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Evaluation of the Organic Acids Ability for Extraction of Anthocyanins and Phenolic Compounds from Different Sources and Their Degradation Kinetics During Cold Storage

Sepideh Hosseini, Maryam Gharachorloo, Babak Ghiassi-Tarzi, Mehrdad Ghavami*

College of Food Science and Technology, Tehran Science and Research Branch, Islamic Azad University, 1477893855 Tehran, Iran

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The study of anthocyanin and phenolic acids has always received much attention due to their extensive range of colors and potential beneficial health effects. In this study extraction of anthocyanins from barberry, eggplant peel and red cabbage was investigated by using different organic solvents. Soluble solid content, antioxidant capacity, total monomeric anthocyanins and total phenolic content were determined and then degradation kinetics of anthocyanin in different solution during freezing process was assayed. In order to examine the effect of different acids on the degree of extraction of anthocyanin and total phenol, varied concentration of hydrochloric, citric and acetic acids were dissolved in a mixture of water and ethanol to prepare acidified aqueous solution. Results indicated that citric acid solution is one of the best solvents for phenolic and anthocyanin extraction which showed the best scavenging activity of DPPH radical. Results from degradation kinetics of total monomeric anthocyanins revealed that stability of anthocyanins in the solution depended on temperature and other ingredients which are present in the medium. Moreover, the present data confirmed that barberry and red cabbage acidified extracts could be one of the more stable natural food colorants based on anthocyanins.

INTRODUCTION

The study of natural colorants has always received much attention due to their potential beneficial health effects. Anthocyanins are most abundant vegetable pigments being used as natural food colorants [Giampieri *et al*., 2014a]. These pigments are harmless, easy soluble in the aqueous phase and might provide different colors (shiny orange, pink, red, violet and blue) in aqueous media, which makes them interesting for food products and beverages but huge variety of anthocyanin and their poor stability limits their application in the food industry [Forbes-Hernández *et al*., 2014].

Several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, and the presence of enzymes, flavonoids, proteins and metallic ions influence the stability of extracted anthocyanins [Rein, 2005]. Since thermal treatments are unavoidable in food industries, many studies have been devoted to the effect of temperature, light, pH, and thermal degradation of anthocyanins [Kamiloglu *et al*., 2013; Howard *et al*., 2014; Shao-Qian *et al*., 2011]. Among the fruits and vegetables that are good sources of anthocyanins, red cabbage has attracted much attention because of its physiological functions and applications. It has a significant amount of anthocyanins with high health-related properties [McDougall *et al*., 2007]. Cyanidin glycosides in most cases, are the main anthocyanin pattern that are followed by pelargonidin glucoside and peonidin glucosides [Charron *et al*., 2009; McDougall *et al*., 2007]. Red cabbage dye is valued in food systems as a natural colorant or in pharmaceutical formulations as a pH indicator [Silva-Pereira *et al*., 2015]. However, there is lack of studies comparing stability of red cabbage extract in different organic solvents.

Recently eggplant (*Solanum melongena*) peel has attracted much attention as an important source of phenolic and flavonoid compounds. Nasunin (delphinidin-3-p-coumaroylrutinoside-5-glucoside) is its major anthocyanin [Gallo *et al*., 2014] which has the strongest antioxidant activity *in vitro* among other anthocyanins [Igarashi *et al*., 1993]. Some studies were carried out on improving anthocyanin extraction from eggplant peel [Boulekbache-Makhlouf *et al*., 2013; Todaro *et al*., 2009]. Their results showed that methanol and tartaric acid 70:30(V/V) were more efficient solvents for anthocyanins extraction.

