Nutrient Solution Concentration Affects Growth, Mineral Composition, Phenolic Acids, and Flavonoids in Leaves of Artichoke and Cardoon

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Abstract. A greenhouse experiment was conducted in Summer and Fall 2011 at the experimental farm of Tuscia University, central Italy, to study the effect of nutrient solution concentration (4, 20, 36, 52, or 68 mequiv·L⁻¹) on biomass production, mineral composition, and concentrations of the major polyphenols in ‘Romolo’ artichoke and ‘Bianco Avorio’ cardoon grown in a floating system. Leaf dry biomass, leaf number, and macromolecular concentrations (nitrogen, potassium, calcium, and magnesium) of artichoke and cardoon increased in response to an increase in the nutrient solution concentration, whereas an opposite trend was observed for the total polyphenols, phenolic acids, and flavonoids (chlorogenic acid, cynarin, caffeic acid, and the flavonoid luteolin). Artichoke and cardoon gave maximum biomass production and leaf number at 45 and 54 mequiv·L⁻¹, respectively. Cardoon showed higher biomass and leaf number (average 1.13 kg·plant⁻¹ and 14.0 n./plant, respectively) than those observed in artichoke (average 1.07 kg·plant⁻¹ and 12.7 n./plant, respectively). The chlorogenic acid, cynarin, caffeic acid, and luteolin concentrations were higher by 204%, 462%, 580%, and 445% in cardoon leaf tissue than in that of artichoke. An improvement of leaf quality (total polyphenols, phenolic acids, and flavonoids) was obtained at the expense of leaf yield through the use of lower fertilizer concentrations in the nutrient solution.

Artichoke (Cynara cardunculus L.) is a complex species, belonging to the Asteraceae family, and native to the Mediterranean Basin. According to Rottenberg and Zohary (1996), it consists of the globe artichoke [var. scolymus (L.) Fiori], the cultivated cardoon (var. altiss DC.), and the wild cardoon, also called an artichoke thistle [var. sylvestris (Lamk) Fiori]. Today artichokes are widely grown around the world with Italy being the leading producer (≈474 kt per year) followed by Spain and France (≈215 and 55 kt per year, respectively) (FAO Statistical Database, 2011). The heads of artichokes, immature flowers, are used as a vegetable all over the world. The leaves of artichokes have been widely used in herbal medicine as a choleric since ancient times (Bruneton, 1995).

The chemical components of artichoke leaves have been studied extensively and have been found to be a rich source of polyphenolic compounds with mono- and dicaffeoylquinic acids and flavonoids as the major chemical components (Dranik et al., 1996; Wagenbreth, 1996). In various pharmacological test systems, artichoke leaf extracts have shown antibacterial, antioxidative, anti-HIV, bile-expelling, hepatoprotective, urinative, and choleric activities as well as the ability to inhibit cholesterol biosynthesis and low-density lipoprotein oxidation (Brown and Rice-Evans, 1998; Dranik et al., 1996; Kraft, 1997; Martino et al., 1999). Leaf extracts have been reported to show antioxidative and protective properties against hydroperoxide-induced oxidative stress in cultured rat hepatocytes (Gebhardt, 1997), to protect lipoprotein from oxidation in vitro (Brown and Rice-Evans, 1998), to inhibit hemolysis induced by hydrogen peroxide, and to inhibit oxidative stress when human cells are stimulated with agents that generate reactive oxygen species: hydrogen, peroxide, phorbol-12-myristate-13-acetate, and N-formyl-methionyl-leucyl-phenylalanine (Perez-Garcia et al., 2000).

The chemical composition and quality of vegetables can be affected by many pre- and postharvest factors (Kader, 2008). The management of mineral nutrition is a key preharvest factor that determines biomass, yield, and quality of vegetable crops. Soilless culture represents an interesting tool, because it permits precise control of plant nutrition (Resh, 1997) leading to the manipulation of target compounds in plants such as phytochemicals and antioxidants. Research has established optimal ranges of total ionic concentration in the nutrient solution for the production of floricultural greenhouse crops (James and van Iersel, 2001; Mak and Yeh, 2001; Rouphael et al., 2008; Van Iersel, 1999). However, there is little information (Falovo et al., 2009a, 2009b; Rouphael and Colla, 2009) about the optimal fertilizer concentrations for producing high-quality vegetables crops in soilless cultures.

