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Scienze e Biotecnologie dei Sistemi Agrari e Forestali

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Indirizzo Produttività delle piante coltivate

Organic management and productions of quality: influence of growing management on nutraceutical compounds and mineral profile in artichoke cv “Spinoso Sardo”

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Ad Angelo, mio marito e compagno di una vita,
per il suo aiuto e il suo sostegno.
Alle mie due bambine, Eleonora e Matilde,
per la pazienza che hanno avuto in questi tre anni.
A mia madre, senza di lei non sarei qui

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ABSTRACT

Recent years have seen a considerable growth of organic food marker. This phenomena is caused by great awareness of consumers as regard food safety and health.

However, several studies have shown different results on organic management, especially as regard the nutritional component (phytochemicals and minerals). The globe artichoke, rich of minerals and polyphenols, has been extensively analysed for the high nutritional value. A large number of research showed that there were considerable variation on minerals and polyphenols according to different management, environmental and biological factors.

The overall objective of this study was to investigate the effects of different crop management on polyphenols profile and mineral component in globe artichoke (Cynara cardunculus var. scolymus)

The experiment was carried out in an experimental field for ten years under organic management, in order to evaluate the applicability of this management in the mid-long term in this crop.

In the Chapter 1 the variation of concentration of major polyphenols has been investigated in relation to different managements, during two growing seasons. In the second one the clones of “Spinoso sardo” micropropagated were introduced to evaluate the genotype influence.

In the Chapter 2 reports the results obtained to quantification of mineral content during two growing seasons to evaluate the effects of different management and environmental condition to uptake of macro e micro minerals. In the second years of trials has been investigated the effect of genotype.

PREFACE

I. Artichoke cv "Spinoso Sardo": organic management and production of quality, is it possible?"

The artichoke is one of the most important crop in Sardinia not only economically but also from historical viewpoint. The intensive cultivation began after the First World War (before it has been in family vegetable gardens) in coastal areas of Cagliari and Sassari. During that period, the farmers followed the natural cycle of crop, until producers of Bosa selected an ecotype of "Spinoso" artichoke that allowed early production bringing forward planting of the crop in summer through use of irrigation. This ecotype, by selection of local farmers, went on to become "Spinoso Sardo". It was precisely in these years that got around the words "Spinoso di Sardegna" especially in a North Italian markets to identify the origin as a quality label. Indeed, the period of major production was locate in the 1960s and 1970s with more 20,000 Ha cultivated (31% of Country field devoted). Thereafter, the countryside progressive phasing out and the loss of market share in favour of other Country Region led Sardinia to the present situation as third producer in Italy, with 13,267 Ha and 1,109,302 q in 2013 (ISTAT 2013).

Even today, the major ecotype grown is "Spinoso Sardo" (avoided PDO mark in the 2011), followed by "Violet of Provence", "Thema 2000" and "Romanesco". The local farmers like local cultivar to the precocity growth by the opportunity to selling at higher price levels, but also by the peculiar organoleptic characteristics, which distinguish it from others.

Furthermore, during the crisis, also the emergence of new producing Counties as Cina, Argentina, Egypt and others of Mediterranean Basin are reducing the market shares on artichoke.

The possible marketing strategy to promote and increase the local product should take to the development of crop also including the organically grown.

In these last decades the consumers, caused by great awareness as regard food safety and health combined with better respect the environment, have been turning to

organic products. Nowadays the organic agriculture is practiced in more than 160 Countries, especially in the developing countries whereas the market is located in industrialised countries (Willer and Kilcher, 2012).

In a comprehensive review, Eva Johansonn and coauthors have well reported several study that observed opposite results about the influence on nutritional compounds, such bioactive molecules (carotenoids, tocopherols and polyphenols) and micronutrient (macro e micro-minerals) growing by organic management or conventional one. (Johansson et al. 2014).

The reasons of these differences are varied : genotype valuated, climate, harvest time, management, soil characteristic and often test material use purchased from marked.

However, in this study the samples analysed come from an experimental field where organic and conventional managements on artichoke cv. “Spinoso Sardo” are compared for almost ten years to evaluate the agronomical and economic feasibility.

In view of this, the main objective of this PhD was to estimate the effects that an organic management have on quality of artichoke cv. “Spinoso Sardo” in the medium term, analysing two of principal component characterising the nutritional value:

- The content of polyphenols (Chapter 1);
- The content of macro and micro-minerals (Chapter 2).

II. Artichoke polyphenols and biological activity

It is well note that the globe artichoke is a source of nutrient and nutraceuticals as reported by several studies. The edible part of artichoke is rich of inuline, fibre, minerals (potassium, calcium, iron, etc.) as shown in a following table:

Table 1: Average nutritional values of globe artichoke for 100 g edible part (font: CRA-NUT)

CHEMICAL COMPONENT	Value for 100 g	CHEMICAL COMPONENT	Value for 100 g
Edible fraction (%):	34	**Potassium (mg):**	376
Water (g):	91.3	**Iron (mg):**	1
Proteins (g):	2.7	**Calcium (mg):**	86
Fat (g):	0.2	**Phosphorus (mg):**	67
Cholesterol (mg):	0	**Magnesium (mg):**	45
Available sugar (g):	2.5	**Zinc (mg):**	0.95
Starch (g):	0.5	**Cupper (mg):**	0.24
Soluble sugar (g):	1.9	**Thiamine (mg):**	0.06
Total fiber (g):	5.5	**Riboflavin (mg):**	0.1
Energy (kcal):	22	**Niacin (mg):**	0.5
Energy (kJ):	92	**Vitamin A retinol eq. (µg):**	18
Sodium (mg):	133	**Vitamin C (mg):**	12

Indeed, among vegetables, the globe artichoke contains the higher content of polyphenols (Brat et al., 2006) to qualify as functional food or nutraceuticals.

These terms used for the first time by Dr. De Felice in the 1989 to identify a series of products with health promoting effects. The nutraceuticals demand has experienced a huge growth in the last years; the global market is estimated about 117

billion dollars and in Europe it is 93 billion of euro (only Italy is worth 2.4 billion EUR in 2014) (www.federfarma. it).

From a chemical point of view, the artichoke's polyphenols can be divided in two categories:

- Caffeoylquininic acids;
- Flavonoids.

Caffeoylquininic acids

These class of compounds, named also hydroxycinnamic acid are present in a several food: green tea, coffee, tomato, cabbage etc. What about globe artichoke they are predominant (mainly 80% of dry matter in heads) and, because of their structures, show scavenging activity against free radicals and reactive oxygen species (ROS) protecting the biological molecules (protein, DNA, lipids) (Kono Y. et all, 1997; Pavlica S & Gebhard R., 2005).

The structure and relative name of most important caffeoylquininic acids, isolated in this study are reported following (Figures 1-4):

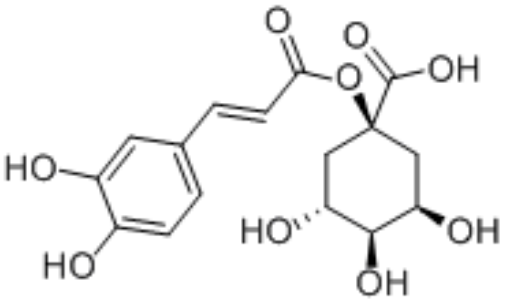

Figure 1: 1-O Caffeoylquininic Acid (CAS 1241-87-8)

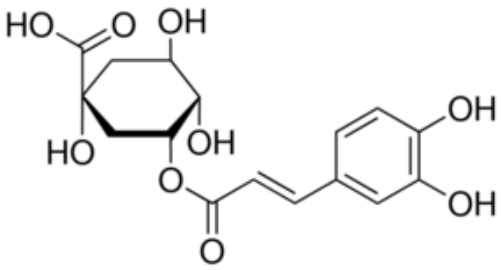

Figure 2: 5-O Caffeoylquininic Acid (Clorogenic Acid) (CAS 906-33-2)

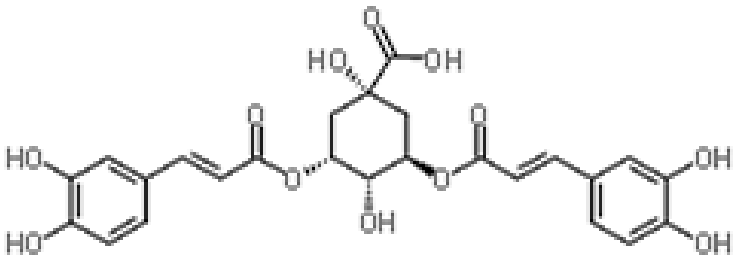

Figure 3: 3,5 O dicaffeoylquininic Acid (Isochlorogenic acid) (CAS 2450-53-5)

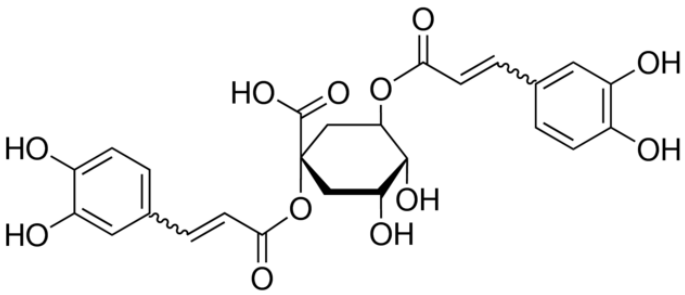

Figure 4 - 1,5 O dicaffeoylquininic Acid (CAS 19870-46-3)

As already mentioned, these compounds have a health promoting effects, known since ancient time. It is well note hepatoprotective (Adzet T. et all. 1987) and choleretic activities linked to presence of cynarine, but also several pharmacological studies have shown an other activities : antioxidant (Gebhardt R. 1997; Brown J. E. & Rice-Evans C. A. 1998, Pérez-Garcìa et all. 2000; Wang et al. 2003), anticancirogenic (Clifford 2000), anti HIV (King et al. 1999).

Also they are response to browning phenomena together with ferritine well explained by Lattanzio et al. 1994.

Flavonoids

A very low concentrations (about 10% or low of total polyphenols) the flavonoids present in artichoke show a high antioxidant activity. They are present linked to glycosides ring as showed above (Figures 5-7):

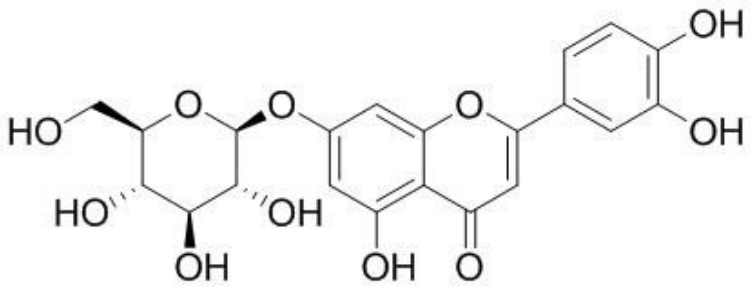

Figure 5: Luteolin 7-O glucoside (Cynaroside) (CAS 5373-11-5)

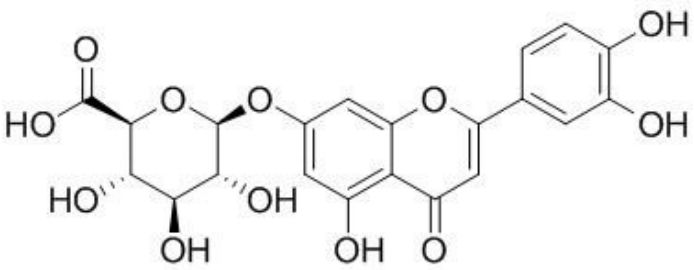

Figure 6: Luteolin 7-O glucoronide (CAS 29741-10-4)

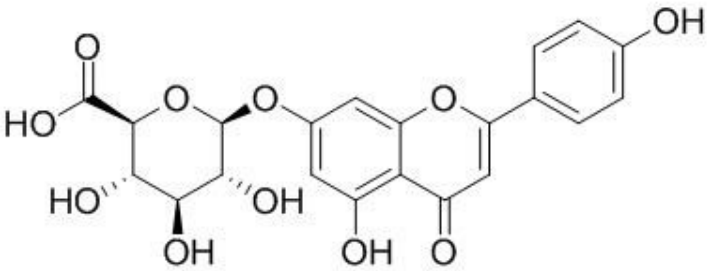

Figure 7: Apigenine 7-O glucoronide (CAS 29741-09-1)

Several studies in the last time have shown important potential interaction between dietary intake of luteoline and apigenine and various desease: most recently the enhancement of anti-proliferative effects of chemotherapeutic drug on human pancreatic cancer cell using them. (Johnson & Gonzalez de Mejia 2013). The apigenine was shown important results on cancer prevent. (Shukla & Gupta 2010)

III. Minerals on artichoke and importance of health

In addition to polyphenols, the globe artichoke is a source of mineral such as K, Ca, Na (López et al. 1997; Romani et al. 2006). If the lack intake of polyphenols no caused pathological diseases, a mineral deficiencies may produce serious health problems and metabolic disorder (Welch et al. 2009). This is due to some macro and micro-minerals are an important components of several enzymes or proteins, essential elements to health. For example, the zinc deficiency leads to decreased body's immune response, or altered or loss of sense of taste; the low levels of iron are present in over half of the World population to different causes and reduce the brain function, the ability to memories and learn. In several studies the variations of minerals were compared between organically and conventionally grown crops (tomato, strawberries, wheat, etc.) but with opposite results (Johansson et al. 2014).

What about globe artichoke there are no founded studies on subject.

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CHAPTER 1

Effect of input management on nutraceutical component in globe artichoke cv "Spinoso Sardo"

1.1. Introduction

In the last years, several study have highlighted the nutritional and pharmacological properties of globe artichoke (Cynara cardunculus ssp. scolymus (L.) Fiori). Artichoke is an important component of the Mediterranean diet and may be considered a functional food (European Commission, 1999) having a low fat content and high minerals, vitamins, inuline, fibres and polyphenols concentrations (Lattanzio et al. 2009).

A recent definition for functional foods was revised to "natural or processed foods that contains known or unknown biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease" (Martirosyan & Singh 2015). In artichoke antioxidant, choleretic, hepatoprotective, bile-enhancing and lipid-lowering effects have been demonstrated (Johnson & Gonzalez de Mejia 2013; Pandino et al. 2011). These therapeutic properties seem to be related to inulin and polyphenolic fraction. The latter phytochemical compounds are also produced by other plants species (e.g. tomato, apple, tobacco) in response to biotic and abiotic stresses: pedo-climatic condition, daylight exposure as well as pathogen attacks (Izaguirre et al. 2007; Tegelberg et al. 2004).

