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Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori)

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Abstract

The analysis of polyphenols of leaves and different parts (outer, intermediate and inner bracts, and receptacle) of heads in five globe artichoke cultivars of Campania region (Italy) and one accession of cultivated cardoon was performed. Data obtained suggest that the edible parts (receptacles with inner and intermediate bracts) of these cultivars of artichoke could represent a good source of health-promoting polyphenols and therefore encourage a nutriceutical use of this species, as an alternative to the more traditional phytopharmaceutical applications of leaf extracts. Moreover, it was demonstrated that single polyphenols accumulate preferentially in specific parts of the heads and in specific genotypes.

Keywords: Antioxidants; Globe artichoke, Nutriceutics; Polyphenols

1. Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori), belonging to the family of Asteraceae (Compositae), is an herbaceous perennial crop, widely cultivated in the Mediterranean area (Bianco, 2005). The heads, i.e., the large immature inflorescences with edible fleshy leaves (bracts) and receptacle, are used worldwide and represent a fundamental ingredient of the Mediterranean diet. The leaves are an herbal medicine and have been recognised since ancient times for their beneficial and therapeutic effects, including promotion of blood circulation, mobilisation of energy reserves, induction of choleresis, inhibition of cholesterol biosynthesis and LDL oxidation, and significant antibacterial, antifungal and antioxidant as well as strong hepatoprotective effects (Adzet, Camarassa, & Laguna, 1987; Gebhardt, 1997; Llorach, Espin, Tomas-Barneran, & Ferreres, 2002; Martino et al., 1999; Thompson Coon & Hernst, 2003; Zhu, Zhang, & Lo, 2004, 2005). Many studies have focused on its health and antioxidant properties and it seems that these actions could be strictly related to the polyphenolic fraction, mainly composed of mono- and dicaffeoylquinic acids and flavonoids. These properties are consistent with the well-known dual role of phenolic compounds as antioxidants and as substrates for oxidative browning reactions, mainly in the presence of high iron concentrations (Lattanzio, Cardinali, Di Venere, Linsalata, & Palmieri, 1994). The chemical activities of polyphenols in terms of their reducing properties, as hydrogen or electron-donating agents, predict their potential effect as free-radical scavengers, (Brent & Rumack, 1993; Knight, 1995).

Globe artichoke is mainly grown in Italy, which accounts for 50% of the world production, followed by Spain and Argentina (FAO, 2005). In Italy, it represents

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the most important horticultural crop after tomato and potato, with a production of about 500 000 tons in the year 2005, mainly from Apulia, Sicily, Campania, Lazio and Tuscany (ISTAT, 2005). Italy is also the richest source of artichoke germplasm, with numerous commercial and local varieties adapted to different environments, which can differ in chemical composition, especially of the polyphenolic fraction, and hence exhibit different nutraceutical and pharmacological properties. Cultivated varieties are mainly classified as early, starting their production in autumn, and late varieties, whose production is dependent on photo- and thermo-periods and starts in late winter or early spring.

The aim of our study was to analyse the polyphenols of leaves and different parts (outer, intermediate and inner bracts, and receptacle) of heads in five cultivars of artichoke and one of cultivated cardoon. The artichokes studied were "Violet de Provence" (an early variety with violet-coloured heads, growing at 400–600 m altitude in Provence), C3 (a commercial late variety belonging to the Castellammare group, typical of the Campania region), and three local varieties from Salerno province in Campania, namely, "Bianco di Pertosa" (a cold-resistant and late variety with light green heads, grown in the Cilento National Park), "Tondo di Paestum" (a late variety grown in the Sele river plain with green, violet-shaded heads, also belonging to the Castellamare group), and "Carciofo di Aquara" (a late variety, with ovate, violet-shaded heads).

2. Materials and methods

2.1. Standards and reagents

Cynarin was purchased from Carl Roth Karlsruhe (Germany); apigenin and luteolin were obtained from Extrasynthese (Genay, France). All other standards were obtained from Sigma Chemicals (St. Louis, MO). Acetonitrile and trifluoroacetic acid were obtained from Carlo Erba Reagenti (Milan, Italy). Acetone, methanol, ethanol and ethyl acetate were purchased from Sigma Chemicals. All reagents were of analytical grade.