Barberry (*Berberis L*.) is one of the traditional Iranian pharmacopoeia which is also consumed as a food additive [Berenji Ardestani *et al*., 2013]. Barberry has been shown to possess some activity against fungal infections, *Candida albicans*, yeast, parasites, and bacterial infections which are attributable to active ingredients including isoquinolone alkaloids, especially berberine [Sharifi & Hassani, 2012; Koncic *et al*., 2010]. *Berberis vulgaris* contains pelargonidin-3-glucoside and cyanidin-3-glucoside as predominating anthocyanins (931.05±21.31 mg/kg fresh fruit), high concentrations of vitamin C and other bioactive substances such as iron,

* Corresponding Author: Tel.: +982144868541; Fax: +982144868541; E-mail: sepidehpaeizan@yahoo.com (Sepideh Hosseini)

zinc, and calcium [Akbulut *et al*., 2009; Berenji Ardestani *et al*., 2013].

In this study, extraction of anthocyanins from barberry, eggplant peel and red cabbage was investigated by using different organic solvents. Soluble solid content, antioxidant capacity, total monomeric anthocyanins and total phenolic content were determined as well. The stability of anthocyanins during storage at ambient and cold temperatures was considered less attractive by scientific research therefore, the degradation kinetics of anthocyanins during freezing processes was evaluated as the second aim of this study.

MATERIAL AND METHODS

Fresh barberry (*Berberis vulgaris*), eggplant (*Solanum melongena*) and red cabbage (*Brassica oleracea*) were purchased from a local market in Tehran, Iran. and used fresh daily. The initial moisture content of the samples in wet basis (%) was 75.47, 89.29 and 93.46 for barberry, eggplant peel and red cabbage respectively. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and Folin–Ciocalteu were purchased from Sigma (Sigma-Aldrich Co. USA). All solvents and other chemicals were obtained from Merck (Merck KGaA, Germany).

Extraction

In brief, 15 g of each sample (red cabbage, barberry and peel of eggplant which was manually removed) were chopped, mixed in a commercial blender (Philips, HR7778–03) with 100 mL of different solvents (water, absolute ethanol, water/ethanol 50:50 (v/v), water/ ethanol/ citric acid, water/ ethanol/ acetic and water/ ethanol/ hydrochloric acid) 50:48:2 (v/v/v) separately for 60 mins in the dark. According to the experiences of previous study, the pH changes could affect the extraction yield [Chandrasekhar *et al*., 2012]. Therefore, the volumes of acids were supposed to be constant and their different concentrations were applied to maintain a constant pH of the medium. The extract was filtered through a 0.45 mm cellulose filter paper (Schleicher & Schuell, Dassel, Germany) and used for analysis. In order to know the efficiency of the extraction medium, the number of extractions was restricted to only one. In other words, after the first extraction the filter cake obtained was discarded although it contained some amount of anthocyanins. The individual filtrates obtained from different extraction media were centrifuged separately at 6000 rpm for about 15 min to remove the fine suspended particles and applied for the following analysis.

Determination of anthocyanin contents

The total monomeric anthocyanin content was determined by the pH differential method [Giusti & Wrolstad, 2003]. Two dilutions of the same sample were prepared using 0.025 mol/L potassium chloride solution and 0.4 mol/L sodium acetate solution adjusted to pH 1.0 and 4.5 with hydrochloric acid, respectively. The absorbance of each dilution was measured at 512 and 700 nm against a distilled water blank using a UV–visible spectrometer (Varian CARY 100 Bio). Anthocyanin content was calculated from the Equation (1) [Sigh *et al*., 2006].

$$\text{Anthocyanin pigment} = \frac{A \times Mw \times DF \times 10^3}{\varepsilon \times I} \quad (1)$$

where: Mw is the molecular weight of anthocyanin (449.2 g/mol), DF is the dilution factor, ε is the extinction coefficient (26,900 L/cm mol) and I is the path length (1 cm) and expressed in cyanidin-3-glucoside equivalents, mg/L.

