furnace at 550±2 °C for 24 h. Then, the samples were left to cool at room temperature in a desiccator. After cooling, samples were weighed to calculate the ash content and then HCl (10%, v/v) was added and the solution was filtered with a Whatman no. 6 filter paper, according to AOAC method [20]. Mineral content was carried out using a Perkin Elmer AAnalyst 200 flame atomic absorption spectrometer (Norwalk, CT) equipped with a multi-element hollow cathode lamp and a deuterium background correction system. All data presented are expressed as g kg^{-1} and mg kg^{-1} of DM, respectively, for macro- and micro-minerals.

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA), based on a randomized blocks experimental design, and means were separated by Tukey's HSD or LSD (least significant difference) tests, when the *F*-test was significant. All data presented are mean values of two independent experiments (n=2).

Results and Discussion

Morphological Characteristics

Little variability was apparent with respect to capitulum size, length/diameter (L/D) or receptacle fresh weight/capitulum fresh weight ratio (Table 1). The mean capitulum fresh weight among the clones was higher (120 *vs*. 94 g) than that of the commercial varieties, with clone 'C_3' achieving a capitulum weight of 156 g. In contrast, the capitula of the clones had a slightly lower percentage (16 *vs.* 17%) of edible fraction. All the accessions produced capitula of cylindrical/conical shape, with an L/D ratio ranging from 1.20 ('Violetto di Sicilia') to 1.43 (clones 'C_1' and 'S_1') (Table 1).

Table 1 Morphological characteristics of the genotypes of globe artichoke under study

Genotype	Capitulum FW[a] (g)	Capitulum L/D ratio[b]	Receptacle FW/capitulum FW (%)
Clones of Violetto di Sicilia			
Clone B3	122b	1.34ac	13.6c
Clone C1	106bc	1.43a	19.2a
Clone C2	108bc	1.31bc	13.0c
Clone C3	156a	1.26cd	17.2b
Clone S1	107bc	1.43a	17.2b
Mean of clones	120	1.35	16.0
Commercial varieties			
Violet de Provence	95c	1.20d	14.1c
Violetto di Sicilia	94c	1.38ab	19.7a
CV[c] (%)	19	6	16

[a] Fresh weight; [b] L: length; D: diameter; [c] coefficient of variation

Different letters within each column indicate significant differences at $P \leq 0.05$

Chemical Characteristics

The two commercial varieties ('Violet de Provence' and 'Violetto di Sicilia') markedly differed from the new clones with respect to their dry matter, total protein, ash, phenolic, inulin and mineral content (Table 2). The data were in substantial agreement with results found in the literature [7, 21]. In particular, the mean dry matter content of the clones was 14.8 g 100 g^{-1}, ranging from 12.4 (clone 'S1') to 16.9 g 100 g^{-1} (clone 'C2') (Table 2). These values were mostly intermediate in comparison with the commercial varieties ('Violetto di Sicilia': 9.9 g 100 g^{-1}, 'Violet de Provence': 16.0 g 100 g^{-1}), with clones 'C_2' and 'C_3' being the only ones with a mean dry matter content over 16.0 g 100 g^{-1} (Table 2). In much the same way, Raigon et al. [22] observed significantly higher dry matter content in eggplant landraces than in commercial varieties. With the exception of clones 'B_3' and 'C_3', the total phenolic content of the clones was lower than that in 'Violetto di Sicilia' (Table 2). This trait was particularly variable (coefficient of variation of 66%), suggesting a substantial reservoir of potentially exploitable genetic variance. The identity of the genotype appeared to exert the strongest single influence over the spectrum and content of polyphenols present in the globe artichoke receptacle [4–6], as it is also the case in a number of other crop plant species [23–26]. Polyphenols have a clear importance to the human diet and have been implicated in the prevention of cancer and cardiovascular disease [27], so food products with a high phenolic content are highly valued by the consumer. Globe artichoke provides a natural source of caffeoylquinic acids, apigenin and apigenin glycosides [4–6], and has been identified as being a major dietary source of 5-*O*-caffeoylquinic acid, 1,5 di-*O*-caffeoylquinic acid and apigenin-7-*O*-glucuronide [28]. Apigenin is not widely distributed in the plant kingdom and have been documented in celery, parsley and certain herbs [29, 30], as well as in globe artichoke. From a processed food perspective, polyphenols are important for their involvement in a number of oxidative reactions during processing, and in particular are responsible for the

