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Fluctuations in the Phenolic Content and Antioxidant Capacity of Nettle (Urtica dioica L.) Drink in Refrigerated Storage

Aziz Homayouni Rad[1], Mahboob Nemati[2], Zeinab Rahimzadeh Sani[1*], Hossein Servat[2], Farnaz Hajieghrari[1]

[1]Department of Food and Technology, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

[*]E-mail: Rahimzadehz@tbzmed.ac.ir

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Abstract

The aim of this study was to analyze the total phenolic content and antioxidant activity of refrigerated nettle drinks stored at 4°C for 3 months. The antioxidant properties determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays and total phenolic contents of the nettle drinks were evaluated using Folin-Ciocalteu method. In addition, correlations between antioxidant activity and total phenolic content were assessed. The results showed a significant increase for the refrigerated nettle drinks ($p<0.05$) in the case of total phenolic content and antioxidant activity in two months. It was concluded that the nettle drink should be treated as a short shelf-life product (2-month).

Keywords: Urtica dioica, nettle drink, antioxidant activity, total phenol.

Introduction

Phenolic compounds are naturally occurring substances in fruit, vegetables, nuts, seeds, flowers, and some herb beverages and are an integral part of the human diet. Phenolics are one of the major dietary components groups that have been associated with atherosclerosis and cancer inhibition (Heim et al., 2002; Teissedre et al.,1996). Flavonoids have powerful antioxidant properties that are related to phenolic hydroxyl group presence in the flavonoid structure. Free radical scavenging plays a important role in the antioxidant activities of such compounds; therefore, leading to the potential use of flavonoid-based drugs for the prevention and therapy of free radical mediated disorders, such as atherosclerosis, ischemia, neuronal degeneration, cardiovascular diseases, and other human burgeoning diseases (Rice-Evans et al., 2001; Pahari et al., 2012).

Polyphenols possess antioxidant activity mostly due to their redox properties. These combinations perform significantly as free radical terminators, hydrogen donors, and metal chelators (Yingming et al., 2007).

Several studies have shown that the antioxidant activities of some fruits and vegetables were highly correlated with their total phenolic (TP) content. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions

(Velioglu et al., 1998; Emmons et al., 1999; Tohma et al., 2010; Katalinic et al., 2013).

Recently, extensive interests have been focused on the addition of polyphenols to functional foods and biological systems, owing to their well-known abilities to scavenge free radicals (that is, antioxidant power). Free radicals generation have major impact on the development of pathological disturbance severity such as atherosclerosis (Steinberg et al., 1988), brain disfunction (Gordon et al., 1996), and cancer (Ames et al., 1983).

Nettle (Urtica dioica L.), an annual and perennial herb, has already been known and therefore consumed for a long time as a medicinal plant. Nettle's isolated major flavonoid glycosides have been determined to be immune stimulatory, anticarcinogenic, anti-inflammatory, anti-oxidant and antiallergenic activities (Yener et al., 2009). The aim of this study was to investigate the relation among the three nettle drinks (ND) antioxidant activity and their TP contents.

Materials and Methods

Plant material and chemicals

Aerial parts of nettle (U. dioica) were collected from Marand city (East Azerbaijan province), Iran in August 2014. A voucher specimen of this plant was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University, Iran. The collected samples were dried and ground to give 40-mesh size powder and stored in the refrigerator (4°C) until they were used in extraction. All chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

Solvent extraction

The polyphenolic compounds were extracted from the dried aerial parts of nettle (20 g) using alcoholic solvent (ethanol/water 80/20, v/v; 100 ml) at 60°C, and contact time was 60 min (Katalini´c et al., 2010). The extract was filtered using Whatman No. 42 filter paper for removal of plant particles and the raw extracted materials were evaporated and solvent was removed by evaporation in the vacuum at 40°C condition and the extracts were stored in a refrigerator (Kamkar et al., 2010).

Nettle soft drink preparation

The filtered-extracted nettle was used in drink formulation according to the recommended formulation standard method. Generally, drink formulation process includes ingredients mixing, packaging, pasteurizing, cooling and storing. To prepare the samples, the ratio of sugar and essence remained unchanged and the ratio of water present in the formulation for rating to 1, 2 and 3% were replaced by the extraction. Treatments were pasteurized in the bath (Memmert Company WNB-10) at 70°C for 20 min; thereafter, they were transferred to 250 ml polyethylene terephthalate (PET) bottles and stored in refrigerator for three months before the analysis procedure.

