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Polyphenol Metabolite Profile of Artichoke Is Modulated by Agronomical Practices and Cooking Method

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ABSTRACT: In this paper artichoke phenolic pattern was characterized using an Orbitrap Exactive Mass Spectrometer at high mass accuracy and conventional HPLC MS/MS. Twenty four phenolic acids and 40 flavonoids were identified, many of them not previously reported in artichoke. Variations in phenolic compounds were investigated in relation to mycorrhization: results showed that inoculation with mycorrhizae greatly influences metabolite profile proving to be a good strategy to enhance the biosynthesis of secondary metabolites in this plant. This practice also caused a different distribution of the main phenolic compounds within head parts.

Both steaming and microwaving cooking treatments caused an increase in antioxidant activity: the lower the initial concentration the higher the effect. A similar trend was observed looking at the phenolic compounds concentration: it increased because of cooking treatments the lower the initial content, the highest the increase. Steamed artichoke showed higher phenols content than microwaved ones.

KEYWORDS: *Cynara cardunculus L. scolymus (L.), mycorrhizal fungi, field location, steaming, microwaving, high-resolution mass spectrometry*

INTRODUCTION

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is a herbaceous perennial plant native to Mediterranean countries. The edible part of the plant is the immature inflorescence (head or capitulum), which is harvested when it is still in rapid growth. It constitutes nearly 35−50% of the fresh weight of the head and consists of the enlarged receptacle and the tender thickened bases of the bracts.[1]

Several studies have described the artichoke's biological actions on humans: the most popular is the hepatoprotection, but also antioxidant, anticarcinogenic, hypocholesterolemic, antiobacterial, anti-HIV, bile-expelling, and diuretic effects have been also reported.[2−4]

These actions has been mainly attributed to artichoke phytochemicals and in particular to the polyphenolic fraction. Caffeic acid derivatives are the main phenolic compounds in artichoke, with a wide range of caffeoylquinic acid derivatives with chlorogenic acid (5-*O*-caffeoylquinic acid) as the most important of these derivatives. Other phenolics such as the flavonoids apigenin and luteolin (both present as glucosides and rutinosides) as well as different cyanidin caffeoylglucoside derivatives have been identified in artichoke tissues.[5−9]

Globe artichoke heads are used worldwide as a fresh, frozen, or canned foodstuff, and they are usually cooked in different ways before consumption. Cooking processes bring about a number of changes in the physical characteristics and chemical composition of all vegetables.[10,11] Steaming and microwaving are popular artichoke cooking methods: they do not require cooking fats, limit the nutritional losses, and have a minimum impact on the organoleptic properties.

From the sustainable production point of view, it is important to consider also artichoke byproducts produced by the processing industry such as leaves, external bracts, and stems. This discarded material accounts for 80−85% of the total biomass, and it can be considered as a source of health-promoting compounds.[12,13] Artichoke leaf extracts have been widely used in herbal medicine as hepatoprotectors and also possess anticarcinogenic, anti-HIV, antioxidative, and diuretic as well as antibacterial properties linked to the presence of flavonoids and phenolic acids.[14,15]

Recent data have shown that mycorrhizal inoculation may represent an efficient and sustainable strategy to enhance plant biosynthesis of secondary metabolites, such as phenolic compounds.[16] Arbuscular mycorrhizal symbioses are mutualistic associations between soilborne fungi and the roots of land plants: they influence primary and secondary metabolism of host plants[17] and induce important changes in physiological mechanisms, leading to the accumulation of secondary metabolites.[18−22]

In this paper, the effects of field location and inoculation with beneficial microorganisms (mycorrhizal fungi and beneficial bacteria) on the profile and concentration of phenolic compounds and on the antioxidant activity of the artichoke extracts were evaluated to optimize agricultural practices aimed at obtaining artichoke tissues with high amounts of polyphenols. Moreover, artichokes from experimental fields and commercial samples were also subjected to steaming and microwaving to investigate cooking effect on health-promoting phytochemicals. Polyphenols quantification was performed also on the leaves and outer bracts with a view to providing useful information considering the possible uses of waste vegetable biomass.

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MATERIALS AND METHODS

Materials. All reagents and solvents of HPLC grade were purchased from Merck (Darmstadt, Germany). 5-*O*-Caffeoylquinic acid and lutein standards were from Sigma (Milano, Italy); 1,3-di-*O*-caffeoylquinic acid standard was from Carl Roth GmbH & Co.

Plant Material, Experimental Site, and Cultural Practices. Plants of 'Romanesco' type cv. C3 were grown in four different fields. The experimental fields were located at Calvi (BN) (41° 4′ N latitude, 14° 51 E longitude, 376 m elevation above sea level), Italy. In fields 1 and 3, the soils were clay loam soils, whereas fields 2 and 4 were characterized by sandy loam soils. In all experimental sites the pH was about 7.0 and the organic matter was always lower than 1%. In all experimental fields, soil was previously cultivated with wheat. At the end of August, soils of different experimental fields were plowed and then disked twice to break up the soil more finely. Micropropagated artichoke plants were transplanted from September 12 to 20, 2011, at a plant density of 8000 plants/ha (1.25 m × 1 m). In all experimental fields, plot size was about 5000 m^2. In experimental fields 3 and 4, part of the micropropagated artichoke plants of 'Romanesco' type cv. C3 were inoculated at transplanting with a commercial inoculum (Aegis Sym Irriga; Italpollina S.p.A., Rivoli Veronese, VE, Italy) containing 700 spores/g of *Glomus intraradices* and 700 spores/g of *Glomus mossae* at a dose of 2 kg ha^{-1}, whereas another part of the plants was inoculated with a commercial inoculum (Endospor Dry Mix; Tecnologías Naturales Internacional, Celaya, Gto., Mexico) containing 132 spores g^{-1} of *G. intraradices* and 2 × 109 CFU/g of beneficial bacteria (*Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus megaterium*, and *Pseudomonas fluorescens*). For the above reasons, three treatments were compared in experimental fields 3 and 4: (1) noninoculated artichoke plants; (2) artichoke plants inoculated with Aegis Sym Irriga; and (3) artichoke plants inoculated with Endospor Dry Mix. Commercial inocula were applied as spore suspension in water directly to the root apparatus of artichoke plantlets at transplanting before the roots were covered with soil. In experimental fields 1, 2, and 4, preplant mineral fertilizers were broadcast (kg/ha; av) and incorporated into the soil at an average rate of 94 kg/ha of N, 51 kg/ha of P, and 95 kg/ha of K, whereas in field 3 manure was broadcast and incorporated at a rate of 40 t/ha. Additional fertilizer (kg/ha; 100 N) was applied at the end of February in experimental fields 1, 2, and 4, whereas 25 kg/ha of N was applied in artichoke field 3. Artichoke plants were sprinkle-irrigated when necessary for 3 weeks after transplantation and drip-irrigated throughout the rest of the season. Crop water needs were determined by a farm manager visually monitoring the soil and the plants. Weeds were controlled with mechanical cultivation and hand hoeing; no pesticide applications were required to control pests and pathogens. Artichokes were harvested at commercial maturity from the beginning of May until the middle of June 2011. They were selected to remove damaged samples and processed on the same day.

