A Comparative Evaluation of Chlorogenic Acid Content and Antioxidant Activity in Artichoke (*Cynara scolymus* L.) Samples Under Different Storage Conditions

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Summary: Artichoke has been used for a long time in the traditional medicine against dyspeptic complaints and as a liver protector due to its choleretic and cholagogue effects. Chlorogenic acid, the main ingredient of artichoke is known to show hepatoprotector, anticancer, anti-obesity and antioxidant activities. In this study, antioxidant activity and chlorogenic acid quantification were performed using the samples of artichoke as fresh, boiled, frozen, boiled frozen, canned, fresh leaf and dry leaf. Potential antioxidant activity was evaluated using DPPH assay. Flavonoid and phenolic contents were determined by Aluminum chloride colorimetry and Folin-Ciocalteu methods. The amounts of colorogenic acid in the samples were determined by HPLC-DAD system. The results have shown that highest chlorogenic acid content was determined in the 10-minute frozen-boiled sample (0.01441 mg/100g), followed by canned (0.01043 mg/100g), 20-minute cooked artichoke (0.001912 mg/100g), fresh artichoke (0.001519 mg/100g) and frozen artichoke (0.0004 mg/100g). The antioxidant activity results have revealed that highest % DPPH capture effect was observed in the cooked artichoke sample (ED_{50} =1.949 mg/ml). The total flavonoid content was equivalent to quercetin (43.859 mg QE/100g) whereas total phenolic content equalled to gallic acid (14.640 mg GAE/100g). The antioxidant activity and the amounts of colorogenic acid were found to be the highest in the cooked artichoke sample whereas the lowest antioxidant activity observed in canned artichoke. The results show that the heat treatment applied for storage can positively affect the amount of chlorogenic acid and antioxidant activity.

Key Words: Artichoke (*Cynara scolymus* L.), Chlorogenic acid, Antioxidant activity, HPLC.

Introduction

Artichoke (*Cynara scolymus* L.) is a herbaceous plant belonging to the family Asteraceae. It grows as a wild form in the Mediterranean Region, South America and Canary Islands, but cultivation is possible in many countries around the world [1]. The most common cultivated cultivars in Turkey are Sakız and Bayrampaşa. The Urla Sakız Artichoke, used in this study, has received geographical indication from 2018 onwards and it has been taken under protection [2]. It has been one of the most popular vegetables and medicinal plants in the Mediterranean since ancient Greek and Roman times. Artichokes are stored following their freezing for further use as a vegetable because its demand as well as production have increased to a large extent. This vegetable is regarded as a medicinal plant as well as a healthy food because of its rich phytochemical composition. Artichoke contains various proteins, minerals, fibers, sesquiterpenes (cynaropicrin, sinaratriol, cinnarcholosite A, B and C). It is rich in phenolic content [cynarine (1,3-di-O-caffeocinic acid), chlorogenic acid (5-O-caffeoylquinic acid), neochlorogenic acid (3-O-caffeoylquinic acid), kryptochlorogenic acid (4-O-caffeoylquinic acid), 1,3-O-dicaffeoylquinic acid, 1,4-O-dicaffeoylquinic acid, and 3,5-O-dicaffeoylquinic acid, luteolin, cynnarocide (luteolin-7-o-glucosid), skolmoside (luteolin7-o-rutinosid)] [3-5]. The infusions, extracts and standardized products prepared from this vegetable are used against liver and biliary diseases because of their choleretic, cholagogue and dyspeptic effects. Antihyperlipidemic [6], diuretic [7], antimicrobial [8], and antioxidant [9] effects have also been proven.

Chlorogenic acid (5-O-caffilic acid) is the ester of caffeic acid and quinic acid. It is a hydroxycinnamic acid derivative and one of the plant-derived phenolic substances showing important biological activities. It has particularly started attracting the attention because of anticancer, antispasmodic [10], antiobesity, antioxidant [11], activities, with positive effect on sugar metabolism as werll as an inhibitory effect on HIV-1 virus [12]. In the light of these beneficial uses chlorogenic acid is included in the supplements list of the Ministry of Food, Agriculture and Animal Husbandry of Republic of Turkey. The list mentions that daily

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optimum intake of chlorogenic acid for an adult should be 180 mg/day. Artichoke is one of the richest source of chlorogenic acid as a vegetable. The definition of chlorogenic acid in artichoke extracts dates back to 1950 [13].