Determination of TP content

Concentration of the phenolic compounds in the extractions was determined according to the Folin-Ciocalteu (FC) method (Singleton et al., 1999; Singh et al., 2002). Also, TP content was measured using calibration curve with the gallic acid equivalent standards. NDs were dissolved in a mixture of methanol and water (2:1 v/v). The samples (50 µl) were mixed with 250 µl of FC reactive and 750 µl 7% Na2CO3 solution. The mixture was stored for 30 min at room temperature after the absorbance was measured using spectrophotometer at 765 nm wavelength (UV-1800 SHIMADZU made in Japans). Methanol solution (80%)was added to the extraction to achieve blank solution instead of 50 µl extraction. Estimation of the phenolic compounds in the NDs was carried out in triplicate, and thereafter, the average of the derived results was used in the examination.

Antioxidant activity assay

Radical-scavenging activity was determined by using stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Koleva et al., 2002). This spectrophotometric assay is based on the reaction between the free DPPH radical and molecules that can donate hydrogen atoms (such as, most antioxidants) (Burits et al., 2000; Szabo et al., 2010). Different concentrations (50 µl) of the studied samples were added to methanolic solution of DPPH (0.15 mM). Pure methanol was used as blank solution and as a control solution, 100 µl of pure water was used instead of 100 µl of the extraction. The absorbance was read against a blank solution at 517 nm wavelength after 30 min incubation at room temperature

condition. The sample extraction's antioxidant capacity was measured using calibration curve prepared with different concentrations (10 to 100 ppm) of gallic acid solution. The percentage inhibition of the DPPH radical (% Inh DPPH) of the free radical DPPH was calculated in percentage using the following equation:

$$\% \text{ Inh DPPH} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A0 stands for the control reaction absorbance and A1 shows the absorbance in the presence of the NDs sample (Duh et al., 1999).

Statistical analysis

In all cases, analyses were performed in triplicate, unless specified elsewhere. Data were presented as mean values ± standard deviation. Experimental data were analyzed using analysis of variance (ANOVA) and differences among means from triplicate analyses at $p < 0.05$ were determined through Duncan's multiple range test (DMRT) using the Statistical Analysis System (SAS).

Results and Discussion

Phenolic content of the NDs

Figure 1 shows the TP values for all of the studied NDs during the 3 months storage at 4°C temperature. Manipulating the regression equation of gallic acid calibration curve ($y = 0.0101x - 0.02365$, $R^2 = 0.9997$), the TP content of each ND was calculated and expressed as gallic acid equivalent (GAE) to facilitate the comparison (Jerez et al., 2004). On the first day of the measurement, TP values ranged from 6.02±0.009 mg/L GAE (drink containing 1% nettle extraction) to 9.06±0.037 mg GAE/10 ml (drink containing 3% nettle extraction).

During the 3 months storage at 4°C, fluctuations were observed in the total phenol content for all studied NDs as shown in Figure 1. In the second month, a significant increase was observed ($p<0.05$) in the TP content for all the samples. Kevers et al. (2007) reported increase in the phenolic content of leek and asparagus in the first days of refrigerated storage. Piljac-Zegarac et al. (2009) also published similar results in the case of dark fruit juices.