In addition, 'Violetto' artichokes and 'Romanesco' artichokes in two different sizes (large and small) were purchased from a local market.

The leaves, external bracts, and edible parts were separated from three plants of each type. Samples were freeze-dried and finely ground.

Cooking Treatments. Samples from field 1, samples from fields 2 and 3 (not inoculated control), and commercial samples were used for cooking treatments. Edible parts of artichokes were quartered and cooked in triplicate. Cooking conditions were optimized by preliminary experiments: the minimum cooking time to reach a similar tenderness for an adequate palatability and taste, according to Italian eating habits, was used.

Steaming treatments were carried out in a steam cooker (Bimby, MOD TM31, Vorwek). Five samples were arranged in a circle (350 g approximately in total) to ensure uniform heating conditions in all samples for each cooking trial. The samples were cooked under atmospheric pressure for 21 min.

Microwaving treatments were carried out in microwave oven (M420 Jet 900W quartz, Whirpool). Five samples were arranged in a circle (350 g approximately in total) inside an airtight container, and 50 mL of water were added. A 900 W power was applied for 5 min.

After cooking, the samples was rapidly cooled on ice, freeze-dried, and used for the following analysis.

Antioxidant Capacity Determinations. One gram of material was extracted by 20 mL of methanol/water (70:30, v/v) and sonicated at room temperature for 30 min. The extraction procedure was repeated twice for each sample. The antioxidant activity of the extracts was determined by using the $ABTS^{\bullet+}$ assay as described by Pellegrini and co-workers.[23] On the same extracts, the total amount of phenolic compounds was measured according to the Folin−Ciocalteu method.[24] In parallel, direct antioxidant capacity determinations were performed by adopting the Quencher procedure,[25] which does not require extraction and allows the determination of the contribution to total antioxidant activity including the insoluble moiety.

Polyphenol Metabolite Profile by Orbitrap High-Resolution Mass Spectrometry (HRMS). One gram of freeze-dried material was extracted with 20 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 Whatman filter paper (Whatman International Ltd., Maidstone, UK), and then used for analysis.

An ultrahigh-performance chromatography (U-HPLC) was performed on a U-HPLC Accela system (Thermo Fisher Scientific, San Jose, CA, USA) consisting of a degasser, a quaternary pump, an autosampler, and a column oven. Chromatographic separation was carried out on a Gemini C18-110A column (150 mm × 2 mm × 5 μm) (Phenomenex, Torrance, CA, USA). The mobile phase composed of a combination of A (0.1% formic acid in water, v/v) and B (0.1% formic acid in acetonitrile, v/v) was used at a flow rate of 200 μL/min 20 °C. After 1 min at 10% B, the linear gradient was from 10 to 90% B at 8 min, held at 90% B to 10 min, back to 10% B at 11 min. The U-HPLC was directly interfaced to an Exactive Orbitrap mass spectrometer (MS) (Thermo Fisher Scientific). The Exactive Orbitrap MS equipped with a heated electrospray interface (HESI) was operated in the negative mode scanning the ions in the m/z range of 65−1000. The resolving power was set to 25000 full width at half-maximum, resulting in a scan time of 1 s. An automatic gain control target was set into high dynamic range, and the maximum injection time was 100 ms. The interface parameters were as follows: the spray voltage was 3.0 kV, the tube lens was at 100 V, the capillary voltage was 15 V, the capillary temperature was 275 °C, and sheath and auxiliary gas flows of 30 and 15 arbitrary units were used, respectively. Three determinations were performed for each sample.

Quantitative Analysis of Main Phenolic Compounds by HPLC-UV and HPLC-MS/MS. One gram of freeze-dried artichoke edible part was extracted as described above.

Chromatographic separation was performed using an HPLC apparatus equipped with two micropumps series 200 (Perkin-Elmer, Shelton, CT, USA), a UV−vis series 200 (Perkin-Elmer) detector set at 330 and 280 nm, and a Prodigy ODS3 100 Å column (250 × 4.6 mm, particle size 5 μm) (Phenomenex). 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), and 90−20% B (3 min), at a constant flow of 0.8 mL/min. The LC flow was split, and 0.2 mL/min was sent to the mass spectrometer. The injection volume was 20 μL.

MS and MS/MS analyses were performed on an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonspray source working in the negative ion mode. The analyses were performed using the following settings: drying gas (air) was heated to 400 °C, capillary voltage (IS) was set at 4000 V, nebulizer gas (air) was set at 12 (arbitrary units), curtain gas (N_2) was set at 14 (arbitrary units), and collision gas (N_2) was set at 4 (arbitrary units).

After peak identification, the phenolics quantification was performed by HPLC as follows: filtered extract (20 μL) was injected into an HPLC (Shimadzu LC 10, Shimadzu, Kyoto, Japan) with a photodiode array detector. Separations were achieved on the same column with the same gradient program. The flow rate was 0.8 mL/min, and chromatograms were recorded at 330 and 280 nm. Monocaffeoylquinic

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Table 1. High-Resolution Mass Spectrometry Identification of Phenolic Acids and Flavonoids Achieved by Orbitrap MS in Artichoke Edible Part