This plant is accepted today as a delicious vegetable with several medicinal benefits. It is generally collected fresh and cooked in different forms. But due to short growing season the vegetable is stored in a frozen form, boiled and frozen form and canned form. In this study, chlorogenic acid quantification and antioxidant activity were performed using the samples of as fresh, boiled, frozen, boiled frozen, canned, fresh leaf and dry leaf. Samples were prepared from the Sakız artichoke grown in Urla-İzmir-Turkey and the chlorogenic acid amounts and antioxidant activities of the samples in different storage conditions were determined and compared.

Experimental

Materials

Sample Preparation

Artichoke samples were collected from Urla-Izmir, Turkey on 5 January 2018.

a-250 g fresh artichoke was used; b-250 g were cooked in 500 ml water for 20 min, until the water evaporates; c-250 g fresh artichoke was frozen; d- 250 g of canned artichoke were used. The samples from a,b, c and d were extracted for 8 hours with 750 ml MeOH: Water (7:3) solution system, all extracts were filtered and concentrated. The samples were also boiled in water for 10 min, filtered, 250 g were frozen and frozen sample was extracted again for 8 hours with the 750 ml MeOH: Water (7:3) solution system. 100 g of fresh and dried leaf samples were extracted with 400 ml MeOH: Water (7:3) solvent system for 8 hours. The extract was filtered and concentrated. All concentrated samples were diluted with 70% MeOH.

HPLC Analysis of Chlorogenic Acid

High-Pressure Liquid Chromatography was used for the determination of amount of chlorogenic acid in the samples. Analysis was performed by using Hewlett Packard 1100 series HPLC system. For the detection a diode array detector (DAD) was used, as the stationary phase, the hichrom column used was 5C18 (25 cm x 4.6 mm). Acetonitrile: Water (containing 0.4% phosphoric acid) (14:86) was applied to the isocratically conducted analysis as the mobile phase. The flow rate was adjusted as 1ml/min. Analysis was performed at room temperature. All samples were filtered through filters with a pore width of 0.45 µm and injected to the system in three replicates with 20µl injection volume.

The retention time of the chlorogenic acid was at the 11th minute. Standard chlorogenic acid (Aldrich) was dissolved in mobile phase to prepare 5 different concentrations (0.25, 0.5, 1, 2, 4 µg/ml). Different concentrations of standard solutions were injected into the HPLC column, the calibration curve was drawn according to the field values and the regression equation was calculated (Fig 1). The detection of chlorogenic acid in the samples was done using retention time and internal standard method. Chlorogenic acid contents of various artichoke samples was performed and the results were computed over sample quantities. For determining the amount of chlorogenic acid, calibration curve was used. For the method validation, performance parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ) and precision were determined. Linearity was defined by drawing a five point calibration curve. Based on the signal-to-noise ratio of S / N = 3/1, the measurement limit and based on the signal-to-noise ratio S / N = 10/1, the detection limit were calculated [14]. Repeatability and relative standard deviation percentages were determined to determine method accuracy. Three concentrations were selected from the calibration curve (0.25,1,4 µg/ml), and repeatability was tested by performing five repetitions on the same day and three repetitions on three different days. Descriptive statistical analysis (correlation coefficient, mean ± SD and% relative standard deviation) was calculated using Microsoft Office Excel.

Determination of Antioxidant Capacity

For this purpose 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical capture activity, phenolic and total flavonoid content analysis were performed.

DPPH Radical Scavenging Activity

Artichoke samples were diluted with MeOH to concentrations in the range of 0.5-1.25 mg / ml. 1 ml of solutions was added over 4 ml of methanolic DPPH (0.004%) solution and left in the dark for 30 min. The absorbance of 517 nm wavelength was measured and percentage DPPH inhibitions calculated using the standard calibration curve, prepared with α-tocopherol. The antioxidant capacity of the samples equivalent to α-tocopherol and their concentrations cleaving 50 % of the free radicals was calculated as ED_{50} [15]. Measurements were done by using Optima SP-3000 Nano Spectrophotometer.