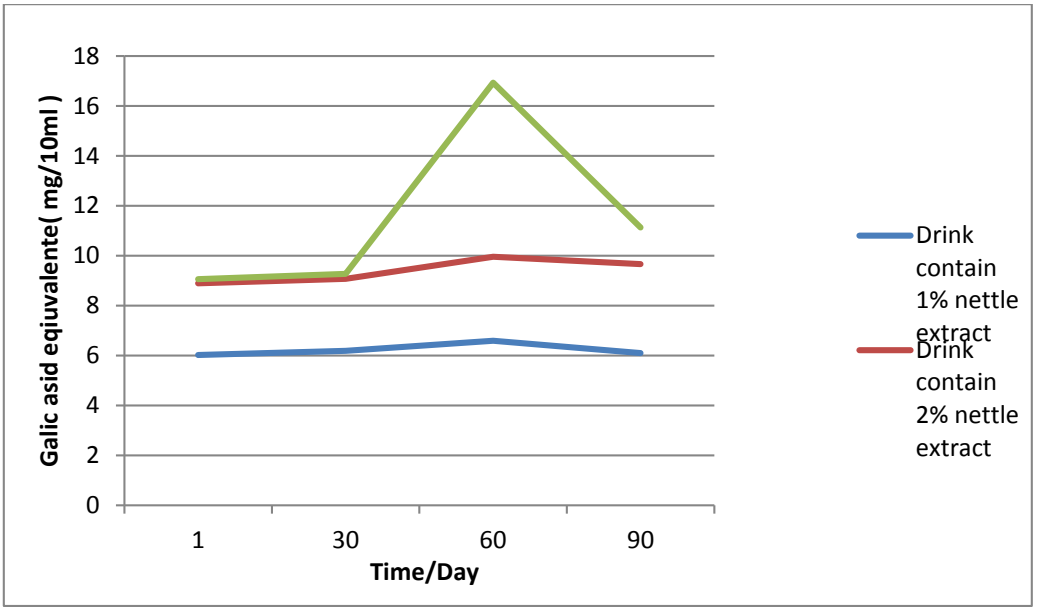

Figure 1: Total phenol content of nettle drinks obtained by Folin–Ciocalteu method, as a function of time.

The initial increase in the phenol content of the samples was associated with a decrease in TP values over the course of next month. The lowest TP values for all of the studied NDs were recorded after 2-month while they were stored in refrigerated condition. However, at the end of the 3-month storage, the final TP values were higher than the initial values for all the NDs. Klimczak et al. (2007) reported that TP content and antioxidant activity of orange juices decreased after 4 months of storage, followed by a significant increase at the end of the storage (6 months). During juice storage, some compounds were formed which reacted with the FC reagent and significantly enhanced the phenolic content. Our results suggested that 3-month refrigerated storage does not lower the amount of phenolic compounds which is in good agreement with the results of Kevers et al. (2007) in stability. Van der Sluis et al. (2005) reported that one month storage of apple juice in a refrigerator or even at room temperature will not decrease the concentration of polyphenolic antioxidants.

Influence of refrigerated storage on antioxidant capacity of NDs

The DPPH radical scavenging capacity in the NDs is as shown in Figure 2. The initial radical scavenging capacities of drinks ranged from 68.24±0.125 (drink with 1% nettle extraction) to 74.91±0.035 (drink with 3% nettle extraction). After the first day in refrigerated storage, the studied NDs revealed an increase in the DPPH radical scavenging capacity until the end of the second month. The observed increase was significant for the all the studied NDs ($p<0.05$).

In the third month of storage, a decrease in the DPPH radical scavenging capacity was observed in all the drink samples. However, at the end of 3-month storage, the DPPH radical scavenging capacities were higher than the initial DPPH radical scavenging capacities for all the NDs.

Increase in the antioxidant activity during storage has been previously reported for the refrigerated celery (Vina et al., 2006) and during the first hours of incubation of apple juice at the elevated temperatures (Van der Sluis et al., 2005) and industrial dark fruit juices during 29-day refrigerated storage (Piljac-Žegarac et al., 2009). Pinelo et al. (2005) also reported an increase in the DPPH antiradical activity of grape extractions during the first days of storage at 22, 37 and 60°C condition. According to Pinelo et al. (2004), increase in the activity may be explained by the strong tendency of polyphenols to undergo polymerization reactions, whereby the resulted oligomers possess larger areas available for charge delocalization.