2.2. Plant material

Five varieties of globe artichoke, Bianco di Pertosa, Carciofo di Aquara, Tondo di Paestum, C3, Violet de Provence, and one accession of cultivated cardoon, were field grown at the Experimental Institute for Vegetable Crops (ISPORT) of the Research Council for Agriculture (CRA) in Pontecagnano (Salerno, Italy) in 2004 under standard growth conditions and management practices. Young leaves and heads at commercial maturity were collected from at least 3 different plants for each variety.

Outer, intermediate and inner bracts of the heads, receptacles and leaves were separated and immediately frozen at −20 °C until extraction and analyses.

2.3. Extraction procedure

The extraction was performed following the method of Fratianni, De Palma, Tucci, and Nazzaro (2007). Briefly, frozen artichoke samples were incubated for 1 h at 4 °C in 5 volumes of acetone: ethanol: methanol (70:15:15). The supernatant was collected and the residue was re-suspended in 5 volumes of a second extraction mixture (ethyl acetate) and incubated at 4 °C for 1 h. The two supernatants were separately concentrated to dryness and re-suspended in methyl alcohol before being pooled together and subjected to polyphenol characterisation.

2.4. Colorimetric analysis of total phenolics

Total phenols were estimated using the Folin–Ciocalteu method (Singleton & Rossi, 1965). To 800 μl of deionised water, 50 μl of Folin–Ciocalteu's phenol reagent and a sample volume ranging from 10 to 50 μl were added and accurately mixed. After 1 min and before 8 min, 100 μl of 20% sodium carbonate solution was added and mixed. This was recorded as time zero. Deionised water was added up to a volume of 1 ml exactly. The solution was thoroughly mixed and total phenols were spectrophotometrically estimated (DU-Beckman, USA) at 760 nm after 2 h incubation. All tests were conducted in triplicate and averaged. Differences within triplicates were always <5%. Quantification was based on the standard curve generated with quercetin.

2.5. Preparation of standards for HPLC analysis

All standards utilised in the experiments were accurately weighed, dissolved in methanol and sonicated for 10 min. The calibration curves were generated with concentrations ranging from 0.01 to 0.5 mM of chlorogenic acid, cumaric acid, ferulic acid, apigenin, luteolin and cynarin.

2.6. Quantitative determination of polyphenols in artichoke by HPLC

The chromatographic determination of polyphenols was performed by RP-HPLC (Fratianni et al., 2007), using a Gold System chromatograph equipped with an UV detector (Beckman, CA, USA). A Khromasil KR 100-5 C18 column (25 cm × 4.6 mm) at room temperature was used for this analysis. The mobile phase included HPLC-grade water (containing 0.1% trifluoroacetic acid; solvent A) and 95 % acetonitrile (containing 0.1% trifluoroacetic acid; solvent B) in the following gradient system: initial 10% B, linear gradient to 50% B in 30 min, linear gradient to 100% B in 5 min, hold at 100% B for 2 min, decreasing gradient to 10% B in 2 min and coming back to the initial steps 10% B for 10 min. The total pre-running and post-running time was 54 min. The flow rate was 1 ml/min, the injection volume was 20 μl, and the detection wavelength was set at 280 nm.

Table 1
Total polyphenolic content in different parts of the heads and in the leaves of five artichoke and one cardoon varieties

Varieties	Receptacle	Inner bracts	Intermediate bracts	Outer bracts	Leaves
Cardoon	0.11 (±0.01)	1.58 (±0.10)	1.02 (±0.01)	0.81 (±0.03)	–
Aquara	3.09 (±0.09)	7.61 (±0.07)	1.54 (±0.01)	0.47 (±0.01)	0.69 (±0.14)
Bianco di Pertosa	1.36 (±0.03)	2.35 (±0.04)	0.61 (±0.02)	0.51 (±0.06)	0.52 (±0.01)
Violet de Provence	1.32 (±0.02)	2.33 (±0.01)	0.50 (±0.04)	0.58 (±0.07)	0.68 (±0.01)
Tondo di Paestum	3.09 (±0.07)	2.95 (±0.04)	0.98 (±0.07)	0.54 (±0.02)	0.60 (±0.1)
C3	1.98 (±0.09)	8.03 (±0.14)	1.74 (±0.01)	1.79 (±0.01)	2.30 (±0.01)

Data are mean values of three determinations and are expressed as mM/g of fresh product. The analysis was not performed on cardoon leaves.