Total Phenolic Content Assay

This paremeter was determined by Folin Ciocalteu method. 2.8 ml of deionized water and 2 ml of 2 percent sodium carbonate were added to the samples (1 mg/ml), these were incubated for 1 minute and 0.1 ml 50 percent Folin Ciocalteu reagent was added to it and left in darkness at 25° C for 30 min. The absorbance of the mixture was measured at 750 nm. Total amount of phenolic content in the samples was calculated as equivalent to gallic acid according to the standard curve prepared using gallic acid [16].

Total Flavonoid Assay

0.1 ml of 10 percent aluminum chloride solution, 1.5 ml of 96 percent ethanol and 2.8 ml of deionized water were added to 0.5 ml of the sample (1 mg/ml). They were left at room temperature in darkness for 40 min. Absorbances were measured at 415 nm. Total flavonoid content was calculated as quercetin equivalent according to standard calibration curve prepared with quercetin [17].

Statistical Evaluations

Analysis were conducted with 3 repetitions. Average value and standard deviations were calculated using Microsoft Office Excel program. ED_{50} values were determined by using Finney probit analysis method.

Results and Discussion

Artichoke is an important medicinal plant containing important ingredients such as chlorogenic acid which is a phenolic compound with high antioxidant activity that can be only obtained from natural plants and fruits. Kholorogenic acid is one of the main ingredients that give the antioxidant activity to the artichoke. Since artichoke can be found at a very short time of the year, it is stored in various ways. How the ingredients of artichokes are affected by various ways of servings and storage conditions is still a matter of curiosity. In our study, chlorogenic acid amounts of artichoke samples prepared under different storage conditions were investigated using HPLC method and validation parameters of the method were examined. For this purpose, linearity studies were performed between 0.25-4 µg / ml and regression equation defined. The limits of detection and quantitation were determined (Table-1).

Table-1: The linearity, the limit of detection and the limit of quantitation.

Standard substance	LOD µg/ml	LOQ µg/ml	Linearity	R^2
Chlorogenic acid	0.042±0.010	0.119±0.010	y=2086.7x+16.264	0.9986

LOD: The limit of detection; LOQ: The limit of quantitation; calibration equation y=ax+b; x=Concentration; y=The peak area

Repeatability applications were performed for an accuracy of the method and the relative standard deviation percentages defined. The results are given in Table-2.

Table-2: Intra-day and inter-day precision results.

Standard substance	Added amount (µg/ml)	Recovery Value Average±SD	%RSD
Chlorogenic acid intra-day (n=5)	0.5	94.2±0.20	4.14
	1	95.4±0.03	1.43
Chlorogenic acid inter-day (n=3)	0.5	95.1±0.04	1.40
	1	96.8±0.10	1.30

n=analysis count; SD: standard deviation; RSD: relative standard deviation

The calibration curve and the regression equation are given in Fig 1.

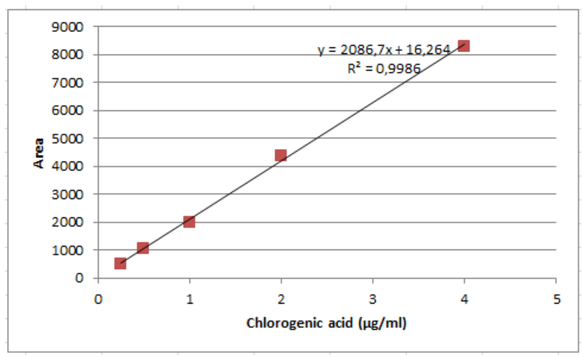

Fig. 1: The calibration curve and regression equation for the chlorogenic acid.

y=area; x=Chlorogenic acid concentration (µg/µl)

Artichokes are generally consumed fresh after being cooked. However, these are also stored for the follow up consumption. The storage methods vary, sometimes untreated fresh artichokes are stored in the freezer but, sometimes these are boiled and then frozen. In recent years there has been an increase in its production and canning industry has come to the forefront. The spectra of HPLC analysis performed to determine the amount of chlorogenic acid of samples prepared under different storage conditions are given in Fig 2-8 and the amounts of chlorogenic acid are given in Table-3.