Total phenolic content

The modified Folin–Ciocalteu procedures [Giampieri *et al*., 2014b] were utilized in this study. Gallic acid stock solution (1 mg/mL) and working standard concentrations of 0, 10, 25, 50, 100, 250 and 500 mg/mL (parts per million, ppm) were prepared in deionized water (DIH_2O). The modified Folin–Ciocalteu procedure consisted in transferring 50 μL of the standard or a sample into a 4–5 mL borosilicate tube, followed by additions of 430 μL of DIH_2O and 20 μL of Folin–Ciocalteu reagent. After mixing the samples, 50 μL of 20% Na_2CO_3 and 450 μL of DIH_2O were added. The sample mixtures were allowed to stand for 1 h at room temperature and were mixed after addition of each reagent. Aliquots of 200 μL were transferred to clear microtitre wells in duplicate and the absorbance was determined at 725 nm using a Biotek Synergy HT. The phenolic content of the samples was reported against the gallic acid (GA) calibration standard (0–500 ppm) [Medina, 2011].

DPPH assay

$DPPH^{\bullet}$ has an intense violet color with a maximum absorbance at 517 nm, but turns colorless as unpaired electrons are scavenged by antioxidants. Reaction mixtures containing 0.1 mL of the sample and 3.9 mL of 200 μmol/L $DPPH^{\bullet}$ (prepared in ethanol) were incubated in a water bath at 37°C for 30 min. After incubation, the absorbance was measured at 517 nm. The percentage inhibition was calculated against a control [Konczak & Zhang, 2004].

Degradation kinetics

In order to evaluate the stability of anthocyanins during the cold storage and investigate the effect of citrate on their degradation kinetics, anthocyanins were extracted from red cabbage, barberry and eggplant peel by water/ethanol/citric acid (50:48:2) and their solvent was evaporated by rotary instruments (Heidolph, Germany). Solid matters were dissolved in water, citrate and glycine hydrochloric acid buffer solutions where the initial concentration of anthocyanin was 30 mg/mL in all solutions. All buffers were prepared in 100-mL volumetric flasks with purified deionized water and the final pH was adjusted to 3.7. The citrate buffer was prepared by adding 37 mL of 0.1 mol/L citric acid solution and 13 mL of 0.1 mol/L sodium citrate solution, then water was added to bring the solution up to the final volume. The glycine hydrochloric acid buffer was prepared by adding 25 mL of 0.2 mol/L glycine solution, 2.5 mL of 1 mol/L hydrochloric acid solution and then adding water to the final volume. The solutions were freshly prepared and pH values were measured at room temperature of 25°C by a portable pH meter (Omega, PHH 222). It is worth mentioning that glycine hydrochloric acid buffer has been applied only to eliminate the effects of pH

on anthocyanins stability results. The thermal stability of anthocyanins was studied at 7 and -20°C for 30 days. Aliquots of 20 mL portions of different solutions were put into plastic tubes, then the sample tubes covered with aluminum foil were well capped to avoid evaporation and placed in an incubator, conventional refrigerator and freezer to a given temperature. Tubes were randomly taken from the storage at regular time intervals and defrosted at ambient temperature. The contents of tubes were analyzed for monomeric anthocyanin content.

Statistical analysis

All extraction processes were conducted with two replications and each treatment extract was analyzed so the results were presented as mean ±SD based on them. Analysis of variance was performed by ANOVA procedure with one factor. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Physical and chemical characteristics of extracts

In order to examine the effect of different kinds of the acids on the degree of extraction of anthocyanins and total phenols, hydrochloric, citric and acetic acids were dissolved in a mixture of water and ethanol to prepare acidified aqueous extractor with constant pH. Soluble solids and pH of mediums are shown in Table 1. In red cabbage and eggplant peel, extraction with hydrochloric acid showed the highest pH between acidified solutions that might be incurred by pectin or protein hydrolysis. Hydrochloric acid is a catalyst for the hydrolysis of most of organic compounds. It seems that in acidic conditions, the liberation of some pectin, sugars, essentially galactose, glucose, and rhamnose happened but not completely. Acidic hydrolysis rates for polypectate (<5% DM) declined as the pH value was raised from 2 to 6. Pectin (35% and 70% dm) hydrolyzed more slowly than polypectate below pH 3.5, but then degradation rates increased because β-elimination became the dominant reaction above pH 3.8 [Garna *et al*., 2004]. The results indicate that carboxylic acids could not catalyze the degradation of cell wall polysaccharides as well as mineral acid since acid-catalyzed hydrolysis is proportional to H+ concentration [Mosier *et al*., 2002].

