Antioxidant Properties of Seeds from Lines of Artichoke, Cultivated Cardoon and Wild Cardoon

Alessandra Durazzo 1*, Maria Stella Foddai 1, Andrea Temperini 2, Elena Azzini 1, Eugenia Venneria 1, Massimo Lucarini 1, Enrico Finotti 1, Gianluca Maiani 1, Paola Crinò 3, Francesco Saccardo 2 and Giuseppe Maiani 1

1 CRA-Ex INRAN (National Research Institute on Food and Nutrition), Via Ardeatina 546, Rome 00178, Italy; E-Mails: foddai@inran.it (M.S.F.); azzini@inran.it (E.A.); venneria@inran.it (E.V.); lucarini@inran.it (M.L.); finotti@inran.it (E.F.); gianluca.maiani@alice.it (G.M.); maiani@inran.it (G.M.)

2 Department of Science and Technologies for Agriculture, Forestry, Nature and Energy (DAFNE), Tuscia University, Via San Camillo De Lellis sncc, Viterbo 01100, Italy; E-Mails: andreatemperini@libero.it (A.T.); migammabeta@yahoo.it (F.S.)

3 Technical Unit for Sustainable Development and Innovation of Agro-Industrial System (UTAGRI), ENEA, Casaccia C.R., Via Anguillarese 301, Rome 00123, Italy; E-Mail: paola.crino@enea.it

* Author to whom correspondence should be addressed; E-Mail: durazzo@inran.it; Tel.: +39-065-149-4651; Fax: +39-065-149-4550.

Received: 7 April 2013; in revised form: 5 June 2013 / Accepted: 6 June 2013 / Published: 17 June 2013

Abstract: The artichoke (Cynara cardunculus L. subsp. scolymus L.), the cultivated cardoon (Cynara cardunculus var. altilis DC.) and the wild cardoon (Cynara cardunculus var. sylvestris L.) are species widely distributed in the Mediterranean area. The aim of this research was to evaluate the antioxidant properties of seeds from lines of artichoke and cultivated and wild cardoon in both aqueous-organic extracts and their residues by FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity) evaluations. Both artichoke and cardoon seeds are a good source of antioxidants. Among artichoke seeds, hydrolysable polyphenols contribution to antioxidant properties ranged from 41% to 78% for FRAP values and from 17% to 37% for TEAC values. No difference between cultivated and wild cardoon in antioxidant properties are reported. Our results could provide information about the potential industrial use and application of artichoke and/or cardoon seeds.
1. Introduction

The artichoke (*Cynara cardunculus* L. subsp. *scolymus* L.), the cultivated cardoon (*Cynara cardunculus* var. *altilis* DC.) and the wild cardoon (*Cynara cardunculus* var. *sylvestris* L.) are species widely distributed in the Mediterranean area. Wild cardoon is the ancestor of both the cultivated forms, which evolved separately as a result of different selection criteria [1,2].

These plants were popular in Greeks and Romans as food and medicine [3]. Nowadays, they are considered as functional foods, owing to their nutritional properties [4–6].

A lot of investigations on bioactive molecules (mainly represented by flavonoids such as apigenin and luteolin and by hydroxycinnamic derivatives such as mono- and di-caffeoylquinic acids) and antioxidant properties of edible part of artichoke and cardoon plant have been carried out [7–11], few studies on antioxidant properties of *Cynara* seeds are present in literature [12,13].

According to Foti *et al.* [14] the chemical composition of artichoke seeds was the following: crude protein 21.6%, crude fiber 17.1%, crude oil 24.05% and ash 3.8%. Raccuia and Melilli [15] have shown like *Cynara* oil is suitable for human consumption.

Saponins, sesquiterpene lactones, flavones, sterols, coumarins and lignans have been identified and quantified in seeds of *Cynara* [12,13]. Georgieva *et al.* [13], by comparing the radical scavenging capacity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) of ethanol extracts prepared from *Cynara scolymus* L. seeds and leaves, have demonstrated that at any studied concentration of the seeds extract the percent of the scavenged DPPH radicals was considerably higher than that calculated for the leaves extract.

