Influence of irrigation on the chemical compounds in leaves in vegetative and reproductive stage and bracts of globe artichoke (*Cynara cardunculus* var. *scolymus* L.)

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Abstract

Artichokes contain antioxidant compounds, principally phenols which are beneficial for health. The content of these active principles can depend on irrigation, genotype and stage of plant's development. This study was aimed at determining the concentration of cynarin and chlorogenic acid, both in leaves in vegetative and reproductive stage and in central and outer bracts of inflorescences. The selected genotypes were 'Oro Verde FCA' (OV), 'Guri FCA' (GU) and 'Gauchito FCA' (GA), all of them produced on the Faculty of Agricultural Sciences, Rosario's National University (UNR). The plants were treated with 194 mm of irrigation by dripping from June to November and the controls did not receive irrigation. The extracts were prepared with 5 leaves from the middle layer of 4 plants in vegetative and reproductive conditions and with bracts of primary heads stored in freezer (-80°C) until the preparation of extracts. Measurements were made at the Mass Spectrometry Laboratory of Scientific and Technology Center (CCT-CONICET Rosario, Argentina). Extracts were analyzed by HPLC using UV spectroscopy (330 nm) and electrospray MS as detectors. The content of cynarin in plants leaves with irrigation was 3052 mg kg^{-1} for GA in vegetative state and 4257 mg kg^{-1} in reproductive state, while in the control the content of cynarin was 1873 and 1599 mg kg^{-1} in each stage. In OV, cynarin was detected in reproductive stage with irrigation (734 mg kg^{-1}) and without irrigation (482 mg kg^{-1}). In the central bracts, the highest content of cynarin was found in irrigated plots of OV and GA, whereas in the outer bracts the same behavior was observed but in GU and GA. The highest content of chlorogenic acid was observed in leaves of irrigated plants in the reproductive stage, with 3445 mg kg^{-1} in OV, 1447 mg kg^{-1} in GU and 1079 mg kg^{-1} in GA. Chlorogenic acid was only detected in the central bracts of irrigated plants, showing the highest concentration in the early cultivars (GA 3405 and OV 1218 mg kg^{-1}). This study demonstrates that irrigation allows to increase the content of active principles in leaves and inflorescences of artichoke crops in which both leaves and inflorescences are harvested.

Keywords: cynarin, chlorogenic acid, antioxidant, HPLC-UV-MS

INTRODUCTION

The generation of products with added value constitutes a challenge taking in account, as important aims, the preservation of the nature and the environmental care too.

Natural resources are excellent primary materials in food production processes and in the determination of certain active compounds present on it, open the possibilities of use of

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all material for both consumption and medicinal purposes.

Among vegetables, the globe artichoke (*Cynara cardunculus* var. *scolymus* L.) plays an important economic role in agriculture and health. Its production is concentrated in the Mediterranean area, especially in Italy, Spain and France. Furthermore, the Food and Agricultural Organization (FAO) reported that artichoke cultivation has spread in South America and the United States, the Middle East, North Africa and China (Food and Agricultural Organization, 2012).

According to FAO data, the production of artichoke in Argentina during 2013 it was 106,325 t, with a yield of 24.93 t ha^{-1}. In the area of La Plata (Buenos Aires), the productive core increased 64% of the total land, seconded by the horticultural ribbon of Rosario (Santa Fe), with 14%. A similar value (14%) is concentrated in the area of Cuyo (the provinces of Mendoza and San Juan), which is mainly oriented to fresh consumption and about 65% goes to industrialization. Traditionally, in the areas of La Plata and Rosario the multiplication was vegetative by using stems rooted whereas in area of Cuyo the multiplication was by cuttings or stumps. More recently they are using hybrids and cultivars multiplied by seeds (García et al., 2015).

In Argentina, the average daily intake of edible part of artichoke is approximately 140 g per person, value far removed 400 g recommended by the World Health Organization (WHO, 2012). In addition to the culinary uses of the chapters, artichoke leaves are used since ancient times in traditional medicine for its choleretic, diuretic and antidiabetic properties (Blumenthal et al., 2000), but the use of artichoke leaves to this purpose is scarcely harnessed in the Argentinean cultivars.

Artichoke has showed high content of phenolic compounds such as cynarin and chlorogenic acid, both in leaves and in edible part of the plant. These compounds have demonstrated to be active as antioxidants, in hepatobiliary diseases and hyperlipidemia, rheumatism and cholesterol metabolism. In consequence, artichoke has been named "functional food" for FuFoSE (European Commission on Functional Food Science in Europe; Cecarelli et al., 2010).

