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Phenolic composition, antioxidant activity and mineral profile in two seed-propagated artichoke cultivars as affected by microbial inoculants and planting time

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A B S T R A C T

The aim of this study was to assess the mineral composition, antioxidant activity, total phenolics and target polyphenols of two-seed propagated artichoke cultivars 'Romolo' and 'Istar' in relation to planting time (September and October), and seed coating with a consortium of arbuscular mycorrhizal fungi and *Trichoderma atroviride* (coated and uncoated seeds during the second planting time). 'Romolo' was found to be richest in K^+, Ca^{2+} and Mg^{2+}. Planting artichoke in October suppressed the antioxidant capacity (DPPH and ABTS) compared to the first planting period. The greatest accumulation of 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid and 1,3-di-*O*-caffeoylquinic acid in primary heads occurred in 'Romolo' during the first planting time. The content of 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, apigenin 7-*O*-glucuronide in primary heads as well 1,5-di-*O*-caffeoylquinic acid in secondary heads increased with seed coating especially in 'Romolo'. These findings can assist growers in selecting cultivars and agronomical practices combining optimal yield with high nutraceutical properties.

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1. Introduction

Consumer perception of *functional* vegetables as pivotal in supporting human health and longevity have been entrenched over the past twenty years (Slavin & Lloyd, 2012). Numerous *in vitro*, pre-clinical and clinical studies suggest that consumption of vegetables can reduce the risk of cardiovascular disease, specific forms of cancer and improve cognitive health (Slavin & Lloyd, 2012). Accordingly, consumers, food scientists, nutritionists and growers are questing for vegetables representing a good source of phytochemicals of considerable antioxidant potential.

As a vegetable, globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori], plays a significant role in human nutrition, as it combines generally pleasing organoleptic properties with a rich source of nutraceutical compounds (Lattanzio, Kroon, Linsalata, & Cardinali, 2009). The health-promoting properties of globe artichoke have been consistently related to phenolic compounds (Pandino, Lombardo, Mauromicale, & Williamson, 2011a) as well as fiber and minerals such as potassium and calcium present in the immature inflorescences (Bonasia, Conversa, Lazzizera, Gamacorta, & Elia, 2010; Pandino, Lombardo, & Mauromicale, 2011b). The main phenolic compounds in artichoke heads are caffeoylquinic acid derivates, particularly 5-*O*-caffeoylquinic acid, 1,5-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid (Lattanzio et al., 2009; Pandino, Courts, Lombardo, Mauromicale,

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& Williamson, 2010; Pandino et al., 2011a). Besides mono- and di-caffeoylquinic acids, other phenolics such as flavones apigenin, luteolin and their conjugates as well as flavanones have been also identified in artichoke heads (Lattanzio et al., 2009; Lombardo et al., 2010).

The quantitative and qualitative variability of secondary metabolites and essential nutrients in the inflorescences depends upon many preharvest factors such as plant genotype, the harvest time as well as the soil microbial (Ceccarelli et al., 2010; Pandino, Lombardo, Lo Monaco, & Mauromicale, 2013).

In literature, several studies showed that genetic material is a major determinant of variation in nutritional quality of globe artichoke (Lombardo, Pandino, Ierna, & Mauromicale, 2012; Pandino et al., 2010, 2011a). However, most of these works were limited to traditional vegetative propagated cultivars, whereas the identification and characterization of potential bioactive compounds in the recently released seed-propagated hybrids is still limited. Moreover, nutritional quality of artichoke heads may also vary depending on the time of harvest (Pandino et al., 2013).

Another key preharvest factor able to enhance plant growth and quality would be through inoculation with beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) and *Trichoderma* (López-Bucio, Pelagio-Flores, & Herrera-Estrella, 2015; Rouphael et al., 2015). Experimental studies showed that AMF can enhance the accumulation of secondary metabolites (phenolics and carotenoids), vitamins (ascorbate and tocopherols), sugars as well as anthocyanins in several vegetables (Battini, Bernardi, Tuttini, Agnolucci, & Giovanetti, 2016; Rouphael et al., 2015; Sbrana, Avio, & Giovannetti, 2014). Such physiological changes during mutualistic association may be ascribed to a transient activation of plant defense reactions in colonized roots leading to the accumulation of secondary metabolites (Rouphael et al., 2015; Sbrana et al., 2014). In a previous paper, Ceccarelli et al. (2010) reported a higher phenolic content and antioxidant activity in artichoke flower heads as affected by mycorrhizal symbiosis. Moreover, *Trichoderma atroviride* MUCL 45362 have been reported to promote plant growth acting by either the production of metabolites with hormone activities like indole-3-acetic acid or auxin analogues or the solubilization of nutrients like Fe through siderophore secretion in the rhizosphere (Colla, Rouphael, Di Mattia, El-Nakhel, & Cardarelli, 2015). Nevertheless, nothing is known about the co-inoculation of the two beneficial fungi AMF and *Trichoderma* on the production of phytochemicals in globe artichoke.

In view of this background, a field trial was conducted to evaluate the effects of planting time and co-inoculation of AMF and *Trichoderma* by seed coating on the productivity, polyphenol pattern, ABTS and DPPH radical scavenging activity and mineral profile of two artichoke seed-propagated hybrids 'Romolo' and 'Istar'. These findings will allow a better understanding of the variation in nutritional quality of new artichoke hybrids tested for the first time. The results will also assist producers to define cultural practices (planting time and microbial inoculants) for achieving optimal yield and high nutritional value of artichoke heads.

2. Materials and methods

2.1. Plant material, experimental design and crop management

The field experiment was carried out during the 2014–2015 growing season at the experimental farm of Grieco located at Calvi-Benevento in south Italy (latitude 41°07′N, longitude 14°87′E, altitude 376 m above sea level). The soil was a clay loam (37% sand, 35% silt, 28% clay), with a pH of 7.02, electrical conductivity of 0.7 dS m^{-1}, organic matter of 0.91% (w/w), total N at 0.07%, available P at 5 mg kg^{-1}, and exchangeable K at 228 mg kg^{-1}.

Two seed-propagated cultivars 'Romolo' and 'Istar' (La Semiorto Sementi, Sarno, Italy) were used. 'Romolo' is a hybrid of *Romanesco* typology with purple head characterized by medium size and globular shape, whereas 'Istar' is a re-flowering hybrid with green head characterized by the absence of thorns on bracts and leaves.