Among the various kinds of natural antioxidants, polyphenols have received much attention. These compounds exhibit a wide range of physiological properties, such as anti-allergic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects [16,17].

Polyphenols exist as easily extractable compounds (free) (compounds solubilized by aqueous organic solvents) and as less extractable types (bound) (compounds that remain in their corresponding extraction residues) [18–25].

Recent investigations have used an alkaline hydrolysis, acid hydrolysis, or enzymatic digestion [26–30]; polyphenols content of plant foods have been underestimated: significant amounts of bioactive compounds remain in the residue from extraction as non extractable polyphenols [31,32].

Arranz *et al.* [23] have concluded, by studying dietary polyphenols in cereals, fruits, vegetables, nuts, and legumes, that non extractable polyphenols are the major part of dietary polyphenols. Studies of non extractable polyphenols are few respect to those on extractable polyphenols [33,34]. No studies addressed to the estimation of antioxidant properties in aqueous-organic extracts and residues of *Cynara* seeds are present in literature.

In this work extraction and isolation of aqueous-organic extracts of foods (extractable polyphenols) and residues (non-extractable polyphenols) of seeds from lines of artichoke and cultivated and wild cardoon were optimized.
The aim of this research was to evaluate the antioxidant properties of seeds from lines of artichoke, cultivated and wild cardoon and hybrids by FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity) evaluations.

The distinction between free and bound antioxidants represents a key element to define the potential of seeds. Thus, a recent increase in serious research on the commercial application of antioxidants, led to the additional focus on the topical relevance of food derived extracts in the present study [35–37].

2. Experimental Section

2.1. Chemicals and Standards

All solvents were of HPLC or optima grade and purchased from Carlo Erba (Milan, Italy). Common reagents and standards were purchased from Sigma–Aldrich srl (Milan, Italy) and were of the highest available grade. Double-distilled water (Millipore, Milan, Italy) was used throughout the study.

2.2. Sample Preparation

The seeds were ground in a refrigerated mill (Janke and Kunke Ika Labortechnik, Germany) and the pulverized samples were sieved to obtain a granulometry of 0.5 mm. The descriptions of selected seeds are reported in Table 1.

Table 1. Description of selected seeds.

| Species                      | Lines             | Abbreviation |
|------------------------------|-------------------|--------------|
| Artichoke-male fertile lines | Brawley North     | BN           |
| Cavi                         | CA                |
| Cyl                          | CYL               |
| F19                          | F-19              |
| Vert de Provence             | VP                |
| White bloomer                | WB                |
| 22BD                         | 22BD              |
| Cultivated Cardoon           | Bianco avorio     | BA           |
| Belgio                       | BE                |
| F.S.                         | F.S.              |
| Madrid                       | MA                |
| Wild Cardoon                 | Cerveteri         | CE           |
| Siena                        | S                 |
| Tarquinia                    | T                 |
Table 1. Cont.

| Cardoon/Artichoke Hybrids | Madrigal | MG |
|---------------------------|----------|----|
| F1 hybrid                 | Romolo   | RO |
| cardoon × artichoke hybrid| F1 103   | F1 103 |
| cardoon × artichoke hybrid| F1 104   | F1 104 |
| F1 hybrid (synthetic variety) | Istar | 1 |

2.3. Extraction for Evaluation of Antioxidant Properties

Total phenolic compounds were extracted as described by Durazzo et al. [38] with minor modifications as follows. Aqueous-organic extracts (extractable polyphenols) and their residues (non-extractable polyphenols) were isolated and studied. In particular in residues, among non-extractable polyphenols, hydrolysable polyphenols (comprising hydrolysable tannins, phenolic acids and hydroxycinnamic acids that are released from the food matrix by strong acidic hydrolysis) were determined.

Aqueous-organic extract. About 3–4 g of samples were placed in a test tube and 20 mL of acidic methanol/water (50:50 v/v, pH 2) were added. The tubes were vortexed at room temperature for 3 min, followed by 1 h shaking in a water bath at room temperature. The tube was centrifuged at 2500 g for 10 min, and the supernatant was recovered. Twenty milliliters of acetone/water (70:30, v/v) were added to residue, then vortexing, shaking and centrifugation were repeated. Both methanolic and acetonic extracts were combined and centrifuged at 3500 g for 15 min. The resulting supernatant was transferred into tubes and directly used for the determination of antioxidant capacity.