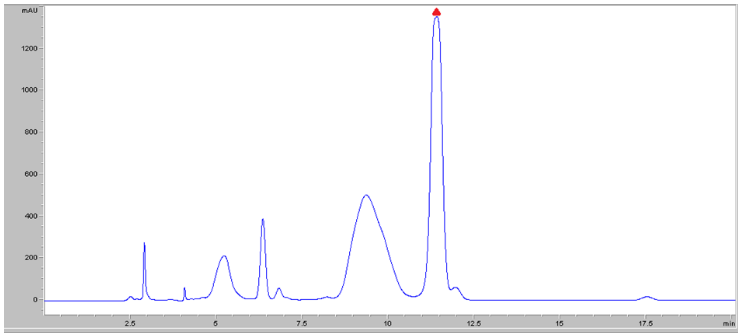

Fig 2 The HPLC spectrum of fresh artichoke.

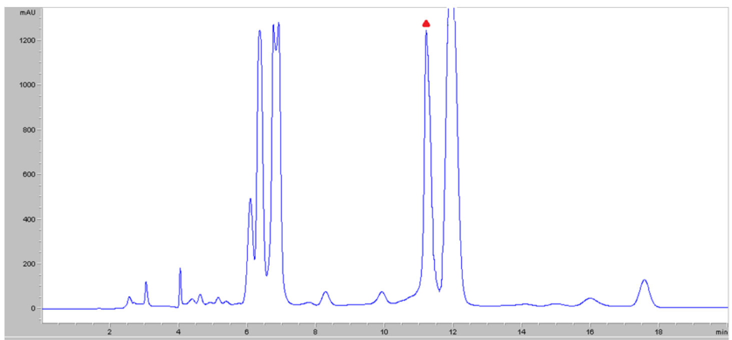

Fig 3 The HPLC spectrum of cooked artichoke.

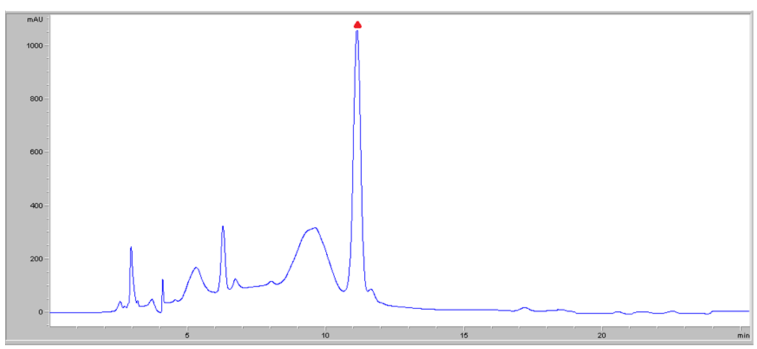

Fig 4 The HPLC spectrum of frozen artichoke.

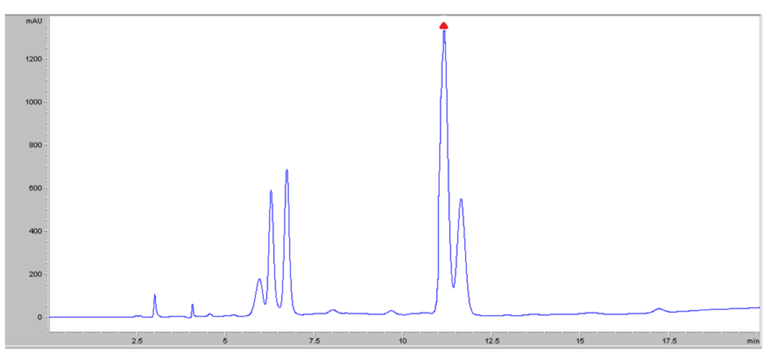

Fig 5 The HPLC spectrum of boiled-frozen artichoke.

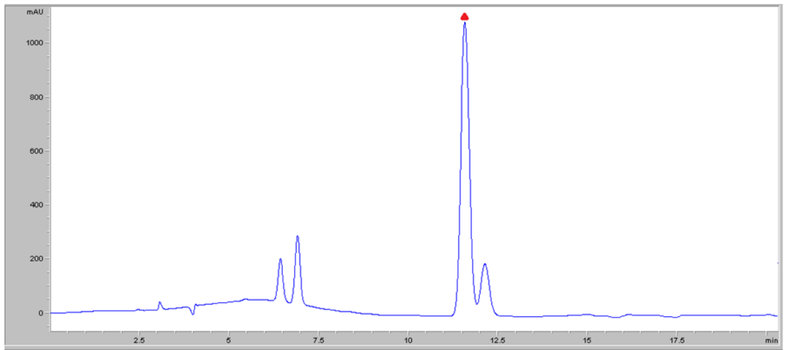

Fig 6 The HPLC spectrum of canned artichoke.