The two artichoke cultivars 'Romolo' and 'Istar' were sown on 25 August (for the September transplanting) and 24 September (for the October transplanting) 2014 in polystyrene plug trays (84 holes) containing peat/perlite mixture in 2:1 volume ratio. During the second sowing date (24 September), half of the seeds were coated with rotary coating machine (6:1 weight ratio between seeds and coating product). Coating product (Coveron, Italpollina S.p.A., Rivoli Veronese, Italy) contained 300 spores g^{-1} of *Rhizophagus intraradices* BEG72, and 200 spores g^{-1} of *Funneliformis mosseae* from leek culture, and 3×10^8 CFU *Trichoderma atroviride* MUCL 45362. This coating product was used in the present study based on previous results, where the co-inoculation of the above mentioned arbuscular mycorrhiza fungi (AMF) and *Trichoderma* strains promote transplant establishment, zucchini and lettuce productivity as well as yield and grain quality of winter wheat (Colla, Rouphael, Bonini, & Cardarelli, 2015; Colla, Rouphael, Di Mattia et al., 2015).

Three weeks after sowing the seed-propagated cultivars 'Romolo' and 'Istar' were transplanted on 15 September (first planting time) and 15 October (second planting time), respectively at a plant density of 8000 plants ha^{-1} (1.25 m $\times$ 1.0 m). Moreover, during the second planting time (15 October) both artichoke plantlets, obtained from uncoated and coated seeds with microbial inoculant, were transplanted.

Randomized complete block design was used in the current experiment with treatments replicated three times. Treatments were defined by a factorial combination of two seed-propagated cultivars ('Romolo' or 'Istar') and three agronomic factors (first planting time on 15 September with transplants obtained from uncoated seeds; second planting time on 15 October with transplants obtained from uncoated seeds or second planting time on 15 October with transplants obtained from coated seeds with beneficial microbial). Each experimental unit consisted of a 14 m^2 plot area containing 20 plants.

Base-dressing organic fertilizer (Italpollina, Italpollina S.p.A., Rivoli Veronese, Italy) containing 4% N, 4% P_2O_5 and 4% K_2O was broadcast at a dosage of 20 t ha^{-1} and incorporated mechanically into the soil. Additional top-dressing fertilizer (60 kg ha^{-1} N and 40 kg ha^{-1} K_2O) was applied three times (30, 120, and 150 days after planting) at equal rates through drip irrigation system using NH_4NO_3 and KNO_3 as sources of N and K. Globe artichoke plants were sprinkle-irrigated when necessary for three weeks after transplanting and drip-irrigated throughout the rest of the growing season. All treatments were given uniform optimal irrigation. Weeds were controlled with hand hoeing and no pesticide applications were required to control pathogens and pests.

2.2. Collection of samples

The artichoke heads were hand-harvested from 10 plants when the heads achieved maximum size, but before the bracts began to spread. The primary and secondary heads of the first planting time were harvested on 27 April and 11 May 2015, respectively (224 and 283 days after planting; DAP). Furthermore, the primary and secondary buds of the second planting time including the ones coming from the coated seeds treatment were harvested on 11 and 29 May, respectively (195 and 213 DAP). The shape index defined as the ratio between length and diameter of the heads was also measured.

A part of the primary and secondary artichoke edible buds was used for determination of dry matter content and mineral

composition. The rest of the fresh samples were instantly frozen in liquid nitrogen and stored at −80 °C for chemical analysis. Both experiments ended on 4 June (263 and 220 DAP for the first and second planting time, respectively).

2.3. Chemicals and standards

(S)-(−)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), 2,2′-azino-bis(3-ethylbenzothiazoline-6′-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride, Folin-Ciocalteau, gallic acid, potassium persulfate, anhydrous sodium acetate, reagent and 2,4,6-tri(2-pyridyl-s-triazine) (TPTZ), were purchased from Sigma (Milan, Italy), as well as 5-caffeoylquinic, luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside.

2.4. Mycorrhizal colonization and quantification of Trichoderma

At the end of the trial, the root colonization by AMF was determined microscopically by the gridline intersect method (Giovannetti & Mosse, 1980). Briefly, the root samples were cleared with 10% potassium hydroxide (KOH), stained with 0.05% trypan blue in lactophenol as reported by Phillips and Hayman (1970). The quantification of *Trichoderma* was conducted using the serial plating soil dilution on a *Trichoderma*-selective agar medium as described by Elad, Chet, and Henis (1981). Each root-substrate sample (15 g) was suspended in sterilized distilled water to give a 1:10 dilution. Serial dilutions were made to 10^{-8}. Ten μl aliquot of each dilution and replicates (three) were spread on the *Trichoderma*-selective agar medium in petri dishes. The petri dishes were then incubated for three days, after that fungal colonies of *Trichoderma* were detected and counted and the number of CFU per g of dry soil was calculated (Elad et al., 1981).

2.5. Surface color measurements

Two color measurements on different points of the primary and secondary heads were performed in the CIELAB (L^* a^* b^*) color space using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd, Osaka, Japan). The measuring aperture diameter was 8 mm and the instrument was calibrated with Minolta standard white plate before sampling globe artichoke heads. L^* (lightness ranging from 0 = black to 100 = white), a^* (ranging from green [−60] to red [+60]), b^* (ranging from blue [−60] to yellow [+60]) readings were transformed to those of the L, a, b color space.

2.6. Dry matter, protein and mineral content analysis

The dry matter content was determined following official method 934.01 of the Association of Official Analytical Chemists (AOAC, 2005). Briefly, triplicates of primary and secondary artichoke samples were oven dried at 65 °C until reaching a constant weight, and weighted using an analytical balance (Denver Instruments, Denver, Colorado, USA).

Dried artichoke-edible heads were ground in a Wiley Mill to pass through an 841 μm screen, and then portions of the dried tissues were used for chemical analyses. Total N concentration in the plant tissues was determined by Kjeldahl method following mineralization with sulphuric acid in the presence of potassium sulfate and low concentration of copper catalyst (Bremner, 1965).

The protein content was determined following official method 976.05 of the Association of Official Analytical Chemists. Briefly, total protein content was assessed by the Kjeldahl method previously described, with nitrogen to protein conversion factor of 6.25.

For the anions (PO_4^{3-}) and cations (K^+, Ca^{2+}, Mg^{2+} and Na^+) analysis, 250 mg of dried material was extracted in 50 ml of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) using a shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 °C for 10 min. The mixture was centrifuged at 6000 rpm for 10 min (R-10M, Remi Elektrotechnik Limited, India), then filtered through a 0.20 μm filter paper (Whatman International Ltd., Maidstone, U.K.). K^+, Ca^{2+}, Mg^{2+} and Na^+ were separated by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) and quantified through an electrical conductivity detector. A conductivity detector with IonPac CG12A (4 × 250 mm, Dionex, Corporation) guard column and IonPac CS12A (4 × 250 mm, Dionex, Corporation) analytical column were used for the analysis of K^+, Ca^{2+}, Mg^{2+} and Na^+ whereas for PO_4^{3-}, an IonPac AG11-HC guard (4 × 50 mm) column and IonPac AS11-HC analytical column (4 × 250 mm) were used.