3. Results

The total phenol content of heads and leaves of five artichoke varieties and one accession of cultivated cardoon is shown in Table 1. The analysis revealed that total polyphenols in heads were higher than in leaves. The lowest amount of phenols was generally found in leaves, and in outer and intermediate bracts, with values ranging from 0.47 mM/g (outer bracts of Aquara) to 2.3 mM/g (leaves of C3). Previous findings also indicated that the heads of globe artichoke varieties Violetto di Toscana have a higher polyphenolic content than the leaves (Romani, Pinelli, Cantini, Cimato, & Heimler, 2006). Receptacles and inner bracts exhibited the highest phenolic concentrations, with values up to 3.09 mM/g for receptacles of Tondo di Paestum and up to 8.03 mM/g for inner bracts of C3.

Generally, the cardoon accession exhibited the lowest phenolic content, while the highest were recorded in C3, Aquara and Tondo di Paestum. These results indicate that artichoke heads could represent an important source of polyphenols with therapeutic activity (Kraft, 1997; Llorach et al., 2002), in addition to leaves, which are widely used for phytopharmaceutical applications (Gebhardt, 1997). Similar conclusions were drawn by Romani et al. (2006), who found that heads of the variety Violetto di Toscana, typical of the Tuscany region, are very rich in phenolic compounds and hence can be regarded as a functional food. Thus, a wider utilisation of the typical Campania varieties Aquara and Tondo di Paestum as well as of the commercial variety C3 should be strongly encouraged both for the fresh market and for the food industry, in view of their ability to supply a high level of these important biomolecules.

4. HPLC analysis

The qualitative and quantitative analysis of some polyphenols in different parts of the heads of the analysed genotypes is reported in Fig. 1 (panels 1–6). We analysed some hydroxycinnamic acids such as chlorogenic, *p*-coumaric and ferulic acid, as well as cynarin (1,3-dicaffeoylquinic acid); the content of the flavonoids apigenin and luteolin was also investigated. On the whole, hydroxycinnamic acids were found to be well represented, mainly in receptacles and inner bracts, with less in intermediate and outer bracts. In the case of chlorogenic acid, the only exception to this general trend was the variety Aquara, while C3 showed a very low variation among the different parts of the heads. A significant amount of chlorogenic acid was found in the inner bracts of Tondo di Paestum, Bianco di Pertosa and Violet de Provence (8.14, 6.79 and 5.5 μM/g, respectively; Fig. 1, panel 1). This result is very important since *in vivo* studies have demonstrated the antioxidant and anticarcinogenic properties of chlorogenic acid (Gonthier, Verny, Besson, Rémésy, & Scalbert, 2003), which is usually poorly absorbed in the small intestine, but capable of providing higher yields of microbial metabolites, active compounds responsible for the biological properties attributed to dietary polyphenols (Gonthier et al., 2003; Kim et al., 1998; Rechner et al., 2002). The high level of chlorogenic acid found in artichoke could be explained by its central role as a substrate for many biochemical reactions producing several phenolic acids (Wittemer et al., 2005), which are highly represented in artichoke. Similarly to chlorogenic acid, coumaric acid accumulated increasingly from receptacles to inner bracts, to decrease towards the outer bracts in most genotypes, with the varieties Tondo di Paestum, Bianco di Pertosa and Violet de Provence showing the highest content of this hydroxycinnamic acid (7.92, 6.87 and 6.12 μM/g respectively; Fig. 1, panel 2). This is very significant, since coumaric acid has received much attention in the last years for its anti-inflammatory in vivo properties (Luceri et al., 2004). As for chlorogenic acid, cultivar C3 showed a very low and constant level of coumaric acid. Ferulic acid (Fig. 1, panel 3), was demonstrated to decrease from receptacles to outer bracts in the varieties Violet de Provence (from 13.66 to 2.43 μM/g, respectively) and Aquara (with values decreasing from 4.11 to 1.05 μM/g, respectively) and in cultivated cardoon (from 8.17 to 1.26 μM/g, respectively). Also in this case, the lowest values were observed for C3 (with quantities ranging from 1.58 μM/g in receptacles to 0.31 μM/g in outer bracts). The content of cynarin, a polyphenolic compound typical of artichoke and important for its choleretic activity (Gebhardt, 1997; Gebhardt & Beck, 1996), was found not to vary significantly in the different parts of the head, except for cultivated cardoon, which showed a high cynarin content in the receptacle (2.5 μM/g) and inner bracts (1.98 μM/g), with a decreasing trend towards the outer bracts (Fig. 1, panel 4).