compound	molecular formula	m/z^- theoretical	m/z^- experimental	mass accuracy (ppm)
(a) Phenolic Acids				
chlorogenic acid	$C_{16}H_{18}O_9$	353.08781	353.08783	0.06
dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.11950	515.11945	−0.10
caffeic acid	$C_9H_8O_4$	179.03498	179.03455	−2.40
caffeic acid hexoside	$C_{15}H_{18}O_9$	341.08781	341.08807	0.76
tricaffeoylquinic acid	$C_{34}H_{30}O_{15}$	677.15119	677.15125	0.09
coumaroyl quinic acid	$C_{16}H_{18}0_8$	337.09289	337.09308	0.56
coumaric acid	$C_9H_8O_3$	163.04007	163.03943	−3.93
coumaric acid glucoside	$C_{15}H_{18}O_8$	325.09289	325.09329	1.23
feruloylquinic acid	$C_{17}H_{20}O_9$	367.10346	367.10367	0.57
feruloylquinic acid hexoside	$C_{23}H_{30}O_{14}$	529.15628	529.15625	−0.06
feruloyl-caffeoylquinic acid[a]	$C_{26}H_{26}O_{12}$	529.13515	529.13605	1.70
ferulic acid	$C_{10}H_{10}O_4$	193.05063	193.05034	−1.50
diferuloylgentiobiose[a]	$C_{32}H_{38}O_{17}$	693.20362	693.20343	−0.27
dimethoxybenzoic acid[a]	$C_9H_{10}O_4$	181.05063	181.05005	−3.20
gentisic acid glucoside[a]	$C_{15}H_{20}O_9$	343.10346	343.10403	1.66
hydroxybenzoic acid glucoside[a]	$C_{13}H_{16}O_8$	299.07724	299.07730	0.20
protocatechuic acid glucoside[a]	$C_{13}H_{16}O_9$	315.07216	315.07162	−1.71
syringic acid[b]	$C_9H_{10}O_5$	197.04555	197.04518	−1.88
digalloyl methyl glucose[a]	$C_{21}H_{22}O_{14}$	497.09368	497.09235	−2.68
gallic acid galloyl glucoside[a,b]	$C_{20}H_{20}O_{14}$	483.07803	483.07593	−4.35
gallic acid gallate[a,b]	$C_{14}H_{10}O_9$	321.02521	321.02469	−1.62
sinapic acid[a]	$C_{11}H_{12}O_5$	223.06120	223.06102	−0.81
rosamarinic acid	$C_{18}H_{16}O_8$	359.07724	359.07733	0.25
trihydroxycinnamic acid[a]	$C_9H_8O_5$	195.02990	195.02982	−0.41
(b) Flavonoids				
luteolin	$C_{15}H_{10}O_6$	285.04446	285.04449	0.11
luteolin hexoside	$C_{21}H_{20}O_{11}$	447.09328	447.09372	0.98
luteolin glucuronide	$C_{21}H_{18}O_{12}$	461.07255	461.07291	0.78
luteolin rutinoside	$C_{27}H_{30}O_{15}$	593.15119	593.15125	0.10
luteolin malonyl glucoside	$C_{24}H_{22}O_{14}$	533.09368	533.09387	0.36
luteolin glucuronide glucoside[a]	$C_{27}H_{28}O_{17}$	623.12537	623.12537	0.00
luteolin diglucoside	$C_{27}H_{30}O_{16}$	609.14611	609.14606	−0.08
luteolin apiosyl malonyl glucoside[a,b]	$C_{29}H_{30}O_{18}$	665.13594	665.13782	2.83
luteolin methyl ether diglucoside[a]	$C_{28}H_{32}O_{16}$	623.16176	623.16205	0.47
apigenin	$C_{15}H_{10}O_5$	269.04555	269.04587	1.19
apigenin hexoside	$C_{21}H_{20}O_{10}$	431.09837	431.09857	0.46
apigenin glucuronide	$C_{21}H_{18}O_{11}$	445.07763	445.07797	0.76
apigenin xyloside[a]	$C_{20}H_{18}O_9$	401.08781	401.08786	0.12
apigenin rhamnoside[a]	$C_{21}H_{20}O_9$	415.10346	415.10519	4.17
apigenin diglucoside[a]	$C_{26}H_{28}O_{14}$	563.14063	563.14087	0.43
apigenin methylglucuronide	$C_{22}H_{20}O_{11}$	459.09328	459.09402	1.61
apigenin rhamnoside rutinoside[a,b]	$C_{33}H_{40}O_{18}$	723.21419	723.21472	0.73
apigenin rutinoside caffeate[a,c]	$C_{36}H_{36}O_{17}$	739.18797	739.18768	−0.39
apigenin acetylglucoside[a]	$C_{23}H_{22}O_{11}$	473.10893	473.10919	0.55
apigenin glucosyllactate[a,b]	$C_{24}H_{24}O_{12}$	503.11950	503.11963	0.26
methylapigenin[a]	$C_{16}H_{12}O_5$	283.06120	283.06158	1.34
quercetin hexoside	$C_{21}H_{20}O_{12}$	463.08820	463.08868	1.04
quercetin glucuronide	$C_{21}H_{18}O_{13}$	477.06746	477.06772	0.54
quercetin glucoside glucuronide[a]	$C_{27}H_{28}O_{18}$	639.12029	639.12054	0.39
quercetin diglucuronide a	$C_{27}H_{26}O_{19}$	653.09955	653.09943	−0.18
quercetin galloylglucoside[a]	$C_{28}H_{24}O_{16}$	615.09916	615.10028	1.82
quercetin acetylglucoside	$C_{23}H_{22}O_{13}$	505.09876	505.09937	1.21
quercetin galloylrutinoside[a]	$C_{34}H_{34}O_{20}$	761.15707	761.15729	0.29
quercetin malonylgalactoside[a]	$C_{24}H_{22}O_{15}$	549.08859	549.08881	0.40
dihydroquercetin rhamnoside[a]	$C_{21}H_{22}O_{11}$	449.10893	449.10956	1.40
naringenin hesperioside	$C_{27}H_{32}O_{14}$	579.17193	579.17212	0.33
naringenin hexoside	$C_{21}H_{22}O_{10}$	433.11402	433.11398	−0.09

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Table 1. continued

compound	molecular formula	m/z^- theoretical	m/z^- experimental	mass accuracy (ppm)
(b) Flavonoids				
naringenin rhamnoside[a,b]	$C_{21}H_{22}O_9$	417.11911	417.12018	2.57
naringenin diglucoside[a]	$C_{27}H_{32}O_{15}$	595.16684	595.16528	−2.62
eriodictyol	$C_{15}H_{12}O_6$	287.05611	287.05643	1.11
eriodictyol diglucoside	$C_{27}H_{32}O_{16}$	611.16176	611.16193	0.28
chrysoeriol glucoside	$C_{22}H_{22}O_{11}$	461.10893	461.10968	1.63
myricetin arabinoside[a]	$C_{20}H_{18}O_{12}$	449.07255	449.07257	0.04
myricetin hexoside	$C_{21}H_{20}O_{13}$	479.08311	479.08310	−0.02

[a]Not previously reported in artichoke. [b]Found only in samples from plants inoculated with beneficial microorganisms. [c]Found only in samples from plants not inoculated with beneficial microorganisms.

