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Seeds and oil polyphenol content of sunflower (*Helianthus annuus* L.) grown with different agricultural management

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Abstract: Using a long term experiment (20 and 11 years of organic cultivation on the same soil), sunflower was cultivated under organic management and in a different part of the same farm under conventional management. Kernels, teguments and oils were analyzed for their polyphenols content. Five caffeoylquinic acids were identified. No qualitative differences were found in the three cases, while quantitative differences have been pointed out and discussed.

1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops. Besides soy and rapeseed oil, sunflower oil is ranking third with a worldwide production of about 44 million tons each year from 2012 to 2016, 28 million tons in the EU (Committee for the Common Organization of Agricultural Markets, 2016). Sunflowers prove to be a protein source of great interest for human nutrition; moreover, the residues originating from oil extraction are rich in phenolic antioxidants, which account for 1-4% of the total mass with chlorogenic acid being the predominant component (Weisz *et al*., 2009). Polyphenols in sunflower seeds were identified and quantified after HPLC analysis (Aramendia *et al*., 2000; Pedrosa *et al.*, 2000). The main phenolic compounds present in both the kernel and hull besides chlorogenic acid are caffeic acid and caffeoylquinic derivatives. Phenolic compounds in sunflower seeds have been shown to exert a high antioxidative potential, which might be beneficial from a biofunctional point of view and may be used as effective antioxidants for sunflower oils (De Leonardis *et al.*, 2003, 2005; Anjum *et al.*, 2012).

Organic agriculture is most widely used and its benefits concern overall the environment and the health of food, which is not contaminated with pesticides and synthetic fertilizers. However organic fertilizer of biological origin may lead in the long term to the "conventionalization of organic farming" (Darnhofer *et al.*, 2010). In order to go deeper in the organic management, the Montepaldi Long Term Experiment (MOLTE) trial in central Italy has been set up to compare three agro-ecosystems with different management: two organic (old organic since 1992 and young organic since 2001) and one conventional (Migliorini, 2014). The aim of this research is to follow the fate of polyphenols in teguments, kernels and oil from sunflower seeds grown under the above-mentioned three different conditions (one conventional and two organic) in order to assess whether such condition may affect the polyphenols profile and which compounds may be regarded as innovative parameters in order to deeper investigate the relation among soil condition, agricultural management and seed characteristics.

2. Materials and Methods

The Montepaldi Long Term Experiment (MOLTE) has been active since 1991 in the farm of the University of Florence (location Montepaldi, San

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Casciano, Val di Pesa, Long. 11°09'08'' E, Lat. 43°40'16'' N) covering a slightly sloping surface of about 15 ha at 90 m a.s.l. The MOLTE experiment has a system approach and includes the following three different micro agro-ecosystem managements:

a) "Old Organic" (OldO) with an area of 5.2 ha, divided into 4 fields under organic since 1992;
b) "YoungOrganic" (YngO) with an area of 5.2 ha, divided into 4 fields (integrated farming) from 1992 to 2000 and converted into organic management since 2001;
c) "Conventional" (Conv) area of 2.6 ha divided into 2 conventional fields, where farming techniques used were those normally used in the territory of the study area for conventional management.

On October 28, 2013, after harvesting the whole sunflower plant, seeds were put in an oven at 60°C for 48 hours and then preserved in paper bags. Seeds were manually dehulled in order to obtain kernel and tegument. Phenolic compounds were extracted for 30 min from kernels (about 2 g) and from teguments (about 1 g) with 70:30 ethanol/water (25 and 15 ml, respectively). The extracts were evaporated under vacuum at room temperature and finally dissolved in 10 ml ethanol/water (70:30). Cold-pressed seeds oils (25 ml) were extracted with 3x25 ml of 70:30 ethanol/water, adjusted to pH 2.0 with formic acid; each step involved an extraction for 30 min at room temperature. The extracts were combined and defatted with 3x50 ml hexane. The defatted extracts were evaporated to dryness under vacuum at room temperature and finally dissolved in ethanol/water (70:30) to a final volume of 4 ml.

Solvents and reagents

All the solvents (HPLC grade) and formic acid (ACS reagent) were purchased from Aldrich Chemical Company Inc. (Milwaukee, Wisconsin, USA). Chlorogenic acid was obtained from Extrasynthese S.A. (Lyon, Nord-Genay, France). The HPLC-grade water was obtained via double-distillation and purification with a Labconco Water Pro PS polishing station (Labconco Corporation, Kansas City, USA).