Flavonoids luteolin and apigenin have diverse pharmacological activities and were suggested to be the bioactive components of different plants (Perez-Garcia, Adzet, &

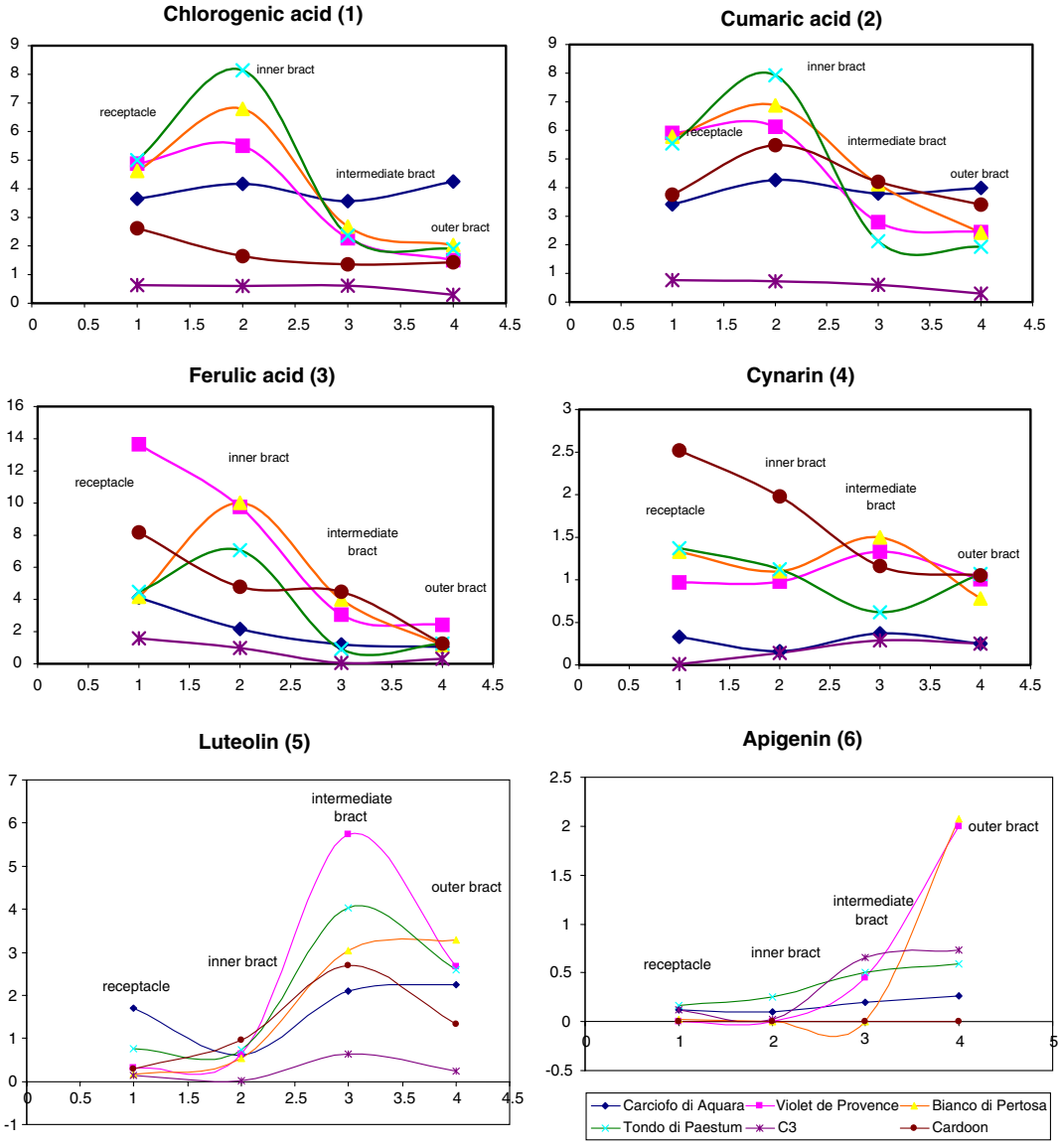

Fig. 1. Quantitative analysis of the major polyphenols in different parts (receptacle and inner, intermediate and outer bracts) of the heads of five artichoke and one cardoon varieties. Values represent the mean of three different analyses of three independent samples.Panel 1: chlorogenic acid; panel 2: coumaric acid; panel 3: ferulic acid; panel 4: cynarin; panel 5: luteolin; panel 6: apigenin. The legend in panel 6 is common to all panels.

Canigueral, 2000). Luteolin is probably synthesized from naringenin, through two main metabolic ways, having as intermediate metabolites apigenin or eriodictiol (see Metabolic pathway of flavonoids, in KEGGS PATHWAY DATABASE http://www.genome.jp/kegg/pathway.html). When the luteolin content was analysed in artichoke heads, it was found to be low in receptacles and inner bracts (ranging from 0.02 to 1.7 μM/g), while the highest levels were observed in intermediate bracts, with the varieties Violet de Provence, Tondo di Paestum and Bianco di Pertosa showing the highest concentration of the flavonoid (5.74, 4.03 and 3.03 μM/g respectively; Fig. 1, panel 5). The presence of luteolin is crucial for the therapeutic effects of artichoke, since it inhibits de novo cholesterol biosynthesis by up to 60% at a concentration of 30 μM and up to 80% at a higher luteolin concentration (Gebhardt & Beck, 1996; Kraft, 1997). The quantitative analysis of apigenin showed a constantly low level for receptacles and inner bracts in all artichoke varieties, which sharply increased in the outer bracts of Bianco di Pertosa (2.07 μM/g) and Violet de Provence (2 μM/g). Cardoon showed an almost zero level of apigenin in all the examined parts of the heads (Fig. 1, panel 6). Leaves, showed an extremely low content of all the analysed hydroxycinnamic acids and flavonoids in all genotypes (data not shown), in contrast to other results reported in the literature, which indicated a higher content of both total phenolics and purified single phenolic compounds in the leaves than in the heads of three different varieties (Wang et al., 2003). This discrepancy might be explained with a different leaf age, since we used very young leaves while the cited authors analysed mature, fully expanded leaves. An effect of the different varieties used in