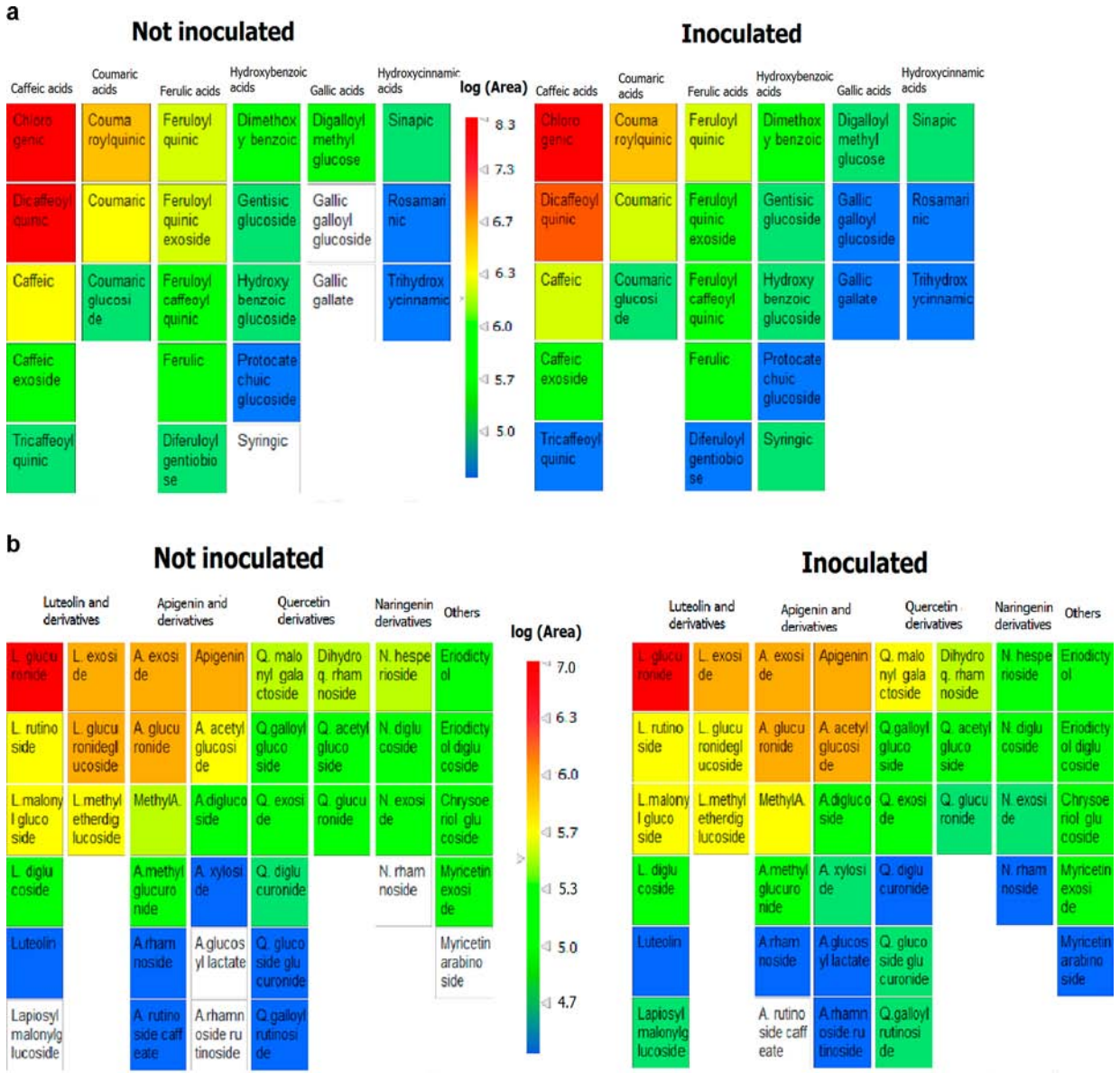

Figure 1. Metabolite profile of the (a) phenolic acid compounds and (b) flavonoid compounds in the edible part of artichoke with and without beneficial microorganism inoculation.

acids were quantified as chlorogenic acid, dicaffeoylquinic acids were quantified as cynarine, and luteolin derivates were quantified as luteolin.

Three injections were performed for each sample.

Statistical Analysis. Differences among samples were determined by analysis of variance and Duncan's multiple-range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Artichoke Polyphenol Metabolite Profile. As shown in Table 1, a total of 24 phenolic acids and 40 flavonoids could be detected using Orbitrap Exactive MS at high mass accuracy

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(<5 ppm). A simple extraction and a fast chromatographic analysis (only 10 min) allowed a complete characterization of the phenolic profile: many compounds that have not been previously reported in artichoke were tentatively identified. The corresponding Figure 1 highlights the metabolite pattern of artichoke edible parts; semiquantification of each tentatively identified compound was based on MS peak areas, and data were reported on a colorimetric scale. As already observed with other analytical techniques,[26,27] the most abundant phenols in artichokes are chlorogenic acid and cynarin (1,5-di-*O*-caffeoylquinic acid). Among flavonoids, only luteolin glucoside is present at significant levels.

Effect of Mycorrhization on the Artichoke Metabolite Profile. As shown in Figure 1, inoculation with beneficial microorganisms greatly influences artichoke metabolite profile. In particular, among phenolic acids, the greatest variations were observed in gallic acid derivatives with a decrease in digalloyl methyl glucose and the appearance of gallic galloyl glucoside and gallic gallate not found in control samples.

Also, the flavonoid metabolite profile of artichoke was significantly modified by inoculation with beneficial microorganisms. It has been suggested that modification of the flavonoid profile in response to biotic stress such as mycorrhizal colonization may be the consequence of a general plant defense response, which is later suppressed.[28] Generally, inoculation of plants results in an overall increase in the production of some new phenolic compounds during the progression of the infection.[29] This evidence was confirmed in artichoke: five flavonoids (namely, luteolin apiosyl malonyl glucoside, apigenin glucosyl lactate, apigenin rhamnoside rutinoside, naringenin rhamnoside, and myricetin arabinoside) were found in inoculated samples and not in control. Among quercetin derivatives, mycorrhization caused increases in glucoside glucuronide and galloyl rutinoside concentrations. On the other hand, corresponding decreases in glucuronide and diglucuronide levels was observed. Similarly, a reduction in luteolin glucuronide glucoside and an increase in apigenin acetylglucoside were also observed in the treated plants.

Quantitative data of the main phenolic compounds showed a different distribution within head parts caused by the two different types of inoculum tested. Results of the quantitative analysis performed by HPLC-UV and MS/MS are summarized in Table 2. In the edible parts of the samples, a percentage decrease in cynarin and a corresponding percentage increase in chlorogenic acid was observed as a result of artichoke plant inoculation with Endospor Dry Mix. On the other hand, an inverse trend was observed in outer bracts: data showed a percentage increase in cynarin and a corresponding percentage decrease in chlorogenic acid using both types of inoculum.

Leaves of the inoculated plant also showed a different phenolic profile with respect to the control ones. According to previous works,[30] the most abundant compound was 5-*O*-caffeoylquinic acid, and it increased (in percentage) in samples with inoculations. 1,5-Di-*O*-caffeoylquinic acid was less abundant in leaves than in heads; however, it increased upon inoculation. Luteolin derivatives are the most typical flavonoids of artichoke leaves,[31] and they showed a percentage decrease in samples with inoculations. These variations were most evident when the inoculation was performed with Endospor Dry Mix, indicating that besides mycorrhizal fungi (*Glomus* spp.) also beneficial bacteria played an active role in changing the phenolic profile of artichoke plants.

