CHARACTERIZATION AND POLYPHENOLIC ANTIOXIDANTS CONTENT OF SUNFLOWER SEEDS AND OILS FROM CONVENTIONAL AND ORGANIC FARMING

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ABSTRACT

The aim of this work is the characterization of polyphenolic antioxidants in different tissues of sunflower seeds (kernels and shells) and their oils, cultivated by organic and conventional techniques, respectively. The presence of radioactivity in seeds via gamma spectrometry technique is also evaluated. The quantitative analysis of antioxidant molecules in the kernel and shell of sunflower seeds shows the presence of hydroxycinnamic acids, such as chlorogenic acid and other caffeoylquinic acid derivatives. There are no specific differences in terms of quality of the antioxidants profile, but the organic samples show higher concentrations of phenol antioxidants in the seed shells with respect to those obtained by conventional cultivation. This study presents an integrated approach of different analytical techniques, focused on the aspect of quality and safety of food products, taking into consideration one of the most important oilseed crops, as sunflower, cultivated in organic and traditional farm, respectively. The seed samples were analyzed also by NaI(Tl) gamma spectroscopy.

Keywords: Organic and Conventional Cultivation, Polyphenol Antioxidants, HPLC/DAD and HPLC/TOF-MS, Gamma-Spectrometry, Radionuclides, Food Quality and Safety.

INTRODUCTION

The main researches on the environmental impacts of organic production (e.g., FAO, 2003; Shepherd et al. 2003) shows that, depending on the products involved, organic farms use 50 to 70% less energy (direct and indirect) per unit of production than conventional farms, mainly as a result of different fertilizer use. Organic production also has clear benefits for biodiversity on agricultural land, although lower yields may mean that a larger land area is required than under conventional production methods (Reisch et al., 2013). Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops. Besides palm, soy and rapeseed oil, sunflower oil is ranking fourth with a worldwide production of about 10.6 million metric tons (mt) in 2006 (FAO-STAT, 2008). 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 G.M. et al., 2009). Sunflower proteins have not been used on an industrial scale due to the high levels of phenols which can affect their quality, causing undesirable browning and structural modifications. Nevertheless, the phenolic compounds have been shown to exert a high antioxidative potential, which might be beneficial from a biofunctional point of view, for instance used as effective antioxidants for sunflower oils (De Leonardis A. et al., 2003).

The plant material has been collected from a flat area in Montepaldi (San Casciano Val di Pesa, Firenze), where there is a farm of the University of Florence which includes the experimental field MOLTE (Montepaldi Long Term Experiment) of about 13 ha, sub-divide in three micro-agrosystems, one conventional (CONV, from 1991) and two organics (ORG1 and ORG2, from 1991 and 2001, respectively).
The plots are regular, each of them of 1.3 ha (260×50 m). The applied rotation is four years in the organic microfarms, and two years in the conventional one. In the organic systems, there is a

management following the European rules for organic farming. The conventional system uses external inputs, in the form of fertilizers and herbicides.
The aim of this work is the HPLC/DAD/ESI-MS characterization of the polyphenolic antioxidants in different tissues of sunflower seeds (kernels and shells) and their respective oils, cultivated by organic and conventional approaches, respectively.
The presence of radioactivity in seeds via gamma spectrometry technique is also evaluated.