In Italy, the artichoke for leaf extract production is traditionally cultivated in soil under high-density plantation and is moved three times per year. Despite a relatively large interest in artichoke biomass production, advanced cultivation techniques such as a floating system have not been sufficiently considered for improving the biomass and quality of this species. The floating system, widely used for leafy vegetable production, can provide an alternative system to the traditional field cultivation of artichoke leaf, to obtain higher productivity and biomass quality, to standardize and control cultural practices, and to provide better water and nutrient supply with high environmental benefits (Falovo et al., 2009a, 2009b).

Few studies (Fratamoni et al., 2007; Lombardo et al., 2010; Wang et al., 2003) have been conducted to investigate the concentration of individual phenolic compounds in relation to genotype of artichoke and cardoon. Moreover, these studies have been focused especially on vegetatively propagated...
germplasm without considering the seeded propagated varieties. Moreover, there is lack of information on the suitability of the different genotypes to be grown in a floating system.

The objective of this study was to determine the effect of nutrient solution concentration (4, 20, 36, 52, and 68 meq/L) on biomass production, mineral composition, and the major polyphenols concentration in 'Romolo' artichoke and 'Bianco Avorio' cardoon cultivars grown in a floating system.

Materials and Methods

Plant material and growth conditions

The experiment was conducted in the Summer and Fall 2011 in a 300-m² polyethylene greenhouse situated on the experimental farm of Tuscia University, central Italy (lat. 42°25′ N, long. 12°08′ E, alt. 310 m above sea level). Plants were grown under natural light conditions. The greenhouse was maintained at daily temperatures between 12 and 30 °C and day/night relative humidity of 55/85%.

Seeds of artichoke (C. cardunculus L. subsp. scolymus (L.) Hegi) cv. Romolo (La Semiorto Sementi, Lavorate di Sarno, Italy) and cardoon (C. cardunculus L. var. altillus DC.) cv. Bianco Avorio (La Semiorto Sementi) were sown on 6 Sept. 2011 into a floating raft growing system. The floating raft system consisted of polystyrene plug trays floating in plastic tanks with a constant volume of nutrient solution, which was continuously aerated with an air compressor to maintain the oxygen concentration above 6.0 mg L⁻¹. All nutrient solutions were prepared using deionized water.

Nutrient solution management

In all treatments the nutrient solution was changed once a week to keep nutrient levels in solution close to the initial concentrations. Consequently, the variation of the electrical conductivity and pH values of the nutrient solution were never greater than 5% of the initial values.

Data collection and analysis

Plant growth measurements. The leaves of artichoke and cardoon from the central part of each plot were harvested five times during the growing cycle (23, 37, 51, 70, and 91 d after sowing). At each harvest, the leaf number per plot was recorded and the leaf tissues were dried in a forced-air oven at 60 °C for 72 h for biomass determination. The leaf dry biomass was expressed in kg m⁻². For each plot, the leaf dry biomass harvested during the growing cycle was mixed together for further mineral and quality analysis.

Mineral analysis. The dried leaf tissues were ground in a Wiley mill to pass through a 20-mesh screen and then 0.5 g of the dried plant tissues were analyzed for the following macro- and micronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B). N concentration was determined after mineralization with sulfuric acid by “Kjeldahl method” (Bremner, 1965). P, K, Ca, Mg, Fe, Mn, Zn, Cu, and B concentrations were determined by dry ashing at 400 °C for 24 h, dissolving the ash in 1:25 HCl, and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy) (Karla, 1998).

Chemicals and standards. Pure caffeoylquinic acids and flavonoids to be determined were purchased as certified reference materials from Sigma (St. Louis, MO). Individual stock solutions (500 μg mL⁻¹) were prepared in pure methanol with sonication and then stored at −30 °C for up to 5 d. Working standard solutions were prepared daily, in amber glass flasks, by diluting combined aliquots of the stock solutions in the high-performance liquid chromatography (HPLC) mobile phase.

All the solvents and reagents used to prepare standard solutions and to extract polyphenols (methanol, ethyl acetate, formic acid) were analytical-grade reagents, whereas methyl alcohol for chromatographic analyses was HPLC-grade; all the solvents were obtained from Merck (Darmstadt, Germany). Formic acid to be used as a mobile phase additive was of HPLC-grade.