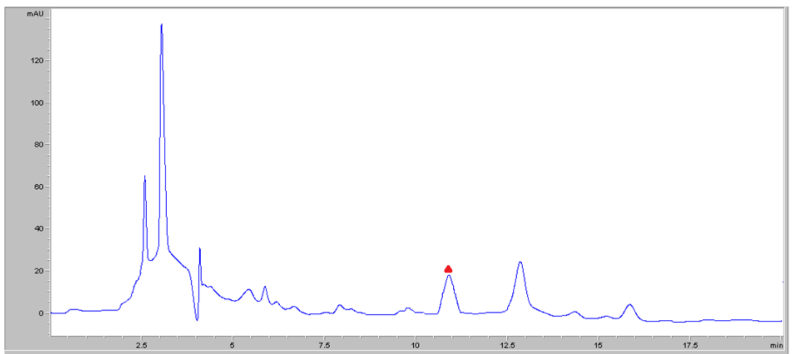

Fig 7 The HPLC spectrum of fresh leaves.

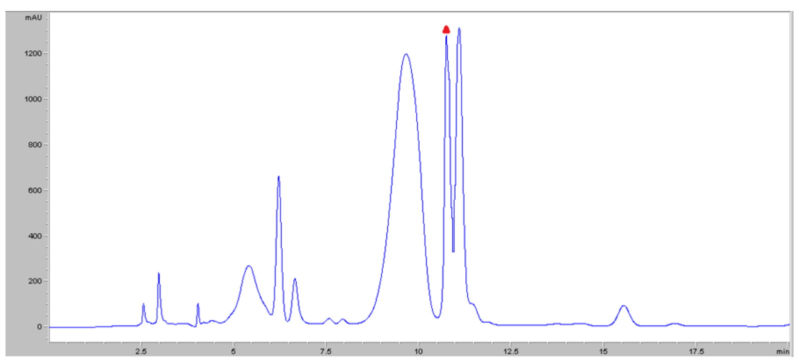

Fig 8 The HPLC spectrum of dry leaves.

Table-3: Chlorogenic acid amounts in the artichoke samples.

Sample	Chlorogenic acid mg/100g
Fresh artichoke	**0.001519±0.0031**
Cooked artichoke	**0.001912±0.0042**
Frozen artichoke	**0.000400±0.0025**
Boiled-frozen artichoke	**0.014410±0.0023**
Canned artichoke	**0.010430±0.0032**
Fresh leaves	**0.000241±0.0019**
Dry leaves	**0.004475±0.0012**

Values are expressed as means ± SE, n =3.

Generally cooked vegetables are subjected to thermal degradation and oxidation. As a result of this there are losses in the nutritional features and antioxidant activities of cooked vegetables [18]. Very few studies have investigated the variation of contents in the artichoke under different serving and storage conditions. All these studies give some conflicting results. Some studies have reported a decrease in the amount of chlorogenic acid in cooked artichoke [9,18] whereas, some have reported that the cooked artichoke samples contain higher amounts of chlorogenic acid than fresh samples [19,20]. Latter studies indicate that cooking process increases the chlorogenic acid content by 1.1 to 4 times. Our analysis results are in accordance with these findings. They show that the amount of chlorogenic acid in cooked samples is higher than fresh samples. The highest amount of chlorogenic acid was found in 10 minute boiled and frozen sample (0.01441 mg / 100g) followed by canned (0.01043 mg / 100g), 20 minute cooked artichoke (0.001912 mg / 100g), fresh artichoke (0.001519 mg / 100g) and frozen artichoke (0.0004 mg / 100g).

The amount of chlorogenic acid was found to be 9.5 times more in the 10-minute boiled and frozen sample than in the fresh sample. Our results have shown the amounts in 10-minute boiled and canned are higher. This increase in the amount of chlorogenic acid is thought to be due to thermal isomerization of dicaffeoylquinic acid derivatives and the release of phenolic acids increases due to the softening of cellular components of the plant cells due to boiling [19, 21]. Moreover, it is known that the solubility of phenolic substances increases with heat [22, 23].