Residue. Residues were left in a ventilating and heating apparatus (max temperature 25 °C), until dryness. Briefly, 300–400 mg of the residue were mixed with 20 mL of methanol and 2 mL of concentrated sulfuric acid (18 M). The samples were gently stirred for 1 min and were shaken in a water bath at 85 °C for 20 h.

The samples were then centrifuged (3000 g for 10 min), and the supernatant was recovered. After two washings with minimum volumes of distilled water and recentrifuging as necessary, the final volume was taken up to 50 mL. The tube was centrifugated at 3500 g for 20 min and was transferred into tubes and directly used for the determination of antioxidant capacity.

2.4. Antioxidant Properties Determination

Antioxidant properties have been determined in both aqueous-organic extracts and their residues using two different assays, Ferric Reducing-Antioxidant Power (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC).

The FRAP method, according to Benzie and Strain [39] and Pulido et al. [40], is based on the reduction of Fe³⁺-TPTZ (2,4,6-tripyridyl-s-triazine) complex to ferrous at low pH through the use of a Tecan Sunrise® plate reader spectrophotometer. The TEAC assay measures the ability of antioxidants to quench radical cations according to Re et al. [41].
2.5. Statistical Analysis

All analyses were performed in triplicate. Data are presented as mean ± Standard Deviation (SD). Statistica for Windows statistical package (release 4.5; StatSoft Inc., Vigonza PD, Italy) was used to perform One-Way Analysis of Variance (ANOVA).

3. Results and Discussion

The chemical diversity of natural phenols makes it difficult to separate, detect, and quantify these molecules from a complex food matrix. For determination of antioxidant capacity of foods, it is important to consider three steps: extraction of antioxidants, antioxidant capacity measurements and expression of results [42–44]. It is generally known that chemical extraction depends on type of solvents, extraction time, and temperature as well as on the chemical compositions and physical characteristics of the sample [44,45].

In Table 2 FRAP values (mmol/kg d.w.) and TEAC values (mmol/kg d.w.) of aqueous-organic extracts (combining two extraction cycles) and the corresponding residues of selected seeds were reported. For artichoke seeds, FRAP values are placed within the range 248.3–344.9 mmol/kg d.w. in aqueous-organic extracts and enlarged range of 185.1–1067.2 mmol/kg d.w. in residues. The type White Bloomer (WB) reached the highest FRAP values both in aqueous-organic extract and residue; TEAC values ranged from 63.3 ± 3.2 to 106.3 ± 7.7 mmol/kg d.w. in aqueous-organic extracts and from 21.5 ± 1.6 to 45.6 ± 7.1 mmol/kg d.w. In our work the hydrolysable polyphenols contribution to antioxidant properties ranged from 41% to 78% for FRAP values and from 17% to 37% for TEAC values; the genotype CYL exhibited the highest contribution both for FRAP and TEAC values. It is interesting to notice that in most of artichoke seeds the hydrolyzable polyphenols presented higher ferric reducing powers than extractable polyphenols. As reported in literature data, non extractable polyphenols are more abundant than extractable polyphenols in many foodstuffs [32]. High antioxidant capacity of hydrolysable phenolics was found in the residues of aqueous-organic extracts in cereals [19] and walnuts [46]. Saura-Calixto et al. [47], studying total polyphenol content of plant foods in the Spanish diet, reported that hydrolysable polyphenols were a quantitatively important fraction of polyphenols in all food groups.