In artichoke previous studies shown significant differences in the levels of polyphenol and presence/absence variation as affected by: i) genotype (Alamanni et al., 2001; Romani et al. 2006; Fratianni et al. 2007; Negro et al. 2012; Lombardo et al. 2009); ii) agronomic practices; and iii) environmental conditions (Palermo et al. 2013; Lombardo et al. 2009; Pandino et al. 2013).

Furthermore, for some years now, Europeans demand high quality fresh products, especially those with high organoleptic quality and nutritional value. A recent survey showed that the demand for organic products by European consumers increased fourfold during the last decade while the area devoted to their cultivation are increase only 0.9% (Willer H & Kilcher L., 2012).

Several reviews show that organically produced vegetables generally have higher levels of micronutrients and health-promoting secondary metabolites, such as phenolics, than conventionally grown vegetables (Raigón et al. 2010). Among others, explanations for these differences include "nutrient dilution" caused by the inverse relationship between yield and micronutrient concentrations, a higher dry matter of organic vegetables compared to conventional ones, and higher levels of accumulation of inducible secondary metabolites responsible of plant defense in organic cultivation (Raigón et al. 2010). However, in the often studies, organic samples coming from the local market were employed, hoping in best management practice by organic farmers.

Since globe artichoke significantly contributes to the Mediterranean agricultural economy with Italy being the leading world producer (about 548 Kt per year, FAO 2013), it seemed interesting to evaluate how contrasting production systems (organic and conventional) affect polyphenolic fraction in an Italian globe artichoke cultivar (''Spinoso sardo''). At present the crop is produced almost exclusively by conventional means that provides monoculture for over ten year with massive mineral fertilizer. However, this techniques system has proved to be energy inefficient and environmentally unsustainable (Burt et al., 2009; Erhart et al., 2007) and economically disadvantageous. In these last years, in Sardinia (the third producer of artichoke in Italy), a huge increase in the land dedicated to organic farming took place (sevenfold in the period, 2000–2010). At present, Sardinia is third region in Italy to organic growing areas (72282 ha) (ISTAT 2015).

Organic farming can be regarded as a system to improve vegetable quality.

The overall objective of this work was to evaluate changes in polyphenol content in organically and conventionally grown globe artichoke quality, so as to provide a product of quality through sustainable management.

1.2. Material and methods

1.2.1. *Experimental site*

This study has been conducted in a trial field in Ottava inside the experimental station of the University of Sassari at 81 m above sea level (40° 46’ 31”N; 8° 29’ 22” E) for two growing seasons: 2012/13 and 2013/14. Previously the experimental site was implanted in July 2006 for the “SIMBIOVEG” (2006-2009, www.aiab.it) a project of the Italian Ministry of University and Scientific Research , with the aim of comparing different organic cultivation techniques of the “Spinoso Sardo” globe artichoke, followed by the “ORWEEDS” national project (2010-2013, www.aiab.it) carried out to date.

As show in the figure 8, the meteorological trends for the 1958-2012 period at the experimental site is typically Mediterranean with 54 years long-term average annual rainfall of 554 mm. The rainfall occurring mostly from October to December.

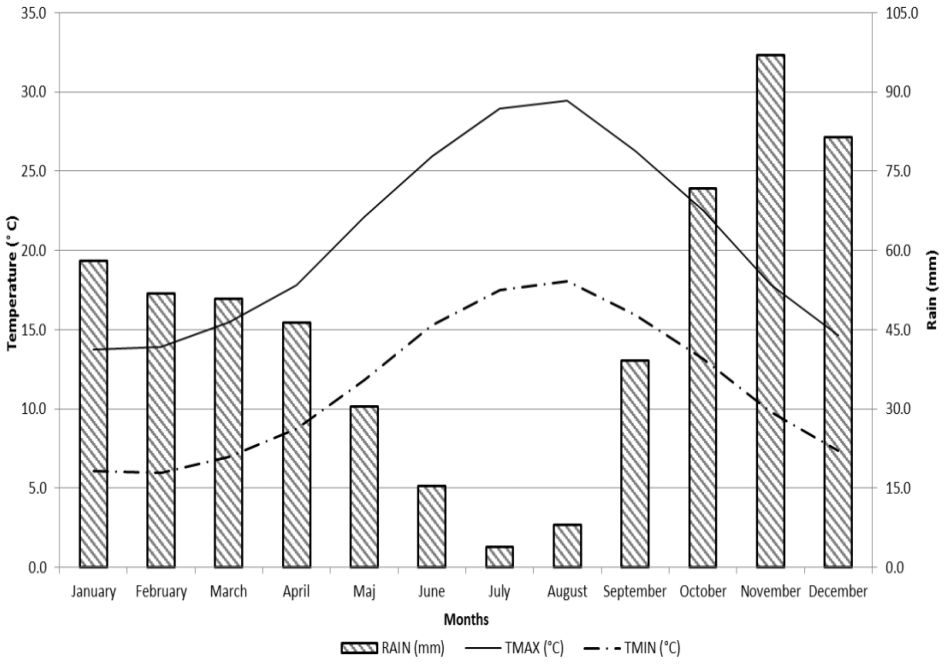


Figure. 8: Long-term meteorological series (1958-2012).

The mean annual temperature varies from 9.9 °C in January and February to 23.7 °C in August. The average of minimum and maximum annual temperatures is 11,4°C and 20,8°C.

Soil type was a sandy-clay-loam (USDA, 2006) belonging to the limestone Xerochrepts group with high calcareus status (Table 2):

Table 2. Physical characteristics of the soil before been planted as experimental field.

	Soil Parameter						
depth (cm)	Sand (g/kg)	Clay (g/kg)	Silt (g/kg)	Coarse (g/kg)	pH	Electrical conducibility (mS cm^{-1})	Field Capacity (% Vol.)
0-20	489.8	196.4	274.6	39.3	7.9	0.29	31.9
20-40	38.7	488.8	198.5	274.1	8.0	0.25	28.9

Before 2006 (beginning of the trials) the area was planted with winters wheat or many years.

1.2.2. Crop management and experimental design

The experiment was based on four factors: cropping system, heads order, harvesting time and genotypes.

The experiment management was different for two year of trial:

- **First year (2012-2013):**

The globe artichoke cv "Spinoso sardo" was arranged in split plot design with three cropping system in main plot, three capitula order in sub plot, and five date of harvesting subsampled at the subplot level. Each treatment was replicated four times.

The cropping system, heads orders and harvesting time compared are listed in Table 3.

Table 3: crop management, heads order and harvesting time compared in the first year of experiment (2012-2013) with related codes.

EXPERIMENTAL FACTORS	CODE
CROPPING SYSTEM	
Conventional	CONV
Biannual Organic	BIA ORG
Intensive Organic	INT ORG
HEADS ORDER	
First	1^{st}
Second	2^{nd}
Third	3^{rd}
HARVEST TIME (days after sowing)	
166	H1
171	H2
179	H3
194	H4
227	H5

- **Second year (2013-2014):**

In the second growing season considered, the experiment was placed in split-split plot design with three cropping system in the main plots, genotypes in sub-plots, and heads' order in sub-sub-plots.

In this second year of trial, two different clones of Spinoso sardo cv. were also considered to evaluate their response to the agricultural system.

The cropping system, heads orders and genotypes compared in second growing season are listed in Table 4.

Table 4: Crop management, heads order and genotypes compared in the second year of experiment (2013-2014) with related codes.

EXPERIMENTAL FACTORS		CODE
CROPPING SYSTEM		
Conventional		CONV
Biannual Organic		BIA ORG
Intensive Organic		INT ORG
HEADS ORDER		
First		1st
Second		2nd
Third		3rd
GENOTYPES		
Spinoso sardo		Ss
Varietal type:	Landrace	
Origin:	Local farmers	
Growing cycle:	Medium-early	
Reproduction system:	Vegetative offshoot	
EFIS		Ef
Varietal type:	Clone	
Origin:	AGRIS	
Growing cycle:	Early	
Reproduction system:	Micropropagated	
SAURO		Sa
Varietal type:	Clone	
Origin:	AGRIS	
Growing cycle:	Late	
Reproduction system:	Micropropagated	

The systems of crop management are described below:

- **“Conventional” crop management (CONV)**

  In according to local farming practices, the main characteristics of this cultivation system were:

  - Globe artichoke in continuous monoculture;
  - incorporation of plants residues into the soil by harrowing up to 20 cm depth at the end of crop cycle;
  - Typically fertilization program used in the zone: 92 kg N, 138 kg P_2O_5 and 150 kg K_2O ha−1.

- **“Intensive Organic” crop management (INT ORG)**

  The main features of this management were:

  - Globe artichoke in continuous monoculture;
  - Organic method of cultivation;
  - The growing cycle was early interrupted to allow introduction of a short-cycle legume species (Phaseolus vulgaris L.). The purpose was to improve the nitrogen concentration in the soil, through the biological fixation of a legume species as French bean;
  - French bean (cv. Rex) was sown at the rate of 100 kg·ha−1 on early June for both years and was ended at the first legume stages, when plants produced the first pods at the end of flowering. This stage occurred on 15 July 2013 and on 17 July 2014;
  - Each year, at the end of April, artichoke fresh residues were chopped and ploughed;
  - The fresh residues from legume specie, were also incorporated into soil by harrowing before artichoke new growing season started.

- **“Biannual Organic” crop management (BIA ORG)**
    - Artichoke was managed in two-year rotations with cauliflower (cv. “Nautilus”, crop duration: 75 days). The purpose was to exploit its fungicidal activity attributed to the chemical breakdown of glucosinolates. Indeed, the cauliflower produces sulfur compounds with allelopathic effects. These compounds are well known to inhibit the development of fungi and bacteria into the soil. A second aim of this rotation with cauliflower was to increase the farmer’s income;
    - Both species were cultivated at the same time in adjacent plots;
    - Both year, cauliflower was transplanted on the plot of the previous rotational artichoke;
    - Each year, at beginning of March, a legume species (*Pisum sativum* L. cv. Attika) was used as cover crop and sown at a rate of 220 kg ha^{-1} in inter-row spaces;
    - As for the INT ORG cultivation system the artichoke fresh residues were incorporated into the soil at the beginning of May.

Each plot covered an area of 440 m^2. In order to prevent any cross-contamination between treatments each plot was separated from neighbouring treatment plots by two rows of untreated artichoke. Blocks were separated by a 3 m artichoke areas.

The main agronomic operations and their timing are listed in the Table 5.

Table 5- The principal agronomic operations in experimental field. **CONV**= Conventional system, **INT ORG**= intensive organic system, **BIA ORG** = Biannual organic crop rotation between artichoke and cauliflower

Agricultural practice	Date of application	2012/13	2013/14	Cultivation system
Seed bed preparation	Early August	Harrowing	Harrowing	All treatments
Fertilization	At planting	Urea 46 N	Urea 46 N	CONV
	At planting	Triple superphosphate 138 P_2O_5	Triple superphosphate 138 P_2O_5	CONV
	At planting	Potassium sulphate 150 K_2O	Potassium sulphate 150 K_2O	CONV
	At planting	—	Chicken manure 3.7 N, 3.6 organic N, 3 P_2O_5	INT ORG, BIA ORG
	During crop growth	Urea 46 N	Urea 46 N	CONV
Irrigation	August to November			All treatments
Planting	August	08/10/2012	8/08/2013	All treatments
Harvesting	From December to March	On a weekly basis		All treatments
Weeding	During crop growth	Mechanical weeding		CONV
Cover crop	March	Pea cv. Attika		BIA ORG
Residues management	April	Globe artichoke broadcast incorporated		INT ORG
		Pea cover crop broadcast incorporated		BIA ORG
French bean Planting/harvest	June/July	French bean cv. Rex		INT ORG
Residues management	July	Globe artichoke broadcast incorporated		CONV
		Green bean broadcast incorporated		INT ORG

1.2.3. Soil analysis and meteorological measurements

In order to determine the soil characteristics, the field was sampled just before been planted with artichokes and before been fertilized at 0-20 cm and 20-40 cm depth for 4 replicates per treatment. All analysis were conducted in the Pedology laboratory of the Department of Agricultural, University of Sassari. The 32 samples were analysed using Standard methods of Società Italiana della Scienza del Suolo (1997, 2000). The following parameters were examined: pH, electrical conductivity; P_2O_5 available (Olsen method), total $CaCO_3$; exchangeable cations and Cation Exchange Capacity (Barium chloride method); total N, total C, organic matter (elemental analyser LECO 628).

Weather data were collected from a weather station located nearby the experimental field (40° 46’ 41”N; 8°29’ 38”E). Figure 9 and 10 show the meteorological conditions for two year of interest.

1.2.4. Chemical analysis

1.2.4.1. Sample preparation

Every season artichoke heads, produced by the five plants per replicate, were weekly collected according to head's order of usual marketing size. From each test plant were withdrawn at last 7 heads.

The samples were disease-free and the length of central global flower buds was approximately 2 mm. From every samples, external bracts were removed and internal bracts and receptacles were washed with distilled water and stored at -80°C, until lyophilization with Heto Lyolab 3000 for 72 h (-56°C). The lyophilized samples were powdered by a blender and stored at - 20°C until they were extracted.

The extraction was performed as reported (Palermo et al. 2013) slightly modified: 80mg of sample was extracted by 4 ml of methanol/water (50:50, v/v) and sonicated at room temperature for 30 min. The mixture were centrifugated 8000 rpm for 15 min at room temperature and filtered through a 0.45 µm Whatman filter paper, and then stored at -20°C until analysis. In consideration of large amount of samples analysed to test the reproducibility of method, duplicate analysis were performed on 20% of all samples. The reproducibility, expressed by confidence limits of the result had a confidence level of 95%. The samples were analysed by laboratory of Biomolecular Institute for Chemistry at the National Research Council of Sassari.

1.2.4.2. Solvents and reagents

LC/MS-grade acetonitrile and methanol were obtained from Fisher Scientific (Loughboroug Leics, UK). Acetic acid and trifluoracetic acid LC/MS grade was purchased from Sigma Aldrich (Milan, Italy).Water was purified by using MIlliQ system (Millipore, Milan). Standards of 5-Caffeoylquinic acid, 1-Caffeoylquinic acid, Luteolin and Apigenin were obtained from Sigma–Aldrich (Seelze, Germany), Luteolin-7-O-Glucoronide, Luteolin-7-O-Glucoside, Luteolin-7-O-Rutinoside, Apigenin-7-O-Glucoside, 1,5-Dicaffeoylquinic acid, 3,5-Dicaffeoylquinic acid Apigenin-7-O-Glucoronide, Apigenin-7-O-Rutinoside were purchased from Extrasynthese (Genay, France).

The purities of all standards were ≥98% and suitable for LC-MS analysis.

From all solid standards concentrated stock solutions were prepared on a weight basis in methanol and stored at −20°C. Different individual solutions containing phenolic compounds were obtained by diluting the concentrate stock solutions with methanol and were stored at −20°C

From these individual stock standard solutions, a stock–standard mixture was prepared in eluent mixture to prepare calibration curves in solvent.