Ceccarelli and co-workers[16] studied the effect of different mycorrizal inoculations on antioxidant activity and total phenol

Table 2. Phenolic Total Content (Grams per 100 g Dry Matter) and Phenolic Profile (Each Component Expressed as Percent of Total) in Parts of Artichoke Plants Grown without Inoculation with Beneficial Microorganisms (Ctr), Inoculated with Aegis Sym Irriga (Aeg) and Inoculated with Endospor Dry Mix (End) (Mean Value ± SE)[a]

	phenolic total content	3-*O*-caffeoylquinic acid	4-*O*-caffeoylquinic acid	5-*O*-caffeoylquinic acid	1-*O*-caffeoylquinic acid	coumaroyl quinic acid	feruloyl quinic acid	luteolin 7-*O*-rutinoside	luteolin 7-*O*-glucoside	3,5-di-*O*-caffeoylquinic acid	1,5-di-*O*-caffeoylquinic acid	4,5-di-*O*-caffeoylquinic acid	luteolin-acetyl-hexoside
Edible Part													
Ctr	2.32 ± 0.52 b	0.2 ± 0.06 a	0.3 ± 0.01 a	19.2 ± 3.48 b	nd	0.1 ± 0.05 a	0.2 ± 0.00 a	nd	0.2 ± 0.06 a	nd	79.4 ± 3.42 a	0.3 ± 0.03 a	nd
Aeg	2.41 ± 0.35 b	0.3 ± 0.05 a	0.3 ± 0.00 a	20.9 ± 2.04 b	nd	0.1 ± 0.01 a	0.1 ± 0.01 a	nd	0.2 ± 0.01 a	nd	77.8 ± 1.98 a	0.2 ± 0.03 b	nd
End	4.55 ± 0.23 a	0.3 ± 0.06 a	0.3 ± 0.05 a	26.5 ± 0.19 a	nd	0.1 ± 0.01 a	0.2 ± 0.00 a	nd	0.3 ± 0.01 a	nd	72.2 ± 0.13 b	0.2 ± 0.03 b	nd
Outer Bracts													
Ctr	1.67 ± 0.30 a	1.1 ± 0.39 a	0.6 ± 0.18 b	41.2 ± 10.10 a	0.1 ± 0.02 c	0.4 ± 0.17 a	0.2 ± 0.03 a	nd	0.4 ± 0.09 b	2.1 ± 0.56 a	52.9 ± 11.80 b	0.9 ± 0.35 a	nd
Aeg	1.78 ± 0.30 a	0.6 ± 0.04 ab	0.9 ± 0.03 a	28.5 ± 0.2 b	0.3 ± 0.09 a	0.4 ± 0.07 a	0.2 ± 0.03 a	nd	1.14 ± 0.57 a	1.3 ± 0.08 a	66.4 ± 0.80 a	0.3 ± 0.05 b	nd
End	2.08 ± 0.87 a	0.5 ± 0.04 b	0.5 ± 0.03b	27.1 ± 0.61 b	0.1 ± 0.02 bc	0.3 ± 0.08 a	0.2 ± 0.01 a	nd	0.7 ± 0.01 b	1.7 ± 0.13 a	68.7 ± 0.67 a	0.6 ± 0.02 ab	nd
Leaves													
Ctr	1.01 ± 0.23 b	nd	nd	52.8 ± 0.53 c	0.9 ± 0.03 a	1.6 ± 0.01 a	0.8 ± 0.02 b	16.0 ± 0.22 a	10.4 ± 0.15 a	nd	8.3 ± 0.14 b	nd	9.0 ± 0.13 a
Aeg	0.47 ± 0.14 c	nd	nd	59.1 ± 1.84 b	0.6 ± 0.01 c	1.0 ± 0.03 b	0.9 ± 0.03 a	8.4 ± 2.73 b	7.5 ± 0.25 b	nd	16.3 ± 0.44 a	nd	6.3 ± 0.24 b
End	1.24 ± 0.82 a	nd	nd	68.4 ± 0.17 a	0.7 ± 0.01 b	0.5 ± 0.00 c	0.6 ± 0.01 c	5.4 ± 0.06 b	4.3 ± 0.04 c	nd	16.6 ± 0.30 a	nd	3.5 ± 0.03 c

[a]Different letters within the same group indicate significant differences at $P < 0.05$.

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content in artichoke bracts and leaves. They reported a large increase in both parameters, but this phenomenon was strongly dependent on the mycorrizal fungal species used.

Our findings were partially in agreement with those of that study; in fact, an increase in antioxidant activity was observed after inoculation (6.81 vs 4.04 mmol Trolox equiv/100 g dry matter for ABTS method; 38.89 vs 19.68 mmol Trolox equiv/100 g dry matter for Quencher method) in artichoke leaves.

In outer bracts, ABTS assay showed increased values in samples inoculated with Endospor Dry Mix (6.63 vs 5.41 mmol Trolox equiv/100 g dry matter in the control). In the edible part, antioxidant capacity did not vary among samples; however, an increase of phenols content was observed with Endospor Dry Mix inoculation (0.96 vs 0.76 g of gallic acid equiv/100 g dry matter in the control without inoculation).

Effect of Field Location on Artichoke Metabolite Profile. Table 3 shows production data of four different experimental fields. Best total yield and highest head weight were observed in field 3, whereas field 2 gave the worst performances. Artichoke plants grown in fields 1 and 4 showed the highest head numbers. In experimental fields 3 and 4, no significant differences in total artichoke yields, head numbers, and head mean weights were recorded among artichoke plants not inoculated or inoculated with Aegis Sym Irriga or inoculated with Endospor Dry Mix (data not shown).

Table 3. Total Yield, Head Number, and Head Mean Weight of Artichoke in Different Experimental Fields[a]

exptl field	yield (t/ha)	head no. (/plant)	head mean wt (g/head)
1	7.5 ab	7.0 a	131.4 ab
2	4.5 b	4.6 b	121.0 b
3	8.3 a	5.8 ab	179.6 a
4	5.7 ab	6.3 a	114.3 b

[a]Different letters within the same group indicate significant differences at $P < 0.05$.

To evaluate the effect of field location on antioxidants, only control samples without inoculation were taken into account for fields 3 and 4. Several authors studied antioxidant activity and phenols content in different artichoke tissues; however, this is the first paper reporting data about field location effect. Data, summarized in Table 4, showed that the edible part had a higher antioxidant activity than external bracts; moreover, significant differences among location of the fields were found. The total antioxidant activity measured by the conventional ABTS assay on the water−methanol extracts was within the range reported in the literature.[32−34] As expected, the Quencher method performed without extraction on the solid freeze-dried material showed higher values than those observed in the soluble fraction, thus suggesting a significant contribution of phenolic compounds bound to the polysaccharide moiety.[35] However, a positive correlation between the two methods was found. This was not the case when the antioxidant activity of outer bracts and in leaves was considered: in this case no correlation between soluble and insoluble antioxidant activity was found. These data could be explained considering that the fiber content is higher in wastes than in the edible parts;[36] therefore, the extractability of antioxidant compounds is lower in the waste material than in the whole sample, leading to an underestimation of the antioxidant capability when it was measured by the conventional ABTS procedure.