2.7. Extraction and preparation for assays

Three grams of freeze-dried head were extracted with 30 mL of methanol/water (70:30, *v*/*v*) by sonication at room temperature for 30 min. The mixtures were centrifuged at 2800*g* for 10 min at room temperature, filtered through a 0.45 μm filter paper (Whatman International Ltd., Maidstone, U.K.), and then used for HPLC analysis, antioxidant activity and total polyphenols (Folin) determinations.

2.8. Identification of phenolic compounds by HPLC-DAD-ESI/MS and quantification by RP-HPLC-DAD

Liquid chromatographic analysis was performed with a Thermo Separation Products Liquid Chromatograph (Model Spectra System, TSP, CA, USA), equipped with HPLC pump (Spectra Series pump P4000), vacuum degasser for liquid chromatography (Solvent degasser SCM 1000), rheodyne and injection valve (Injection volume: 50 μl). System parameters were controlled with system controller (SN 4000) and chromatographic data were collected and recorded using the PC 1000 system software. A reversed phase column Gemini C18 (250 × 4.6 mm i.d., particle size 5 μm; Phenomenex, Torrance, CA, USA) was employed. The eluents were (A) 0.2% formic acid in water and (B) acetonitrile/methanol (60:40 v/v). The gradient program was as follows: 20–30% B (6 min), 30–40% B (10 min), 40–50% B (8 min), 50–90% B (8 min), 90–90% B (3 min), 90–20% B (3 min), at a constant flow of 0.8 mL min^{-1}. Diode array detection was between 200 and 600 nm and absorbance was recorded at 280 and 330 nm.

Caffeoylquinic acids and flavones, were separated using HPLC and identified by their retention times, comparison with commercially available standards, their UV/Vis spectra and available data in the literature (Lombardo et al., 2010; Pandino et al., 2011a, 2011b). Amounts of mono- and di-caffeoylquinic acids were calculated at 330 nm using 5-caffeoylquinic acid and as reference. Apigenin derivatives were quantified at 330 nm using apigenin-7-*O*-glucoside as references. All data are presented as mean ± standard deviation (n = 3), and expressed as mg kg^{-1} of dry matter (DM).

2.9. Determination of total phenolics

Total phenolics content was determined using the Folin-Ciocalteu colorimetric method as previously reported (Gallo, Ferracane, Graziani, Ritieni, & Fogliano, 2010). Briefly, artichoke extracts (100 μL) were mixed with Folin-Ciocalteu reagent (0.2 mL) and H_2O (2 mL), and incubated at room temperature for 3 min. Total polyphenols were determined after 1 h of incubation at room temperature, by adding of 20% sodium carbonate (1 mL) to the mixture. The absorbance of the resulting blue color was measured at 765 nm with a UV–VIS spectrophotometer (Gallo et al., 2010). Quantification was done with respect to the standard

curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), milligrams per 100 g of dry weight. Total phenolics analysis was performed in triplicate.

2.10. Assay of antioxidant capacity

The antioxidant capacity of our artichoke head extracts assay was assessed using an UV–VIS recording spectrophotometer (Shimadzu, Japan) by the improved ABTS$^{\cdot+}$ method as previously reported (Gallo et al., 2010; Re et al., 1999). ABTS$^{\cdot+}$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23 °C) in dark for 16 h. The ABTS^{+} solution was diluted with ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. The filtered sample was diluted with 70% methanol to give 20–80% inhibition of the blank absorbance with 0.1 mL of sample. ABTS^{+} solution (1 mL, with absorbance of 0.7 ± 0.05) was added to the tested samples (0.1 mL) and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 2.5 min and the absorbance was immediately recorded at 734 nm. Trolox® standard solution (final concentration 0–15 μM) in methanol was prepared and assayed at the same conditions (Gallo et al., 2010). The absorbance of the resulting oxidized solution was compared to that of the calibrated Trolox® standard. Results were expressed in terms of Trolox® equivalent antioxidant capacity (TEAC, mmol Trolox® equivalents per 100 g dw).

The DPPH radical-scavenging activity was also assessed using the method as previously reported (Gallo et al., 2010; Yen & Chen, 1995). Briefly, 100 μM DPPH was dissolved in ethanol (96%). The DPPH solution (1 mL) was added to the polyphenol extract (1 mL) with ethanol (3 mL). The mixture was shaken vigorously and then allowed to stand for 10 min in the dark at room temperature. The decrease in absorbance of the resulting solution was monitored at 517 nm for 10 min (Gallo et al., 2010). The results were corrected for dilution and expressed in μmol Trolox® per 100 g dw. The DPPH analysis was performed in triplicate.

2.11. Statistics

Analysis of variance (two way-ANOVA) of the experimental data was performed using the SPSS 20 software package. To separate treatment means within each measured parameter, the Duncan's Multiple Range Test was performed at $P \leq 0.05$. Quality traits were subjected to Principal Component Analysis (PCA) to explore relationships among variables and treatments and to determine which quality traits were the most effective in discriminating between cultivars, planting time and microbial inoculants. The PCA outputs include variable loading to each selected component and treatment component scores.

3. Results and discussion

3.1. Yield, yield components, arbuscular mycorrhizal fungi root colonization and quantification of Trichoderma spp

The yield characteristics were mainly affected by agronomic factors ($P < 0.001$) and to a lesser extent by cultivar ($P < 0.05$) whereas no significant interactions were found between cultivar and agronomic factors for yield and yield components of primary, secondary and total artichokes (data not shown). When averaged over agronomic factors, the highest primary and total head fresh weight was recorded in 'Romolo' (avg. 188.2 and 158.8 g head^{-1}, respectively) rather than in 'Istar' (avg. 154.2 and 140.3 g head^{-1}, respectively). Irrespective of cultivars, the primary and total head fresh weight as well as the secondary and total number of buds coming from uncoated seeds of the first planting time and also from coated seeds of the second planting period (avg. 187.3, 159.3 g head^{-1}, 4.6 and 5.6 heads plant^{-1}, respectively) were significantly higher in comparison to those from uncoated seeds of the second planting time (avg. 139.1, 129.4 g head^{-1}, 3.3 and 4.3 heads plant^{-1}, respectively; data not shown). Similar to yield components, the primary and total yields of globe artichoke were significantly higher by 22.8% and 20.4% in 'Romolo' (avg. 1.51, and 6.78 t ha^{-1}, respectively) than in 'Istar' (avg. 1.23, and 5.63 t ha^{-1}, respectively; Fig. 1), whereas no significant differences among cultivars were found for the secondary yield (avg. 4.84 t ha^{-1}). Furthermore, the primary, secondary and total yields of globe artichoke were significantly influenced by agronomic factors with the highest values recorded in non-inoculated artichoke planted in September (i.e. first planting time) and in inoculated plants planted in October (i.e. second planting time) (avg. 1.50, 5.56, and 7.06 t ha^{-1}, respectively; Fig. 1), whereas the lowest values were observed in non-inoculated plants coming from the second planting time (avg. 1.13, 3.40, and 4.53 t ha^{-1}, respectively; Fig. 1).