Statistical analysis

Analysis of variance of the data were calculated using the software package, SPSS "for Windows, 2001 (SPSS Inc., Chicago, IL). Orthogonal polynomial contrasts were used to compare nutrient solution concentration effects on selected parameters (Gomez and Gomez, 1983). Regression analyses were conducted to identify relationships between total phenolic compounds in each extract was then determined by the Folin-Ciocalteu method. Aliquots (250 μL) of each artichoke and cardoon extract were mixed with 1 mL of pure water, 500 μL of Folin-Ciocalteu reagent (Sigma), and 1.5 mL of 1 M sodium carbonate. Absorbance was recorded at 765 nm after 40 min at 20 °C in the dark. A calibration curve was prepared using aliquots of gallic acid ethanolic solutions and the results were expressed as gallic acid equivalents (milligrams gallic acid per gram of dry weight).

Determination of target polyphenols. Both the target caffeoylquinic acids (cynaric, caffee, chlorogenic, and ferulic acid) and the flavonoids (apigenin, luteolin, silybin, and miricetin) were determined by liquid chromatography followed by tandem mass spectrometry with an electrospray ionization source (LC-ESI/MS/MS). A 1200 series liquid chromatograph system equipped with a quaternary pump, electrospray ionization system, and coupled to a G6410A triple quadrupole mass spectrometer detector (all from Agilent Technologies, Santa Clara, CA) was used. The instrument was operating in the positive mode, and each target analyte was quantified by the external standard method.

The plant material (1 g) was extracted with Ultra-Turrax in 10 + 5 + 5 mL of a methanol + ethyl acetate (4:1 v/v) mixture. After centrifugation (503 g for 8 min), the extract was diluted with the mobile phase, filtered through a 0.45-μm cellulose membrane, and then analyzed by reversed phase LC/MS/MS using a Kinetex C18 column (75 × 2.1 mm, 2.6 μm) from Phenomenex (Torrance, CA). The solvent system used water (Solvent A) and methanol (Solvent B); formic acid was added to both mobile phases at the concentration of 0.1% v/v. Data handling was performed by the MassHunter software under Multiple Reaction Monitoring (MRM) acquisition.

The gradient was designed to decrease Solvent A from 45% at 0 min to 40% at 3 min, held until 4 min, and a post-run time of 2 min was adopted. The flow was 0.22 μL min⁻¹, the injection volume was 5 μL, and the drying gas was N at the temperature of 340 °C and fluxing at 9 L min⁻¹. Capillary voltage was set to 4000 V and the nebulizer was operating at 20 psi. Dwell time was 70 ms and resolution was set too wide for both precursor and product ions in each MRM transition.

The instrument was calibrated during each batch of analyses through the injection of standard solutions at four concentrations in triplicate in the range of concentration from 0.2 to 2 mg kg⁻¹. A calibration curve was built for each analyte and a linear fitting was adopted without forcing to origin; each curve was accepted when the coefficient of determination was greater than 0.99.

Statistical analysis

Analysis of variance of the data were calculated using the software package, SPSS "for Windows, 2001 (SPSS Inc., Chicago, IL). Orthogonal polynomial contrasts were used to compare nutrient solution concentration effects on selected parameters (Gomez and Gomez, 1983). Regression analyses were conducted to identify relationships between total phenolics expressed as gallic acid equivalents,
chlorogenic acid, cyanarin, luteolin, caffeic acid, and the concentration of the nutrient solution.

Results

Biomass production. The leaf dry biomass of artichoke and cardoon at 23 and 70 d after sowing (DAS) and the total leaf biomass were significantly affected by crop and nutrient solution concentration but not by their interaction, whereas the leaf dry biomass at 37, 51, and 91 DAS was only affected by nutrient solution concentration but not by crop and the crop × nutrient solution concentration interaction (Table 1). Increasing the nutrient solution concentration from 4 to 68 mequiv L⁻¹ caused a highly statistically significant quadratic trend in total leaf dry biomass with the optimum value recorded at 53.9 mequiv L⁻¹ (y = −0.0004x² + 0.0362x + 0.4804 where y = leaf dry biomass in kg m⁻² and x is the nutrient concentration in mequiv L⁻¹; R² = 0.96). Moreover, irrespective of the nutrient solution concentration, the leaf dry biomass at 23 and 70 DAS and the total biomass were significantly higher by 11.1%, 17.6%, and 5.6%, respectively, in cardoon in comparison with those recorded in globe artichoke (Table 1).