As expected, we failed to show any significant difference in the barberry extracts which would be due to lack of the complex polysaccharide and protein in barberry cell wall that could be extensively hydrolyzed by acid and increased pH.

Brix degrees or total soluble solids are mostly used in the fruit industry to evaluate the concentration of liquids. Brix degrees in various solutions are significantly different ($p<0.05$) and ethanol has a great potential effect on soluble solids extraction (Table 1), but results in the Tables 2, 3 and 4 indicate that Brix is not an appropriate scale for predicting phenolic compounds concentration.

Phenolic and anthocyanin contents

Acidified aqueous mixtures of ethanol, methanol or acetone are commonly used for the extraction of phenolics and anthocyanins from fruits and vegetables [Astadi *et al*., 2009; Chandrasekhar *et al*., 2012]. Methanolic extraction is more effective than ethanolic but in food technology methanol is not preferred due to its toxicity [Kapasakalidis *et al*., 2006]. Results of previous studies showed that hydrochloric acid has a potential ability to accelerate extraction of total phenols and anthocyanins [Castaneda-Ovando *et al*., 2009; Li *et al*., 2013].

There are also reports that the organic acids like acetic or formic acids might destroy the cell membranes, simultaneously dissolve the phenolics and anthocyanins and stabilize them [Todaro *et al*., 2009; Mosier *et al*., 2002]. The effects of solvents on anthocyanins extracted from red cabbage (*Brassica oleracea*), eggplant peel (*Solanum melongena*) and barberries (*Berberis vulgaris*) are discussed in the following sections. Since the pH of acidified solvent highly influences the final content of anthocyanins, the extraction is conducted at constant initial pH (pH=3.49).

Red cabbage (*Brassica oleracea*)

The content of phenolic compounds in the red cabbage extract ranged from 529.60 to 724.14 mg /100g of FW in ethanol and citric acid solvents, respectively. It is known that phenolic compounds are polar molecules, thus extraction is highly influenced by polarity. Among the non-acidified solvents, water was more efficient than ethanol due to higher polarity (dipole momentum of water and ethanol are 1.95 and 1.69 D, respectively). As presented in Table 2, citric acid ensured the highest content of the total phenolics among the six media. It might be attributed to high hydrolyzing and polarity activity and also

TABLE 1. pH of red cabbage, barberry and eggplant peel extracts.

Extract	pH*	Red cabbage		Barberry		Eggplant peel	
		pH**	Brix (%)	pH**	Brix (%)	pH**	Brix (%)
Distilled water (100)	5.75	6.30	2.00	3.46	2.80	5.24	1.97
Ethanol (100)	7.80	6.85	21.50	4.90	21.09	6.17	20.11
Water/ethanol (50:50)	6.90	6.64	14.22	3.99	17.25	4.98	14.93
Water/ethanol/citric acid (50:48:2)	3.49	4.59	14.83	3.79	17.71	4.15	14.32
Water/ethanol/acetic acid (50:48:2)	3.49	4.38	15.12	3.78	17.76	4.14	15.10
Water/ethanol/hydrochloric acid (50:48:2)	3.49	5.60	15.17	3.78	16.47	5.33	15.56

*Initial pH of solvent; ** pH of extractions.

TABLE 2. Concentration of total phenols, anthocyanins and DPPH scavenging capacity in red cabbage extracts.