1.2.4.3. LC/MS system

An Agilent Technologies (Palo Alto, CA, USA) 1100 series LC/MSD equipped with a diode-array detector and an autosampler (G1313A) was used for LC separation. A ChemStation HP A.10.03 was used for data analysis. Chromatographic separation was achieved using a Gemini C18 (100 mm × 2.1 mm, 2.6µm, 100 A°) (Phenomenex, Torrance, CA, USA). The column temperature was maintained at 37 ◦C. The mobile phase consisted of Eluent A, water with 0.01% acetic acid an 0.01% TFA, and Eluent B, acetonitrile. The gradient elution was performed at the flow rate of 0.4 mL/min and a run time of 60 min. Time program = 0 min, 95% A/; 20.0 min, 85% A/25% B; 40 min, 70% A/30% B; 50 min, 30% A/70% B; Ten minutes of post-run time was used to equilibrate the column. The injection volume was 10 µl, and the autosampler compartment was set to 10 °C. The Diode Array Detector was set at 270, 320 and 520 nm.

LC/MS analyses in both negative electrospray ionization (ESI−) and positive electrospray ionization (ESI+) modes were performed using Agilent G1946 (MSD 1100) single stage quadrupole instrument equipped with an electrospray atmospheric pressure ionization (ES-API) source. The interface voltage for (ESI−) and (ESI+) modes were 3400 V and 3800 V, respectively. Mass scan range, 150–800 amu. The following ES-API conditions were applied: drying gas (nitrogen) heated at 350 ◦C at a flow rate of 9.5 L/min; nebulizer gas (nitrogen) at a pressure of 42 psi; fragmentor voltage at 70eV in Pos mode and 50 eV in Neg mode; dwell time was 460 ms. Tuning and mass calibration were performed by infusing a Agilent ESI calibration mix solution.

1.2.5. Statistical analysis

Analysis of variance was performed to evaluate the influence of cropping systems, heads' order, genotypes and harvesting times on mineral composition and their interaction. The variance was analyzed using PROC GLM (SAS software 9.02, SAS institute Ltd, USA) for both the years separately. The difference between means was compared with Fisher's least significant difference test (LSD) at 5% probability level.

Pearson linear coefficients of correlation (r) among studied variables were calculated from regression analyses between pairs of traits. Principal component analysis (PCA) was performed on 2012-2013 soil physical-chemical parameters, polyphenolic pool of first, second and third heads order.

1.3. RESULTS AND DISCUSSION

1.3.1. Soil and weather conditions

The figures 9 and 10 show the main thermo-pluviometric trend at experimental site, for two year of interest.

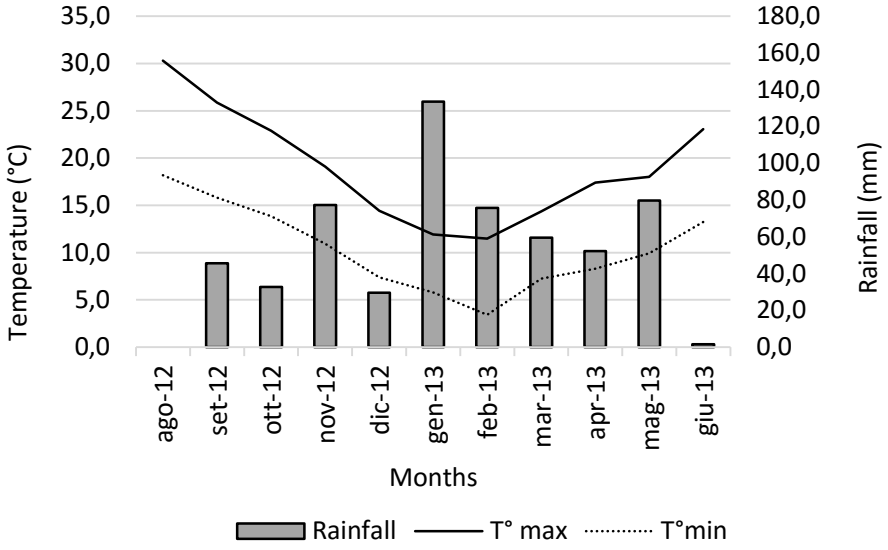


Figure 9: Monthly maximum (solid line) and minimum (dashed line) mean temperatures and rainfall (bar chart) during the first year (crop cycle 2012-2013).

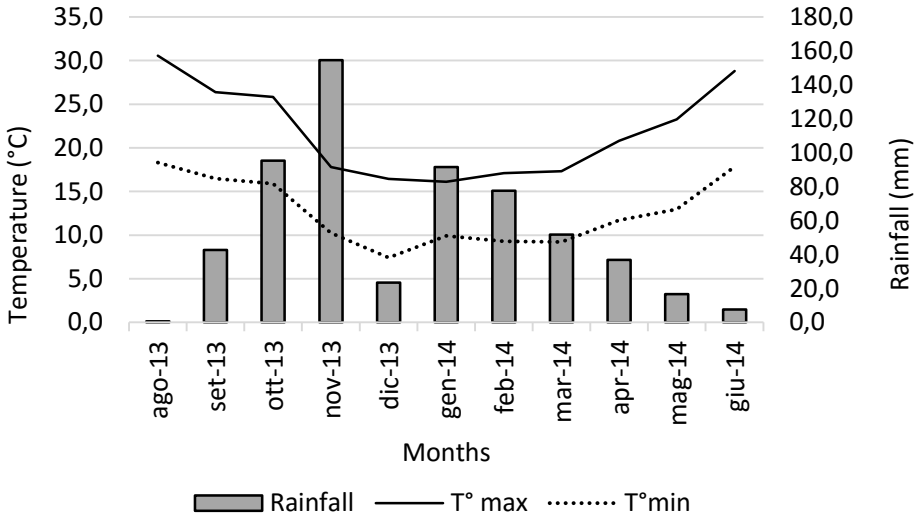


Figure 10: Monthly maximum (solid line) and minimum (dashed line) mean temperatures and rainfall (bar chart) during the second year of analysis at the experimental site (crop cycle 2013-2014).

The total rainfall was similar within season (588.8 mm vs 598.4mm on average) but showed a differently distributed. In the first season the rainiest month was January with 133,6 mm followed by May (79.8 mm), November (77.4 mm) and February (75.8 mm), while in the second season the wettest month was November with 154.5 mm followed by October (95.3 mm) and January (91.6 mm). As regard temperature were registered in the 2013/14 extreme values higher by comparison with the first.

	Maximum Air Temperature (°C)		**Minimum Air Temperature (°C)**	
	2012/2013	**2013/2014**	**2012/2013**	**2013/2014**
August	34.0	39.5	14.0	15.6
September	32.0	32.0	11.0	13.0
October	33.0	31.0	6.0	11.5
November	23.5	25.0	6.0	2.0
December	18.0	19.0	3.0	4.0
January	18.0	19.0	-2.0	7.0
February	**16.0**	**24.1**	**-1.0**	**6.0**
March	**17.0**	**24.1**	**0.0**	**5.5**
April	27.0	24.9	4.0	6.8
May	23.0	32.5	6.0	9.5
June	30.0	36.0	9.0	12.0

Table 6: Maximum Air temperature and Minimum Air Temperature during the growth seasons 2012/13 and 2013-2014 at the experimental site.

As shown the table 6 the maximum air temperature on February and March were very different: 16.0 °C vs 24.1°C and 17.0°C vs 24.1°C respectively. The same trend was recorded about minimum air temperature (-1.0°C vs 6.0°C and 0.0°C vs 5.5°C)

The chemical characterization of the soil is reported in table 7.

The chemical analysis highlights differences among managements and between growing seasons within treatments. The limestone content was lower in BIA ORG than in CONV and INT ORG and between two seasons it showed a decrease in INT ORG and in BIA ORG. The P_2O_5 available followed a similar trend: in CONV the content of phosphorous was similar in two growing seasons while there was a decrease in INT ORG and BIA ORG between the first and the second season.

Table 7: Soil values during the growth seasons at the experimental site.

Characterization	CONV				INT ORG				BIA ORG			
	2012		2013		2012		2013		2012		2013	
Depth	(0-20)	(20-40)	(0-20)	(20-40)	(0-20)	(20-40)	(0-20)	(20-40)	(0-20)	(20-40)	(0-20)	(20-40)
pH (H_2O)	7,9	7,9	7,9	7,9	7,9	7,9	7,9	7,9	8,1	8,1	7,9	7,9
E.C., mS cm^{-1} in H_2O 5/1	0,25	0,23	0,22	0,22	0,23	0,23	0,22	0,23	0,20	0,21	0,21	0,21
Total limestone, g kg^{-1}	207	205	216	214	287	187	147	141	109	117	95	79
Organic Carbon, g kg^{-1}	21	22	20	23	17	20	20	20	18	18	16	16
Organic Matter, g kg^{-1}	36	38	35	36	30	34	35	34	31	32	27	27
Tot N, g kg^{-1}	1,8	1,8	1,6	1,5	1,8	1,7	1,6	1,6	1,7	1,8	1,7	1,6
C/N	12	13	13	14	10	11	13	12	11	10	10	10
P_2O_5 available, mg Kg^{-1}	98	86	91	88	89	86	66	55	84	83	63	57
Ca^{++}exchangeable ' meq 100 g^{-1}	26,42	27,55	24,14	24,00	25,35	24,84	23,87	23,80	22,92	22,70	23,47	24,26
Mg++ exchangeable, meq 100 g^{-1}	1,86	1,87	1,99	1,93	2,24	2,12	2,63	2,50	1,99	1,96	2,40	2,31
Na+ exchangeable , meq 100 g^{-1}	0,36	0,34	0,54	0,60	0,49	0,43	0,73	0,99	0,54	0,51	0,58	0,56
K+ exchangeable , meq 100 g^{-1}	0,27	0,27	0,72	0,22	0,40	0,37	0,89	0,85	1,03	1,03	0,93	0,84
Sum of exchangeable bases, meq 100 g^{-1}	28,91	30,02	27,39	26,74	29,35	28,57	28,12	28,14	26,48	26,20	27,38	27,97
C.E.C., meq 100 g^{-1}	30,1	30,2	27,4	26,7	30,7	30,1	29,2	29,0	27,4	27,7	28,8	29,4
G.S.B. (%)	96	100	100	100	96	95	96	97	97	95	95	95
Exchangeable acidity, meq 100 g^{-1}	1,2	0,1	0,0	0,0	1,4	1,5	1,1	0,9	1,0	1,5	1,4	1,4

1.3.2. Characterization of polyphenols in artichoke cv " Spinoso sardo"

The Table 7 lists the 10 compounds separated and identified using LC/MS (Peak number refer to Figure 9). Each polyphenols were characterized with the retention time values, UV/Vis spectra, their MS full scan and Ms information. Individual phenolic compounds were quantified using calibration curves of respective reference compounds. The calibration curves of peak area against amount showed a coefficient of correlation r^2>0.999.

Table 7: Compound identified in globe artichoke cv. "Spinoso Sardo" reporting with properties after LC/MS.

Peak*	Compounds	t_R (min)	LC-DAD (nm)	[M+H] $(m/z)^+$	[M-H] (m/z)	Limit of Detection
1	5-Caffeoylquinic acid	10.34	320	355	353	> LQD
2	1-Caffeoylquinic acid	11.74	320	355	353	> LQD
3	Luteolin-7-O-Glucoronide	27.34	270	463	461	> LQD
4	Luteolin-7-O-Glucoside	28.14	270	449	447	> LQD
5	Luteolin-7-O-Rutinoside	29.06	270	595	593	**< LQD**
6	Apigenin-7-O-Glucoside	29.76	270	447	445	**< LQD**
7	1,5-Dicaffeoylquinic acid	30.78	320	517	515	> LQD
8	3,5-Dicaffeoylquinic acid	31.01	320	517	515	> LQD
9	Apigenin-7-O-Glucoronide	31.68	270	433	431	> LQD
10	Apigenin-7-O-Rutinoside	31.92	270	579	577	**< LQD**

*****Peak number refer to Figure 9. All metabolites are reported to the recommended IUPAC numbering system.

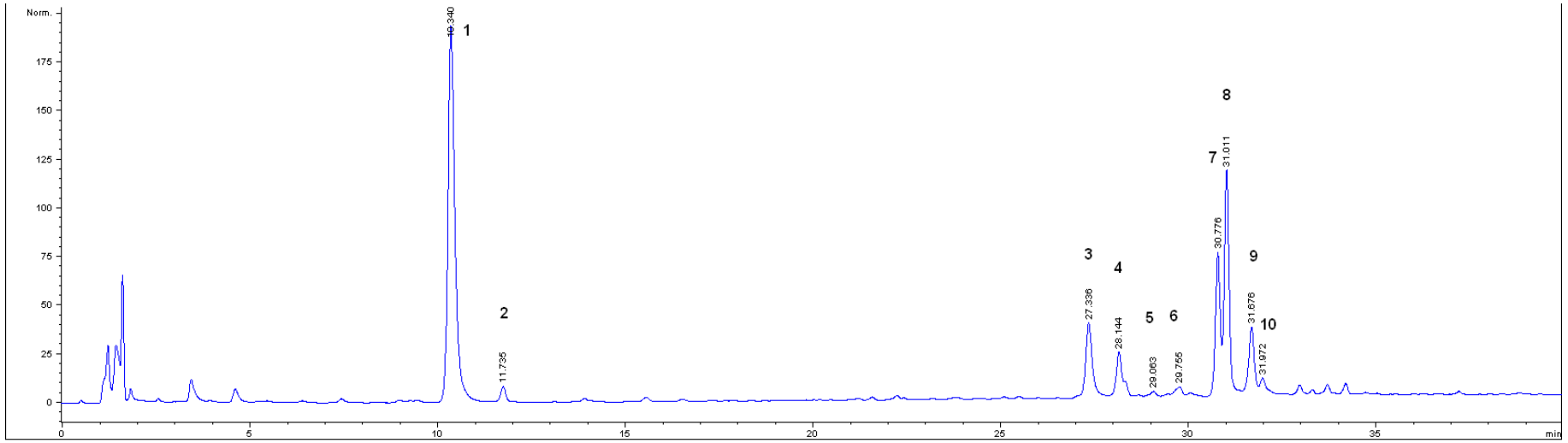


Figure 9: HPLC chromatogram at 270 nm of globe artichoke head extract. Identified compounds: (1) 5-Caffeoylquinic acid (Chlorogenic acid); (2) 1-Caffeoylquinic acid; (3) Luteolin-7-O-Glucoronide; (4) Luteolin-7-O-Glucoside; (5) Luteolin-7-O-Rutinoside; (6) Apigenin-7-O-Glucoside; (7) 1,5-Dicaffeoylquinic acid; (8) 3,5-Dicaffeoylquinic acid; (9) Apigenin-7-O-Glucoronide;(10) Apigenin-7-O-Rutinoside

Our results on the qualitative profile were not consistent to previous works on globe artichoke cv. “Spinoso Sardo” (Alamanni et al. 2001, Alamanni & Cossu, 2003). In these works were identified only five polyphenols (chologenic acid, cynarine, caffeic acid, cynaroside and scolimuside) and a group of iso chlorogenic acids. In the present study, were not detected caffeic acid and scolimuside (Luteolin-7-O rutinoside) showed lower concentration than detection limit. The iso-chlorogenic acids were identified as 1,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid. These differences may largely be related to various factors as different analytical methods, different extraction techniques and mainly the high degree of heterozygosity of globe artichoke (Lanteri et al. 2001).