The leaf number of artichoke and cardoon had a similar trend to the leaf dry biomass with a significantly higher values recorded in cardoon than in artichoke cultivars (Table 2). When averaged over nutrient solution concentration, the number of leaves per plant at 23, 37, and 91 DAS and the total number of leaves were significantly higher by 7.6%, 14.2%, 23.1%, and 10.2%, respectively, in cardoon in comparison with those recorded in globe artichoke (Table 2). Finally, increasing the nutrient solution concentration from 4 to 68 mequiv L⁻¹ caused a highly statistically significant quadratic trend in total number of leaves per plant with the highest values recorded at 53.9 mequiv L⁻¹ (y = −0.0017x³ + 0.1832x + 9.8831 where y = leaf number in n. plant⁻¹ and x is the nutrient concentration in mequiv L⁻¹; R² = 0.96) (Table 2).

Mineral composition. The macro- and microelements concentrations in cardoon and artichoke leaf tissue as a function of crop and nutrient solution concentration are displayed in Tables 3 and 4. The concentration of N, K, Ca, and Mg in leaf tissue was significantly affected by nutrient solution concentration but not by crop and the crop × nutrient solution concentration interaction (Table 3). Moreover, no significant difference among treatments was observed for P concentration (average 9.55 g kg⁻¹ dry weight). The concentration of N and Ca increased quadratically and linearly, whereas the concentration of K and Mg increased only linearly in response to an increase in nutrient solution concentration (Table 3).

The effect of nutrient solution concentration on tissue micronutrients concentration (Fe, Mn, Cu, and B) was highly significant (Table 4). The tissue concentrations of Fe, Cu, and B declined linearly and those of Mn decreased linearly and quadratically as the external nutrient concentration increased. Finally, irrespective of nutrient solution, the concentrations of Fe, Mn, and Zn were significantly higher by 10.2%, 19.4%, and 18.2%, respectively, in cardoon leaf tissue in comparison with artichoke, whereas an opposite trend was observed for B (Table 4).

Total and target polyphenols in leaves. Although chromatographic resolution was limited, the analysis of target polyphenols was robust thanks to the highly specific and sensitive detection by tandem mass spectrometry. Indeed, the specificity arising from MRM in tandem MS acquisition provided selective analyses for each target compound. The linear dynamic range was also adequate, being the coefficient of determination higher than 0.99 in all cases over a 0.2 to 2 mg L⁻¹ interval.

### Table 1. Mean effects of crop and nutrient solution concentration on leaf dry biomass.

| Treatment               | Leaf dry biomass (kg m⁻²) |
|-------------------------|---------------------------|
| **Crop**                |                           |
| Artichoke               | 23 DAS<sup>2</sup> | 37 DAS<sup>2</sup> | 51 DAS | 70 DAS | 91 DAS | Total |
| Cardoon                 | 0.18                     | 0.32                  | 0.17      | 0.17         | 0.23      | 1.07  |
| Cardoon                 | 0.20                     | 0.31                  | 0.19      | 0.20         | 0.20      | 1.13  |
| Solution concentration  |                           |                       |           |             |           |       |
| (mequiv L⁻¹)            |                           |                       |           |             |           |       |
| 4                       | 0.12                     | 0.18                  | 0.07      | 0.13         | 0.10      | 0.60  |
| 20                      | 0.16                     | 0.30                  | 0.20      | 0.20         | 0.25      | 1.12  |
| 36                      | 0.21                     | 0.36                  | 0.19      | 0.20         | 0.25      | 1.21  |
| 52                      | 0.26                     | 0.38                  | 0.23      | 0.20         | 0.31      | 1.39  |
| 68                      | 0.20                     | 0.34                  | 0.22      | 0.18         | 0.24      | 1.19  |
| **Significance**        |                           |                       |           |             |           |       |
| Crop (C)                | *                        | NS                    | NS        | *            | NS        |       |
| Solution concentration (S) | ***                      | ***                   | ***       | ***          | ***       |       |
| Linear                  | *                        | NS                    | NS        | *            | NS        |       |
| Quadratic               | NS                       | NS                    | NS        | NS           | NS        |       |
| C × S                   | NS                       | NS                    | NS        | NS           | NS        |       |

<sup>2</sup>DAS = days after sowing.