BACKGROUND

Different genotypes of sunflower seeds were previously studied and the phenolic compositions of their kernel and hull were characterised by HPLC and MS techniques (Pedrosa et al., 2000). The main phenolic compounds present in both the kernel and hull are chlorogenic acid, caffeic acid and caffeoylquinic derivatives. The concentration of the main phenol compounds present in the kernel ranged from 94.6% to 99.3% of the total phenolic compounds of the whole seed. Therefore, the dehulling of the seed seems scarcely improve its nutritional value.
A first pilot study to produce a food antioxidant from sunflower seed shells (*Helianthus annuus*) by De Leonardis et al. (2005) reports a laboratory procedure to obtain an antioxidant from sunflower seeds, rich in phenols (1–4 wt-%), especially chlorogenic acid. Protein recovery from sunflower seeds is limited by the native chlorogenic acid, which causes an undesirable browning of the cakes and a significant reduction in the availability and digestibility of the proteins. The residue originating from sunflower oil recovery is a much more suitable material for polyphenol extraction, due to its high amounts and consumer acceptance, demanding sustainable agricultural production. Since sunflower kernels contain about 50% oil, the residues of oil production amount to approximately 10.6 million mt, as can be estimated from the annual sunflower oil production (FAO-STAT, 2008). Assuming a total phenolic content of 3%, as determined in a previous study (Weisz G.M. et al., 2009), the recovery of up to 300,000 mt from the by-products of the sunflower oil extraction would be possible, which might then be used for natural ingredients of functional or enriched foods or as natural food antioxidant components.

MAIN FOCUS OF THE PAPER

Aim, Issues, Controversies, Problems

The present study aims to verify the phenolic antioxidants content in sunflower kernels and shells in crops cultivated under conventional and organic agronomical procedures, with particular regard also to the obtained oils. Raw seeds have been also measured by a Na(Tl)I gamma spectrometer.

Method or Approach

For each sample, kernel and shell have been weighed separately, then extracted by hydroalcoholic solution, and, finally, analyzed via HPLC/DAD and HPLC/TOF-MS methods. A similar extraction and fractionation procedure has been applied for the related oil samples. The quantitative analysis has been performed via HPLC, with specific calibration curves, built with standards and reference molecules at different levels of concentration, considering the chromatographic profile at the maximum wavelength of absorbance of each molecule or subclass of interest.
A method for checking the radiological properties of the samples under study was adopted, by means of a NaI(Tl) gamma spectrometer (Ortec Food Guard). The instrument consists of a thallium-doped sodium iodide 3”×3” detector, enclosed in a low-background lead shield (30 mm thick), an analog-to-digital converter (ORTEC DigiBase) integrated in an all-in-one spectrometer, and a controlling computer. The internal stabilization electronics and an internal check source (K-40 4500 Bq/kg) allow the system to be stabilized. The main control and analysis software was the ORTEC ScintiVision-32. An independent Radon sensor was adopted to monitor the radon concentration in real time during the analyses by the NaI(Tl) system, and the whole background radiation was subtracted from the spectra to derive the fnal results.

The data were acquired, saved and summed up every 1800 s. The centroid of the photopeak of the K-40 at 1461 keV was kept always corresponding to the channel 559.20+/- 0.20.

The energy calibration of the NaI(Tl) system was done in several steps (Calin 2011, Graaf 2011) taking into account the K-40 photopeak and the following peaks from the natural background content of U-238 and Th-232: the Tl-208 peak at 2614 keV, the Bi-214 peak at 1765 keV the Ac-228 peak at 969 keV, the Tl-208 peak at 511 keV and the Pb-212 at 239 keV. Furthermore, three reference sources were adopted: Ba-133 (53 and 81 keV peaks), Cd-109 (88 keV peak) and Cs-137 (662 keV peak).

RESULTS AND IMPLICATIONS

Figure 1 reports, as an example, the chromatographic profiles of organic sunflower kernel registered at 330 nm, maximum wavelength of absorbance of caffeic acid and its derivatives, are reported. Each identified compound is listed in the caption. HPLC/DAD and HPLC/TOF-MS analyses have allowed to obtain a quali-quantitative evaluation of each polyphenolic compounds in the different tissues of sunflower samples (kernel and shell) and in the respective oils. Data are reported in Table 1 for kernels an shells of sunflower cultivated under organic (ORG 1 and ORG 2) and conventional agronomical conditions.