The data of Table 4 show that the antioxidant activity of artichoke tissue was dependent on the location field, confirming previous findings on other crops and the strict correlation with total phenolic compound concentration.[37−41] The absolute values of antioxidant activity and phenolic compounds were within the ranges reported in the literature, with the highest values in the edible part.[42−45] It is worth noting that there is a significant interaction between field location and antioxidant activity (AA) and total polyphenol content of artichoke tissues. The edible part of artichoke heads harvested in fields 1 and 3 showed a higher AA (Quencher method) than in those harvested from fields 2 and 4. Moreover, the value of antioxidant activity measured on the soluble fraction gave the highest antioxidant activity in the edible part of artichoke harvested in field 1, whereas artichoke heads harvested from field 4 gave the lowest value. Intermediate values were observed in the edible part of artichoke heads harvested from fields 2 and 3. A similar trend was recorded for total polyphenol content in

Table 4. Antioxidant Activity (AA) and Total Polyphenol Content in Parts of Artichoke Plants Grown in Different Experimental Fields (Mean Value ± SE)[a]

	AA (mmol Trolox equiv/100 g dm)		
field	Quencher method	ABTS method	total polyphenol content (g gallic acid equiv/100 g dm)
		Edible Part	
1	70.23 ± 5.64 a	14.62 ± 1.62 a	1.01 ± 0.10 ab
2	45.08 ± 3.66 b	10.42 ± 1.17 bc	0.77 ± 0.03 bc
3	56.93 ± 4.84 ab	11.32 ± 0.65 b	0.84 ± 0.04 bc
4	49.52 ± 4.31 b	8.35 ± 0.58 c	0.76 ± 0.06 c
		Outer Bracts	
1	21.21 ± 3.93 a	5.18 ± 0.72 ab	0.30 ± 0.03 ab
2	16.99 ± 3.19 a	4.09 ± 0.94 b	0.24 ± 0.05 b
3	23.13 ± 2.13 a	6.02 ± 0.23 a	0.33 ± 0.01 a
4	23.86 ± 1.96 a	5.74 ± 0.30 a	0.33 ± 0.02 a
		Leaves	
1	30.30 ± 1.52 bc	8.33 ± 0.63 a	0.38 ± 0.06 a
2	47.08 ± 1.25 a	5.80 ± 0.19 b	0.36 ± 0.09 a
3	31.55 ± 3.37 b	5.78 ± 0.54 b	0.27 ± 0.02 a
4	23.88 ± 2.22 c	4.51 ± 0.43 b	0.27 ± 0.02 a

[a]Different letters within the same group indicate significant differences at $P < 0.05$.

Table 5. Antioxidant Activity and Total Polyphenol Content in Artichokes Taken from the Market (Romanesco Large Size, Romanesco Small Size, and Violetto) and Harvested in Experimental Fields 1, 2, and 3 (Mean Value ± SE)[a]

		AA (mmol Trolox equiv/100 g dm)		total polyphenol content (g gallic acid equiv/100 g dm)
variety	cooking treatment	Quencher method	ABTS method	Folin−Ciocalteu method
Romanesco (large)	raw	64.66 ± 0.37 b	23.59 ± 0.77 b	1.25 ± 0.08 ab
	steaming	69.32 ± 0.67 a	23.57 ± 0.38 b	1.45 ± 0.00 a
	microwaving	62.94 ± 1.05 b	36.31 ± 0.19 a	1.08 ± 0.04 b
Romanesco (small)	raw	62.36 ± 7.32 a	31.19 ± 1.30 a	1.30 ± 0.02 b
	steaming	75.67 ± 13.43 a	30.95 ± 0.38 a	1.44 ± 0.02 a
	microwaving	58.11 ± 1.00 a	27.56 ± 0.38 a	1.46 ± 0.07 a
Violetto	raw	65.22 ± 7.97 a	27.70 ± 1.59 a	1.30 ± 0.01a
	steaming	60.42 ± 1.54 a	29.19 ± 0.91 a	1.07 ± 0.03 b
	microwaving	61.12 ± 2.59 a	30.24 ± 1.01 a	1.30 ± 0.05 a
field 1	raw	84.59 ± 4.32 a	21.72 ± 0.30 c	1.06 ± 0.01 a
	steaming	91.75 ± 3.41 a	24.81 ± 0.34 b	1.08 ± 0.04 a
	microwaving	88.02 ± 3.48 a	31.45 ± 0.06 a	1.01 ± 0.01 a
field 2	raw	42.83 ± 3.13 b	10.01 ± 0.09 c	0.48 ± 0.00 b
	steaming	103.71 ± 13.15 a	33.14 ± 0.84 a	1.35 ± 0.012 a
	microwaving	68.49 ± 4.30 ab	23.43 ± 0.28 b	1.19 ± 0.01 a
field 3	raw	79.34 ± 8.13 a	26.16 ± 0.05 b	1.31 ± 0.02 a
	steaming	105.92 ± 9.19 a	32.51 ± 0.14 a	1.48 ± 0.01 a
	microwaving	77.07 ± 2.58 a	34.49 ± 0.79 a	1.44 ± 0.13 a

[a]Different letters within the same group indicate significant differences at $P < 0.05$.

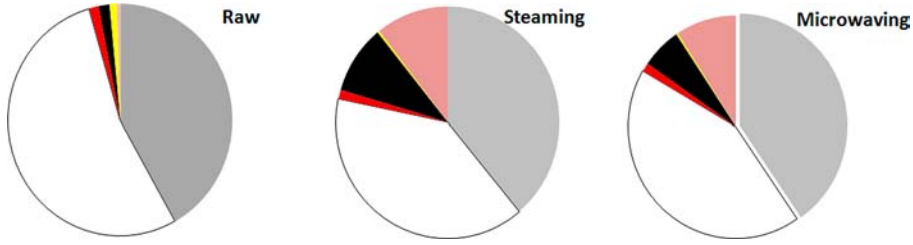

Figure 2. Percentage composition of phenolic fraction in raw, steamed, and microwaved artichoke (mean values). White, 1,5-di-*O*-caffeoylquinic; gray, 5-*O*-caffeoylquinic acid; black, 4-*O*-caffeoylquinic acid + 1-*O*-caffeoylquinic acid; pink, 4,5-di-*O*-caffeoylquinic; red, 3-*O*-caffeoylquinic acid; yellow, sum of minor compounds.

the edible part of artichoke heads. Antioxidant activity (Quencher method) was higher in leaves harvested from fields 1, 2, and 3 than in those from field 4, whereas the highest AA was recorded in leaves harvested from field 1 when the ABTS method of AA analysis was used. The above findings may be related to the different characteristics of the soils in the experimental fields. The high content of clay in field 1 may have increased the antioxidant activity of the edible part and leaves of artichokes as a result of the great root availability of macrocations. Fanasca and co-workers[46] found that antioxidant activity and caffeic acid were enhanced in tomato fruits when Mg availability was increased in the root zone.