Table 2 Chemical characteristics of the receptacle of the globe artichoke genotypes under study

Genotype	Dry matter (g 100 g^{-1})	Total protein (g kg^{-1} DM[a])	Ash (g kg^{-1} DM)	Total phenolics (g kg^{-1} FW[b])	Inulin (g kg^{-1} DM)
Clones of Violetto di Sicilia					
Clone B3	14.6c	213bc	101ab	12.7a	258ab
Clone C1	13.8c	254ab	94bc	6.2bc	309a
Clone C2	16.9a	240ab	94bc	3.1cd	237ab
Clone C3	16.1ab	240ab	106a	8.2b	264ab
Clone S1	12.4d	260ab	104ab	1.2d	203b
Mean of clones	14.8	241	100	6.3	254
Commercial varieties					
Violet de Provence	16.0b	269a	76d	2.5bc	185b
Violetto di Sicilia	9.9e	189c	88c	6.6cd	265ab
CV[c] (%)	17	12	11	66	17

[a] Dry matter; [b] Fresh weight; [c] coefficient of variation

Different letters within each column indicate significant differences at $P \leq 0.05$

Table 3 Macro-mineral content of the receptacle of the globe artichoke genotypes under study

Genotype	K	Ca	Mg	Na	Na/K
	–g kg^{-1} DM[a]–				
Clones of Violetto di Sicilia					
Clone B3	10.4c	4.9c	1.9a	0.9bc	0.086b
Clone C1	24.7a	5.4bc	1.2bc	0.7ce	0.028de
Clone C2	24.7a	6.0b	1.1bc	0.6de	0.024e
Clone C3	24.0a	6.2b	1.3b	1.0b	0.042cd
Clone S1	14.0bc	5.4bc	1.9a	1.7a	0.121a
Mean of clones	19.6	5.6	1.5	1.0	0.060
Commercial varieties					
Violet de Provence	18.6ab	3.4d	0.8c	0.6e	0.032de
Violetto di Sicilia	16.4bc	7.3a	1.4b	0.9bd	0.055c
CV[b] (%)	30	22	30	40	–

[a] Dry matter; [b] coefficient of variation

Different letters within each column indicate significant differences at $P \leq 0.05$

degradation of food flavour, texture, colour and a number of other sensory attributes [31]. Among the clones investigated, 'B_3' and 'C_3' would appear to be well suited for fresh consumption as a result of their notably high TPC (respectively, 12.7 and 8.2 g kg^{-1} of FW) and might be regarded for future breeding programmes to enhance the healthy characteristics of the capitulum. In contrast, clone 'S_1' had a TPC of 1.2 g kg^{-1} of fresh weight, thus it is probably better suited for the processed food industry. In common bean, Saha et al. [32] were able to identify accessions showing both a high total protein and a high mineral content, and suggested that these are valuable parents for improvement programmes. An evaluation of the content of key phenolics and flavonoids in the present set of globe artichoke clones has now been initiated, and the early indications are that 1,5 di-*O*-caffeoylquinic acid and apigenin-7-*O*-glucuronide are highly represented in clone 'C_1', while their content in clone 'S_1' is much diminished. The latter clone also scored poorly with respect to the content of the fructose polymer inulin (203 g kg^{-1} of DM; Table 2), a substance which cannot be digested by the small intestine, and is instead fermented in the colon by bifidobacteria [33, 34]. These micro-organisms have the beneficial effect of increasing ion absorption while simultaneously decreasing cholesterol and serum lipid levels [35, 36]. In addition, ingested inulin has little effect on blood glucose, and so has been recommended for patients suffering from diabetes mellitus [37]. Clone 'C_1', with its inulin content of 309 g kg^{-1} of DM (Table 2), could be regarded as a nutraceutical product if consumed fresh. On average, the inulin content of the clones was similar to that of 'Violetto di Sicilia' (254 *vs.* 265 g kg^{-1} of DM), but also it was 1.4 fold above that of 'Violet de Provence'. The total protein content of the clones, ranging from 213 (clone 'B_3') to 260 g kg^{-1} of DM (clone 'S_1'), was intermediate between those of 'Violetto di Sicilia' and 'Violet de Provence' (respectively, 189 and 269 g kg^{-1} of DM), and therefore were uniformly above that of 'Violetto di Sicilia' (Table 2). Finally, with respect to ash content, the clones out-performed both commercial varieties, with clone 'C_3' in particular achieving 106 g kg^{-1} of DM (Table 2).