The total phenolic content of about 1700 - 1900 mg / 100 g on dry matter basis in the whole seed (kernel and shell), makes the sunflower seeds an interesting source of antioxidant compounds that may be recovered and used for the development of functional ingredients in food products. The main difference in polyphenol composition between the seed tissues is the presence of dicaffeoylquinic derivatives in the kernels. Table 2 reports the phenolic content of the oils obtained by processing the sunflower seeds (organic and conventional, respectively). Regarding the polyphenol content, there are no particular differences between the two techniques of cultivation. It is worth of noting that in one organic sample (ORG 2) the total phenol content seems lower, but with the highest values of cholorogenic acid concentrations.

Organic and conventional samples, from the radiological point of view are very similar, with some difference in the Potassium contents, that can explain the different values of K-40 (Table 3). The “conventional” sample shows its K-40 content very clearly after background subtraction (Table 3 and Figure 2), probably because of more abundant K fertilization. The low Tl-208 values of both samples, if confirmed, could be due to natural sources.

FUTURE RESEARCH DIRECTIONS

This study presents an integrated approach of different analytical techniques, focused on the aspect of quality and safety of food products, taking into consideration one of the most important oilseed crops, such as the sunflower, cultivated in organic and traditional farm, respectively. Further researches are needed to collect data on different years of production, to obtain the relative phenolic profiles, which hopefully, will depend also on the cultivation conditions. The agronomical features of the different samples will be deeply investigated to possibly identify selected phenolic markers that can characterize not only selected plant tissue (kernel and shell), but also if the crop is cultivated under conventional and organic conditions.

CONCLUSION

Sunflower seeds and the cake after the oil extraction can be interesting sources of phenolic antioxidants. Since the protein recovery from sunflower seeds is limited by the native chlorogenic acid, which causes an undesirable browning of the cakes and a significant reduction in the availability and digestibility of the proteins, the recovery of these molecules becomes increasingly important and suggest important applications for the antioxidant properties of polyphenols. The residue originating from sunflower oil recovery is then a much more suitable material for polyphenol extraction, in particular using sustainable technological procedures, as reported in a paper presented in this Conference (Romani et al., *AISME 2016*), where an example of circular economy is given, with a process based on green technologies, in order to obtain biologically active compounds from by-product of food processing.

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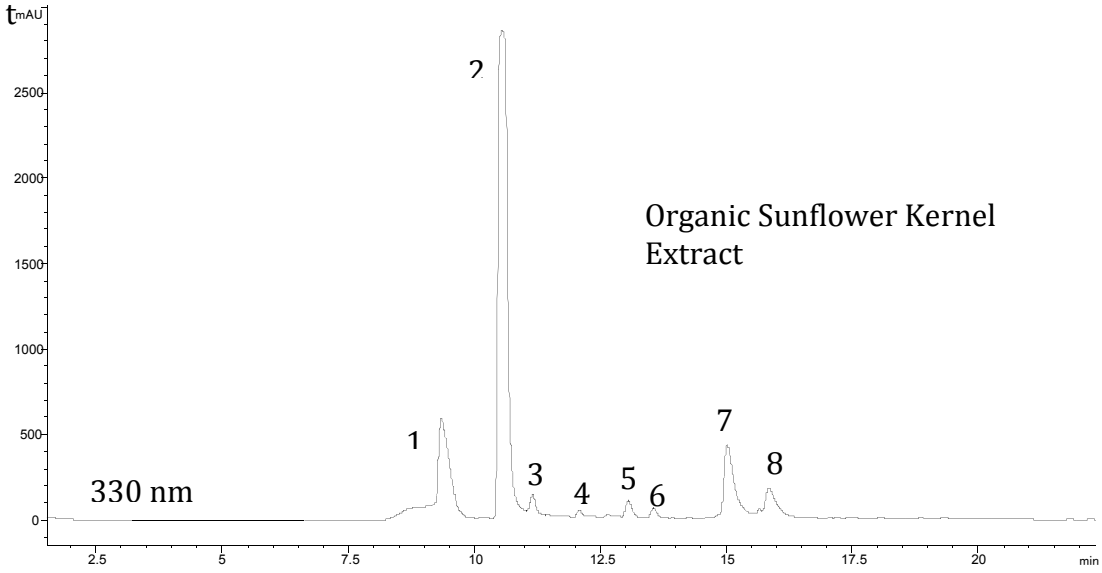