The percentage of AMF root colonization at the end of the trial was affected by cultivar and agronomic factors with no significant cultivar × agronomic factors interaction, whereas the number of *Trichoderma* colonies recovered from the soil was only affected by agronomic factors (Table 1). Irrespective of agronomic factors, the percentage of AMF infection was significantly higher in 'Romolo' (avg. 21.3%) than in 'Istar' (avg. 14.9%) (Table 1). Moreover, when averaged over cultivars, artichoke plants from the coated seeds displayed significant increase in mycorrhizal root colonization percentage and *Trichoderma* colonies (avg. 30.5% and 1.4×10^5 CFU g^{-1}, respectively) compared to those from the uncoated seed treatment (avg. 11.9% and 2.8×10^4 CFU g^{-1}, respectively). The lowest crop performance recorded in uncoated plants coming from the second planting period could be attributed to the lower air temperature during the vegetative stage (the first 100 days after transplanting) (Supplementary Fig. S1), which may have negatively affected the canopy growth thus reducing the photosynthates available for head development.

Concerning the effect of microbial inoculants on crop productivity, our results indicated that the increase in yield induced by the co-inoculation of AMF and *Trichoderma atroviride* during the second planting time could be associated to several growth enhance-

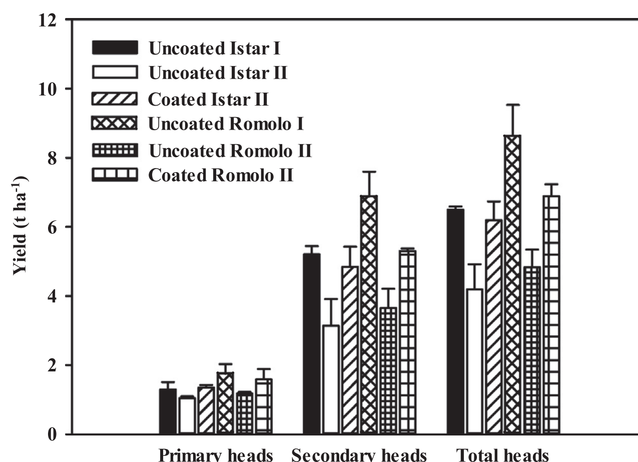

Fig. 1. Primary, secondary and total yields of two seed-propagated cultivars 'Romolo' and 'Istar' planted in two periods (I = 15 September and II = 15 October) using transplants from uncoated or coated seeds with 'Coveron' containing beneficial microbial *Rhizophagus intraradices* BEG72, *Funneliformis mosseae* and *Trichoderma atroviride* MUCL 45362. All data are expressed as mean ± standard deviation, n = 3.

Table 1
Effects of cultivar and agronomic factors (planting time and seed coating with beneficial microbial) on mycorrhizal root colonization and *Trichoderma* spp. population in roots of globe artichoke.

Cultivar	Agronomic factors	Mycorrhiza root colonization (%)	*Trichoderma* spp. ($\times 10^4$ CFU/g)
Istar	I planting – uncoated	10.8 ± 5.6	2.1 ± 1.3
	II planting – uncoated	8.7 ± 2.7	2.0 ± 1.7
	II planting – coated	25.3 ± 1.8	8.3 ± 3.3
Romolo	I planting – uncoated	16.7 ± 3.8	4.9 ± 3.5
	II planting – uncoated	11.4 ± 1.5	2.3 ± 2.6
	II planting – coated	35.7 ± 3.7	19.2 ± 11.1
Significance			
Cultivar (C)		**	ns
Agronomic factors (A)		***	***
C × A		ns	ns

ns, **, *** Nonsignificant or significant at $P \leq 0.01$, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).
Seed coating with 'Coveron' containing 300 spores g^{-1} of *Rhizophagus intraradices* BEG72, and 200 spores g^{-1} of *Funneliformis mosseae* from leek culture and 3×10^8 CFU *Trichoderma atroviride* MUCL 45362.

ment mechanisms such as (1) increasing nutrient uptake and translocation through greater effective root area as well as to the colonization level of AMF (Table 1), (2) solubilization of trace elements by *Trichoderma* and also (3) throughout the production in the root zone of volatiles, small peptides and metabolites with hormone activities (i.e., indole-3-acetic acid) or analogues (for *Trichoderma* spp; López-Bucio et al., 2015; Rouphael et al., 2015).

3.2. Colorimetric attributes, dry matter, protein content and mineral profile

Among the physical characteristics of globe artichoke that strongly influence the consumer preference is the outer bract color. In the current study, the genetic material resulted as the predominant factor (P < 0.001) influencing the color changes of the primary and secondary heads, whereas the influence of agronomic factors (planting time and co-inoculation with beneficial microorganisms) as well as cultivar × agronomic factors interaction were not significant (data not shown). The deepest coloration [highest redness (a^* = 5.9 and 4.4 in primary and secondary heads); lowest yellowness (b^* = 9.4 and 5.0 in primary and secondary heads) and brightness (L^* = 27.2 and 32.1 in primary and secondary heads)] was observed in 'Romolo', whereas the lightest coloration [lowest redness (a^* = −5.2 and −3.6 in primary and secondary heads), highest yellowness (b^* = 26.2 and 18.8 in primary and secondary heads); highest lightness (L^* = 45.7 and 46.2 in primary and secondary heads)] was recorded in 'Istar'. The highest redness and yellowness values observed in 'Romolo' (violet color) and 'Istar' (green color) could be expected since these two seed-propagated cultivars are characterized by the high presence of pigments (anthocyanins and chlorophyll, respectively) involved in outer bract coloration.

Artichoke fruit shape index (SI) constitutes another important morphometric trait predominantly governed by genotype and little affected by environmental or cultural factors. This was the case in the current study, since the SI was significantly affected by cultivars (P < 0.001), but not by agronomic factors and cultivar × agronomic factors interaction (data not shown). The SI of the primary and secondary heads ranged from 0.81 to 0.96 and from 0.94 to

Table 2
Effects of cultivar and agronomic factors (planting time and seed coating with beneficial microbial) on dry matter percentage, protein content and mineral concentration of primary and secondary artichoke heads. All data are expressed as mean ± standard deviation, n = 3.