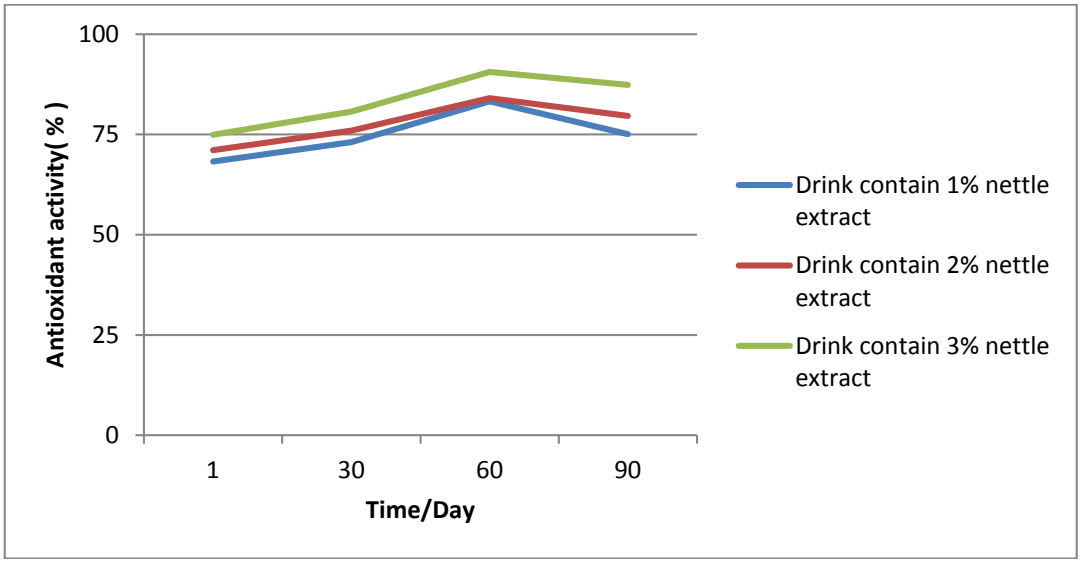

Figure 2: Antioxidant capacity of nettle drinks obtained by the DPPH radical scavenging assay, as a function of time

When the degree of polymerization was violated from a critical value, the increased molecular complexity and steric hindrance reduced the availability of hydroxyl groups in reaction with DPPH radicals which caused a resultant decrease in the antiradical capacity. This may be explained by the observed decrease in the antiradical activity of the NDs in this study. This followed after the initial transient increase. Antioxidant activity of spinach, broccoli and leek also decreased more than 50% after 30 days in refrigerated storage (Kevers et al., 2007).

Correlation between TP content and antioxidant activity of NDs

In the present study, a significant linear correlation was observed between TP content and antioxidant activity against DPPH ($r2=0.547$, $p<0.05$). Many authors have reported that TP content is strongly linear correlated with antioxidant activity (Meda et al., 2005; Buratti et al., 2007; Socha et al., 2009). Cheung et al. (2003) found a correlation between higher antioxidant activity and large amount of TPs in mushroom extractions. Similar results were also found in nocino liqueur studies (Alamprese et al., 2005). Our results showed that the contents of TP in the NDs can demonstrate its antioxidant activity. Amakura et al. (2000) observed no correlation between TP and radical scavenging activity in berry species. Piljac- Zegaracet al. (2009) also observed no correlation between TP and radical scavenging activity in dark fruit juices.

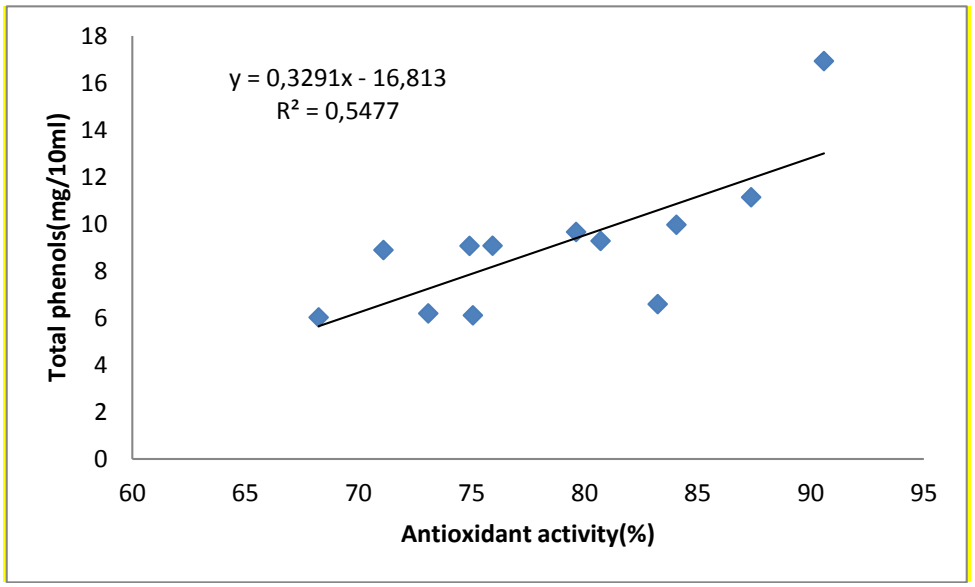

Figure 3: Correlation between total phenol and antioxidant activity

Conclusions

In this study, all the analyzed NDs showed substantial fluctuations in the TP content and antioxidant capacity during 3-month refrigerated storage. At the end of the refrigerated storage, the TP values revealed a greater stability and even an increase in the ND samples. According to the obtained results, it can be suggested that it will be better that the NDs should be treated as short shelf-life product (2-month) and consumed within the first couple of days after they are opened. The authors propose further studies to optimize the storage time and temperature of such products in relation to functional properties and to identify the compounds formed by the co-pigmentation phenomenon since they are probably compounds with high antioxidant activity.

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