Figure 1. Peaks: 1. 3-O-caffeoylquinic acid; 2. Chlorogenic acid; 3. 4-O-caffeoylquinic acid; 4-6. Caffeic acid derivatives; 7. 3,5- O-dicaffeoylquinic acid; 8. Ac. 4,5- O-dicaffeoylquinic acid

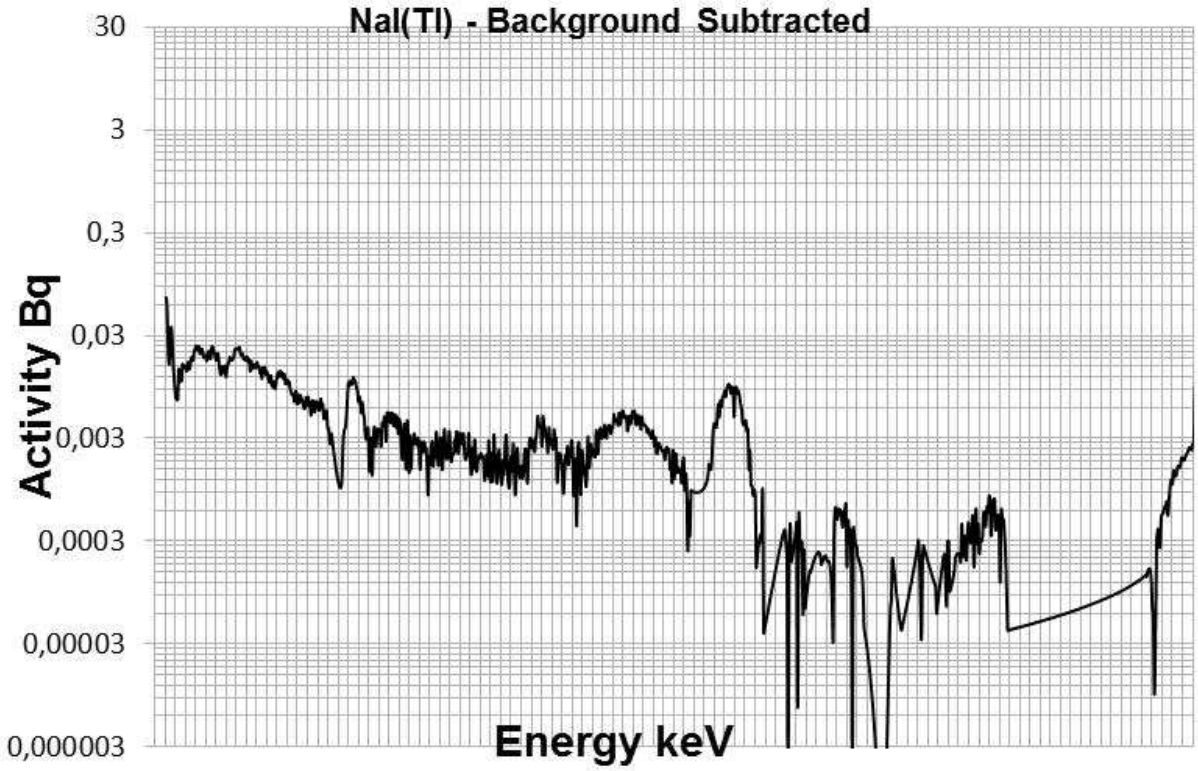

Figure 2. The NaI(Tl) gamma spectrum of the Sunflower seed sample (traditional cultivation), whose content of K-40 (1460 keV) is made clear by the background subtraction.