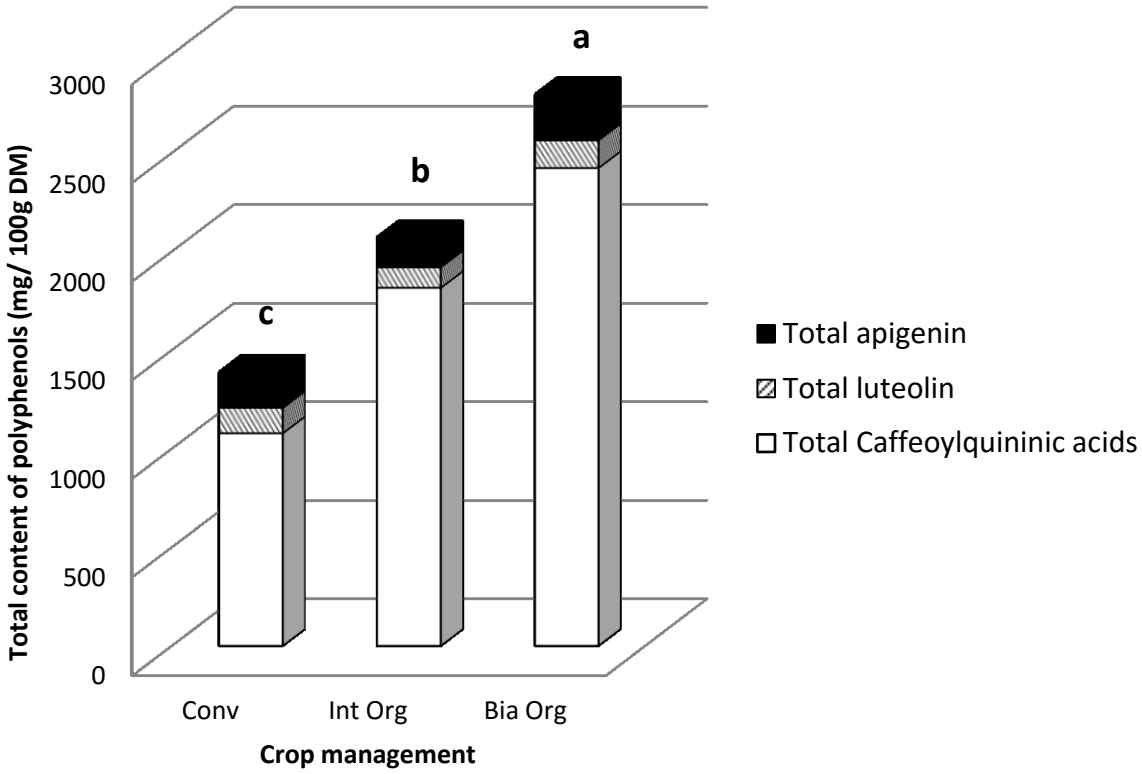
According to different experimental designs, for each year, statistical analysis were carried out separately.

1.3.3. Trial A: First growing season 2012/2013

1.3.3.1. Effect of managements

As shown in a Figure 11 the total measures of polyphenols, calculated as sum of identified phenolic compounds, were affected by different management resulting significantly higher in the Biannual Organic System (BIA ORG) than in the other ones. The Intensive Organic System (INT ORG) achieved an intermediate levels. Our results were partially in agreement with these reported by Lombardo and coauthors 2009 and 2015.

The hydroxycinnamic acids were predominant compounds followed to flavonoids. The group of luteolin showed minor variability among managements.



Values are expressed in mg $100g^{-1}$ of DM. Different letters indicate statistical significance at P≤ 0.05 according to LDS test.

Figure 11: Profile of total polyphenols present in each treatments for the 2012-13 growing season.

As reported in Table 8, also the analysis of each polyphenol, showed different results.

Table 8: Polyphenols content (mg 100 g^{-1} DM) of artichoke heads for the different levels of treatments tested for 2012-13 growing season

COMPOUNDS	MANAGEMENTS		
	CONV	**INT ORG**	**BIA ORG**
5 CQ ac	846.5 **c**•	1225.9 **b**	1558.5 **a**
1 CQ ac	12.18 **a**	10.62 **b**	9.32 **c**
1,5 di CQ ac	56.45 **c**	166.64 **b**	234.02 **a**
3,5 di CQ ac	162.51 **c**	412.01 **b**	620.73 **a**
Total Caffeoylquininic acids	**1077.64 c**	**1815.17 b**	**2422.57 a**
Lut glc	67.18 **a**	44.57 **b**	58.61 **a**
Lut rut	< LQD*	< LQD	< LQD
Lut glr	61.67 **b**	59.71 **b**	82.01 **a**
Total luteolin	**128.85 ab**	**104.28 b**	**140.62 a**
Api glr	182.43 b	153.23 c	234.37 a
Api rut	< LQD	< LQD	< LQD
Api glc	< LQD	< LQD	< LQD
Total apigenin	**182.43 b**	**153.23 c**	**234.27 a**

*< LQD = below the quantification limit of method. •Different letters indicate statistical significance at P≤ 0.05 according LDS test

Note: **5 CQ ac:** 5-O-caffeoylquininic acid, **1 CQ ac:** 1-O-caffeoylquininic acid; **Lut glr:** Luteolin-7- Glucoronide; **Lut glc:** Luteolin-7- Glucoside; **Lut rut:** Luteolin-7-Rutinoside; **Api glc:** Apigenin-7- Glucoside; **1,5 di CQ ac:** 1,5-dicaffeoylquininic acid; **3,5 di CQ ac:** 3,5-dicaffeoylquininic acid; **Api glr:** Apigenin-7- Glucoronide ; **Api rut:** Apigenin-7- Rutinoside

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Concerning the caffeoylquininic acids the Chlorogenic (**5 CQ ac**) was noteworthy in all managements (78% in CONV, 67% in INT ORG and 64% in BIA ORG of total amount of caffeoylquininic acids) whereas the **1 CQ ac** was lowest and founded in higher quantity in the CONV than BIA ORG one (12.18 mg $100g^{-1}$Vs 9.32 mg $100g^{-1}$ respectively). The dicaffeoylquininic acids showed the similar trend among management with **3,5 di CQ ac** present in a major quantity (15% in CONV, 23% in INT ORG and 26% in BIA ORG of total amount of caffeoylquininic acids) . These last results were in contrast with precedent study (Palermo et al. 2013, Lombardo et al. 2009, 2015 Pandino et al. 2012) where **1,5 di CQ ac** often showed the major amount. This result is very important in light of recent knowledge on neuroprotective effects of **3,5 di CQ ac** as possible drug to neurodegenerative disease (Kim et al.2005).

As far as flavonoids, **Api glur** was the prevalent for all growing system followed by **Lut glr** and **Lut gluc**. These results were consistent to few works (Pandino et al. 2010, 2012) and in contrast with other (Romani et al., 2006; Negro et al., 2012) demonstrating how is difficult to compare available data.

It is interesting to note that the behavior of **Lut glr** and **Lut glc** was different as among management as within these.

The **Lut glr** was present in highest amount in a BIA ORG followed to CONV and INT ORG which were non funded significantly differences. On the other hand **Lut glc** presented values comparable in BIA ORG and CONV, but lowest in INT ORG. It is interesting to note how behavior of luteolines was different also within each treatments: in Conventional system were registered similar amount (52% **Lut glc** and 48% **Lut glr**) while in Organic managements the **Lut glr** was present in a higher concentrations than **Lut glc** (43% **Lut glc** and 57% **Lut glr** in INT ORG and 42% **Lut glc** and 58% **Lut glr** in BIA ORG).

1.3.3.2. Effect of heads' order and day after planting

As shown in a Table 9, we not observed significant differences in heads order.

Table 9: Mean values (mg 100 g^{-1} DM) for the polyphenols content traits of artichoke heads for the different levels of treatments, heads' order, and days after planting based on the three compared cropping systems tested for 2012-13 growing season

Factor	5 CQ ac	1 CQ ac	Lut glc	Lut glr	1,5 di CQ ac	3,5 di CQ ac	Api glr
Treatment (T)							
CONV	846.5 c •	12.18 a	67.18 a	61.67 b	56.45 c	162.51 c	182.43 b
INT ORG	1225.9 b	10.62 b	44.57 b	59.70 b	166.64 b	412.01 b	153.23 c
BIA ORG	1558.5 a	9.32 c	58.61 a	82.01 a	234.02 a	620.73 a	234.37 a
Heads' order (H)							
1st	1283.0	9.94	56.00	78.40	183.49	410.25	174.19
2nd	1267.2	10.18	54.69	62.67	157.10	413.74	181.83
3rd	1307.1	10.86	51.55	72.17	190.79	511.66	202.88
Days after planting (D)							
166	1295.5	9.67 ab	57.94 a	88.17 a	212.82 a	506.58	166.97 ab
171	1315.0	10.16 ab	53.64 a	66.79 a	188.57 ab	430.60	171.10 ab
179	1487.2	8.50 b	26.70 b	38.07 b	221.78 a	443.39	133.13 b
194	1362.2	9.76 ab	52.50 a	41.40 b	142.94 b	431.03	189.55 a
227	1221.0	11.09 a	55.18 a	80.59 a	173.44 ab	448.60	198.30 a
LSD interaction (P≤0.05)							
(T) X (H)	ns	ns	ns	ns	ns	ns	ns
(T) X (D)	ns	ns	ns	ns	ns	ns	ns

• Different letters indicate statistical significance at P≤ 0.05, according to the LSD test; ns = not significant

Note: **5 CQ ac:** 5-O-caffeoylquininic acid; 1 **CQ ac:** 1-O-caffeoylquininic acid; **Lut glr:** Luteolin-7- Glucoronide; **Lut glc:** Luteolin-7-Glucoside; **1,5 di CQ ac:** 1,5-dicaffeoylquininic acid (Rt= 30.78 min); **3,5 di CQ ac:** 3,5-dicaffeoylquininic acid (Rt= 31.01 min); **Api glr:** Apigenin-7- Glucoronide.

Despite of results obtained to head' order, the effect of harvest time showed significant differences to some polyphenols (table 9). The lowest amounts of **1CQ ac** (in a figure 12)**, Lut glc, Lut glr** and **Api glr** were observed in 179 day after planting. The variations were strongest in luteolins as shown in a figure 13.

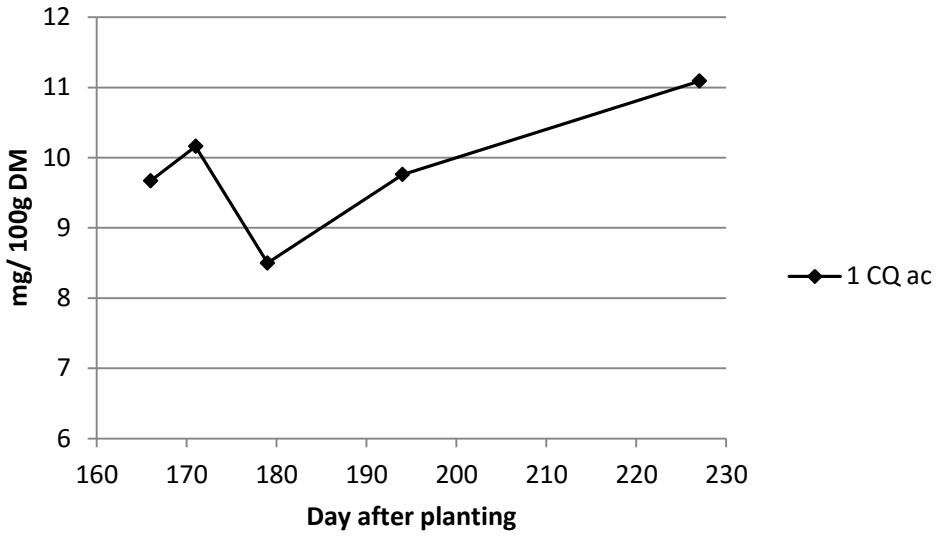

Figure 12: Trend of the **1CQac** on days after planting during 2012-13 growing season.

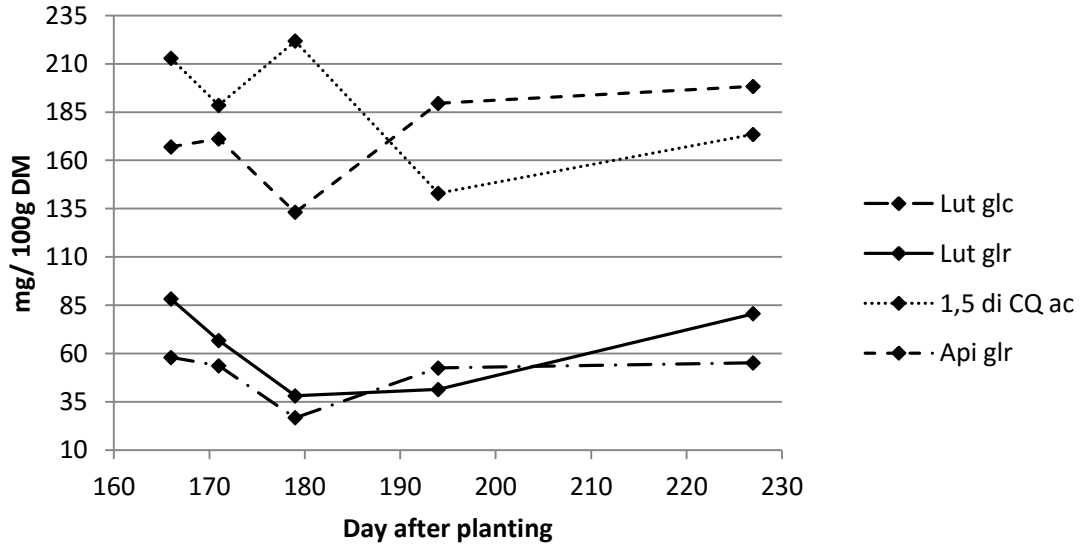

Figure 13: Trend of the **Lut glc**, **Lut glr**, **1,5 di CQ ac** and **Api glr** on days after planting during 2012-13 growing season.