For cardoons, FRAP values ranged from 255.4 ± 22.4 to 358.8 ± 18.30 mmol/kg d.w. for aqueous-organic extracts and from 206.6 ± 80.1 to 733.8 ± 56.4 mmol/kg d.w for residues, whereas TEAC values ranged from 75.3 ± 5.1 to 119.0 ± 1.3 mmol/kg d.w. for aqueous-organic extracts and from 19.1 ± 3.1 to 42.0 ± 2.2 mmol/kg d.w. for residues; no difference in antioxidant properties between cultivated and wild cardoon are reported. Among cultivated cardoons, in both fractions the lowest FRAP and TEAC values were found for type Madrid (MA). Among wild forms, the genotype Siena (S) reached the highest TEAC values in both fractions and the highest FRAP values in residues, and this genotype exhibited the hydrolysable polyphenols contributions of 68% for FRAP values and of 31% for TEAC values.

Among hybrids, FRAP values ranged from 231.3 ± 18.0 to 331.8 ± 12.3 mmol/kg d.w. in aqueous-organic extracts and from 159.8 ± 39.4 to 391.5 ± 30.1 mmol/kg d.w. in residues; the hydrolysable polyphenols contribution to antioxidant properties ranged from 37% to 61% for FRAP values and from 13% to 25% for TEAC values.
Table 2. Ferric Reducing Antioxidant Power (FRAP) values (mmol/kg d.w.) and Trolox Equivalent Antioxidant Capacity (TEAC) values (mmol/kg d.w.) of selected seeds *.

| Crop          | Aqueous-organic extract | Residue | Aqueous-organic extract | Residue |
|---------------|-------------------------|---------|-------------------------|---------|
| Artichoke     |                         |         |                         |         |
| BN            | 278.5 ± 18.8 a          | 249.3 ± 51.0 a | 71.4 ± 3.8 b            | 31.5 ± 11.7 ab |
| CA            | 262.3 ± 17.0 a          | 185.1 ± 6.6 a  | 106.3 ± 7.7 c           | 21.5 ± 1.6 a |
| CYL           | 248.3 ± 16.3 a          | 898.4 ± 65.4 b | 63.3 ± 3.2 a            | 36.7 ± 6.2 ab |
| F-19          | 310.2 ± 28.7 b          | 333.7 ± 69.3 a | 87.9 ± 5.6 b            | 25.4 ± 5.6 a |
| VP            | 344.9 ± 17.9 c          | 616.3 ± 39.4 c | 87.4 ± 9.6 b            | 45.6 ± 7.1 b |
| WB            | 320.0 ± 25.2 b          | 1067.2 ± 173.1 d | 72.85 ± 2.5 a           | 36.6 ± 2.7 ab |
| 22BD          | 273.6 ± 7.2 ab          | 704.3 ± 57.0 c  | 64.3 ± 2.1 a            | 34.4 ± 8.1 ab |
| Cultivated Cardoon |                  |         |                         |         |
| BA            | 352.3 ± 22.2 b          | 708.9 ± 73.4 b  | 96.2 ± 4.1 b            | 30.0 ± 1.8 ab |
| BE            | 326.1 ± 34.1 b          | 298.6 ± 76.2 a  | 104.4 ± 7.0 b           | 38.7 ± 14.9 b |
| F.S.          | 333.1 ± 23.0 b          | 206.6 ± 80.1 a  | 119.0 ± 1.3 c           | 19.1 ± 3.1 a |
| MA            | 255.4 ± 22.4 a          | 265.4 ± 98.6 a  | 85.8 ± 2.0 a            | 29.7 ± 11.0 ab |
| Wild Cardoon  |                         |         |                         |         |
| CE            | 340.9 ± 24.2            | 301.3 ± 20.0 a  | 78.2 ± 1.7 a            | 23.1 ± 0.8 a |
| S             | 343.2 ± 8.8             | 733.8 ± 56.4 c  | 93.6 ± 9.0 b            | 42.0 ± 2.2 c |
| T             | 358.9 ± 18.3            | 629.2 ± 52.7 b  | 75.3 ± 5.1 a            | 28.7 ± 1.8 b |
| Cardoon/Artichoke Hybrids |                |         |                         |         |
| MG            | 231.3 ± 18.0 a          | 159.8 ± 39.4 a  | 95.8 ± 3.6 b            | 18.6 ± 1.9 a |
| RO            | 252.7 ± 20.6 ac         | 391.5 ± 30.1 b  | 94.9 ± 2.5 a            | 32.1 ± 2.0 c |
| F1 103        | 292.2 ± 22.6 bc         | 219.9 ± 33.8 a  | 110.7 ± 2.4 b           | 19.6 ± 0.6 a |
| F1 104        | 280.2 ± 10.5 c          | 166.5 ± 30.9 a  | 123.5 ± 2.6 c           | 17.9 ± 3.3 a |
| I             | 331.8 ± 12.3 d          | 238.0 ± 47.2 a  | 122.3 ± 7.2 e           | 24.5 ± 0.7 b |