Extract	Total phenols (mg GAE /100 g) FW	Anthocyanins (mg/100 g) FW	DPPH (%) inhibition
Water (100)	657.06±12.78[b]	53.23±2.68[e]	71.56±0.82[c]
Ethanol (100)	529.60±8.19[d]	78.09±1.58[c]	56.82±1.81[f]
Water/ ethanol (50:50)	549.12±18.67[d]	61.78±2.83[d]	76.40±1.39[d]
Water/ ethanol/citric acid (50:48:2)	724.14±11.99[a]	115.43±5.94[a]	89.81±1.63[a]
Water/ ethanol/acetic acid (50:48:2)	593.95±14.17[c]	71.64±3.59[c]	81.45±1.08[b]
Water/ ethanol/hydrochloric acid (50:48:2)	587.20±10.93[c]	90.37±2.53[b]	64.13±2.66[e]

Notes: Values are averages ± standard deviation of duplicate analysis; different letters in same column indicate significant difference ($P<0.05$; Duncan's multiple test). FW: Fresh Weight. GAE: Gallic Acid Equivalent.

some individual characteristics of chelation ability of metal ions. One of the main characteristics of anthocyanins and anthocyanidins with o-di-hydroxyl groups in the B ring, is their ability to form metal-anthocyanin complexes [Boulton, 2001] which could be carried out in fruits and vegetables like red cabbage. Whereas citric acid has a strong chelating ability to anthocyanins; co-pigment can dissolve the anthocyanins, and stabilize them. Therefore, higher phenol and anthocyanin contents were obtained. The contents of phenolic compounds in the extracts obtained by acetic and hydrochloric acids did not differ significantly ($p<0.05$). Despite the fact that acetic acid (1.74 D) has a higher dipole momentum than hydrochloric acid (1.08 D), but hydrochloric acid has a higher hydrolyzing ability of cell membrane would lead to closely similar results.

The content of anthocyanins in red cabbage is given in Table 2. High variability ($p<0.05$) in anthocyanin concentrations was observed among red cabbage extracts. The contents of extracted anthocyanins are as follow: citric acid 115.43, hydrochloric acid 90.37, ethanol 78.09, acetic acid 71.64, water/ethanol 61.78, and water 53.23 mg/100 g FW. Based on these results the acidified solutions exhibit higher amounts of anthocyanins. In acidic conditions, the flavylium cation (red color) is a predominant species. Increasing pH hydrated this ion slowly to purple quinonoidal bases that these compounds are labile. In the presence of strong acids like hydrochloric and sulfuric acid, hydrolysis reactions are carried out and a glycosyl group in the location of glycosidic residues is replaced by a hydrogen atom and aglycone is produced [Fleschhut *et al*., 2006]. Thus extraction is facilitated by weak acid [Holzwarth *et al*., 2012].

The greatest anthocyanin contents were obtained by citric acid. As mentioned earlier, it would be associated with its chelating ability. At pH conditions typically used for extraction (pH 4–6), four structural forms of the anthocyanins coexist: flavylium cation, anhydrous quinoidal base, colorless carbinol base and the pale yellow chalcone [Chen *et al*., 2013]. The counter-ion of the flavylium cation would be co-pigments to organic acid which could lead to increased extraction efficiency and higher stability.

However, there are many studies which demonstrated that anthocyanins are polar pigments but they usually may not exhibit high hydrophilicity. As an example, cyanidin 3-glucoside which is the predominant anthocyanin of red cabbage [McDougall *et al*., 2007] indicates log p 0.386–0.5 (log p means the logarithm of the molecular 1-octanol-water partition coefficient of compounds). Positive log p of cyanidin 3-glucoside indicates that this material also tends to non-polar solvents. In this respect, citric acid is significantly better extractor than others.

Barberry (*Berberis vulgaris*)

As it can be seen in Table 3, the aquatic extract of barberry exhibited good total phenolic and anthocyanin contents, as a consequence had higher DPPH scavenging ability which followed by citric acid, water/ethanol, acetic acid and hydrochloric acid ($p<0.05$), that might be explained by the structure of specific type of cell wall of barberry which does not require hydrolysis to release phenolic compounds. In the absence of the results which were obtained by citric acid, the polarity of the solvents probably is the most important factor defining the extraction of phenolic and anthocyanins compounds.