Cooking Treatments. Data shown in Table 5 demonstrate that different cooking treatments caused an increase in total antioxidant activity. This trend was confirmed in almost all of our samples, with minor differences between the two methods, but not in artichokes having the highest antioxidant activity in the raw material. Differences between steaming and microwaving were not always significant, although interactions between cooking methods and types of artichoke were found in some cases. Interestingly, it can be observed that the lower the initial polyphenol content, the higher the increase caused by the cooking treatments. With few exceptions, steamed artichokes showed higher phenol content than microwaved ones (Table 5).

Ferracane and co-workers[47] ascribed this increase in total phenolics to the increase of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acid. In this study, we observed this phenomenon only in artichokes with a low concentration of total phenolics, and again it can be mainly attributed to the increase in the concentration of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acid (fields 1 and 2). Similar trends were also observed for luteolin-7-rutinoside and luteolin-7-glucoside, whereas cooking caused a decrease of the concentrations of coumarylquinic and feruloylquinic acids.

In Figure 2 the percentage composition of the various phenolic compounds as influenced by cooking procedure is reported: the main phenomenon is the reduction of 5-*O*-caffeoylquinic concentration. According to Slanina and co-workers,[48] artichoke heat treatment caused the intramolecular transesterification of caffeoylquinic acids, resulting in the increase of 4-*O*-caffeoylquinic

and 1-*O*-caffeoylquinic acids after cooking. The data also suggest that in the same manner 1,5-di-*O*-caffeoylquinic acid was converted in 4,5-di-*O*-caffeoylquinic acid. Both phenomena were more evident in steamed artichokes than in microwaved artichokes.

A percentage reduction of all the minor phenolic compounds was observed in cooked artichokes, also in agreement with the previous study.[47]

In conclusion, data confirmed that artichoke is a very good source of bioactive phytochemicals and that agronomical practices as well as cooking treatments can deeply influence the concentration and tissue distribution of the various phytochemicals. Field location can significantly influence antioxidant activity and phenol content in each part of the plant. For this reason, depending on the cultivation area, artichokes might be positioned on the market stressing their nutraceutical potential or used for the extraction of health-promoting substances, in particular from nonedible parts of the plant. Inoculation of artichoke transplant with beneficial microorganisms can change plant secondary metabolism, determining quantitative and qualitative variation of the phenolic pathway. In this respect it can be important to select the right type of inoculation depending on the artichokes' final intended use.

Finally, cooking treatments can maximize the bioaccessibility of artichoke phytochemicals, particularly when extractability from the raw tissue is very low. The data on the cooking effects emphasize the importance of selecting the right type of starting material depending on the artichokes' final destination.

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Notes

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REFERENCES

(1) Bianco, V. V. Carciofo. In *Orticoltura*; Bianco, V. V., Pimpini, F., Eds.; Patron Ed.e: Bologna, Italy, 1990; pp 209−251.

(2) Kukić, J.; Popović, V.; Petrović, S.; Mucaji, P.; Ćirić, A.; Stojković, D.; Sokovic, M. Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. *Food Chem.* **2008**, *107*, 861−868.

(3) Lattanzio, V.; Kroon, P. A.; Linsalata, V.; Cardinali, A. Globe artichoke: a functional food and source of nutraceutical ingredients. *J. Funct. Foods* **2009**, *1*, 131−144.

(4) Shen, Q.; Dai, Z.; Lu, Y. Rapid determination of caffeoylquinic acid derivatives in *Cynara scolymus* L. by ultra-fast liquid chromatography/tandem mass spectrometry based on a fused core C column. *J. Sep. Sci.* **2010**, *33*, 3152−3158.

(5) Aubert, S.; Foury, C. In *Studi sul Carciofo*; Marzi, V., Lattanzio, V., Eds.; Industrie Grafiche Laterza: Bari, Italy, 1981; pp 57−76.

(6) Lattanzio, V. Attuali conoscenze sui polifenoli del carciofo In *Studi sul Carciofo*; Marzi, V., Lattanzio, V., Eds.; Industrie Grafiche Laterza: Bari, Italy, 1981; pp 13−32.

(7) Lattanzio, V.; Van Sumere, C. F. Changes in phenolic compounds during the development and cold storage of artichoke (*Cynara scolymus* L.) heads. *Food Chem.* **1987**, *24*, 37−50.

(8) Lattanzio, V.; Linsalata, V.; Palmieri, S.; Van Sumere, C. F. The beneficial effect of citric and ascorbic acid on the phenolic browning reaction in stored artichoke (*Cynara scolymus* L.) heads. *Food Chem.* **1989**, *33*, 93−106.

(9) Lattanzio, V.; Cardinali, A.; Di Venere, D.; Linsalata, V.; Palmieri, S. Browning phenomena in stored artichoke (*Cynara scolymus* L.) heads: enzymic or chemical reactions? *Food Chem.* **1994**, *50*, 1−7.

(10) Rehman, Z. U.; Islam, M.; Shah, W. H. Effect of microwave and conventional cooking on insoluble dietary fibre components of vegetables. *Food Chem.* **2003**, *80*, 237−240.

(11) Miglio, C.; Chiavaro, E.; Visconti, A.; Fogliano, V.; Pellegrini, N. Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *J. Agric. Food Chem.* **2008**, *56*, 139−147.

(12) Llorach, R.; Espin, J. C.; Tomas-Barberan, F. A.; Ferreres, F. Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *J. Agric. Food Chem.* **2002**, *50*, 3458−3464.

(13) Lattanzio, V.; Cicco, N.; Linsalata, V. Antioxidant activities of artichoke phenolics. *Acta Hortic.* **2005**, *681*, 421−428.

(14) Gebhart, R. Antioxidative and protective properties of extracts from leaves of the artichoke against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* **1997**, *144*, 279−286.

(15) Coon, J. S. T.; Ernst, E. Herbs for serum cholesterol reduction: a systematic view. *J. Fam. Pract.* **2003**, *52*, 468−478.

(16) Ceccarelli, N.; Curadi, M.; Martelloni, L.; Sbrana, C.; Picciarelli, P.; Giovannetti, M. Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leale and flower heads two years after field transplant. *Plant Soil* **2010**, *335*, 311−323.

(17) Schliemann, W.; Ammer, C.; Strack, D. Metabolic profiling of mycorrhizal roots of Medicago truncatula. *Phytochemistry* **2008**, *69*, 112−146.

(18) Walter, M. H.; Fester, T.; Strack, D. Arbuscular mycorrhizal fungi induce the non-mevalonate methylerythriol phosphate pathway of isoprenoid biosynthesis correlated with accumulation of the "yellow pigment" and other apocarotenoids. *Plant J.* **2000**, *21*, 571−578.

(19) Lambais, M. R.; Rios-Ruiz, W. F.; Andrade, R. M. Antioxidant responses in bean (*Phaseolus vulgaris*) roots colonized by arbuscular mycorrhizal fungi. *New Phytol.* **2003**, *160*, 421−428.