* mean ± S.D.; a–d: Anova, Tukey HSD Test: within samples of each type of seeds, by columns, means followed by different letters are significantly different (p < 0.05).

Among hybrids, FRAP values for aqueous-organic extracts can be placed in the following decreasing order of concentration: I > F1 103 > F1 104 > RO > MG, whereas the type Romolo (RO) reached the highest FRAP values in residue; this type reached the highest TEAC values in residue too, exhibiting the highest contribution of hydrolysable polyphenols to antioxidant properties both for FRAP and TEAC values.

4. Conclusions

Both artichoke and cardoon seeds represent a rich source of antioxidants. These seeds could represent a good source of health-promoting bioactive compounds and therefore encourage a nutraceutical use of this species. Among artichoke seeds, hydrolysable polyphenols contribution to antioxidant properties ranged from 41% to 78% for FRAP values and from 17% to 37% for TEAC values. No difference between cultivated and wild cardoon in antioxidant properties are reported.
The results of this study contribute to the better knowledge of biochemical composition of the artichoke/cardoon seeds. The high antioxidant content of seeds makes this vegetable a potential useful source in nutritional as well as industrial field (agro-industrial applications).

Acknowledgments

This work was undertaken within the project CARVARVI, financed by Italian Ministry of the Agriculture, Food and Forestry.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Raccuia, S.A.; Mainolfi, A.; Mandolino, G.; Melilli, M.G. Genetic diversity in Cynara cardunculus revealed by AFLP markers: Comparison between cultivars and wild types from Sicily. Plant Breed 2004, 123, 280–284.
2. Acquadro, A.; Portis, E.; Albertini, E.; Lanteri, S. M-AFLP-based protocol for microsatellite loci isolation in Cynara cardunculus L. (Asteraceae). Mol. Ecol. Notes 2005, 5, 272–274.
3. Sonnante, G.; Pignone, D.; Hammer, K. The domestication of artichoke and cardoon: From Roman times to the genomic age. Ann. Bot. 2007, 100, 1095–1100.
4. Lattanzio, V.; Kroon, P.A.; Linsalata, V.; Cardinali, A. Globe artichoke: A functional food and source of nutraceutical ingredients. J. Funct. Foods 2009, 1, 131–144.
5. Ceccarelli, N.; Curadi, M.; Picciarelli, P.; Martelloni, L.; Sbrana, C.; Giovannetti, M. Globe artichoke as functional food. Mediter. J. Nutr. Metab. 2010, 3, 197–201.
6. Christaki, E.; Bonos, E.; Florou-Paneri, P. Nutritional and functional properties of Cynara crops (globe artichoke and cardoon) and their potential applications: A review. Int. J. Appl. Sci. Technol. 2012, 2, 64–70.
7. Jiménez-Escrig, A.; Dragsted, L.O.; Daneshvar, B; Pulido, R.; Saura-Calixto, F. In vitro antioxidant activities of edible artichoke (Cynara scolymus L.) and effect on biomarkers of antioxidants in rats. J. Agric. Food Chem. 2003, 51, 5540–5545.
8. Di Venere, D.; Linsalata, V.; Calabrese, N.; Cardinali, A.; Sergio, L. Biochemical characterization of wild and cultivated cardoon accessions. Acta Hort. 2005, 681, 523–528.
9. Kukić, J.; Popović, V.; Petrović, S.; Mucaji, P.; Ćirić, A.; Stojković, D.; Soković, M. Antioxidant and antimicrobial activity of Cynara cardunculus extracts. Food Chem. 2008, 107, 861–868.
10. Velez, Z.; Campinho, M.A.; Guerra, A.R.; García, L.; Ramos, P.; Guerreiro, O.; Felício, L.; Schmitt, F.; Duarte, M. Biological Characterization of Cynara cardunculus L. Methanolic extracts: Antioxidant, anti-proliferative, anti-migratory and anti-angiogenic activities. Agriculture 2012, 2, 472–492.
11. Pandino, G.; Lombardo, S.; Williamson, G.; Mauromicale, G. Polyphenol profile and content in wild and cultivated Cynara cardunculus L. Ital. J. Agron. 2012, 7, e35.
12. Falleh, H.; Ksouri, R.; Chaieb, K.; Karray-Bouraoui, N.; Trabelsi, N.; Boulaaba, M.; Abdely, C. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *C. R. Biol.* 2008, **331**, 372–379.

