SUPPLEMENTARY MATERIAL

Antioxidant activity of *Cynara scolymus* L. and *Cynara cardunculus* L. extracts obtained by different extraction techniques

Eleni Kollia^a, Panagiota Markaki^a, Panagiotis Zoumpoulakis^b and Charalampos Proestos^{a*}

^aDepartment of Chemistry, Laboratory of Food Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou 15784 Athens, Greece.

^bInstitute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48, Vas. Constantinou Ave., 11635 Athens, Greece.

Abstract

Extracts of different parts (heads, bracts and stems) of *Cynara cardunculus* L. (cardoon) and *Cynara scolymus* L. (globe artichoke), obtained by two different extraction techniques (Ultrasound-Assisted Extraction (UAE) and classical extraction (CE)) were examined and compared for their total phenolic content (TPC) and their antioxidant activity. Moreover infusions of the plant's parts were also analyzed and compared to aforementioned samples. Results showed that cardoon's heads extract (obtained by Ultrasound-Assisted Extraction) displayed the highest TPC values (1.57 mg Gallic Acid Equivalents (GAE) g⁻¹ fresh weight (fw)), the highest DPPH[•] scavenging activity (IC₅₀; 0.91mg ml⁻¹) and the highest ABTS^{•+} radical scavenging capacity (2.08 mg Trolox Equivalents (TE) g⁻¹ fw) compared to infusions and other extracts studied. Moreover Ultrasound-Assisted Extraction technique proved to be more appropriate and effective for the extraction of antiradical and phenolic compounds.

3. Experimental

3.1. Sampling and treatment

Cynara cardunculus L. or commonly named cardoon (variety: Large Smooth) was collected from fields in the area of Thebes (Central Greece) and globe artichoke (*Cynara scolymus* L.) originated from Argos (Peloponnese, Greece, (Variety artichoke Argitiki, aka Green Argos) was purchased from a local market in Athens, Greece. A voucher specimen was deposited at the crop science

department, Agricultural University of Athens, Greece. Samples were washed with water, dried with filter paper, chopped and separated into different parts (heads, bracts and stems). Every part of the plant was homogenized before analysis.

3.2. Apparatus

Ultrasound-Assisted Extraction (UAE) procedure was performed using Sonics and Material Inc., Vibra-Cell VCX750 (20 kHz, 750 W) ultrasonics processor equipped with piezoelectric converter and 13 mm diameter probe fabricated from titanium alloy Ti-6Al-4V (Inc. USA). For the evaporation of extracts a rotary evaporator (Heidolph, Laborota 4000 efficient, WB eco) was used. For the spectrophotometric analyses a UV-vis spectrophotometer (Novaspek III visible spectrophotometer, Amersham Biosciences, USA) was used.

3.3. Reagents

Folin–Ciocalteu's phenol reagent were obtained from Merck KGaA (Germany) and ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] from Tokyo Chemical Industry Co Ltd (Tokyo, Japan). DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) free radical and gallic acid were obtained from Alfa Aesar GmbH and Co KG (Germany) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). L-ascorbic acid was obtained from Fisher Chemical, UK. Methanol (pro analysis) were from Merck (Darmstadt, Germany).

3.4. Statistical analysis

Data were analyzed by one-way ANOVA and t-test. The mean differences which are significantly different were examined by using the Tukey test (Fowler and Cohen 1997).

3.5. Classical extraction

The fresh samples (10 g) were repeatedly (3 times) extracted by stirring with 40 mL of MeOH:H₂O (80:20 v/v) at room temperature (25°C) for 48h and filtered through Whatman no 1 filter paper. All extracts, were evaporated to dryness at

40°C and re-dissolved in 40 mL of MeOH:H₂O (80:20 v/v). The extracts were stored at 4°C and analysed within a week.

3.6. Ultrasound-Assisted Extraction (UAE, probed)

10g of samples (fresh artichokes) were repeatedly extracted (3 times) with 40 mL of MeOH:H₂O (80:20 v/v). The homogenized samples and the solvent were placed in a 250 mL three-neck vessel within an icebath in order to keep the temperature below 40°C during the whole extraction time. The extracts were sonicated for 15 min, using 80% amplitude and pulse sonication sequence set to 10s ON and 5s OFF. All obtained extracts, were then evaporated to dryness at 40°C and re-dissolved in 40 mL of MeOH:H₂O (80:20 v/v). The extracts were stored at 4°C and analysed within a week (Petrović et al. 2014).

3.7. Herbal infusions

Ten grams of the samples were extracted by stirring 40 mL of hot water (80°C) for 3min, finally filtered through Whatman no 1 filter paper. The extracts were analysed the same day of preparation.

3.8. Determination of Total Phenolic Content

The total phenolic content (TPC) of each sample was determined by Folin–Ciocalteu's colorimetric assay (Singleton and Rossi 1965). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per g of fresh weight (FW). The quantification was done on the basis of the standard curve of gallic acid using 25–300 mg GAE L⁻¹. (Lantzouraki et al. 2015). The experiment was performed in triplicate.

3.9. DPPH radical scavenging assay

Radical scavenging activity of each sample was evaluated using the stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH[•]) according to the slightly modified method of Brand-Williams et al. (1995). 100µL of each extract and 1500 µL of methanolic solution of DPPH[•] (100 µM) were added in a cuvette and the absorbance at 516 nm (till stabilization – plateau) was measured using a UV-vis spectrophotometer. The antiradical activity (AA) of samples was expressed as mg L-Ascorbic Acid Equivalents (AAE) per g of fresh weight, using a standard

curve with 20.0–1500.0 $\mu\text{g AAE mL}^{-1}$. The solution concentration with 50% inhibition (IC_{50}) of DPPH $\cdot$ was calculated from the plot of the inhibition % against sample concentrations and was expressed in mg mL^{-1} (Lantzouraki et al. 2015)

3.10. Trolox Equivalent Antioxidant Capacity Assay (TEAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) assay is based on the scavenging of the [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical (ABTS $^{•+}$). The ABTS free radical-scavenging activity was determined according to a modification of the method described by RE et al. (1999) and described in details by (Lantzouraki et al. 2015).

References

- Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol* 28:25–3.
- Fowler J, Cohen L. 1997. Practical statistics for field biology. Chichester, England: John Wiley and Sons.
- Lantzouraki DZ, Sinanoglou VJ, Zoumpoulakis PG, Glamoclija J, Ćirić A, Soković M, Heropoulos G, Proestos C. 2015. Antiradical–antimicrobial activity and phenolic profile of pomegranate (*Punica granatum* L.) juices from different cultivars: a comparative study. *RSC Adv* 5:2602.
- Petrović J, Papandreou M, Glamoclija J, Ćirić A, Baskakis C, Proestos C, Lamari F, Zoumpoulakis P, Soković M. 2014. Different extraction methodologies and their influence on the bioactivity of the wild edible mushroom *Laetiporus sulphureus* (Bull.) Murrill. *Food and Funct* 5(11):2948-60.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231–1237.
- Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Am J Enol Vitic* 16:144–158.