HPLC analysis

HPLC/DAD Analysis. The HPLC/DAD analyses were performed with an HP 1100L liquid chromatograph equipped with HP DAD (Agilent Technologies, Palo Alto, CA, USA). A Kinetex C18 column 100×2.1 mm, 5 µm (Phenomenex) operating at 30°C was used. The eluents were H_2O adjusted to pH 3.2 by formic acid and acetonitrile. A four-step linear solvent gradient was performed starting from 100% water up to 100% acetonitrile, with a flow rate of 0.2 ml/min for a 30-min period (Table 1).

Table 1 - Linear solvent gradient system used in HPLC-DAD and HPLC-MS analysis

Time (min)	A	B
0.10	100.0	0.0
5.00	80.0	20.0
7.00	80.0	20.0
13.00	70.0	30.0
18.00	70.0	30.0
22.00	30.0	70.0
26.00	30.0	70.0
30.00	0.0	100.0

Solvent A= H_2O adjusted to pH 3.2 by HCOOH. Solvent B= CH_3CN.

HPLC/ESI-MS Analysis. The HPLC-MS analyses were performed using an HP 1100L liquid chromatograph equipped with a DAD and 1100 MS detectors. The interface was an HP 1100 MSD API-electrospray (Agilent Technologies). Mass spectrometer operating conditions were the following: gas temperature 350°C at a flow rate of 10.0 l/min, nebulizer pressure 30 psi, quadrupole temperature 30°C and capillary voltage 3500 V. The mass spectrometer operated in positive and negative ionization mode at 80-120 eV, for both ionization modes.

3. Results and Discussion

In figure 1, the chromatographic profile of organic sunflower kernel, registered at 330 nm, maximum wavelength of absorbance of caffeic acid and its derivatives, is reported. Eight compounds have been identified: 3-*O*-caffeoylquinic acid, chlorogenic acid, 4-*O*-caffeoylquinic acid, three derivatives of caffeic acid, 3,5-*O*-dicaffeoylquinic acid, and 4,5-*O*-dicaf-

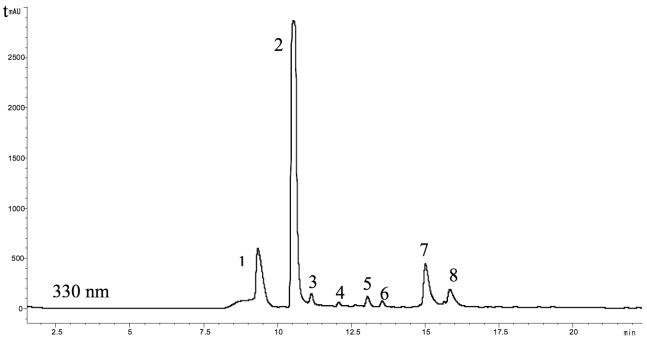

Fig. 1 - HPLC chromatogram of sunflower kernel extract recorded at 330 nm. Peaks= 1. 3-O-caffeoylquinic acid; 2. chlorogenic acid; 3. 4-O-caffeoylquinic acid; 4-6. caffeic acid derivatives; 7. 3,5- O-dicaffeoylquinic acid; 8. Ac. 4,5- O-dicaffeoylquinic acid.