### Table 2. Mean effects of crop and nutrient solution concentration on leaf number.

| Treatment               | Leaf number (n. plant⁻¹) |
|-------------------------|---------------------------|
| **Crop**                |                           |
| Artichoke               | 23 DAS<sup>2</sup> | 37 DAS<sup>2</sup> | 51 DAS | 70 DAS | 91 DAS | Total |
| Cardoon                 | 3.9                       | 2.1                    | 1.6     | 2.5         | 2.6        | 12.7  |
| Cardoon                 | 4.2                       | 2.4                    | 1.7     | 2.6         | 3.2        | 14.0  |
| Solution concentration  |                           |                       |           |             |           |       |
| (mequiv L⁻¹)            |                           |                       |           |             |           |       |
| 4                       | 3.8                       | 1.8                    | 1.1     | 2.2         | 1.5        | 10.6  |
| 20                      | 3.9                       | 2.3                    | 1.7     | 2.6         | 2.6        | 13.0  |
| 36                      | 4.0                       | 2.3                    | 1.7     | 2.8         | 3.0        | 13.8  |
| 52                      | 4.4                       | 2.5                    | 1.7     | 2.7         | 3.9        | 15.3  |
| 68                      | 3.9                       | 2.4                    | 2.1     | 2.5         | 3.4        | 14.3  |
| **Significance**        |                           |                       |           |             |           |       |
| Crop (C)                | *                        | **                    | NS        | NS          | ***        | ***   |
| Solution concentration (S) | ***                      | ***                   | ***       | ***          | ***       | ***   |
| Linear                  | NS                       | *                    | ***       | NS           | **        | **   |
| Quadratic               | NS                       | **                    | NS        | NS           | ***       | ***   |
| C × S                   | NS                       | NS                    | NS        | NS           | NS        | NS   |

<sup>2</sup>DAS = days after sowing.

### Table 3. Mean effects of crop and nutrient solution concentration on macronutrient composition of leaves.

| Treatment               | Macronutrients (g kg⁻¹ dry wt) |
|-------------------------|-------------------------------|
| **N**                   | P                             | K                             | Ca                             | Mg                             |
| **Crop**                | Artichoke                     | Cardoon                       |                                |                                |
| Cardoon                 | 45.0                          | 9.6                           | 26.9                           | 11.4                           | 3.0 |
| Cardoon                 | 45.9                          | 9.5                           | 25.9                           | 11.1                           | 2.8 |
| Solution concentration  | (mequiv L⁻¹)                  |                                |                                |                                |     |
| 4                       | 31.2                          | 8.4                           | 22.5                           | 8.9                            | 2.6 |
| 20                      | 46.4                          | 9.8                           | 25.4                           | 12.2                           | 2.6 |
| 36                      | 48.2                          | 9.9                           | 28.1                           | 11.9                           | 2.8 |
| 52                      | 48.9                          | 10.0                          | 27.5                           | 11.5                           | 3.1 |
| 68                      | 49.9                          | 9.7                           | 28.7                           | 11.7                           | 3.2 |
| **Significance**        |                                |                                |                                |                                |     |
| Crop (C)                | NS                            | NS                            | NS                             | NS                             | NS |
| Solution concentration (S) | ***                      | NS                            | NS                             | NS                             | NS |
| Linear                  | NS                            | **                            | NS                             | NS                             | NS |
| Quadratic               | NS                            | **                            | NS                             | NS                             | NS |
| C × S                   | NS                            | NS                            | NS                             | NS                             | NS |

N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium.
Precision, expressed as repeatability, was below 6.2% (n = 5) and extraction efficiency was higher than 93%.

The total polyphenols were highly (P < 0.001) influenced by nutrient solution concentration, crop, and their interaction (data not shown). The relationship resulting from regression analysis of the total polyphenols expressed as gallic acid equivalents over nutrient solution concentration was best described by a power function (Fig. 1). The gallic acid concentration in both cardoon and artichoke decreased with increasing the nutrient solution concentration from 4 to 68 mequiv-L\(^{-1}\). Moreover, when averaged over nutrient solution concentration, the highest values of gallic acid were recorded in cardoon than in artichoke (average 13.3 vs. 9.9 g·kg\(^{-1}\)).