Eggplant (*Solanum melongena*) peel

Table 4 shows the total phenolic contents of eggplant peel extracts, which are significantly different ($p<0.05$). Todaro *et al*. [2009] found that the phenolic content of acidified ethanolic extract of fresh eggplant peels was 188.73±73 μg GAE/mL of extract. In turn, Nisha and his collaborators [2009] have reported 49.02±1.3 mg GAE/100 g DE in the methanolic extract and Eun-Ju *et al*. [2011] have found 55.19±1.3 mg GAE/100 g DE in 70% ethanolic extract of fresh eggplant peel. These differences in phenolic contents might be due to the extraction condition (time and temperature).

The total anthocyanin contents of eggplant peel varied in the different extracts (Table 4). The highest level has been detected in citric acid extract (51.40 mg /100 g FW), followed by acetic acid, hydrochloric acid, ethanol and water extracts (43.27, 37.29, 16.66, 9.38 and 5.61 mg /100 g FW, respectively).

DPPH radical scavenging capacity

The measured inhibition of DPPH radical, for all red cabbage extracts are shown in Table 2. Citric acid shows the highest antioxidant activity followed by acetic acid, water, hydrochloric acid and ethanol. These variations are mostly attributed to anthocyanin contents. Some extracts demonstrated the lowest antioxidant activity even with high total phenolic contents. There was a significant variation in the DPPH radical-scavenging ability of barberry extracts (52.24–96.60%) (Table 3). Correlations between the scavenging activity obtained from

TABLE 3. Concentration of total phenols, anthocyanins and DPPH scavenging capacity in barberry extracts.

Extract	Total phenols (mg GAE /100 g) FW	Anthocyanins (mg/100 g) FW	DPPH (%) inhibition
Water (100)	2636.16±11.86[a]	47.69±1.39[a]	96.60±1.03[a]
Ethanol (100)	2250.04±29.89[f]	35.12±2.24[b]	82.46±2.89[e]
Water/ ethanol (50:50)	2502.27±7.81[c]	25.48±1.49[c]	90.00±1.91[b]
Water/ ethanol/citric acid (50:48:2)	2595.42±7.84[b]	33.76±3.00[b]	77.78±2.17[a]
Water/ ethanol/acetic acid (50:48:2)	2424.25±9.52[d]	20.12±0.26[d]	64.92±1.71[c]
Water/ ethanol/hydrochloric acid (50:48:2)	2391.27±13.58[e]	15.36±1.47[e]	52.24±2.99[d]

Notes: Values are averages ± standard deviation of duplicate analysis; different letters in same column indicate significant difference (P<0.05; Duncan's multiple test). FW: Fresh Weight. GAE: Gallic Acid Equivalent.

TABLE 4. Concentration of total phenols, anthocyanins and DPPH scavenging capacity in eggplant peel extracts.

Extract	Total phenols (mg GAE /100 g) FW	Anthocyanins (mg/100 g) FW	DPPH (%) inhibition
Water (100)	66.06±2.71[f]	9.38±1.38[f]	24.09±2.80[f]
Ethanol (100)	184.26±4.86[d]	16.66±1.44[d]	47.65±0.94[d]
Water/ ethanol (50:50)	125.17±2.44[e]	5.61±1.06[e]	37.31±1.88[e]
Water/ ethanol/citric acid (50:48:2)	217.32±2.12[b]	51.40±2.43[a]	71.68±2.01[a]
Water/ ethanol/acetic acid (50:48:2)	207.79±4.20[c]	43.27±1.24[b]	56.72±0.097[c]
Water/ ethanol/hydrochloric acid (50:48:2)	230.02±3.12[a]	37.29±2.32[c]	63.86±2.19[b]

Notes: Values are averages ± standard deviation of duplicate analysis; different letters in same column indicate significant difference (P<0.05; Duncan's multiple test). FW: Fresh Weight. GAE: Gallic Acid Equivalent.

all barberry extracts are total phenols and anthocyanins are 0.95–0.43% respectively. It means that the free radical scavenging activity depends mostly on the total phenols content. In fact, the presence of bioactive compounds that have a high antioxidant activity such as berberine, vitamin C and phenolic acids, affect more $DPPH^{\bullet}$ radical scavenging than anthocyanins do. Therefore, anthocyanins are not considered the most effective antioxidants in barberry extracts.