As regard **1,5 di CQ ac**, the trend was opposite: the acid showed the highest level at the 179 D. It is interesting to note that the **1,5 di CQ ac** development was opposite to **Api glu**.

1.3.3.3. Correlation

Strong correlations between soil physical-chemical parameters and polyphenols were detected (Table 10). It is interesting to note the positive correlations identified between **5 CQ ac** in all heads order and exchangeable Na. The same trend emerged for each Dicaffeolylquininic acids.

Significant positive correlations were found between **5 CQ ac**, **1,5 di CQ ac** and **3,5 di CQ ac** in 1st and 2nd heads' order and exchangeable Mg while **Lut glc** proved to be negatively associated with both Nitrogen in 1st heads' order and exchangeable Mg in 2nd heads' order .

Moreover **Apiglc** was negatively correlated with electrical conductivity in 1st heads' order.

Finally, **5 CQ ac** showed significant negative correlations with organic C and exchangeable Ca although only in the 1st heads' order.

	Coarse	sand	silt	clay	pH	E.C.	$CaCO_3$	C	SOM	N	C/N	P_2O_5	Ca	Mg	Na	K	S.E.B.	C.E.C.	GSB	E. A.
5 CQac 1st	-.272	.227	-.521	.450	.240	-.678**	-.289	-.417	-.381	.067	-.594*	.126	-.640*	.576*	.891**	.574*	-.347	-.085	-.484	.464
1 CQ ac 1st	-.275	.355	.082	-.333	-.139	.468	.152	.670**	.662**	-.130	.813**	.067	.884**	-.181	-.427	-.526	.728**	.381	.681**	-.674**
Lutglc 1st	-.186	.359	-.112	-.240	-.474	-.233	.023	.123	.086	-.321	.277	.411	.163	-.593*	-.240	-.149	-.060	-.196	.151	-.158
Lutglr 1st	-.161	.189	-.183	.060	-.363	-.497	-.037	-.457	-.467	-.716**	-.144	.152	-.151	-.377	.065	.216	-.181	-.081	-0.24	.218
1.5 diCQac 1st	-.202	.110	-.518	.550*	.303	-.580*	-.333	-.368	-.344	.138	-.601*	.139	-.551*	.626*	.891**	.485	-.227	.086	-.523	.511
3.5 diCQ ac 1st	-.276	.164	-.487	.514	0.28	-.584*	-.385	-.299	-.282	.167	-.538*	.182	-.498	.572*	.875**	.469	-.210	.084	-.497	.480
Apiglc 1st	-.496	.700**	-.251	-.293	-.340	-.705**	.003	-.272	-.241	-.554*	.017	.261	-.287	-.320	.178	.399	-.414	-.593*	.103	-.150
5 CQac 2nd	-.492	.344	-.367	.347	.268	-.489	-.407	-.095	-.068	.213	-.302	.115	-.298	.561*	.813**	.419	-.074	.108	-.310	.282
1 CQ ac 2nd	.157	.202	.220	-.677	-.237	.526	.508	.554*	.557*	-.0002	.660*	-.002	.631*	-.394	-.740**	-.630*	.357	-.142	.835**	-.818**
Lutglc 2nd	-.536*	.573*	.076	-.387	-.547*	-.059	.065	.335	.334	-.423	.715**	.166	.189	-.580*	-.393	.088	-.094	-.345	.326	-.332
Lutglr 2nd	-.663**	.452	.040	-.044	-.659*	-.349	-.124	-.058	-.053	-.454	.283	-.125	-.198	-.369	.027	.387	-.292	-.136	-.331	.322
1.5 diCQac 2nd	-.458	.378	-.496	.388	.189	-.587*	-.293	-.245	-.204	.012	-.364	.086	-.413	.578*	.849**	.495	-.139	.064	-.352	.330
3.5 diCQ ac 2nd	-.541*	.321	-.440	.501	.292	-.565*	-.528	-.130	-.114	.150	-.311	.235	-.369	.549*	.880**	.541*	-.128	.121	-.420	.393
Apiglc 2nd	-.810**	.668**	-.243	.064	.0005	-.538*	-.509	.187	.188	.041	.170	.428	-.120	.040	.495	.441	-.169	-.196	-.049	.006
5 CQac 3rd	-.468	.205	-.313	.477	.437	-.616*	-.636*	-.262	-.254	.110	-.410	.304	-.427	.443	.832**	.642*	-.245	-.027	-.418	.372
1 CQ ac 3rd	.727**	-.579*	.387	-.253	.420	.647*	.270	.115	.097	.216	.016	-.106	.472	-.065	-.560*	-.563*	.376	.120	.491	-.483
Lutglc 3nd	-.291	.002	.653*	-.355	.152	.394	-.315	.505	.477	.488	.394	-.140	.496	-.172	-.275	-.214	.255	.100	.261	-.296
Lutglr 3nd	-.157	-.007	.291	-.119	-.406	-.420	-.128	-.520	-.531	-.363	-.307	-.221	-.349	-.448	-.005	.296	-.459	-.252	-.471	.434
1.5 diCQac 3nd	-.395	.197	-.295	.398	.395	-.647*	-.503	-.391	-.366	.036	-.492	.179	-.536*	.449	.807**	.681**	-.342	-.142	-.412	.366
3.5 diCQ ac 3nd	-.388	.213	-.419	.490	.476	-.630	-.556*	-.292	-.278	.094	-.464	.330	-.432	.519	.871**	.598	-.210	-.016	-.367	.325
Apiglc 3nd	-.520	.486	-.183	-.019	.167	-.443	-.446	.120	.106	.023	.062	.510	.068	-.036	.355	.209	-.019	-.143	.126	-.174

EC: Electrical conductivity, $CaCO_3$: total limestone, C: organic C, SOM: soil organic matter, N: total N, SEB: sum of exchangeable basis, CEC: cation exchange capacity, EA: exchangeable acidity

Table 10 Partial Pearson's Correlation coefficients and significance correlation among soil parameters and polyphenols.

Pairwise correlation between analysed polyphenols are reported in table 11. As expected, many significant correlations were found in all orders. Similarly, significant correlations were found between all hydroxycinnamic acids although at different extents.

The **5CQ ac** showed a positive correlation with **1,5 di CQ ac** and **3,5 di CQ ac** in all heads' order, whereas **1CQ ac** proved to be negatively associated with **5CQ ac, 1,5 di CQ ac and 3,5 di CQ ac.**

Apiglc was found to be positively correlated with **1,5 di CQ ac** in the 2nd and 3rd heads' order.

	5 CQac 1st	1 CQ ac 1st	Lutglc 1st	Lutglr 1st	1.5 diCQac 1st	3.5 diCQ ac 1st	Apiglc 1st	5 CQac 2nd	1 CQ ac 2nd	Lutglc 2nd	Lutglr 2nd	1.5 diCQac 2nd	3.5 diCQ ac 2nd	Apiglc 2nd	5 CQac 3rd	1 CQ ac 3rd	Lutglc 3nd	Lutglr 3nd	1.5 diCQac 3nd	3.5 diCQ ac 3nd	Apiglc 3nd
5 CQac 1st	-																				
1 CQ ac 1st	-.559*	-																			
Lutglc 1st	-.198	.338	-																		
Lutglr 1st	.196	.187	.512	-																	
1.5 diCQac 1st	.972**	-.526	-.143	.206	-																
3.5 diCQ ac 1st	.953**	-.448	-.034	.251	.988**	-															
Apiglc 1st	.333	.129	.336	.606*	.174	.223	-														
5 CQac 2nd	.880**	-.171	-.080	.261	.875**	.914**	.398	-													
1 CQ ac 2nd	-.793**	.541*	.200	-.414	-.837**	-.820**	-.109	-.707**	-												
Lutglc 2nd	-.499	.497	.386	.154	-.624*	-.571*	.489	-.368	.538*	-											
Lutglr 2nd	.073	.183	.334	.619*	-.009	.047	.618*	.198	-.238	.619*	-										
1.5 diCQac 2nd	.933**	-.249	-.157	.336	.901**	.909**	.463	.963**	-.750**	-.349	.248	-									
3.5 diCQ ac 2nd	.887**	-.211	-.032	.312	.895**	.933**	.375	.979**	-.776**	-.343	.240	.952**	-								
Apiglc 2nd	.469	.192	.394	.348	.414	.524	.683**	.719**	-.281	.289	.506	.622*	.730**	-							
5 CQac 3rd	.833**	-.233	-.128	.330	.814**	.847*	.440	.924**	-.776**	-.314	.205	.896**	.947**	.712**	-						
1 CQ ac 3rd	-.615*	.178	-.347	-.491	-.569*	-.621*	-.575*	-.637*	.548*	-.205	-.748**	-.670**	-.691**	-.714**	-.554*	-					
Lutglc 3nd	-.299	.533*	.030	-.100	-.304	-.203	.081	.124	.212	.245	.154	-.083	.045	.402	.146	.126	-				
Lutglr 3nd	.249	-.072	.279	.789**	.212	.261	.571*	.331	-.515	.104	.712**	.348	.329	.374	.396	-.528	.199	-			
1.5 diCQac 3nd	.860**	-.340	-.310	.280	.793**	.793**	.494	.876**	-.761**	-.303	.202	.902**	.878**	.600*	.960**	-.506	.062	.402	-		
3.5 diCQ ac 3nd	.884**	-.273	-.149	.300	.871**	.890**	.409	.931	-.768**	-.404	.092	.922**	.949**	.653*	.985**	-.511	.031	.307	.954**	-	
Apiglc 3nd	.369	.322	.493	.452	.367	.486	.614	.661	-.222	.104	.264	.532*	.653*	.896**	.680**	-.452	.460	.375	.530	.647*	-

Table 11 Partial Pearson's Correlation coefficients and significance correlation among polyphenols

1.3.3.4. PCA analysis

Respectively, PC1 and PC2 contributed to 38.5 % and 17.7 % of the total variation (Figure 14). Conventional cropping system was clearly separated from the rest of the cropping systems. Furthermore, a strong heads' order effect was observed across all cropping systems with 1st and 2nd heads' order clustering separately from 3rd heads' order. The separation of 1st and 2nd heads' order was attributed to the content of **1,5 diCQ ac**, and **3,5 diCQac** in the 1st heads' order and **5 CQ ac**, and **Lut glc** in the 2nd heads' orders.

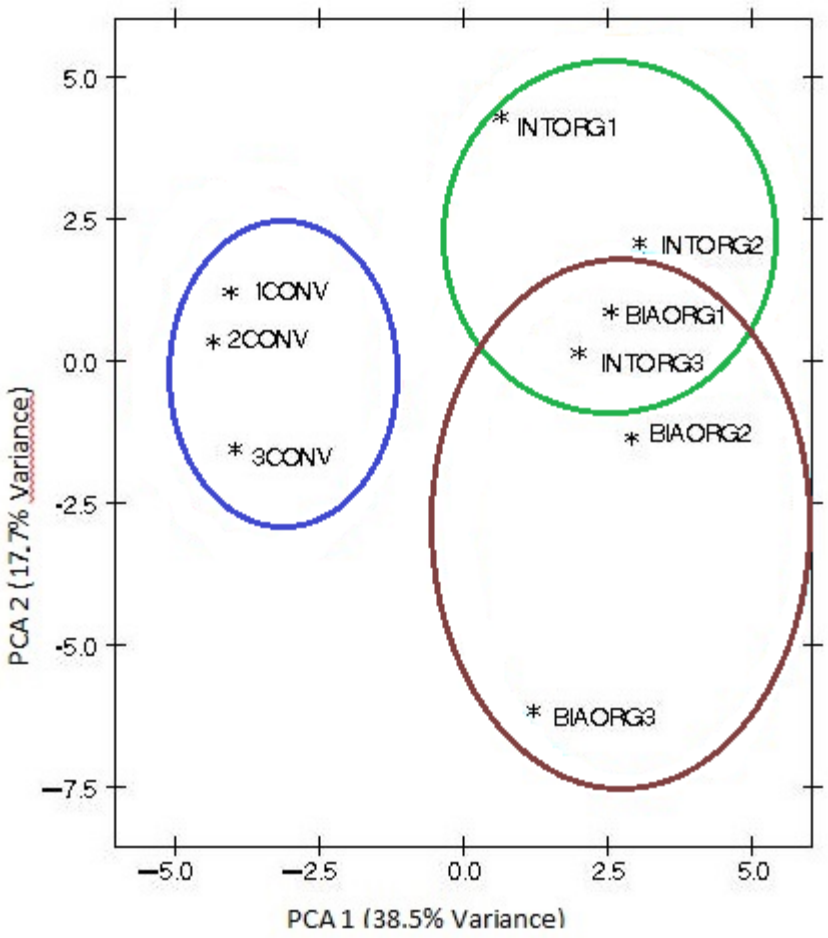

Figure 14 Principal component analysis (PCA) of polyphenols data.

1.3.4. Trial B: Second growing season 2013/2014

1.3.4.1. Effect of managements

In the second years of trials, the introduction of clones and the important differences on climate significantly affected the development of polyphenols profile. As observed in the table 12 the amount of polyphenols was considerably above respect to first season, except to the luteolins, where the increase was less pronounced respect to others.

Among the total caffeoylquininic acids CONV and BIA ORG showed the highest amount (3646.66 mg 100 g^{-1} and 3442.97 mg 100g^{-1} respectively) respect to INT ORG (3114.41 mg 100g^{-1}).

Table 12: Mean values (mg 100 g^{-1} DM) for the polyphenols content traits of artichoke heads for the different levels of treatments tested for 2013-14 growing season.