Concerning the quantitative and qualitative profiles of the individual polyphenols (flavonoids and caffeoylquinic acids), significant differences among crops, nutrient solution concentration, and the crop × nutrient solution concentration interaction were found (Fig. 2). Among the caffeoylquinic acid derivatives, the chlorogenic acid was the predominant compound followed by cynarin, and finally caffeic acid, whereas the ferulic acid was not detected. Besides caffeoylquinic acid derivatives, other phenolics belonging to the flavonoid class such as the flavones luteolin have been identified in the artichoke and cardoon leaves, whereas apigenin, silybin, and miricetin were not detected. The relationship resulting from regression analysis of chlorogenic acid, cynarin, caffeic acid, and luteolin concentrations over nutrient solution concentration was best described by a power function (Fig. 2). The phenolic acids and flavonoids in both cardoon and artichoke leaves decreased with increasing the nutrient solution concentration, especially from 4 to 20 mequiv-L\(^{-1}\). Finally, when averaged over nutrient solution concentration, the highest chlorogenic acid, cynarin, caffeic acid, and luteolin concentration were observed with cardoon (average 1043.1, 426.6, 2.9, and 72.6 mg·kg\(^{-1}\) dry weight, respectively), whereas the lowest concentration was recorded in artichoke (average 512.3, 92.3, 0.5, and 16.3 mg·kg\(^{-1}\) dry weight, respectively).

### Discussion

It is well known that crop growth and yield are negatively affected by excessive high or low concentrations of nutrient solutions (Savvas and Adamidis, 1999). In the current experiment, dry shoot biomass and leaf number of artichoke and cardoon were significantly reduced especially at low (4 and 20 mequiv-L\(^{-1}\)) and high (68 mequiv-L\(^{-1}\)) fertilizer concentrations, probably as a result of nutrient deficiencies and osmotic stress, respectively. The reduction of crop growth resulting from osmotic effect has been reported previously for many vegetable crops (Colla et al., 2006a, 2006b, 2008, 2010, 2012; Rouphael et al., 2006, 2012). The main effects of high or low nutrient solution concentration were the reduction of growth rate as a result of smaller leaves, shorter stature, and fewer leaves. These results are consistent with the findings of Fallovi et al. (2009a) who reported that the marketable fresh yield, dry shoot biomass, and leaf area index of leafy lettuce were reduced at low (4 and 20 mequiv-L\(^{-1}\)) and high (68 mequiv-L\(^{-1}\)) solution concentrations. Moreover, the results of the present study suggest that artichoke and cardoon gave maximum biomass production and leaf number at 45 and 54 mequiv-L\(^{-1}\), respectively.

In our experiment, marked differences emerged in the plant growth parameters (leaf dry biomass and number) of the tested crops (Tables 1 and 2). The cardoon showed higher biomass and leaf number (average 1.13 kg·m\(^{-2}\) and 14.0 n./plant, respectively) than those observed in artichoke (average 1.07 kg·m\(^{-2}\) and 12.7 n./plant, respectively). The marked differences found between cardoon and artichoke crops suggest the possibility of exploiting this kind of variability in breeding programs with the aim of selecting phytochemical crops with higher biomass production.

The effect of crop on macro- and micronutrient concentration in leaves was less pronounced than the effect caused by the nutrient solution concentration (Tables 3 and 4). Among the macroelements studied, N was the mineral with a higher concentration with a mean index of leafy lettuce were reduced at low (4 and 20 mequiv-L\(^{-1}\)) and high (68 mequiv-L\(^{-1}\)) solution concentrations. Moreover, the results of the present study suggest that artichoke and cardoon gave maximum biomass production and leaf number at 45 and 54 mequiv-L\(^{-1}\), respectively.

**Fig. 1.** Relationship between total polyphenols expressed as gallic acid equivalents and nutrient solution concentration in leaves of artichoke and cultivated cardoon. Vertical bars indicate ± SE of means, their absence indicates the size was less than the symbol.