13. Georgieva, E.; Karamalakova, Y.; Nikolova, G.; Grigorov, B.; Pavlov, D.; Gadjeva, V.; Zheleva, A. Radical scavenging capacity of seeds and leaves ethanol extracts of *Cynara scolymus* L.—A comparative study. *Biotechnol. Biotechnol. Equip.* 2012, 151–155, doi:10.5504/50yrtimb.2011.0028.

14. Foti, S.; Mauronicale, G.; Raccuia, S.A.; Fallico, B.; Fanella, F.; Maccarone, E. Possible alternative utilization of *Cynara* spp. I. Biomass, grain yield and chemical composition of grain. *Ind. Crop. Prod.* 1999, **10**, 219–228.

15. Raccuia, S.A.; Melilli, M.G. Biomass and grain oil yields in *Cynara cardunculus* L. genotypes grown in a Mediterranean environment. *Field Crop Res.* 2007, **101**, 187–197.

16. Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: Food source and bioavailability. *Am. J. Clin. Nutr.* 2004, **79**, 727–747.

17. Scalbert, A.; Manach, C.; Morand, C.; Remesy, C. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* 2005, **45**, 287–306.

18. Hartzfeld, P.W.; Forkner, R.; Hunter, M.D.; Hagerman, A.E. Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *J. Agric. Food Chem.* 2002, **50**, 1785–1790.

19. Perez-Jimenez, J.; Saura-Calixto, F. Literature data may underestimate the actual antioxidant capacity of cereals. *J. Agric. Food Chem.* 2005, **53**, 5036–5040.

20. Saura-Calixto, F.; Goni, I. Antioxidant capacity of the Spanish Mediterranean diet. *Food Chem.* 2006, **94**, 442–447.

21. Diaz-Rubio, M.E.; Perez-Jimenez, J.; Saura-Calixto, F. Dietary fiber and antioxidant capacity in *Fucus vesiculosus* products. *Int. J. Food Sci. Nutr.* 2009, **2**, 23–34.

22. Goni, I.; Diaz-Rubio, M.E.; Perez-Jimenez, J.; Saura-Calixto, F. Towards an update methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. *Food Res. Int.* 2009, **42**, 840–846.

23. Arranz, S.; Silván, J.M.; Saura-Calixto, F. Non extractable polyphenols, usually ignored, are the major part of dietary polyphenols: A study on the Spanish diet. *Mol. Nutr. Food Res.* 2010, **54**, 1646–1658.

24. Rufino, M.S.; Alves, R.E.; de Brito, E.S.; Pérez-Jimenez, J.; Saura-Calixto, F.; Mancini-Filo, J. Bioactive compounds and antioxidant capacity of 18 non-traditional tropical fruits from Brazil. *Food Chem.* 2010, **121**, 996–1002.

25. Kristl, J.; Slekovec, M.; Tojnko, S.; Unuk, T. Extractable antioxidants and non-extractable phenolics in the total antioxidant activity of selected plum cultivars (*Prunus domestica* L.): Evolution during on-tree ripening. *Food Chem.* 2011, **125**, 29–34.