Cultivar	Agronomic factors	Dry matter (%)		Protein (g kg^{-1} DW)		Mineral elements (g kg^{-1} DW)							
						K^+		Ca^{2+}		Mg^{2+}		Na^+	
		Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads
Istar	I planting – uncoated	11.8 ± 0.8	14.0 ± 0.2[c]	193.0 ± 10.6[b]	176.6 ± 21.8[a]	29.6 ± 1.7[b]	26.3 ± 8.9	1.05 ± 0.30[b]	1.18 ± 0.43	0.94 ± 0.10[bc]	0.94 ± 0.32	0.55 ± 0.03	0.55 ± 0.46
	II planting – uncoated	13.4 ± 1.1	18.7 ± 0.8[a]	185.8 ± 5.6[b]	113.3 ± 5.0[b]	28.7 ± 3.1[bc]	21.4 ± 1.2	1.01 ± 0.35[b]	0.55 ± 0.17	0.91 ± 0.17[c]	0.71 ± 0.13	0.51 ± 0.18	0.54 ± 0.24
	II planting – coated	12.5 ± 0.6	16.2 ± 1.0[b]	185.9 ± 9.4[b]	130.9 ± 13.7[b]	20.6 ± 0.9[b]	23.8 ± 4.5	1.00 ± 0.07[b]	0.46 ± 0.37	0.98 ± 0.12[b]	0.60 ± 0.28	0.75 ± 0.35	0.44 ± 0.33
Romolo	I planting – uncoated	13.7 ± 0.6	16.3 ± 1.0[b]	143.7 ± 26.2[b]	143.2 ± 5.6[ab]	25.9 ± 2.0[c]	19.7 ± 6.9	0.89 ± 0.32[b]	0.75 ± 0.21	0.84 ± 0.21[c]	0.93 ± 0.31	0.45 ± 0.05	0.40 ± 0.14
	II planting – uncoated	12.0 ± 0.8	14.1 ± 1.1[c]	210.0 ± 17.5[a]	171.8 ± 40.6[a]	36.3 ± 1.6[a]	29.3 ± 1.9	1.76 ± 0.32[a]	0.94 ± 0.20	1.25 ± 0.19[ab]	1.17 ± 0.03	0.57 ± 0.27	0.56 ± 0.25
	II planting – coated	11.7 ± 0.8	17.1 ± 0.6[ab]	211.9 ± 12.5[a]	128.1 ± 20.0[b]	35.0 ± 2.0[a]	25.8 ± 1.0	1.48 ± 0.20[ab]	0.60 ± 0.31	1.31 ± 0.17[a]	0.89 ± 0.27	0.74 ± 0.18	0.26 ± 0.02
Significance													
Cultivar (C)		ns	ns	ns	ns	**	ns	*	ns	*	ns	ns	ns
Agronomic factors (A)		ns	*	**	ns	***	ns	ns	ns	*	ns	ns	ns
C × A		ns	***	***	**	***	ns	*	ns	ns	ns	ns	ns

Means within columns separated using Duncan's multiple range test, P = 0.05.
ns, *, **, ***Nonsignificant or significant at P < 0.05, 0.01 or 0.001, respectively.
n.d. not detected.
Seed coating with product 'Coveron' containing 300 spores g^{-1} of *Rhizophagus intraradices* BEG72, and 200 spores g^{-1} of *Funneliformis mosseae*, and 3×10^8 CFU *Trichoderma atroviride* MUCL 45362.

1.04, respectively for 'Romolo' (spherical heads), whereas for 'Istar' the SI ranged from 1.03 to 1.07 and from 1.13 to 1.14 (for primary and secondary buds, respectively) indicating a more cylindrical shape (data not shown). Moreover, the dry matter (DM) contents recorded in the primary and secondary inflorescences ranged from 11.7% to 13.7% and from 14.0% and 18.7%, respectively (Table 2). The highest DM content of the secondary buds was recorded in uncoated 'Istar' and coated 'Romolo' plants from the second planting period, whereas the lowest values were observed in uncoated 'Istar' and 'Romolo' coming from the first and second planting period, respectively (Table 2). Our results on 'Romolo' and 'Istar' DM content were proximate to the 15–20% of DM value reported by Ceccarelli et al. (2010).

ANOVA highlighted that the cultivar × agronomic factors interaction was significant for proteins of primary and secondary heads, with the lowest values recorded in 'Romolo' and 'Istar' planted especially in September and October, respectively (Table 2). Our findings on proteins content (143.7–211.9 and 113.3–176.6 g kg^{-1} dw in primary and secondary inflorescences, respectively) were proximate to the 189 and 269 g kg^{-1} dw reported by Pandino, Lombardo, and Mauromicale (2011c) on two commercial varieties 'Violetto di Sicilia' and 'Violet de Provence'.

It is well established that most nutritional disorders are generated by diets deficient in minerals and vitamins. Fruits and vegetables contribute normally by 35%, 7% and 24% to the human dietary intake of total potassium, calcium and magnesium, respectively (Levander, 1990). Even if annual per capita consumption of artichoke is limited to 0.3 kg, a recent study conducted by Dosi et al. (2013) indicated that global artichoke consumption increased by 44% over the last two decades. Therefore, it expected that the contribution of artichoke to the human dietary intake of minerals and vitamins will be more significant in the coming years. Among the macro-cations studied, K^+ was the main mineral constituent of the primary and secondary buds of 'Romolo' and 'Istar', followed by Ca^{2+}, Mg^{2+} and finally Na^+ (Table 2). This trend was confirmed in previous works on artichoke mineral profiling conducted by Pandino et al. (2011b) on nine cultivars and by Bonasia et al. (2010) on three seed-propagated hybrids (Madrigal, Opal and Tempo).

Concerning the influence of cultivar and agronomic factors on mineral profiling, significant variation was observed for K^+, Ca^{2+} and Mg^{2+} contents in the primary heads, with the highest values recorded in 'Romolo' during the second planting time in both coated and uncoated treatments (Table 2). Moreover, PO_4^{3-} content in primary artichoke heads was significantly affected by cultivar ($P < 0.05$) and agronomic factors ($P < 0.001$) with no significant cultivar × agronomic factors interaction (data not shown). When averaged over agronomic factors, the content of PO_4^{3-} was significantly higher by 13.1% in 'Romolo' (15.6 g kg^{-1} dw) than in 'Istar' (13.8 g kg^{-1} dw). Moreover, the coated seeds inoculated with beneficial microorganisms enhanced the PO_4^{3-} content in the primary heads by 56.7% compared to uncoated seeds treatment in both growing periods (17.8 and 11.3 g kg^{-1} dw, respectively). The mutualistic symbiosis of AMF and cultivated plants is particularly important for enhancing the availability and uptake of the relatively immobile and insoluble phosphate ions in soil (Rouphael et al., 2015).

3.3. Total phenolics and antioxidant capacity

Polyphenols represent a large family of secondary metabolites acting as primary antioxidants in the neutralization of free radicals (Grace, 2005). The total polyphenol (TP) content of the primary and secondary heads recorded in the present study was cultivar-dependent, with the highest values observed in 'Romolo' (Table 3). The strong influence of genetic factors on the TP has been previously demonstrated in other vegetable species (Reddivari, Hale, & Miller 2007; Vallverdú-Queralt et al., 2011). The TP in the primary heads coming from uncoated seeds of the first planting time was significantly higher than those coming from the second planting, due to the lower temperature during the head formation, which may have enhanced the biosynthesis of these antioxidant molecules. The biosynthesis of phenolic compounds has been described as actively involved in plant's response to various stressors including low temperature (Pandino et al., 2013). Moreover, all inoculated field grown plants showed higher TP in the edible parts compared to the control plants, with the highest values found in the main more than in the secondary flower heads (Table 3).