the two studies cannot be ruled out, although it seems unlikely, since both Wang et al. (2003) and the present study examined several varieties and each set of varieties showed the same, contrasting trend of polyphenol accumulation.

Taken together, our data suggest that the edible parts of artichoke (receptacles with inner and intermediate bracts) are a good source of health-promoting polyphenols and therefore encourage a nutriceutical use of these species, as an alternative to the more traditional phytopharmaceutical applications of leaf extracts. Moreover, we have demonstrated that single polyphenols accumulate preferentially in specific parts of the heads and in specific genotypes. Therefore, conservation of the genetic variability found in the artichoke germplasm as well as a wider cultivation of local varieties seem extremely desirable (Alamanni & Cossu, 2003; Romani et al., 2006), since they represent an invaluable source of specific bioactive compounds, both for their use in nutraceutical and pharmacological applications and for breeding new varieties with a higher quality in terms of beneficial and therapeutic effects.

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References

Adzet, T., Camarassa, J., & Laguna, C. J. (1987). Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl_4 toxicity in isolated rat hepatocytes. *Journal of Natural Products, 50*, 612–617.

Alamanni, M. C., & Cossu, M. (2003). Antioxidant activity of the extracts of the edible part of artichoke (*Cynara scolymus* L.) var. spinoso sardo. *Italian Journal of Food Science, 15*, 187–195.

Bianco, V. V. (2005). Present situation and future potential of artichoke in the Mediterranean basin. *Acta Horticulturae, 681*, 39–55.

Brent, J. A., & Rumack, B. H. (1993). Role of free radicals in toxic hepatic injury. I. Free radical biochemistry. *Journal of Toxicology, Clinical Toxicology, 31*, 139–171.