The quality of artichoke measured in the content of polyphenols in the plant, depends on the genetic diversity, the phenologic stage and the growing conditions of the cultivars (Lombardo et al., 2012). The content of water is critic for the growing, production and quality of the crops. The hydric stress during the harvest of artichoke can produce small plants, delay the stages of flowering and reproduction or modify the content of active principles.

Since no data exist on the effect of drip irrigation in three cultivars generated in the Faculty of Agricultural Sciences, Rosario's National University, this study was conducted in order gain insight in the identification and quantification of chlorogenic acid and cynarin, in leaves and inflorescences of artichoke under irrigation conditions in vegetative and reproductive stages.

MATERIALS AND METHODS

Plant material

The selected genotypes were 'Oro Verde FCA' (OV) (Figure 1), 'Gauchito FCA' (GA) (Figure 2) and 'Guri FCA' (GU) (Figure 3), all of them developed on the Faculty of Agricultural Sciences (FCA), Rosario's National University (UNR), in the town of Zavalla (33°01'S; 60°53'O). The plants were identified by Dra. Stella Maris García, Horticulture Area (FCA, UNR), and were registered in the National Registry of Cultivars with the following numbers and characteristics:

1. 'Oro Verde FCA' Nº6814.

Globular, compact artichoke, light-green with slight purple pigmentation at the base of bracts, with an approximate weight of 195 g, harvest starts on September 25; high-yielding (13 t ha^{-1}).

Figure 1. 'Oro Verde FCA'.

2. 'Gauchito FCA' Nº9330.

Globular, compact, glossy light-green head with an approximate weight of 223 g; harvest starts on September 23; high-yielding (17 t ha^{-1}).

Figure 2. 'Gauchito FCA'.

3. Gurí FCA Nº9331.

Globular, compact, variegated (purple-green), glaucous green foliage, head weighs about 220 g; harvest starts on October 10; high-yielding (15 t ha^{-1}).

Plant spacing was 1.4 m between rows and 0.8 m in the row (9125 plants ha^{-1}). Each batch was deposited at the herbarium of the FCA-UNR and identified as OV FCA, GA FCA and GU FCA.

Figure 3. 'Guri FCA'.

Irrigation treatment

Irrigation was applied using drip irrigation system. The plants were treated with 194 mm (54 mm by rain and 140 mm by dripping), from June to November (2012). The control plant only received 54 mm by rain irrigation.

The leaves and bracts of the plants were collected over two periods of 2012: August

and November.

Preparation of extracts

The extracts were prepared with 5 leaves (Figure 4) from the middle layer of 4 plants in vegetative and reproductive stages or with 10 bracts of primary heads, considering outer bracts the first 10 in centripetal way, then 11 to 20 were wasted and the selected medium bracts were the 21 to 30. They were stored in freezer (-80°C) until the preparation of extracts. Each vegetal sample (10 g) was macerated with methanol (100 mL, 30 min, 230 rpm ×2) (Figure 5). The obtained extracts were filtered (Figure 6) and analyzed by HPLC without posterior treatment, using UV and ESI-MS as detectors. These measurements were made in the Laboratory of Mass Spectrometry in CCT-CONICET-Rosario (Argentina).

Figure 4. Leaves for the preparation of extracts.

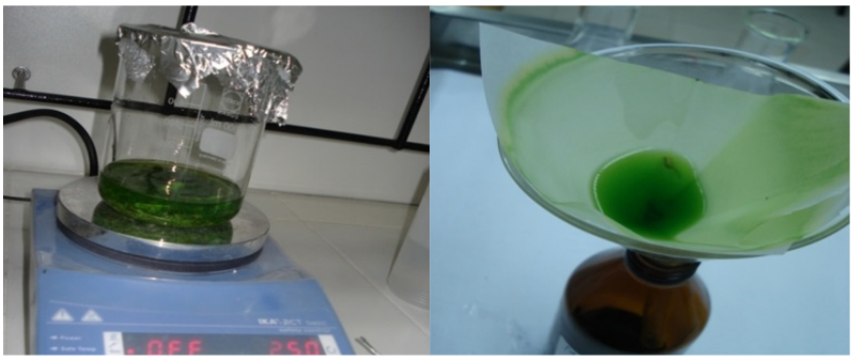

Figure 5. Maceration of leaves with methanol (100 mL ×2) and filtration of the extract.