In the current study, two commonly used *in vitro* assays ABTS and DPPH, were adopted to evaluate the antioxidant activities in the primary and secondary heads of artichoke. The antioxidant activity is an important trait in assessing the quality of fruits and vegetables, since antioxidant molecules has a significant role in ensuring plant growth and development under biotic and abiotic stress conditions as well as promoting health properties in human diet (Colonna, Rouphael, Barbieri, & De Pascale, 2016). The ABTS and DPPH radical scavenging activities were significantly affected by cultivar and agronomic factors with a significant interaction

Table 3
Effects of cultivar and agronomic factors (planting time and seed coating with beneficial microbial) on antioxidant activity by 2,2′-azino-bis(3-ethylbenzothiazoline-6′-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods and total phenolic compounds of primary and secondary artichoke heads. All data are expressed as mean ± standard deviation, n = 3.

Cultivar	Agronomic factors	Antioxidant capacity				Total phenolics (mg gallic acid eq. 100 g^{-1} DW)	
		ABTS (mmol Trolox eq. 100 g^{-1} DM)		DPPH (μmol Trolox eq. 100 g^{-1} DM)			
		Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads
Istar	I planting – uncoated	14.2 ± 0.49^{c}	9.2 ± 0.27^{e}	15.4 ± 0.49^{c}	15.0 ± 0.67^{b}	8287.6 ± 249.1^{c}	3174.8 ± 78.5^{d}
	II planting – uncoated	11.7 ± 0.45^{d}	11.2 ± 0.39 c^{d}	10.9 ± 0.39^{d}	6.1 ± 0.22^{d}	7564.7 ± 280.3^{d}	3616.3 ± 117.0^{c}
	II planting – coated	18.5 ± 0.38^{a}	12.0 ± 0.35^{c}	12.7 ± 2.50^{d}	6.6 ± 0.07^{d}	9432.8 ± 191.4^{b}	3378.8 ± 117.1 c^{d}
Romolo	I planting – uncoated	16.5 ± 0.36^{b}	10.5 ± 1.37^{de}	19.7 ± 0.31^{b}	16.7 ± 0.31^{a}	9517.8 ± 182.0^{ab}	3513.9 ± 175.1^{c}
	II planting – uncoated	14.4 ± 0.12^{c}	14.8 ± 1.07^{b}	17.2 ± 1.38^{c}	14.7 ± 0.06^{b}	8681.0 ± 215.3^{c}	5309.8 ± 323.9^{a}
	II planting – coated	18.4 ± 0.76^{a}	16.8 ± 0.41^{a}	22.6 ± 0.78^{a}	13.8 ± 0.29^{c}	9861.6 ± 289.1^{a}	4799.7 ± 209.2^{b}
Significance							
Cultivar (C)		***	***	***	***	***	***
Agronomic factors (A)		***	***	***	***	***	***
C × A		***	**	**	***	*	***

Means within columns separated using Duncan's multiple range test, P = 0.05.
ns, *, **, ***Nonsignificant or significant at P < 0.05, 0.01 or 0.001, respectively. n.d. not detected.
Seed coating with product 'Coveron' containing 300 spores g^{-1} of *Rhizophagus intraradices* BEG72, and 200 spores g^{-1} of *Funneliformis mosseae*, and 3× 10^{8} CFU *Trichoderma atroviride* MUCL 45362.

between the two (Table 3). The ABTS and DPPH scavenging activities in primary and secondary heads were higher in 'Romolo' than 'Istar' especially in non-inoculated treatments, which implies that 'Romolo' could be considered a potential rich source of natural antioxidants. Moreover, except for the DPPH in the secondary heads, planting globe artichoke in October using non inoculated transplants suppressed the antioxidant capacity compared to the first planting period (September; Table 3). Furthermore, in the second planting time, co-inoculation with endophytic fungi enhanced the ABTS (for primary and secondary heads) and DPPH (only for primary head) scavenging activities of both cultivars compared to control plants (Table 3), as defense response of artichoke crop to root colonization of AMF and *Trichoderma* (Rouphael et al., 2015). Finally, irrespective of treatments the positive correlation between TP and ABTS scavenging activity was highly significant ($P < 0.01$; Pearson's coefficient = 0.956 and 0.881 for the primary and secondary heads), whereas the correlation coefficient between TP and DPPH was not significant (data not shown). The same type of correlation between antioxidant activity and phenolic concentration has been demonstrated in different artichoke genotypes as well as their byproducts (i.e., leaves) (Lombardo, Pandino, & Mauromicale, 2015; Rouphael et al., 2016)

3.4. Phenolic profile

The HPLC-DAD-ESI/MS^n analysis of the methanolic extracts of artichoke provides a qualitative and quantitative evaluation of phenolic profile. After HPLC separation, six compounds were indentified belonging to hydroxycinnamate and flavone groups (Table 4, Supplementary Fig. S2). Irrespective of treatments, 1,5-di-*O*-caffeoylquinic acid (1,5-diCQA) was the most abundant compound within the caffeoylquinic derivates, followed by 5-*O*-caffeoylquinic acid (5-CQA), 3-*O*-caffeoylquinic acid (3-CQA), and finally 1,3-di-*O*-caffeoylquinic acid (1,3-diCQA) (Table 4), which is in agreement with the findings of Lattanzio et al. (2009). Furthermore, among the flavonoids, apigenin 7-*O*-glucuronide (Api glr) was the predominant compound in artichoke heads, while apigenin 7-*O*-glucoside (Api glc) was present in rather smaller concentrations.

Remarkable differences among cultivars and agronomic factors were found for the target phenolic compounds, as reflected by a significant cultivar × agronomic factors interaction (Table 4). In uncoated treatments, the greatest accumulation of 3-CQA, 5-CQA and 1,3-diCQA in primary heads occurred in 'Romolo' during the first planting time. In addition, the highest concentrations of 1,5-diCQA, Api glr and Api glc in primary heads were also found during the first planting time irrespective of the cultivars. The greatest accumulation of hydroxycinnamates and to a lesser degree flavonoids during the first planting could be attributed to the fact that plants react to the climatic-induced changes (temperature and solar radiation) modifying the biosynthesis of their secondary metabolites (Pandino et al., 2013). Pandino et al. (2013) demonstrated that polyphenol content of the re-flowering cultivar 'Violetto di Sicilia' respond positively to low temperature exposure and solar radiation increase, reflecting a build-up of ROS activity. The enhancement of bioactive composition observed in the current study is very important, since several clinical and pre-clinical studies have demonstrated the hepatoprotective, antioxidant, antibacterial, vasorelaxant, and anticarcinogenic properties of caffeoylquinic acids (chlorogenic acid and cynarin) and flavones (de Falco, Incerti, Amato, & Lanzotti, 2015).