26. Iqbal, S.; Bhanger, M.I.; Anwar, F. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem.* 2005, **93**, 265–272.

27. Gorinstein, S.; Vargas, O.J.M.; Jaramillo, N.O.; Salas, I.A.; Ayala, A.L.M.; Arincibia-Avila, P.; Toledo, F.; Katrich E.; Trakhtenberg, S. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. *Eur. Food Res. Technol.* 2007, **225**, 321–328.
28. Bennet, R.N.; Shiga, T.M.; Hassimoto, N.M.A.; Rosa, E.A.S.; Lajolo, F.M.; Cordenunsi, B.R. Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (Musa acuminata Juss.) Cultivars. J. Agric. Food Chem. 2010, 54, 1646–1658.

29. Delgrado-Andrade, C.; Conde-Aguilera, J.A.; Haro, A.; de la Cueva, S.P.; Rufian-Henares, J.A. A combined procedure to evaluate the global antioxidant response of bread. J. Cereal Sci. 2010, 52, 239–246.

30. Taberner, M.; Venema, K.; Maathuis, A.J.H.; Saura-Calixto, F.D. Metabolite production during in vitro colonic fermentation of dietary fiber: Analysis and comparison of two European diets. J. Agric. Food Chem. 2011, 59, 8968–8975.

31. Pérez-Jiménez, J.; Torres, J.L. Analysis of nonextractable phenolic compounds in foods: The current state of the art. J. Agric. Food Chem. 2011, 59, 12713–12724.

32. Saura-Calixto, F. Concept and health-related properties of nonextractable polyphenols: The missing dietary polyphenols. J. Agric. Food Chem. 2012, 60, 11195–11200.

33. Arranz, S.; Saura-Calixto, F.; Shah, S.; Kroon, P.A. High contents of non extractable polyphenols in fruits suggest that polyphenol contents of plant foods have been underestimated. J. Agric. Food Chem. 2009, 57, 7298–7303.

34. Tarascou, I.; Souquet, J.M.; Mazauric, J.P.; Carrillo, S.; Coq, S.; Canon, F.; Fulcrand, H.; Cheynier, V. The hidden face of food phenolic composition. Arch. Biochem. Biophys. 2010, 501, 16–22.

35. Fukumoto, L.R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agric. Food Chem. 2000, 48, 3597–3604.

36. Peschel, W.; Sanchez-Rabaneda, F.; Diekmann, W.; Plescher, A.; Gartzia, I.; Jimenez, D.; Lamuela-Raventos, R.; Buxaderas, S.; Codina, C. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem. 2006, 97, 135–150.

37. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; et al. Phenol-Explorer: An online comprehensive database on polyphenol contents in foods. Database 2010, bap024, doi:10.1093/database/bap024.

38. Durazzo, A.; Turfani, V.; Azzini, E.; Maiani, G.; Carcea, M. Phenols, lignans and antioxidant properties of legume and sweet chestnut flours. Food Chem. 2013, 140, 666–671.

39. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The assay. Anal. Biochem. 1996, 239, 70–76.

40. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 2000, 48, 3396–3402.

41. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999, 26, 1231–1237.

42. Reşat Apak, R.; Gorinstein, S.; Böhm, V.; Schaich, K.M.; Özyürek, M.; Kubilay Guclü, K. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure Appl. Chem. 2013, 85, 957–998.

43. Schlesier, K.; Harwat, M.; Bohm, V.; Bitsch, R. Assessment of antioxidant activity by using different in vitro methods. Free Radic. Res. 2002, 36, 177–187.
44. Perez-Jimenez, J.; Arranz, S.; Tabernero, M.; Diaz-Rubio, M.E.; Serrano, J.; Goni, I.; Saura-Calixto, F. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurements and expression of results. *Food Res. Int.* **2008**, *41*, 274–285.

45. Luthria, D.L. Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. *J. Sci. Food. Agric.* **2006**, *86*, 2266–2272.

46. Arranz, S.; Perez-Jimenez, J.; Saura-Calixto, F. Antioxidant capacity of walnut (*Juglas regia* L.): Contribution of oil and defatted matter. *Eur. Food Res. Technol.* **2011**, *227*, 425–431.

47. Saura-Calixto, F.; Serrano, J.; Goni, I. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chem.* **2007**, *101*, 492–501.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).