COMPOUNDS	MANAGEMENTS		
	CONV	**INT ORG**	**BIA ORG**
5 CQ ac	2457.0	2070.8	2185.9
1 CQ ac	24.85	24.02	21.63
1,5 di CQ ac	36.01 b	50.90 a	36.46 b
3,5 di CQ ac	1128.80 ab	968.69 b	1198.97 a
Total Caffeoylquininic acids	**3646.66 a**	**3114.41 b**	**3442.97 a**
Lut glc	59.52	56.69	56.35
Lut glr	56.24 b	86.18 a	58.29 b
Total luteolin	**115.76 b**	**142.87 a**	**114.64 b**
Api glr	242.74	262.84	272.81
Total apigenin	**242.74**	**262.84**	**272.81**

Different letters indicate statistical significance at P≤ 0.05, according to the LSD test

Concerning the hydroxycinnamic acids, **5 CQ ac** and **1 CQ ac** showed no significant differences among managements. It is interesting to note the behaviour of dicaffeoylquininc acids. Both showed significant differences but the trends were dissimilar: **1,5 di CQ ac** was present in the highest amount in INT ORG followed to BIA ORG and CONV (50.90 mg $100g^{-1}$ Vs 36.46 and 36.01 mg $100g^{-1}$ respectively), while **3,5 di CQ ac** was richest in BIA ORG followed to CONV and INT ORG (1198.97 Vs 1128,80 and 968.69 mg $100g^{-1}$, respectively). It is interesting to observe that **3,5 di CQ ac** was more abundant than **1,5 di CQ ac in** all treatment and it was consistent with previous results obtained in the first year. What about flavonoids, the **Api glc** was the main compound but the levels on all management were comparable. The same behaviour was founded in **Lut glc**. However, **Lut glr** showed higher concentration in INT ORG (50.90 mg $100g^{-1}$ of DM) respect to CONV and BIA ORG. Between last treatments the amounts of **Lut glr** were similar (56.24 and 58.29 mg $100g^{-1}$ of DM). Analysing the total amount of luteoline the ratio between each composts into managements is quite different: in CONV and BIA ORG treatments **Lut glc** and **Lut glr** showed the same trend (52% and 48% in CONV and 49% and 51% in BIA ORG, respectively) whereas in INT ORG **Lut glc** was 39% vs 61 % of **Lut glr.**

1.3.4.2. Effect of heads' order and genotypes.

As shown in a table 13, in the heads' order were founded significant differences only in **1,5 di CQ ac** where the first order was richest to second and third (58.58, 39.72 and 26.49 mg $100g^{-1}$ of DM, respectively). In the other polyphenols the high variability within heads' order have no identified differences.

As concerned genotypes, remarkable differences were found in accordance previous finding (Fratianni et al. 2007, Lombardo et al. 2010, Pandino et al. 2001, 2012) except that in **1,5 di CQ ac,** the hydroxycinnamic acid influenced by management and heads' order.

The SAURO clone (Sa), to medium-late crop, contained the highest levels of **5 CQ ac** (2605.5 mg $100g^{-1}$ DM) and **3,5 di CQ ac** (1316.38 mg $100g^{-1}$ DM) while was the lowest content of **1 CQ ac**.

What about flavonoids, the most abundant was **Api glc** and its highest concentration was founded in Sa followed by Ef and Ss (258.72 and 221.26 mg $100g^{-1}$ DM, respectively). The **Lut glc** showed highest content in Sa and the lowest in Ss (67.98 mg $100g^{-1}$ DM Vs 46.69 mg $100g^{-1}$ DM) while the Ef had a middle amount (57.18 mg $100g^{-1}$ DM). Instead the Ef genotype contained the most content of **Lut glr** (80.38 mg $100g^{-1}$ DM) and the Ss the minor one (52.25 mg $100g^{-1}$ DM).

These variations were attributed not only at genotypic differences (Moglia et al. 2008) but also at plant vigour of Sauro clone. This, in fact, creates a condition of so-called intraspecific competition well described by Lombardo and coauthors 2009 where reported higher level polyphenols linked to higher planting density.

Table 13 - Mean values (mg 100 g^{-1} DM) for the polyphenols content traits of artichoke heads for the different levels of treatments, heads' order, and genotypes based on the three compared cropping systems tested for 2013-14 growing season.

Factors	5,CQ ac	1,CQ ac	Lut glc	Lut glr	1,5 di CQ ac	3,5 di CQ ac	Api glc
Treatment (T)							
CONV	2457.0	24.85	59.52	56.24 b	36.01 b	1128.80 ab	242.74
INT ORG	2070.8	24.02	56.69	86.18 a	50.90 a	968.69 b	262.84
BIA ORG	2185.9	21.63	56.35	58.29 b	36.46 b	1198.97 a	272.81
Heads' order (H)							
1st	2284.1	22.70	69.10	103.10	54.58 a	1143.4	306.49
2nd	2134.1	27.13	51.05	60.06	39.72 ab	1072.7	236.85
3rd	2232.5	19.75	53.26	40.97	26.49 b	1126.5	250.24
Genotypes (G)							
Ss	1996.9 b	27.66 a	46.69 b	50.25 b	38.47	978.42 b	221.26 b
Ef	2044.3 b	21.60 b	57.18 ab	80.38 a	44.49	1046.33 b	258.72 b
Sa	2605.5 a	19.91 b	67.98 a	68.70 ab	38.37	1316.38 a	309.09 a

Different letters among management for each compounds indicate statistical significance at P≤0.05 according to the LSD test.

1.4. CONCLUSIONS

In recent years, the demand for organic products became particularly significant. Such a trend led producers to evaluate the real feasibility of organic crop management. However, although organic farming represents an established technique, the nutritional components of the resulting cultivated crops has received only little attention. The globe artichoke is an important crop due to its nutritional and pharmacological properties and, for this reason, it is today considered a "functional food".

The proposed agricultural system ensured a high level of polyphenols that are responsible for the properties described above.

Moreover, the results of this study highlighted the applicability of an organic management to the cultivation of globe artichoke.

In the first trial, the crop showed higher amounts of nutraceutical compounds for all the used organic managements, especially in the "Biannual Organic".

In the second trial, differences were less strong but still in favour of organic ones.

Genetic variability of the used genotypes resulted in a variety of polyphenols profiles, as previously reported in literature. Indeed such an observation will push producers toward the selection of better clones as source of polyphenols.

Further, the globe artichoke cv "Spinoso sardo" proved to produce a noticeable amounts of 3,5 dicaffeoylquininc acid. The documented involvement of such a molecule in the treatment of several degenerative diseases may boost the commercial value of this local cultivar.

Finally, some of the analysed polyphenols showed significant correlation with a few soil microelements. As a way of example, positive association emerged with exchangeable Na, indicating a possible stress due to Na as possible cause of increase of polyphenols amount.

The PCA evidently separates different managements between organic and conventional, indicating that this technique may be a powerful tool for the chemometric classification of organically and conventionally cultivated artichokes.

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CHAPTER 2

Variation of mineral composition in globe artichoke cv "Spinoso sardo": organic vs conventional managements

2.1 Introduction

Artichoke, *Cynara cardunculus*, is a dietary and medicinal plant species with a long tradition of use dating back to the ancient Egyptians, Greeks, and Romans. It comprises the globe artichoke, *C. cardunculus* subsp. *scolymus*, the cultivated cardoon, *C. cardunculus* subsp. *altilis*, and the wild cardoon, *C. cardunculus* subsp. *sylvestris*. Globe artichoke is cultivated for its large immature flower heads. The edible parts are the tender inner bracts and the receptacle that constitute nearly 35–55% of the fresh weight of the head (De Falco et al., 2015).

Globe artichoke is described as a functional food because of its particular composition of different bioactive compounds which is linked to the prevention of certain types of cancer, and cardiovascular pathologies (Ceccarelli et al., 2010). In addition, heads provides relevant quantities of some minerals, such as K, Ca and Na.

As reported in previous studies, mineral content in fruits and vegetables is affected by factors as pedo-climatic conditions, and genotypes; organic vegetables, in particular, are usually considered to have higher mineral content (Jolly, 1991).

Due to its high nutritional requirements and susceptibility to pests and diseases, globe artichoke is conventionally cultivated by largely adopting chemical fertilizers and pesticides (Pisanu et al., 2009). As a result there is a lack of knowledge on the variation between organic and conventional management for the mineral composition of globe artichoke.

In the last few years, there has been a lot of discussion about sustainability and environmental development, and proportionally there has been an increase on the demand for organic foods. These ones are widely perceived as being tastier and healthier than the products produced in the conventional way, moreover the production process is less harmful to the environment, what is explained by the position organics are taking in the global food market and consumption pattern (Zanoli et al., 2012; de Souza Araujo et al., 2014). Worldwide production of organic food might still grow substantially, being often considered as one of the largest growing markets in the food (de Souza Araujo et al., 2014). Italy is the country within the EU with more land dedicated to organic farming, followed by Germany and UK. In Sardinia, organic farming started about 30 years ago and nowadays is a quite promising sector, with about 72282 ha being cultivated in 2013 (ISTAT, 2015).

There are growing number of studies comparing organic and conventional farming food concerning the physicochemical and microbiological composition, phenolic compounds, minerals in plant and soil, especially in some vegetables as broccoli (Valverde et al., 2015), zucchini (Montemurro et al., 2013), pepper (Lopez et al., 2013), Chinese cabbage (Kim et al., 2014) and tomato (Hernandez et al., 2014).

However, there is still controversy about the higher nutritional value of organic foods when they are compared to the conventional foods, especially the minerals.

The aim of this paper is to assess the influence of the agricultural management system, including organic and conventional, on the mineral composition of a widely used Italian cultivar ("Spinoso sardo"). The reported study is part of larger research project (SIMBIOVEG and ORWEEDS) in which a long term field trial is being carried out to set up weeds, and nutrient management under organic systems.

2.2 Materials and Method

2.2.1 Experimental site

This study, as part of a larger research project (SIMBIOVEG 2006 – 2009; and ORWEEDS 2010 - 2013) was established in summer 2006 on field which had previously been under winter cereals for over 10 years. The field trial was carried out at the experimental station of the University of Sassari (40° 46' N; 8° 29' E; 81 m asl), North West Sardinia, Italy, over 2012-13 and 2013-14 growing seasons.

The site has a typical Mediterranean climate with a mean annual rainfall (1958–2004) of 554 mm that mainly occurs from October to December and a mean annual air temperature of 16.2 °C (Figure 1). Weather data during the growing seasons were collected from a meteorological station close to the experimental site. Soil type was characterized as sandy-clay-loam belonging to the limestone Xerochrepts group and with high calcareous status (Table 1).

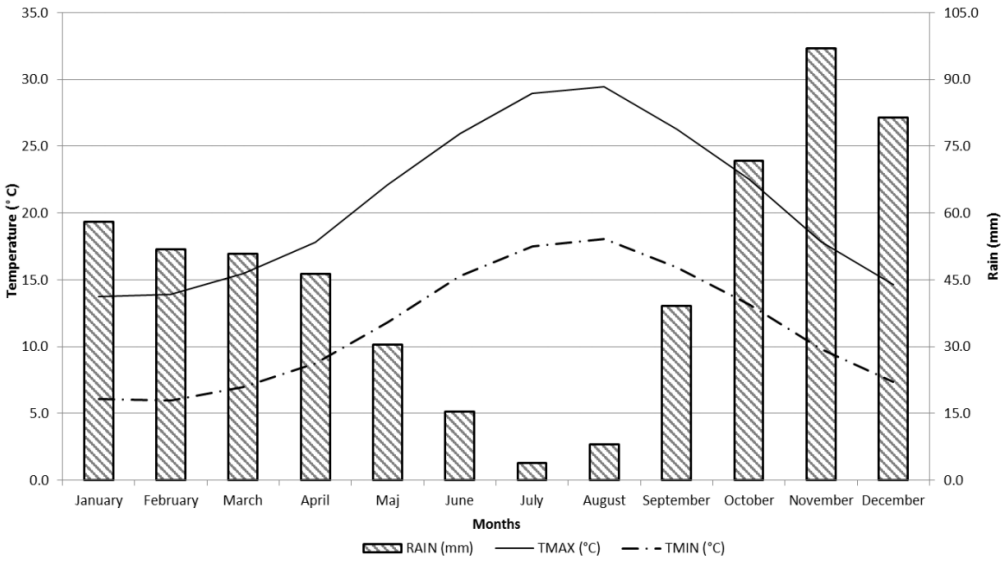

Figure 1 Long-term meteorological (1958–2004) series.

Table 1 Initial soil characterization at two depths (0 – 20 cm and 20 – 40 cm) and before the beginning of the crop cycle.

Depth (cm)	Sand (g/kg)	Clay (g/kg)	Silt (g/kg)	Coarse fraction (g/kg)	pH	Electrical conductivity (mS cm^{-1})	Field capacity (% vol.)
0-20	489.8	196.4	274.6	39.3	7.9	0.29	31.9
20-40	38.7	488.8	198.5	274.1	8.0	0.25	28.9

2.2.2 Crop management and experimental design

The experiment was based on four experimental factors: cropping system, heads order, harvesting time, and genotypes.

In the first year, the globe artichoke cv. 'Spinoso sardo' was arranged in split plot design with three cropping systems in main plots, three heads' order in sub plots, and five harvesting times subsampled at the subplot level. Each treatment was replicated four times. The cropping systems were organic (O), conventional (C) and intensive organic (I); heads orders were first (1^{st}), second (2^{nd}), and third (3^{rd}); and harvesting times corresponded to 166 (H1), 171 (H2), 179 (H3), 194 (H4), and 227 (H5) days after planting.

In the 2013-14 growing season, two different clones of Spinoso sardo cv were also considered to evaluate their response to the agricultural system (Table 2). The experiment was laid out in split-split plot design with three cropping systems in the main plots, genotypes in sub-plots, heads' order in sub-sub-plots and harvesting times sub-sampled at the sub-sub-plot level. The three cropping systems were O, C, and I; the three genotypes were Spinoso sardo landrace (Ss), Efis clone (Ef), and Sauro clone (Sa); the three heads orders were first (1^{st}), second (2^{nd}), and third (3^{rd}).

Table 2 Genotypes used in 2013-14 growing season for each of the two varietal type tested (landrace and clones).

Genotypes	Varietal type	Origin	Growing cycle	Reproduction system
Spinoso sardo (Ss)	Landrace	Local farmers	Medium early growing	Vegetative by offshoot
Efis (Ef)	Clone	AGRIS	Early growing	Micropropagated
Sauro (Sa)	Clone	AGRIS	Late growing	Micropropagated

Each plot covered an area of 440 m2. In order to prevent any cross-contamination between treatments each plot was separated from neighbouring treatment plots by two rows untreated artichoke. Block were separated by a 3 m artichoke areas.

In both years, semi-dormant offshoots ("ovoli") were hand-planted within the first ten days of August; at a planting density of 1 plant m^{-2} (plants spaced 1.5 m between rows and 0.7 m within rows).

In the second year and before planting, a pelletized poultry manure (7.3 N – 3 P) was also applied at rates of 8.8 tons ha^{-1} in organic treatment plots (I, and O). Poultry manure was applied only twice over the entire lasting of the experiment started in 2006.

In order to allow an earlier establishment of the crop, drip irrigation was applied (when accumulated daily evaporation reached 35 mm, 100% of maximum evapotranspiration), from date of planting, and carried out until first rainfalls occurred. Crop management practice were listed in Table 3.

In particular, C was managed (according to local farming practices) in continuous monoculture and with incorporation of plants residues into the soil by harrowing up to 20 cm depth at the end of crop cycle. The fertilization program commonly used in the area for globe artichoke was adopted (92 kg N, 138 kg P_2O_5 and 150 kg K_2O ha^{-1}).