feoylquinic acid. In the shells the same compounds with the exception of 4-*O*-caffeoylquinic acid and the two dicaffeoylquinic acids were identified. The same caffeoyl and dicaffeoylquinic acid derivatives were already found by Aramendia *et al*. (2000), Pedrosa *et al*. (2000), and Weisz *et al*. (2009). The qualitative pattern does not change along with the agricultural management. As concerns quantitative data, Table 2 reports the quantitative data of kernel and teguments. Total polyphenols amount is the lowest, in the case of kernel, when the plant grows on the old organic field, while it is almost the same with the other two managements; in the case of teguments, on the contrary, the lowest amount is observed in the case of conventional management. It should however be noted that in the case of teguments, polyphenols content is very low changing from about 10% to 2% with respect to kernel content. Chlorogenic acid is the main compound and it accounts for about 90% in tegument. Chlorogenic acid in our samples, both kernel and tegument, is about ten times more abundant with respect to the quantitative data reported by Aramedia *et al.* (2000) and Pedrosa *et al*. (2000). The comparison of quantitative data is however difficult since different techniques and sample treatments are involved (Weisz *et al*., 2009). Polyphenols content along with agriculture management has recently reviewed and in most vegetables grown under organic conditions, a higher content of polyphenols has been found; on the other hand a higher soil nitrogen availability decreases polyphenols content (Heimler *et al.*, 2017). Our data support previous findings that indicate how old managed organic soil was the most efficient in term of C and N storage (Migliorini *et al*., 2014). Furthermore, when the individual compounds are taken into account the relative percentages of compounds from new organic and conventional managed soils are similar, while in the case of the old managed organic soil, notwithstanding the lowest total polyphenol content, a higher dicaffeoylquinic acids content was found (17% with respect to 12%). Chlorogenic acid and dicaffeoylquinic acids derive from the phenylpropanoid pathway. Generally, this pathway is induced by biotic and abiotic stress such as wounding, UV irradiation, or pathogen attack (Moglia *et al.*, 2008). No information is available on the regulation of dicaffeoylquinic acids in any plant species, even if the same regulation of chlorogenic acid synthesis could be foreseen (Moglia *et al.*, 2008).

In Table 3, the data of sunflower oil are reported. Oil has been obtained by means of a mechanical

Table 2 - Polyphenols (mg/g, fresh weight) in sunflower kernel and tegument

Compound	Agricultural management					
	Kernel			Tegument		
	OldO	YoungO	CO	OldO	YoungO	CO
3-*O*-caffeoylquinic acid	2.51(0.37)	4.57(0.82)	4.74(0.76)	0.08(0.01)	0.08(0.01)	0.05(0.01)
chlorogenic acid	6.25(1.12)	11.39(1.94)	11.82(1.42)	0.95(0.18)	0.96(0.18)	0.41(0.07)
4-*O*-caffeoylquinic acid	0.44(0.08)	0.8(0.11)	0.83(0.13)	0.03(0.005)	traces	traces
caffeic acid derivatives	0.21(0.04)	0.38(0.04)	0.39(0.06)	traces	n.d.	traces
3,5-*O*-dicaffeoylquinic acid	1.3(0.24)	n.d.	n.d.	n.d.	n.d.	n.d.
4,5-*O*-dicaffeoylquinic acid	0.77(0.11)	2.36(0.34)	2.45(0.27)	n.d.	n.d.	n.d.
Total polyphenols	11.48	19.5	20.23	1.06	1.04	0.46

OldO= old organic; YoungO= young organic; CO= conventional (see experimental section). ND= not determined. Standard deviation within brackets.

Table 3 - Polyphenols (mg/l) in sunflower oil

Compound	Agricultural management		
	OldO	YoungO	CO
3-*O*-caffeoylquinic acid	0.232(0.192)	0.35(0.09)	0.176(0.057)
chlorogenic acid	1.96(0.393)	2.43(0.11)	1.936(0.12)
p-coumaroylquinic acid	2.36(0.318)	0.112(0.022)	0.592(0.079)
4-*O*-caffeoylquinic acid	0.048(0.005)	0.104(0.011)	0.08(0.02)
caffeic acid derivatives	2.28(0.644)	0.328(0.079)	3.216(0.415)
not identified polar compounds	0.504(0.011)	1.2(0.045)	1.456(0.429)
Total polyphenols	7.384	4.368	7.456

OldO= old organic; YoungO= young organic; CO= conventional (see experimental section). ND= not determined. Standard deviation within brackets.

press, which is the only technology that allows the maintenance of polyphenols high content (Bendini *et al.*, 2011). Oil yields are different (22.2% for old organic soil, 29.8% for young organic soil and 24.7% for conventional management) and, the young organic soil, with the highest yield, exhibits the lowest total polyphenol content. Sunflower oil-amount of 10 mg kg^{-1} has been reported (De Leonardis *et al*., 2005). Polyphenols content of the three seeds oils (Table 3) are almost in accordance with such amount. Young managed organic soil gave rise to an oil with the smallest polyphenols content notwithstanding the high polyphenols content of almond and teguments.

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