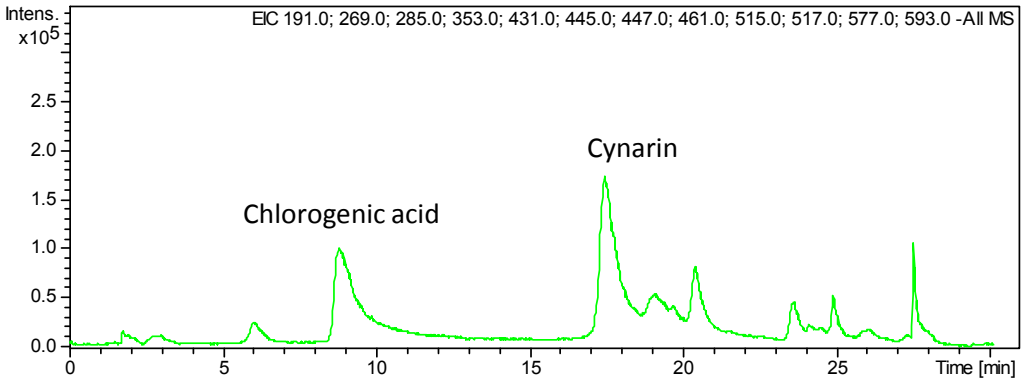

Figure 6. HPLC-HRMS chromatograms of central bract's extracts of GA, with drip irrigation showing the active compounds.

Reference compounds

Chlorogenic acid (1) and cynarin (2) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the purities of these reference compounds was determined by HPLC-UV-HRMS prior to injection of the rest of samples into the HPLC system.

HPLC-UV-MS-MS/MS analyses

HPLC-UV-ESI-MS/MS analyses of all batches of *GA*, *GU* and *OV* extracts were carried out by using a MicroTOFQ II instrument (Bruker Daltonics, MA,USA), equipped with an ESI ion source with nitrogen as nebulizing gas (4 psi) and drying gas (8 L min^{-1}, 200°C); capillary 4500 V and end plate offset at 500 V. Mass accuracy was verified by infusing a 10-mM solution of Na-formiate (Sigma-Aldrich) dissolved in MeOH:H_2O (50:50). First, the markers were characterized by direct infusion to ESI using a syringe pump (Harvard Apparatus11 Plus) recording both MS and MS/MS spectra. Because of the improved performance reached using direct infusion to ESI, we report only MS and MS/MS data obtained in the negative mode. Markers and extracts were introduced in the HPLC (5 µL) using an autosampler (Agilent HiP-ALSSL+) at 30°C in a C_{18}-RP column Symetry Waters (150×4.6 mm, 5 µm). Flow rate was set to 1 mL min^{-1} propelled by an Agilent 1200 series G1312B SL binary pump, using ultra-pure water or HPLC-MS grade ACN Carlo Erba with 0.1% formic acid (solutions A and B respectively), in gradient for Method 1: 8% B in the first minute and changing to 25% B within the following 20 min. Then the composition was changed in 1 min to 100% B and it was held 3 min, returning to 8% B in 2 min and keeping this condition for additional 3 min to achieve the column's stabilization before the next run (total run time was 30 min). Method 2: 8% B in the first minute and changing to 25% B within the following 20 min. Then the composition was held 10 min, changing to 100% B within 2 min; then the composition was held 3 min, returning to 8% B in 1 min and keeping this condition for additional 3 min to achieve the column stabilization before the next run (total run time was 40 min). Eluted compounds were monitored at 330 nm and 1/3 of the HPLC flow was introduced into the ESI source of the mass spectrometer. For the analyses of chromatograms and mass spectra, the Data Analysis 4.0 SP1software (Bruker Daltonik GmbH, Germany) was used. The detected compounds in the extracts were identified by comparing their retention times (Rt), HRMS and MS/MS with those corresponding to reference compounds or those reported in literature. The content of caffeoylquinic acids was quantified in the extracts using the HPLC-UV-MS-MS/MS chromatograms. The calibration curves of the reference compounds 1 and 2, were prepared with five appropriate dilutions of stock MeOH solutions by triplicate. To monitor the samples, the wavelength selected (330 nm) was chosen according to absorption's maxima of reference compounds.