Macro and Micro-Mineral Content

Here, we have also reported the macro- and micro-mineral content of the globe artichoke receptacle. Of the former, the most prevalent mineral was K in both the commercial varieties and the new clones, as has been similarly documented elsewhere [2, 3, 38]. As already reported, K is the nutrient most absorbed by artichoke plants during the growing cycle [39]. On average, the K content of the clones was above that of the commercial varieties (19.6 *vs.* 17.5 g kg^{-1} of DM). Clones 'C_1', 'C_2' and 'C_3' accumulated

Table 4 Micro-mineral content of the receptacle of the globe artichoke genotypes under study

Genotype	Fe	Zn	Mn	Cu
	–mg kg^{-1} DM[a]–			
Clones of Violetto di Sicilia				
Clone B3	30.8a	23.4b	7.0bc	6.2bc
Clone C1	26.6b	25.0b	5.4d	6.6bc
Clone C2	24.4b	19.6c	5.0d	5.6cd
Clone C3	30.2a	28.6a	8.1b	8.2a
Clone S1	30.8a	28.7a	7.8b	8.4a
Mean of clones	28.6	25.1	6.7	7.0
Commercial varieties				
Violet de Provence	25.8b	19.0c	5.8cd	4.5d
Violetto di Sicilia	31.4a	28.8a	10.2a	7.5ab
CV[b] (%)	10	17	26	21

[a] Dry matter; [b] coefficient of variation

Different letters within each column indicate significant differences at $P \leq 0.05$

the highest concentration of K (mean of 24.5 $g kg^{-1}$ of DM), and their Na/K ratios were low compared with those in clones 'B_3' and 'S_1'. This latter parameter is important, since high values are associated with an increased risk of elevated blood pressure and cardiovascular disease [40]. Similarly, the other macro-minerals (Ca, Mg, Na) tended to be present in higher concentrations in the clones than in the commercial varieties (Table 3). Micro-mineral content behaved rather differently. Thus, for example, the content of Mn in the commercial varieties was greater than in the clones (8.0 *vs.* 6.7 mg kg^{-1} of DM, on average), whilst for Fe no overall difference was observed. The coefficient of variation was 22–40% for the macro-minerals, but only 10–26% for the micro-minerals (Tables 3 and 4), perhaps because macro-mineral content (as also TPC) has a higher heritability than micro-mineral content.

Conclusions

The combined morphological and chemical analysis of the clones and commercial varieties of globe artichoke has highlighted the potential beneficial effect of the cloning procedure on a number of human health-related traits. Thanks to their elevated content of total phenolics, minerals and inulin, clones 'B_3' and 'C_3' represent suitable genotypes for the development of varieties suitable for fresh consumption, while clone 'S_1', in contrast, appears to be better suited for the food processing industry due to its low content of both total phenolics and inulin. The clones as a whole expressed a relevant level of genetic variation with a view to a programme aimed at the enhancement of Sicilian globe artichoke germplasm, both as a mean of combating genetic erosion and providing inputs into possible improvement programmes through breeding. Finally, the globe artichoke has clear and interesting nutritional qualities in the context of the human diet. Hence, combined with its popular taste and utilization in many recipes, the global consumption of globe artichoke can be expected to increase over the coming years and over the Mediterranean Basin.

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