Chromatographic analysis of the six target compounds, in primary and secondary heads from artichoke plants coming from the second planting time, revealed that most caffeoylquinic acids and apigenin derivates were significantly higher with AMF and *T. atroviride* inoculated plants than in non-inoculated ones (Table 4).

Table 4
Effects of cultivar and agronomic factors (planting time and seed coating with beneficial microbial) on target phenolic contents of primary and secondary artichoke heads. All data are expressed as mean ± standard deviation, n = 3.

Cultivar	Agronomic factors	Phenolic acids (mg kg^{-1} DM)								Flavonoids (mg kg^{-1} DM)			
		3-*O*-caffeoylquinic acid		5-*O*-caffeoylquinic acid		1,3-di-*O*-caffeoylquinic acid		1,5-di-*O*-caffeoylquinic acid		Apigenin 7-*O*-glucoside		Apigenin 7-*O*-glucuronide	
		Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads	Primary heads	Secondary heads
Istar	I planting – uncoated	268.2 ± 48.2 c^{d}	263.0 ± 51.7^{b}	3017.5 ± 26.4^{bc}	1607.1 ± 194.8	3.36 ± 0.40^{c}	n.d.	5311.5 ± 199.9	2610.4 ± 196.2^{c}	34.4 ± 3.7^{b}	40.8 ± 2.1^{f}	60.5 ± 7.1	32.3 ± 1.5^{e}
	II planting – uncoated	193.7 ± 10.9^{d}	273.3 ± 1.6^{b}	2343.4 ± 193.6^{d}	1779.7 ± 19.1	2.69 ± 0.28^{c}	n.d.	4737.9 ± 158.0	2404.1 ± 43.7^{c}	25.4 ± 1.0^{c}	61.7 ± 0.1^{c}	39.3 ± 0.8	23.0 ± 0.1^{f}
	II planting – coated	219.3 ± 13.9^{d}	174.5 ± 2.7^{c}	3254.6 ± 57.3^{b}	1957.2 ± 11.5	5.95 ± 0.49^{b}	n.d.	5582.3 ± 107.9	3583.0 ± 49.8^{b}	33.2 ± 1.2^{b}	57.0 ± 3.5^{d}	45.5 ± 1.0	41.3 ± 3.0^{d}
Romolo	I planting – uncoated	682.6 ± 20.2^{a}	68.6 ± 1.7^{d}	4138.4 ± 111.9^{a}	2286.6 ± 155.0	7.25 ± 0.44^{a}	n.d.	5254.5 ± 329.3	4417.3 ± 268.2^{a}	45.4 ± 4.4^{a}	46.4 ± 1.7^{e}	120.8 ± 15.3	68.7 ± 6.6^{b}
	II planting – uncoated	288.0 ± 46.6^{c}	162.7 ± 3.2^{c}	2995.3 ± 58.8^{c}	2561.8 ± 14.3	5.46 ± 0.18^{b}	n.d.	4956.0 ± 133.4	3800.8 ± 343.7^{b}	30.3 ± 0.9^{b}	83.5 ± 1.1^{a}	91.5 ± 3.4	100.3 ± 0.8^{a}
	II planting – coated	430.1 ± 15.5^{b}	405.5 ± 2.8^{a}	3070.4 ± 188.4^{bc}	2675.2 ± 4.0	7.62 ± 0.68^{a}	n.d.	5920.7 ± 474.0	4662.8 ± 73.7^{a}	30.6 ± 0.9^{b}	79.1 ± 1.3^{b}	114.4 ± 1.9	52.0 ± 0.9^{c}
Significance													
Cultivar (C)		***	*	***	***	***	–	ns	***	**	***	***	***
Agronomic factors (A)		***	***	***	***	***	–	***	***	***	***	***	***
C × A		***	***	***	ns	**	–	ns	*	**	***	ns	***

Means within columns separated using Duncan's multiple range test, $P = 0.05$.
ns, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01 or 0.001, respectively.
n.d. not detected.
Seed coating with 'Coveron' containing 300 spores g^{-1} of *Rhizophagus intraradices* BEG72, and 200 spores g^{-1} of *Funneliformis mosseae*, and 3×10^8 CFU *Trichoderma atroviride* MUCL 45362.

Remarkably, 3-CQA (in primary and secondary heads), 5-CQA (in primary heads), 1,5-diCQA (in secondary heads) as well as Api glr (in primary heads) increased sharply in 'Romolo' compared to 'Istar' (Table 4) indicating that phenolic profile of globe artichoke was cultivar-depended, as reported previously by several authors (Romani, Pinelli, Cantini, Cimato, & Heimler, 2006; Wang et al., 2003). Furthermore, the content of 1,3-diCQA, 1,5-diCQA and Api glu in primary heads incurred significant increase with seed coating treatment relative to uncoated seeds in the second planting period (Table 4). Endophytic fungi, in particular AMF, symbioses can affect secondary metabolism of colonized roots by enhancing antioxidant enzymatic systems activity, mevalonate, malonyl-CoA as well as polyphenols biosynthesis (Sbrana et al., 2014). Our results are in agreement with the findings from Palermo, Colla, Barbieri, and Fogliano (2013), which reported that inoculation with beneficial microorganisms (AMF alone or in combination with bacteria) greatly influences artichoke Romanesco type cv. C3 flavonoid metabolite profile.

3.5. Principal component analysis

To obtain a broad view on the artichoke quality changes that occurred in the two seed-propagated cultivars as affected by microbial inoculants and planting time, the whole data set was subjected to principal component analysis (PCA). The first three PCs were associated with eigenvalues greater than 1 and explained 85.4% of the total variance, with PC1 accounting for 45.6%, PC2 for 22.7% and PC3 for 17.1% (Supplementary Table S1). PC1 was positively and strongly (>0.6) correlated with a*, DPPH, Api glr, 1,3-diCQA, TP, and Mg^{2+} in primary and secondary buds. It was