RESULTS AND DISCUSSION

The qualitative and quantitative analysis of extracts of leaves and bracts of cultivars, showed by HPLC-UV-MS-MS/MS caffeoylquinic acids such as chlorogenic acid (5-caffeoylquinic acid) and cynarin (1,3-dicaffeoylquinic acid) (Figure 6); the presence of flavonoids apigenin and luteolin was also investigated. On the whole, caffeoylquinic acids were found well represented, mainly in leaves and central bracts of cultivars.

Leaves

1. Cynarin (1,3-dicaffeolylquinic acid).

GA was the only cultivar with content of cynarin in vegetative stage (Figure 7). This cultivar showed higher amount of this active compound in irrigated plants (3052 mg kg^{-1}) respect to the control (1873 mg kg^{-1}). It represented an increase of 63%.

In reproductive stage, GA and OV showed presence of higher levels of cynarin in irrigated plants (Figure 7). The content of this compound in the controls was 1599 and 482 mg kg^{-1}, respectively. Whereas these values were increased with irrigation to 4257 (166%) and 734 mg kg^{-1} (52%), respectively.

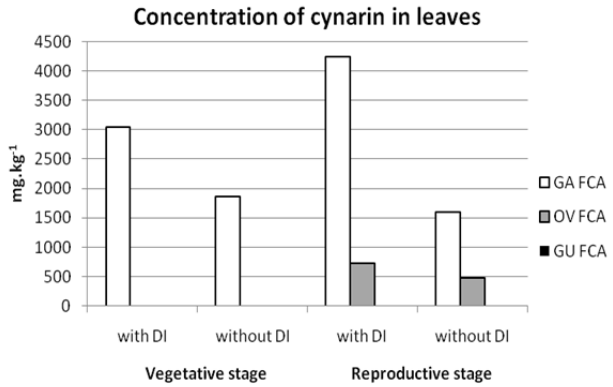

Figure 7. Concentration of cynarin (mg kg^{-1}) in leaves extracts of GA, OV and GU in vegetative and reproductive stages, with and without drip irrigation.

2. Chlorogenic acid (5-caffeolylquinic acid).

In vegetative stage, the three cultivars showed lower amount of this active compound in irrigated plants than in the controls (Figure 8). In GU, the content of chlorogenic acid decreased from 1509 to 200 mg kg^{-1}, in OV 2738 to 863 mg kg^{-1} and GA 717 to 562 mg kg^{-1} when drip irrigation was applied.

On the contrary, in reproductive stage GA, OV and GU showed higher levels of chlorogenic acid in irrigated plants than in the controls (Figure 8). The higher increase was manifested by OV (305 to 3445 mg kg^{-1}), followed by GU (194 to 1447 mg kg^{-1}) and GA (894 to 1079 mg kg^{-1}).

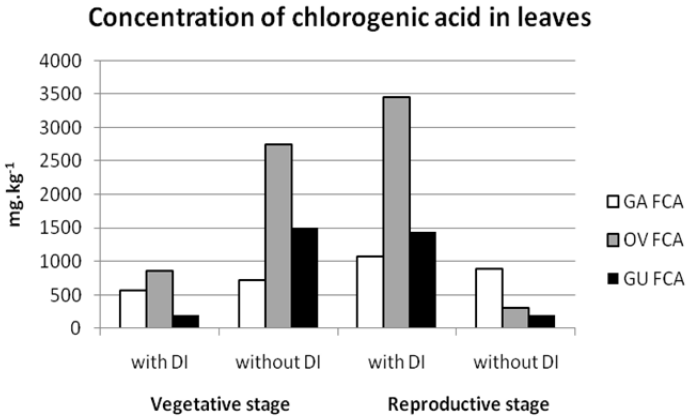

Figure 8. Concentration of chlorogenic acid (mg kg^{-1}) in leaves of GA, OV and GU in vegetative and reproductive stages, with and without drip irrigation.

Bracts

1. Cynarin.

This active compound is present in the central bracts of the three cultivars in study (Figure 9). The irrigation treatment produces an increase of concentration in OV and GA with respect to the controls (6790 to 9007 mg kg^{-1} and 6949 to 9797 mg kg^{-1}, respectively), whereas in GU the content of cynarin decreased with the irrigation (4571 to 298 mg kg^{-1}).

In the outer bracts, the evaluated cultivars showed the lowest levels of cynarin (Figure 9). With irrigation, this compound was absent in OV, it was remained in GA while it increased 100% in GU (1056 to 2422 mg kg^{-1}).