Intensive organic system (I) combined organic methods with artichoke monoculture system. This treatment provided that the artichoke growing cycle was early interrupted to allow introduction of a short-cycle legume species (*Phaseolus vulgaris* L.). Introducing a

short-cycle legume species, as French bean, might improve biological nitrogen fixation. Each year, at the end of April, artichoke fresh residues were chopped and ploughed. French bean (cv. Rex) was sown at the rate of 100 kg·ha^{-1} on early June for both years. The French bean was ended at the first legume stages, when plants produced the first pods at the end of flowering. This stage occurred on 15 July 2013 and on 17 July 2014. Also, fresh residues from this crop were incorporated into soil by harrowing before artichoke new growing season started.

In organic cropping system (O), artichoke was managed in two-year rotations with cauliflower (cv. "Nautilus", crop duration: 75 days). Since cauliflower have been shown to possess fungicidal activity attributed to the chemical breakdown of glucosinolates. Both species were investigated at the same time by using adjacent plots. Both year, cauliflower was transplanted on the plot of the previous rotational artichoke. Each year, at beginning of March, a legume species (*Pisum sativum* L. cv. Attika) was used as cover crop and sown at a rate of 220 kg ha^{-1} in inter-row spaces.

At the end of each growing season (early May), artichoke, cauliflower, and pea fresh residues were chopped and ploughed (to a 15–20 cm depth). Three to four weeks after incorporation, the beds in all plots were rotary tillered for the next cauliflower or artichoke production cycle.

Table 3 Crop management program of the cultivation systems.

Agricultural practice	Date of application	2012/13	2013/14	Cultivation system
Seed bed preparation	Early August	Harrowing	Harrowing	All treatments
Fertilization	At planting	Urea 46 N	Urea 46 N	C
	At planting	Triple superphosphate 138 P_2O_5	Triple superphosphate 138 P_2O_5	C
	At planting	Potassium sulphate 150 K_2O	Potassium sulphate 150 K_2O	C
	At planting	—	Chicken manure 3.7 N, 3.6 organic N, 3 P_2O_5	I; O
	During crop growth	Urea 46 N	Urea 46 N	C
Irrigation	August to November	Drip irrigation system		All treatments
Planting	August	08/10/2012	8/08/2013	All treatments
Harvesting	From December to March	On a weekly basis		All treatments
Weeding	During crop growth	Mechanical weeding		C
Cover crop	March	Pea cv. Attika		O
Residues management	April	Globe artichoke broadcast incorporated		I
		Pea cover crop broadcast incorporated		O
French bean Planting/harvest	June/July	French bean cv. Rex		I
Residues management	July	Globe artichoke broadcast incorporated		C
		French bean broadcast incorporated		I

2.2.3 Data collection and measurements

2.2.3.1 Soil analysis

At the beginning of the two-year experiment, soils samples were taken from the entire field, being uniformly managed, at 0-20 cm and 20-40 cm depth for 4 replicates. The samples were oven dried, ground to pass a 2-mm sieve and then analysed for determining the main soil chemical characteristics. Analysis were conducted in the Pedology Laboratory of Department of Agricultural, University of Sassari according to standard methods of Società Italiana della Scienza del Suolo (1997, 2000). The following parameters were examined: pH, electrical conductivity; P_2O_5 available (Olsen's method), total $CaCO_3$; exchangeable cations and Cation Exchange Capacity (Barium chloride method); total N, total C, organic matter (elemental analyser LECO 628).

2.2.3.2 Heads artichokes samples collection and preparation

Artichoke heads were weekly collected on permanent sampling areas (5 plants each) chosen in the middle of each plot and throughout the harvest period. At the end of each growing seasons, an average of 7 heads per plant were collected, and systematically separated according to heads order and harvesting time. Samples were disease-free and the length of central global flower buds was approximately 2 mm. From every samples, external bracts were removed and internal bracts and receptacles were washed with distilled water and stored at -80 °C, until lyophilization with Heto Lyolab 3000 for 72 h (-56°C). The lyophilized samples were powdered by a blender and stored at -20 °C until they were extracted. The extraction was performed as reported by Pandino et al. (2011) and according to AOAC official method. A lyophilized sample (1.0 g) was placed in muffle at 550 °C for 24 h. The samples were reported at room temperature in desiccator. After cooling the sample were weighted to evaluate humidity grade; HCl (10%, v/v) were added and filtered 0.45 µm Whatman filter paper. The samples were analysed by Pedology laboratory of the Department of Agricultural, University of Sassari.

2.2.3.3 Mineral analysis

Mineral content was analysed using PerkinElmer AAnalyst 200 flame atomic absorption spectrometer (Nordwalk, CT, USA) equipped with a multi-element hollows cathode lamp and a deuterium background correction system. The quantification of each minerals were carried out by calibration curves . In consideration of large amount of samples analysed to test the reproducibility of method, duplicate analysis were performed on 15% of all samples. The reproducibility, expressed by confidence limits of the result had a confidence level of 95%. The mean value are expressed as g kg^{-1} of DM for macrominerals and mg kg^{-1} of DM for microminerals.

2.2.4 Statistical analysis

Analysis of variance was performed to evaluate the influence of cropping systems, heads' order, genotypes and harvesting times on mineral composition and their interaction. The variance was analyzed using PROC GLM (SAS software 9.02, SAS institute Ltd, USA) for both the years separately. The difference between means was compared with Fisher's least significant difference test (LSD) at 5% probability level. Pearson linear coefficients of correlation (r) among studied variables were calculated from regression analyses between pairs of traits. Principal component analysis (PCA) was performed on 2012-2013 soil physical-chemical parameters, mineral composition of first, second and third heads order.

2.3 Results and discussion

2.3.1 Effect of cultivation system

As regard the effect of cropping system on heads macrominerals composition, artichoke grown under C system showed higher K concentrations (Tables 4 and 5). The organic heads (both I and O) had a higher Mg and Na content than the conventional ones. The values differ by between 50 – 55%. Levels of Ca in the first year were insensitive to cropping system and in the range 1.57 – 1.91 g kg^{-1} DM (Table 4). In second year, Ca content were only slightly higher, ranging from 1.55 to 1.96 g kg^{-1} but with significant differences among treatments (Table 4).

In the first year, copper content was not significantly different ($p > 0.05$) among cropping systems (Table 6). However, in the second year, Cu exhibited a four-fold higher ($p < 0.05$) concentration in I artichoke, with significant differences between conventional and organic treatments (Table 7). Artichoke grown in C method had higher values of Zn than those grown under organic cultivation method (38.87 vs 29.82 mg kg^{-1} DM in the first year, and 37.65 vs 27.24 mg kg^{-1} DM in the second year). In the first year, organic artichoke heads shown high levels of Fe (69.47 and 62.78 for I and O, respectively) (Table 6). These results were consistent to the significantly higher values for Fe that were found in O treatment in the second year (Table 7). However, in the second year, Fe content was two-fold higher in C cultivation method, and markedly lower in I treatment when compared to the previous year results.

Table 4 Growing season 2012-13: Effect of cropping systems (C, I, and O), heads' order (1st, 2nd, and 3rd), and harvesting time (H1, H2, H3, H4, and H5) on macromineral composition (g kg^{-1} DM) of artichoke heads.

Factors	Ca	Mg	Na	K
Cropping systems (C)				
C	1.82 a	0.70 c	1.12 c	25.27 a
I	1.57 a	1.71 a	3.13 a	17.88 b
O	1.91 a	1.18 b	2.13 b	22.97 c
Heads' order (Hd)				
1st	1.96 a	1.23 a	2.35 a	23.24 a
2nd	1.99 a	1.36 a	2.34 a	22.91 a
3rd	1.36 a	1.20 a	2.13 a	18.96 b
Harvesting time (DAP)				
H1	1.88 ab	1.49 ab	2.83 ab	22.37 a
H2	2.01 ab	1.02 b	1.73 b	24.08 a
H3	2.24 a	1.84 a	3.65 a	19.67 a
H4	2.00 ab	1.30 ab	2.13 b	23.85 a
H5	1.50 b	1.14 b	2.03 b	20.35 a
LSD interaction (P≤0.05)				
C x Hd	n.s.	n.s.	n.s.	n.s.
C x DAP	n.s.	n.s.	n.s.	n.s.
Hd x DAP	n.s.	n.s.	n.s.	n.s.

Different letters in the same column show significant differences between means according to LSD test ($P < 0.05$).

Table 5 Growing season 2013-14: Effect of cropping systems (C, I, and O), heads' order (1st, 2nd, and 3rd), and genotypes (Ss, Ef, and Sa) on macromineral composition (g kg^{-1} DM) of artichoke heads.

Factors	Ca	Mg	Na	K
Cropping systems (C)				
C	1.96 a	1.58 a	2.39 b	24.39 a
I	1.55 b	0.97 b	2.67 b	14.71 c
O	1.94 a	1.48 a	3.19 a	18.38 b
Genotypes (G)				
Ss	1.64 b	1.18 b	2.44 b	15.74 b
Ef	2.02 a	1.34 a	3.01 a	19.18 a
Sa	1.76 b	1.42 a	3.12 a	20.06 a
Heads' order (Hd)				
1st	1.80 a	1.08 b	2.60 a	18.18 a
2nd	1.98 a	1.38 a	2.79 a	18.80 a
3rd	1.63 a	1.37 a	3.04 a	17.51 a
LSD interaction (P≤0.05)				
C x G	n.s.	n.s.	n.s.	n.s.
C x Hd	n.s.	n.s.	n.s.	n.s.
G x Hd	n.s.	n.s.	n.s.	n.s.

Different letters in the same column show significant differences between means according to LSD test ($P < 0.05$).

Table 6 Growing season 2012-13: Effect of cropping systems (C, I, and O), heads' order (1st, 2nd, and 3rd), and harvesting time (H1, H2, H3, H4, and H5) on micromineral composition (mg kg^{-1} DM) of artichoke heads.

Factors	Fe	Mn	Zn	Cu
Cropping systems (C)				
C	35.02 b	7.67 a	38.87 a	15.86 a
I	69.47 a	5.59 b	30.43 ab	21.26 a
O	62.78 ab	7.61 a	29.22 b	16.80 a
Heads' order (Hd)				
1st	54.83 a	6.72 ab	34.90 a	18.30 a
2nd	67.71 a	7.88 a	38.55 a	19.24 a
3rd	50.96 a	5.80 b	23.22 b	17.25 b
Harvesting time (DAP)				
H1	65.94 a	5.26 b	32.68 a	18.18 a
H2	44.24 a	8.37 a	37.13 a	20.40 a
H3	63.28 a	8.10 a	41.93 a	23.87 a
H4	78.33 a	8.01 a	34.91 b	14.93 a
H5	50.16 a	6.36 ab	28.37 b	18.36 a
LSD interaction (P≤0.05)				
C x Hd	n.s.	n.s.	n.s.	n.s.
C x DAP	n.s.	n.s.	n.s.	n.s.
Hd x DAP	n.s.	n.s.	n.s.	n.s.

Different letters in the same column show significant differences between means according to LSD test ($P < 0.05$).

2.3.2 *Effect of heads' order*

When the effect of heads' order was considered, in the first year 1st and 2nd heads' order had higher mean values of K than 3rd, whereas for the other macrominerals there were no significant differences (Table 4). In 2013-14 growing season, the only significant difference found was for Mg. Second and 3rd heads' order had a higher Mg content than 1st; for other macrominerals there were no significant differences (Table 5). As regard microminerals content, in 2012-13, 1st and 2nd heads' order had significantly higher content of Mn, Zn, and Cu (Table 6). In the second growing season, only Mn content was significantly different among heads' order (Table 7).

2.3.3 *Effect of harvesting time*

Harvesting time significantly affected Ca, Mg, and Na concentrations. In particular, these macrominerals recorded a peak value in the middle of the growing cycle (H3), and then significantly decreased from H4 to H5 (Table 4). As regard micromineral compositions, Fe and Cu were not affected by the harvesting time (Table 5). Zinc decreased as the season progressed with significant differences among the first three harvesting date (H1, H2, H3) and H4 and H5. The Mn concentration increased by the first harvesting date (H1) reaching a plateau value among H2 and H4 (8.37 – 8.01 mg kg^{-1} DM).

2.3.4 *Effect of genotypes*

Significant differences in the macromineral content were found among different artichoke genotypes (Table 5). Clones showed the highest values for Ca, Mg, Na and K. Efis was also the genotype with the highest Ca content, and Sauro was the genotype with the highest Mg, Na and K. A different micromineral accumulation was observed

for the three genotypes (Table 7). Sauro was the most effective accumulator of Mn, Zn and Cu. By contrast, no significant differences were found among the three genotypes considered for Fe content.

Table 7 Growing season 2013-14: Effect of cropping systems (C, I, and O), heads' order (1st, 2nd, and 3rd), and genotypes (Ss, Ef, and Sa) on micromineral composition (mg kg^{-1} DM) of artichoke heads.

Factors	Fe	Mn	Zn	Cu
Cropping systems				
C	70.72 b	11.01 a	37.65 a	8.31 a
I	41.74 c	7.72 b	22.42 c	5.34 b
O	96.39 a	10.93 a	32.06 b	5.98 b
Heads' order				
1st	74.34 a	8.58 b	30.07 a	5.50 a
2nd	69.71 a	10.63 a	31.60 a	6.63 a
3rd	68.66 a	9.41 ab	27.45 a	6.03 a
Genotypes				
Ss	69.72 a	8.86 b	27.66 b	4.76 c
Ef	72.20 a	9.57 b	27.28 b	6.62 b
Sa	70.42 a	10.82 a	34.19 a	7.46 a
LSD interaction (P≤0.05)				
C x G	n.s.	n.s.	n.s.	n.s.
C x Hd	n.s.	n.s.	n.s.	n.s.
G x Hd	n.s.	n.s.	n.s.	n.s.

Different letters in the same column show significant differences between means according to LSD test ($P < 0.05$).

2.3.5 Correlation

Many significant correlations among traits were detected (Table 8). Most of correlations detected were positive correlations among soil parameters and among plant minerals. Significant correlations involved a positive correlation of Fe in 2nd heads' order with K^{+}, and exchangeable acidity; Mg in 1st heads' order with Mg^{++} and Na^{++}; and Zn in 1st heads' order with organic C, soil organic matter, and C/N ratio. By contrast, significant negative correlations were found for Fe in 2nd heads' order with electrical conductivity, organic C, soil organic matter, total N, and Ca^{++} (Table 8).