(20) Fester, T.; Wray, V.; Nimtz, M.; Strack, D. Is stimulation of carotenoid biosynthesis in arbuscular mycorrhizal roots a general phenomenon? *Phytochemistry* **2005**, *66*, 1781−1786.

(21) Marulanda, A.; Porcel, R.; Barea, J. M.; Azcon, R. Drought tolerance and antioxidant activities in lavander plants colonized by native drought-tolerant of drught-sensitive *Glomus* species. *Microb. Ecol.* **2007**, *54*, 543−552.

(22) Toussaint, J. P.; Smith, F. A.; Smith, S. E. Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza* **2007**, *17*, 291−297.

(23) Pellegrini, N.; Re, R.; Yang, M.; Rice-Evans, C. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,21-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid radical cation decolorization assay. *Methods Enzymol.* **1999**, *299*, 379−389.

(24) Box, J. D. Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511−525.

(25) Gokmen, V.; Serpen, A.; Fogliano, V. Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. *Trends Food Sci. Technol.* **2009**, *20*, 278−288.

(26) Sanchez-Rabaneda, F.; Jauregui, F. O.; Lamuela-Raventos, R. M.; Bastida, J.; Viladomat, F.; Codina, C. Identification of phenolic compounds in artichoke waste by high performance liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2003**, *1008*, 57−72.

(27) Moglia, A.; Lantieri, S.; Comino, C.; Acquadro, A.; Vos, R.; Beekwilder, L. Stress-induced biosynthesis of dicaffeoylquinic acids in globe artichoke. *J. Agric. Food Chem.* **2008**, *56*, 8641−8649.
(28) Volpin, H.; Elland, Y.; Okon, Y.; Kapulnik, Y. A vesicular arbuscular mycorrhizal fungus (*Glomus intraradix*) induces a defence response in alfalfa roots. *Plant Physiol.* **1994**, *104*, 683−689.
(29) Devi, M. C.; Reddy, M. N. Phenolic acid metabolism of groundnut (*Arachis hypogaea* L.) plants inoculated with VAM fungus and *Rhizobium*. *Plant Growth Regul.* **2002**, *37*, 151−156.
(30) Colla, G.; Rouphael, Y.; Cardarelli, M.; Svecova, E.; Rea, E.; Lucini, L. Effects of saline stress on mineral composition, phenolic acids and flavonoids in leaf of artichoke and cardoon genotypes grown in floating system. *J. Sci. Food Agric.* **2012**, *93*, 1119−1127.
(31) Negro, D.; Montesano, V.; Grieco, S.; Crupi, P.; Sarli, G.; De Lisi, A.; Sonnante, G. Polyphenol compounds in artichoke plant tissues and varieties. *J. Food Sci.* **2012**, *77*, 244−252.
(32) Gouveia, S. C.; Castilho, P. C. Phenolic composition and antioxidant capacity of cultivated artichoke, Madeira cardoon and artichoke-based dietary supplements. *Food Res. Int.* **2012**, *48*, 712−724.
(33) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812−2819.
(34) Cefola, M.; D'Antuono, I.; Pace, B.; Calabrese, N.; Carito, A.; Linsalata, V.; Cardinali, A. Biochemical relationships and browning index for assessing the storage suitability of artichoke genotypes. *Food Res. Int.* **2012**, *48*, 397−403.
(35) Serpen, A.; Capuano, E.; Fogliano, V.; Gokmen, V. A new procedure to measure the antioxidant activity of insoluble food components. *J. Agric. Food Chem.* **2007**, *55*, 7676−7681.
(36) Femenia, A.; Robertson, J. A.; Waldron, K. W.; Selvendran, R. Cauliflower (*Brassica oleracea* L), globe artichoke (*Cynara scolymus*) and chicory witloof (*Cichorium intybus*) processing by-products as sources of dietary fibre. *J. Sci. Food Agric.* **1998**, 511−518.
(37) Mpofu, A.; Sapirstein, H. D.; Beta, T. J. Genotype and environmental variation in phenolic content, phenolic acid composition and antioxidant activity of hard spring wheat. *J. Agric. Food Chem.* **2006**, *54*, 1265−1270.
(38) Yu, L.; Perret, J.; Harris, M.; Haley, S. Antioxidant properties of bran extracts from 'Akron' wheat grown at different locations. *J. Agric. Food Chem.* **2003**, *51*, 1566−1570.
(39) Lombardo, S.; Pandino, G.; Ierna, G. Variation of polyphenols in a germplasm collection of globe artichoke. *Food Res. Int.* **2012**, *46*, 544−551.
(40) Connor, A. M.; Luby, J. J.; Tong, C. B. S. Genotypic and environmental variation in antioxidant activity, total phenolic content and anthocyanin content among blueberry cultivars. *J. Am. Soc. Hortic. Sci.* **2002**, *127*, 89−97.
(41) Emmons, C. L.; Peterson, M. D. Antioxidant activity and phenolic content of oat as affected by cultivar and location. *Crop Sci.* **2001**, *41*, 1676−1681.
(42) Fratianni, F.; Tucci, M.; De Palma, M.; Pepe, R.; Nazzaro, F. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). *Food Chem.* **2007**, 1282−1286.
(43) Romani, A.; Pinelli, P.; Cantini, C.; Cimato, C.; Heimler, D. Characterization of Violetto di Toscana, a typical Italian variety of artichoke (*Cynara scolymus* L.). *Food Chem.* **2006**, *95*, 221−225.
(44) Pandino, G.; Lombardo, S.; Mauromicale, G.; Williamson, G. Profile of polyphenols and phenolic acids in bracts and receptacles of globe artichoke (*Cynara cardunculus* var. *scolymus*) germplasm. *J. Food Compos. Anal.* **2011**, *24*, 148−153.
(45) Lombardo, S.; Pandino, G.; Mauro, R.; Mauromicale, G. Polyphenol content in globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] as affected by genotype, head part and harvest time. In *Proceedings of the 24th International Conference on Polyphenols*, 2008; pp 563−564.
(46) Fanasca, S.; Colla, G.; Maiani, G.; Venneria, E.; Rouphael, Y.; Azzini, E.; Saccardo, F. Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition. *J. Agric. Food Chem.* **2006**, *54*, 4319−4325.
(47) Ferracane, R.; Pellegrini, N.; Visconti, A.; Graziani, G.; Chiavaro, E.; Miglio, C.; Fogliano, V. Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. *J. Agric. Food Chem.* **2008**, *56*, 8601−8608.
(48) Slanina, J.; Taborska, E.; Bochorakova, H.; Slaninova, I.; Humpa, O.; Robinson, W. E., Jr.; Schrame, K. H. New and facile method of preparation of the anti-HIV-1 agent, 1,3-dicaffeoylquinic acid. *Tetrahedron Lett.* **2001**, *42*, 3383−3385.