In the case of leaf samples, the dry leaves have shown much higher chlorogenic acid content than fresh ones. This may be attributed to the fact that dry leaves loose their water as such the sample size is greater than the same amount of fresh leaves.

Artichoke is high antioxidant effect depending on rich phenolic content. In our study, it was investigated how antioxidant activity would be affected by different storage conditions. The antioxidant capacity of the samples was determined by analyzing 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical capture activity, total phenolic and total flavonoid content. The results of are given in Table 4.

Table-4: Results of antioxidant capacity together with total phenolic and flavonoid content analysis.

Sample (µg/ml)	DPPH ED_{50} (mg/ml)	Total flavonoid content mg QE/100g ±SD	Total phenolic content mg GAE/100g ±SD
Fresh artichoke	**11.9510**	**25.920±0,0020**	**9.0088±0,0015**
Cooked artichoke	**1.94920**	**43.859±0,0011**	**14.640±0,0013**
Frozen artichoke	**16.5247**	**18.227±0,0024**	**6.4696±0,0021**
Boiled and frozen artichoke	**12.3473**	**11.492±0,0037**	**6.4344±0,0034**
Canned artichoke	**20.0336**	**2.7340±0,0012**	**5.7472±0,0021**
Fresh leaves	**28.0336**	**2.5320±0,0014**	**7.2280±0,0012**
Dry leaves	**1.97100**	**269.36±0,0360**	**37.784±0,0038**

QE: Quercetin Equivalent; GAE: Gallic Acid Equivalent; SD: Standard Deviation.

The highest DPPH capture activity (ED_{50}=1.949 mg/ml), the total flavonoid content equivalent to quercetine (43.859 mg QE/100g) and the total phenolic content equivalent to gallic acid (14.640 mg GAE/100g) were observed in the cooked artichoke sample. The lowest DPPH capture activity (ED50=20.034 mg/ml), the total flavonoid content equivalent to quercetine (2.734 mg QE/100g) and the total phenolic content equivalent to gallic acid (5.747mg GAE/100g) were observed in the canned artichoke sample. Therefore, it can be concluded that canning adversely affect the antioxidant capacity of samples. Highest effects after cooked artichoke found in fresh sample. DPPH capture activity ED50=11.951mg/ml, the total flavonoid content equivalent to quercetine 25.920 mg QE/100g and the total phenolic content equivalent to gallic acid 9.009 mg GAE/100g were found. This is followed by frozen artichokes and boiled and frozen artichokes. Dry artichoke leaf samples showed very high activity compared to fresh ones and the other samples (DPPH capture activity ED50=1.971mg/ml, the total flavonoid content equivalent to quercetine 269.36 mg QE/100g and the total phenolic content equivalent to gallic acid 37.784 mg GAE/100g). This result is attributed to the fact that dry samples contain more material than the same amount of fresh samples. Antioxidant activity studies have mostly done on dry leaves, some studies have even stated that they could not achieve measurable results in fresh leaves. [24].

Some researchers working on the antioxidant activity of artichokes have reported that the total phenolic content varies between 4.55 and 23.38, and the DPPH capture activities between 5.20-69.91 [19, 25]. There are conflicting results related to the antioxidant activity just like chlorogenic acid quantification. Some researchers have argued that fresh samples show higher activity [26,27], but some say that heat treatment increases antioxidant activity [25,28]. Our results suggest that the components, responsible for antioxidant activity, are activated by heat. This result is likely due to the matrix softening effect and the increased extractability of the compounds with heat [29]. In addition, new derivatives may be formed as a result of hydrolia with the effect of heat.

Conclusions

In our case study highest amount of chlorogenic acid was detected in the samples heated to about 10 min and the highest antioxidant activities was determined in the sample heated upto 20 min. This shows that antioxidant activity as well as chlorogenic acid content of *Cynara scolymus* increase with the heat treatment.

These results show that some common storage conditions can increase the nutritional value of artichoke and facilitate bioavailability of health promoting ingredients.

Conflict of interest

The authors declare that there is no conflict of interest.

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