Table 8 Pearson's correlation coefficients and significance of correlations among all the chemical parameters analyzed

	Coarse	Sand	Silt	Clay	pH	EC	$CaCO_3$	C	SOM	N	C/N	P_2O_5	Ca^{++}	Mg^{++}	Na^{++}	K^{+}	SEB	CEC	GSB	EA	Ca 1st	Mg 1st	Na 1st	K 1st	Fe 1st	Cu 1st	Mn 1st	Zn 1st	Ca 2nd	Mg 2nd	Na 2nd	K 2nd	Fe 2nd	Cu 2nd	Mn 2nd	Zn 2nd
Coarse	-																																		
Sand	**-.730*****	-																																	
Silt	.301	**-.550***	-																																
Clay	-.202	-.210	-.472	-																															
pH	.152	-.367	-.101	.496	-																														
EC	.326	-.390	.525	-.267	.033	-																													
$CaCO_3$	.459	.127	.003	**-.657***	**-.560***	.313	-																												
C	-.419	.341	.062	-.147	.010	**.614***	-.020	-																											
SOM	-.431	.388	.025	-.169	-.012	**.602***	.033	**.996*****	-																										
N	.015	-.297	.385	.046	.463	.464	-.302	.471	.456	-																									
C/N	-.501	.505	.022	-.270	-.321	.474	.133	**.858*****	**.859*****	-.007	-																								
P_2O_5	-.275	.303	**-.547***	.369	.404	-.524	-.518	.007	-.026	-.217	.028	-																							
Ca^{++}	.039	.066	.208	-.345	.012	**.779*****	.276	**.762*****	**.752*****	.189	**.724*****	-.116	-																						
Mg^{++}	-.162	.045	-.481	**.571***	**.550***	.071	-.177	.200	.239	.434	-.127	-.035	-.013	-																					
Na^{++}	-.454	.250	**-.652***	**.731*****	.445	**-.601***	**-.546***	-.183	-.162	.112	-.374	.372	-.521	**.715*****	-																				
K^{+}	-.484	.146	-.318	**.589***	.194	**-.782*****	**-.673*****	-.453	-.457	-.201	-.369	.423	**-.796*****	.116	**.687*****	-																			
SEB	.017	.047	-.075	-.016	.167	**.727*****	.240	**.704*****	**.711*****	.225	**.582***	-.144	**.894*****	.391	-.186	**-.691*****	-																		
CEC	-.035	-.219	-.070	.426	.203	**.610***	-.052	.481	.474	.298	.305	-.261	**.562***	**.561***	.103	-.376	**.813*****	-																	
GSB	.096	.385	-.038	**-.632***	-.008	.394	.507	.530	**.553***	-.028	**.567***	.112	**.725*****	-.112	-.470	**-.655***	**.556***	-.031	-																
EA	-.086	-.389	.021	**.644***	-.018	-.351	-.469	-.500	-.522	.030	**-.538***	-.142	**-.700*****	.137	.458	**.620***	-.514	.081	**-.997*****	-															
Ca 1st	**-.721*****	.518	-.309	.265	.317	**-.658***	**-.694*****	.018	.016	.017	.008	**.639***	-.349	.115	**.612***	**.732*****	-.372	-.385	-.114	.060	-														
Mg 1st	.072	-.103	-.475	**.549***	.317	-.111	-.036	-.137	-.102	.343	-.464	-.146	-.314	**.846*****	**.702*****	.132	.099	.407	-.387	.417	-.089	-												
Na 1st	-.097	.027	-.444	.495	.309	-.160	-.113	-.059	-.020	.423	-.406	-.146	-.314	**.863*****	**.766*****	.187	.076	.362	-.371	.392	.069	**.973*****	-											
K 1st	-.374	.499	.032	-.396	-.410	-.431	.012	-.168	-.167	**-.657***	.234	.239	-.007	**-.637***	-.214	.207	-.270	-.500	.208	-.253	.411	**-.721****	**-.634***	-										
Fe 1st	-.064	.223	-.472	.197	-.019	-.232	.233	-.184	-.123	.095	-.367	-.303	-.251	**.671*****	**.597***	.031	.092	.260	-.209	.228	-.052	**.838*****	**.872*****	-.316	-									
Cu 1st	**-.532***	.341	.080	-.052	-.405	.231	.119	.401	.444	.263	.359	**-.623***	.126	.347	.154	-.068	.252	.409	-.148	.178	-.038	.281	.404	-.085	.492	-								
Mn 1st	-.367	-.019	**.595***	-.183	.177	.069	-.460	.150	.132	.237	.193	-.073	.041	-.223	-.122	.277	-.179	-.225	-.010	-.038	.478	**-.532***	-.388	.404	-.470	.149	-							
Zn 1st	-.315	.113	.210	-.060	-.328	.497	.038	**.566***	**.551***	-.137	**.812*****	-.112	.474	-.248	-.465	-.155	.346	.282	.205	-.170	-.157	-.523	**-.542***	.150	**-.582***	.248	.289	-						
Ca 2nd	-.017	.127	-.014	-.155	-.368	-.277	.368	-.476	-.411	-.311	-.275	-.513	**-.542***	.048	.092	.318	-.454	-.357	-.250	.253	-.047	.146	.160	.136	.353	.357	.106	-.050	-					
Mg 2nd	.385	-.377	-.338	.504	.226	.035	.106	-.258	-.241	.229	**-.536***	-.230	-.233	**.643***	.442	-.066	.140	.475	-.414	.454	-.414	**.910*****	**.806*****	**-.765*****	**.709*****	.103	**-.698*****	-.488	.041	-				
Na 2nd	.210	-.239	-.488	.623	.281	-.029	-.038	-.125	-.117	.233	-.418	-.022	-.179	**.695*****	**.560***	-.001	.207	**.534***	-.388	.427	-.259	**.912*****	**.824*****	**-.736*****	**.682*****	.088	**-.726*****	-.446	-.135	**.962*****	-			
K 2nd	-.282	.204	.469	-.480	-.351	-.112	-.027	-.068	-.071	-.338	.278	-.086	-.038	**-.653***	-.422	.210	-.361	**-.533***	.121	-.161	.293	**-.817*****	**-.727*****	**.774*****	**-.561***	.036	**.762*****	.421	.352	**-.880*****	**-.944*****	-		
Fe 2nd	-.207	-.053	-.267	**.555***	-.133	**-.703*****	-.404	**-.667*****	**-.682*****	**-.557***	-.413	.262	**-.757*****	-.163	.399	**.816*****	**-.668*****	-.275	**-.761*****	**.746*****	.308	.032	-.010	.274	-.025	-.156	-.013	-.045	.336	.056	.073	.181	-		
Cu 2nd	.369	-.378	-.317	.503	-.039	.070	.224	-.375	-.367	-.443	-.234	-.177	-.117	.233	.076	.014	.160	.444	-.339	.386	**-.538***	.366	.179	-.304	.218	-.077	**-.547***	.116	.222	**.584***	.526	-.375	.419	-	
Mn 2nd	.336	-.215	.245	-.260	.478	**.565***	.233	.342	.372	**.696*****	-.014	-.356	.358	.495	-.075	-.477	.425	.217	.440	-.433	-.219	.302	.327	**-.549***	.209	.122	.115	-.181	.004	.207	.115	-.260	**-.789*****	-.239	-
Zn 2nd	.353	-.284	.203	-.132	-.264	.471	.391	.141	.137	-.068	.273	-.252	.110	-.225	**-.549***	-.256	.041	.003	.116	-.068	**-.561***	-.209	-.349	-.313	-.445	-.115	-.152	**.613***	.186	-.042	-.131	.084	-.052	.358	.075	-

EC: Electrical conductivity, $CaCO_3$: total limestone, C: organic C, SOM: soil organic matter, N: total N, SEB: sum of exchangeable basis, CEC: cation exchange capacity, EA: exchangeable acidity

2.1.1 PCA analysis

The first and second components of the PCA described 28 % and 23 % of the total variation.

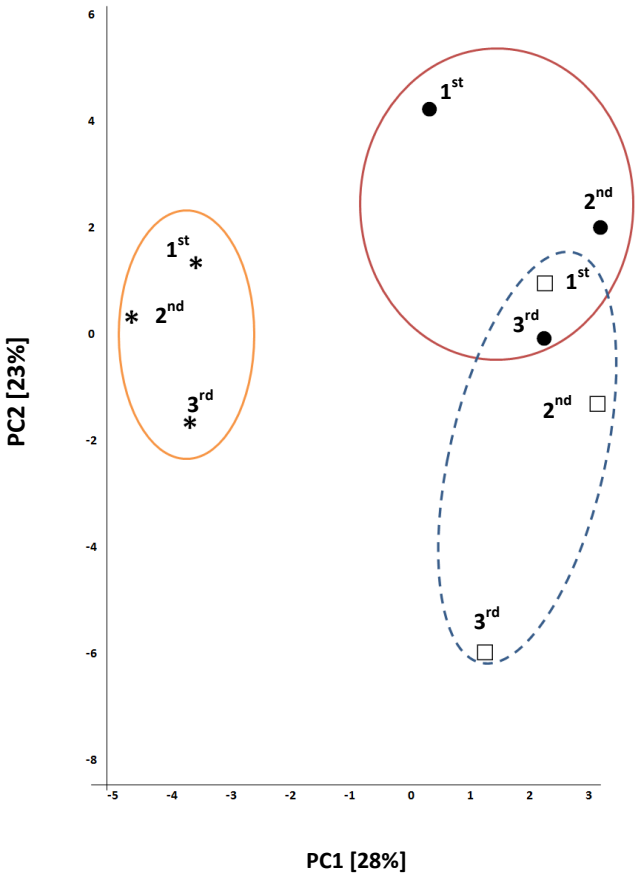

Figure 2 Principal component analysis (PCA) of mineral data obtained by analysis of the heads' order, and soil parameters of three cropping systems conventional (*), intensive organic (●) and organic (□).

The projections of the combinations of cropping systems, heads' order, soil chemical parameters on the PCA graph clearly show that the first component mostly separates cropping systems, with conventional having negative values and organic cropping systems having positive values (Figure 2). The second component clearly separated heads' order, such that in each cropping system, values for 1st and 2nd heads' order were higher than 3rd for this second component.

2.1 Discussions

The materials used in this paper come from conventional and organic plots in which no differences in yield were observed between cultivation methods. Colla et al. (2002) and Raigòn et al. (2010) found similar results in tomato and eggplants, respectively. This is important for this study, as the differences found here in composition between organic and conventional cultivation methods cannot be attributed to differences in yield. It is also important to highlight that yields in 2012-13 were lower than 2013-14, which may be attributed to differences in environmental conditions that resulted in reduced growth and development, and consequently lower yield in 2012-13. The lower yield may result in differences between years in compositions, as there is a well-known relationship between yield and the concentrations of some nutrients.

All macrominerals were within the ranges found in literature. The results showed that the content of macrominerals were significantly impacted by cropping systems.

Potassium concentration (mean value of all cropping systems) was 20.6 g kg^{-1} DM, while the value reported in literature was 18.8 g kg^{-1} DM and (Pandino et al., 2010). In general, heads K concentrations were significantly higher in C plants than in organically grown plants, as conventionally managed plots had a higher level of K fertilization than those managed according to organic agriculture; and being K the nutrient most absorbed by artichoke during the crop cycle (Rincon et al., 2007). Calcium concentration was notably lower if compared to Ca concentrations reported by Pandino et al. (2010), but consistent with the findings of Lopez et al. (1997) and Romani et al. (2006) whose stated that in globe artichokes heads Na level was higher than Ca. In our study, organically grown artichokes had a higher Na, and Mg content than artichokes from conventional plots. This may be explained from the slightly higher Na levels in the soil of organically grown plots (both I and O treatments). Elia and Conversa (2007) also found that the mineral element content in artichoke heads was affected by soil type and crop management.

Among the microminerals, Fe was the most abundant. In particular, Fe content was significantly higher in organically grown cropping systems. Lattanzio et al. (1979) and Romani et al. (2006) also report similar Fe content values in conventionally grown artichoke. In this study conventional artichoke heads exhibited high level of Zn. Conventional and organic intensive (I) cropping systems were the most effective accumulator of Mn, while Cu was insensitive to the cropping systems in the first year and recorded a two-fold lower levels in C and I in the second year. Mean Cu concentration was in the range 15.9 mg kg^{-1} DM – 21.3 mg kg^{-1} DM (2012-13) and 5.3 mg kg^{-1} DM – 8.3 mg kg^{-1} DM (2013-14), while the range reported by other studies was 4.7 mg kg^{-1} DM - 9.2 mg kg^{-1} (Pandino et al., 2010). Pandino et al. (2010) studied the nutrient composition of 9 artichokes varieties over two different locations and two growing seasons and found a wide range of variability in the nutrient content.

In this study, we also investigated the influence of heads' order, harvesting times and genotypes, on the mineral composition. Differences in mineral compositions among harvesting times were found for Ca, Mg, Na, Mn and Zn contents. In particular, these elements showed a similar trend, by increasing until H3. This pattern was probably influenced by differences in the climate conditions between the first harvesting time that occurred in January (133 mm rainfall, 5.8 °C minimum temperature, and 11.9 °C maximum temperature) and the last harvesting time in March (59.6 mm rainfall, 7.2 °C minimum temperature and 14.4 °C maximum temperature).

In relation to genotypes, significant differences among genotypes have been found for all of the minerals studied, confirming published data suggesting considerable genetic diversity in artichoke heads composition (Pandino et al., 2010). In particular, Efis and Sauro clones were more efficient macro- and microminerals accumulators than landrace. Genotypes x cultivation method interactions are very frequent in the comparison of organic and conventional cultivation methods. In our experiment no significant genotypes x cultivation method interactions were found, suggesting that artichoke cultivars are insensitive to growing in either organic or conventional cultivation methods.

The differences in mineral accumulation experienced under the two growing season also depended on the weather conditions, just as it affects the mineral assimilation rate by plants. Minerals accumulation (particularly Mg, Na, Fe and Mn) was higher in 2013-14 than in 2012-13. The 2013-14 growing season showed evenly distributed rainfall and relative milder minimum and maximum temperature with respect to the previous growing season which may have promoted minerals availability for plant uptake.

Mineral content were positively and negatively correlated to soil characteristics, indicating that different soil growing conditions may increase or reduce the accumulation of minerals in artichoke heads. The PCA clearly separates artichoke heads grown under organic conditions from those that have grown under conventional conditions, indicating that PCA may be a powerful tool as a chemometric classification procedure for discriminating organically and conventionally cultivated artichokes.

2.2 Conclusions

In the present study, macro- and microminerals of globe artichoke grown within two different organic cropping systems were compared with the results obtained from conventional grown artichoke. This study is the first contribution to the efforts to comprehensively elucidate the influence of organic cultivation on globe artichoke mineral content. Though our work can be considered a preliminary study, it shows some differences in the qualities of conventionally and organically produced artichoke heads. Our findings suggest that the contents of various minerals of artichoke depend not only on growing management practices but also on the pedo-climatic conditions. Finally, the assessment of the mineral content of plants under different management systems underlines the importance of evaluating more than one genotype in the comparison between organic and conventional farming methods.

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