FAO Statistical Database, (2005): http://faostat.fao.org/.

Fratianni, F., De Palma, M., Tucci, M., Nazzaro, F., (2007). Analisi del profilo polifenolico di varietà locali di carciofo (*Cynara scolymus*). In (Chiriotti eds) Ricerca e Innovazione nell'industria alimentare Italiana, Vol VII, pp. 652–656, Pinerolo, Italy.

Gebhardt, R. (1997). Antioxidative and protective properties of extracts from leaves of the artichoke against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicology and Applied Pharmacology, 144*, 279–286.

Gebhardt, R., & Beck, H. (1996). Differential effects of garlic-derived organosulfides on cholesterol byosinthesis in primary rat hepatocyte cultures. *Lipids, 31*, 1269–1276.

Gonthier, M. P., Verny, M. A., Besson, C., Rémésy, C., & Scalbert, A. (2003). Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *Journal of Nutrition, 133*, 1853–1859.

Kim, D. H., Jung, E. A., Sohng, I. S., Han, J. A., Kim, T. H., & Han, M. J. (1998). Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. *Archives of Pharmacology Researches, 21*, 17–23.

Knight, J. A. (1995). Diseases related to oxygen-derived free radicals. *Annals of Clinical and Laboratory Science, 25*, 111–121.

Kraft, K. (1997). Artichoke leaf extracts. Recent findings reflecting effects on lipids metabolism, liver and gastrointestinal tracts. *Phytomedicine, 4*, 369–378.

ISTAT, (2005). <http://www.istat.it>.

Lattanzio, V., Cardinali, A., Di Venere, D., Linsalata, V., & Palmieri, S. (1994). Browning phenomena in stored artichoke (*Cynara scolymus* L.) heads: enzymic or chemical reaction? *Food Chemistry, 50*, 1–7.

Llorach, R., Espin, J. C., Tomas-Barneran, F. A., & Ferreres, F. (2002). Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *Journal of Agricultural and Food Chemistry, 50*, 3458–3464.

Luceri, C., Guglielmi, F., Lodovici, M., Giannini, L., Messerini, L., & Dolara, P. (2004). Plant phenolic 4-coumaric acid protects against intestinal inflammation in rats. *Scandinavian Journal of Gastroenterology, 39*, 1128–1133.

Martino, V., Caffini, N., Philippson, J. D., Lappa, A., Tchernitchin, A., Ferraro, G., et al. (1999). Identification and characterization of antimicrobial components in leaf extracts of globe artichoke (*Cynara scolymus* L.). *Acta Horticulturae, 501*, 111–114.

Perez-Garcia, F., Adzet, T., & Canigueral, S. (2000). Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. *Free Radicals Research, 33*, 661–665.

Rechner, A. R., Kuhnle, G., Bremner, P., Hubbard, G. P., Moore, K. P., & Rice-Evans, C. A. (2002). The metabolic fate of dietary polyphenols in humans. *Free Radicals Biology and Medicine, 33*, 220–235.

Romani, A., Pinelli, P., Cantini, C., Cimato, C., & Heimler, D. (2006). Characterization of Violetto di Toscana, a typical Italian variety of artichoke (*Cynara scolymus* L.). *Food Chemistry, 95*, 221–225.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolibdic phosphotungstic acid reagent. *American Journal of Enology and Viticulture, 16*, 144–158.

Thompson Coon, J. S., & Hernst, E. (2003). Herbs for serum cholesterol reduction: a systematic view. *Journal of Family Practise, 52*, 468–478.

Wang, M., Simon, J. E., Aviles, I. F., He, K., Zheng, Q. Y., & Tadmor, Y. (2003). Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *Journal of Agricultural and Food Chemistry, 29*, 601–608.

Wittemer, S. M., Ploch, M., Windeck, T., Muller, S. C., Drewelow, B., Derendorf, H., et al. (2005). Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of Artichoke leaf extracts in humans. *Phytomedicine, 12*, 28–38.

Zhu, X. F., Zhang, H. X., & Lo, R. (2004). Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *Journal of Agricultural and Food Chemistry, 52*, 7272–7278.

Zhu, X. F., Zhang, H. X., & Lo, R. (2005). Antifungal activity of *Cynara scolymus* L. extracts. *Fitoterapia, 76*, 108–111.