**Fig. 2.** Among the caffeoylquinic acid derivatives, the chlorogenic acid was the predominant compound followed by cynarin, and finally caffeic acid, whereas the ferulic acid was not detected. Besides caffeoylquinic acid derivatives, other phenolics belonging to the flavonoid class such as the flavones luteolin have been identified in the artichoke and cardoon leaves, whereas apigenin, silybin, and miricetin were not detected. The relationship resulting from regression analysis of chlorogenic acid, cynarin, caffeic acid, and luteolin concentrations over nutrient solution concentration was best described by a power function (Fig. 2). The phenolic acids and flavonoids in both cardoon and artichoke leaves decreased with increasing the nutrient solution concentration, especially from 4 to 20 mequiv-L\(^{-1}\). Finally, when averaged over nutrient solution concentration, the highest chlorogenic acid, cynarin, caffeic acid, and luteolin concentration were observed with cardoon (average 1043.1, 426.6, 2.9, and 72.6 mg·kg\(^{-1}\) dry weight, respectively), whereas the lowest concentration was recorded in artichoke (average 512.3, 92.3, 0.5, and 16.3 mg·kg\(^{-1}\) dry weight, respectively).

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such as flavonoids and phenolic acids (including caffeoylquinic acids). These are widely studied as a result of their multiple biological activities (Holst and Williamson, 2008). In the genus *Cynara*, mono- and dicaffeoylquinic acids and luteolin and apigenin derivatives have been documented as the major compounds (Schütz et al., 2004). Among the caffeoylquinic acid derivatives, the chlorogenic acid was the predominant compound in artichoke and cardoon leaf tissue with a mean concentration of 778 mg·kg⁻¹ dry weight followed by cynarin (average 259 mg·kg⁻¹ dry weight), and finally by caffeic acid (average 1.7 mg·kg⁻¹ dry weight). In contrast, lowest quantities were observed for flavonoid in particular luteolin (average 44.5 mg·kg⁻¹ dry weight).

It is known that the concentration of flavonoids and phenolic acids varies as a function of many factors such as environment, cultural practices, biotic and abiotic stresses, and genotype (Pandino et al., 2010). In general, increased nutrient solution concentration reduces the yield of vegetable crops but may improve their nutritional quality, as observed in plants grown in both soil and soilless culture (Francois and Maas, 1994; Rouphael et al., 2006, 2010). In this study, increasing nutrient solution concentration from 4 to 68 mequiv·L⁻¹ decreased the quality aspects of leaves by decreasing the total polyphenols, chlorogenic acid, cynarin, caffeic acid, and luteolin concentration, especially when the nutrient solution concentration increased from 4 to 20 mequiv·L⁻¹ (Figs. 1 and 2). The reduction in phenolic acids and flavonoids in leaves of artichoke and cardoon with the increase of nutrient concentration suggests that the secondary metabolism of plant was promoted by low nutrient availability as observed by Kraus et al. (2004).

The concentration of polyphenols in artichoke and cardoon leaves appears strongly influenced by genetic factors as previously demonstrated in other crops including blackberry (Connor et al., 2005), potato (Reddivari et al., 2007), garlic (Beato et al., 2011), faba bean (Chaieb et al., 2011), and tomato (Vallerdu´-Queralt et al., 2011). In the current study, the highest amount of chlorogenic acid was found in cardoon ‘Bianco Avorio’ compared with the artichoke cv. Romolo (Fig. 2). This result is very important because in vivo rat studies have demonstrated the antioxidant and anticarcinogenic properties of chlorogenic acid (Gonthier et al., 2003). The chlorogenic acid is usually poorly absorbed in the small intestine but capable of providing higher yields of microbial metabolites, active compounds responsible for the biological properties attributed to dietary polyphenols (Gonthier et al., 2003). The high level of chlorogenic acid found in cardoon cultivar Bianco Avorio could be explained by its central role as a substrate for many biochemical reactions producing several phenolic acids (Wittemer et al., 2005), which are highly represented in artichoke. Similar to chlorogenic acid, the highest concentration of cynarin, a polyphenolic compound typical of *C. cardunculus* and important for its choleretic activity (Gebhardt, 1997; Gebhardt and Beck, 1996), was also observed in cardoon (Fig. 2). Finally, the highest concentration of flavonoid luteolin was also observed in cardoon.

**Conclusions**

Increasing fertilizer concentrations linearly or quadratically increased plant growth.
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