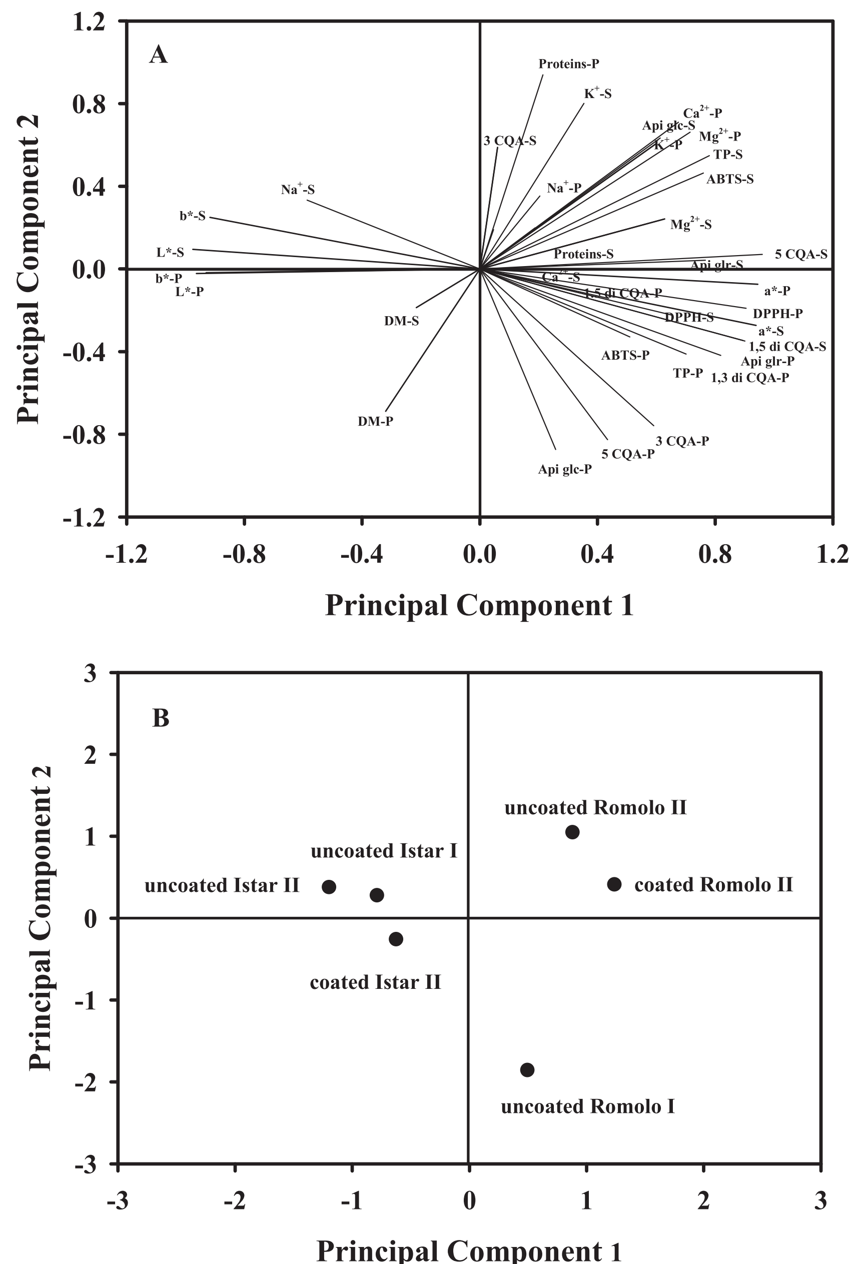

Fig. 2. (A) Principal component loading plot and (B) scores of principal component analysis of quality traits of globe artichoke primary (P) and secondary (S) heads as a function of coating seed treatment (coated and uncoated with beneficial microbial), planting time (I = 15 September and II = 15 October) and cultivar (Romolo and Istar). TP, total phenolics; 3-CQA, 3-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 1,3-diCQA, 1,3-di-*O*-caffeoylquinic acid; 1,5-diCQA, 1,5-di-*O*-caffeoylquinic acid; Api glr, apigenin 7-*O*-glucuronide; Api glc, apigenin 7-*O*-glucoside; DM, dry matter.

negatively correlated with L* and b* in both heads. Moreover, PC2, was negatively correlated with Api glc, 5-CQA, 3-CQA and DM in primary heads, whereas PC3 was positively correlated with Ca^{2+}, proteins and DM in the secondary heads (Supplementary Table S1).

The first two PCs account for relevant variance; therefore, only PC1 and PC2 were retained and interpreted (Fig. 2). The loading plot (Fig. 2A) illustrates the relationships among variables (i.e., quality traits) were two vectors with an angle <90° are positively correlated and two vectors with an angle >90° are not correlated. For instance, the variation in 5-CQA in the secondary heads was most closely aligned to that of Api glr, and variation in DPPH in the primary buds was more strongly correlated to 1,5-diCQA rather than 1,3-diCQA content. Similarly, 3-CQA in the primary heads was more strongly correlated to 5-CQA than to Api glc, whereas 3-CQA in the secondary buds was not correlated to 1,5-diCQA.

Several scientists have demonstrated the effectiveness of PCA plotting in species classification, evaluation of fruit and vegetable quality as well as postharvest studies (Colonna et al., 2016; Finnengan & O'Beirne, 2015; Kyriacou, Soteriou, Rouphael, Siomos, & Gerasopoulos, 2016). This was the case in the current study, since PCA scores separate and categorize treatment populations into four groups, enabling interpretation of results on the basis of all parameters examined. The positive side of PC1 (quadrants 2 and 4) included uncoated and coated 'Romolo' coming from the first and second planting period. The treatments from the upper right quadrant were characterized by high proteins, cations (K^+, Ca^{2+} and Mg^{2+}), TP, ABTS, 5-CQA and Api glr contents especially in the secondary heads. The cluster in the lower right quadrant represents artichoke (mainly primary heads) characterized by high DPPH and ABTS scavenging activities as well as high concentrations of 3-CQA, 1,3-diCQA, 5-CQA, 1,5-diCQA and Api glc (Fig. 2B). Moreover, the negative side of PC1 (quadrants 1 and 3) corresponded to 'Istar' cultivar picked from uncoated and coated plants coming from the first and second planting. The treatments of the upper left quadrant were characterized by high L*, b* in the secondary heads, whereas those of the lower quadrant depicted the treatment of highest dry matter content (Fig. 2B). The results of the PCA may provide the basis for a more in-depth approach to elucidate the effects of genetic variation, microbial inoculation and planting time on the quality of artichoke.

4. Conclusions

The constant pressure on farmers and agro-food industry to produce high quality vegetables with nutraceutical properties represents a strong stimulus for food nutritionists and scientists to propose sustainable tool for the improvement of vegetable quality, in order to meet the consumer demand. In the present study, the configuration of artichoke quality was analyzed in a multifactorial approach accounting for the effects of cultivars and agronomic factors such as planting time and microbial inoculant. The great accumulation of health-promoting compounds in 'Romolo' heads (higher antioxidant capacity and caffeoylquinic acids) fortified by early planting, confers to this seed-propagated cultivar a high value as a component of a healthy diet. Our results also demonstrated that yield and nutritional value in terms of antioxidant activity, total phenolics, caffeoylquinic acids and flavonoids could be enhanced especially with 'Romolo' by coating seeds with a consortium of beneficial fungi like *R. intraradices*, *F. mosseae* and *T. atroviride*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.04.175.

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