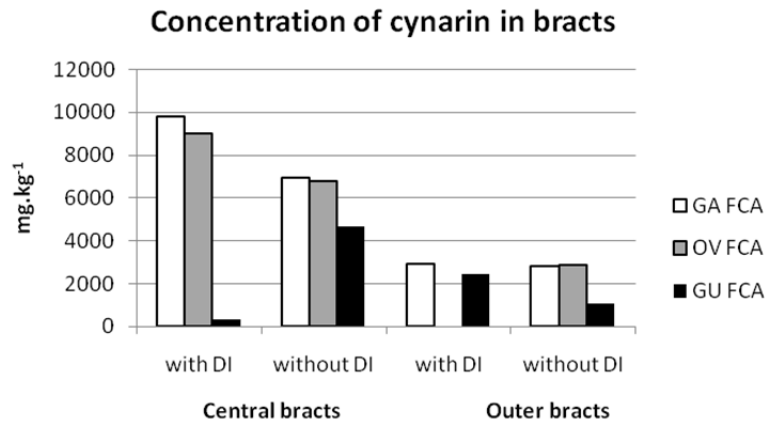

Figure 9. Concentration of cynarin (mg kg^{-1}) in central and outer bracts of GA, OV and GU, with and without drip irrigation.

2. Chlorogenic acid.

The studied cultivars did not show 5-caffeolylquinic acid in the outer bracts, with or without irrigation (Figure 10).

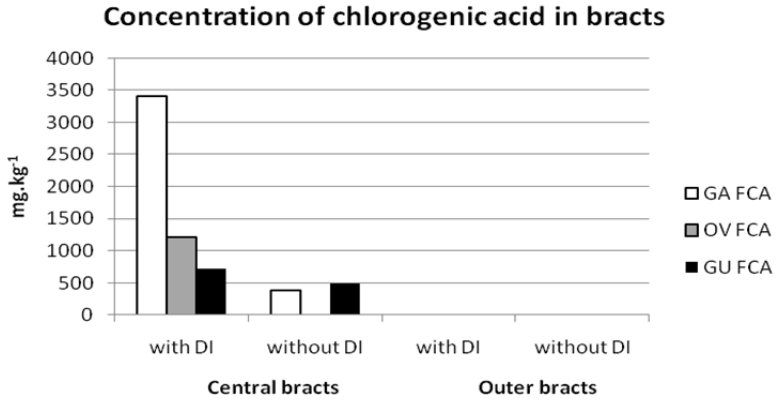

Figure 10. Content of chlorogenic acid (mg kg^{-1}) in central and outer bracts of GA, OV and GU, with and without drip irrigation.

In the central bracts, GU's control showed 490 mg kg^{-1} of this active compound which was increased to 719 mg kg^{-1} with the irrigation of plants (Figure 10). Whereas in GA this effect was improved (381 to 3406 mg kg^{-1}), as well as in OV (1218 mg kg^{-1}).

In a whole, these results show that irrigation allows increasing the content of active principles in the leaves and inflorescences of artichoke crops, whereas each genotype responds in a different way front to the irrigation.

These results are in concordance with those reported by Curadi et al. (2005), Di Venere et al. (2004), Wang et al. (2003), Llorach et al. (2002) and Gil-Izquierdo et al. (2001). All of them mention that phenolic content in artichoke heads varies with cultivar, growing season, head maturity, storage and processing. Some of them reviewers that the increase of phenolic content in artichoke heads by deficit irrigation may be a plant defense response against drought stress as shown in other vegetables (English-Loeb et al., 1997). In contrast, with regard to water availability conditions are coincidences among our results and those obtained by Fonseca et al. (2006), who evaluated a kind of chamomile (*Tanacetum parthenium* Sch. Bip.), with increases in the concentration of phenolic compounds when subjected to frequent water intake.

CONCLUSIONS

The effect of irrigation generates increased concentration of cynarin in leaves of cultivars GA, OV and GU, in both vegetative and reproductive stages, not responding in the same way in all genotypes.

The effect of irrigation generates increased concentration of chlorogenic acid in leaves only in reproductive stage, not responding in the same way in all cultivars.

The effect of irrigation generated increased concentration of cynarin mainly on central bracts, not responding in the same way in all cultivars.

The effect of irrigation led to increased concentration of chlorogenic acid only in central bracts, not responding in the same way in all cultivars.

The production of artichoke cultivars can be developed with dual purpose of harvesting: chapters (inflorescences) for fresh consumption and leaves for medicinal use, since the active compound concentration remains in the leaves after the reproductive stage of the plant.

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