The DPPH scavenging capacity of eggplant peel varied significantly ($p<0.05$) between 24.09% and 71.68% (Table 4). The free radical scavenging activity of eggplant peel extracts was highly dependent on anthocyanins contents with a correlation coefficient of 0.97. It can be concluded that anthocyanins are the largest class of compounds with the antioxidant activity in the peel of eggplant.

Degradation kinetics of total monomeric anthocyanins

Maintenance of naturally colored pigments in processed and stored foods is the major challenge in food processing. Therefore, the stability of anthocyanins as food colorants in aqueous solutions was evaluated and the anthocyanin contents of solution during cold storage were plotted with a regular interval of 5 days. The degradation of monomeric anthocyanins of the solution followed the first order reaction kinetics with respect to temperature. This kinetic type is expressed by the Equation (2).

$$C = C_0 \times \exp(\pm k_1 \times t) \quad (2)$$

where C_0 is the initial anthocyanin content and C is the anthocyanin content after time t (min) of heating at the given temperature while k_1 (min^{-1}) is the first order rate constant. Half-life ($t_{1/2}$) which is the time needed for 50% degradation is calculated by the Equation (3).

$$t_{1/2} = -ln\ 0.5/k_1 \quad (3)$$

where $t_{1/2}$ is the half-life and k_1 is the first order degradation rate constant (h^{-1}).

The effect of temperature on the degradation rate constants is expressed by the linearized Arrhenius equation by plotting ln k against 1/T in which the temperature dependence of k is quantified by the activation energy Ea according to Equation (4).

$$\ln k = \ln A_0 - E_a/RT \quad (4)$$

where k is the rate constant (min^{-1}), A_0 is the frequency factor (min^{-1}), Ea is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/mol/K) and T is the absolute temperature (Kelvin, K). The Ea value is calculated from the slope of the straight lines given by Equation (4) [Cao *et al*., 2011; Jing *et al*., 2012; Kara & Ercelebi, 2013].

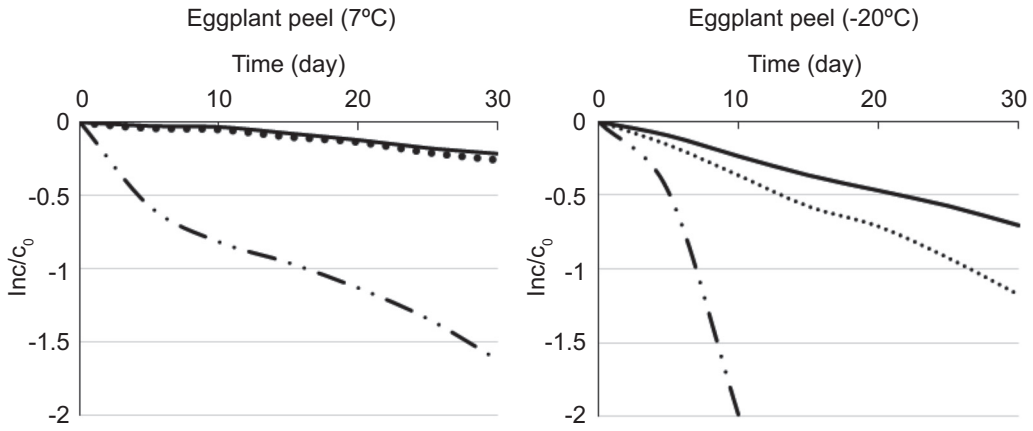

FIGURE. 1. Evolution of the concentration of monomeric anthocyanins of eggplant peel with time at the different temperatures (– ·· – Water, — glycine HCl buffer and ···· citrate buffer).

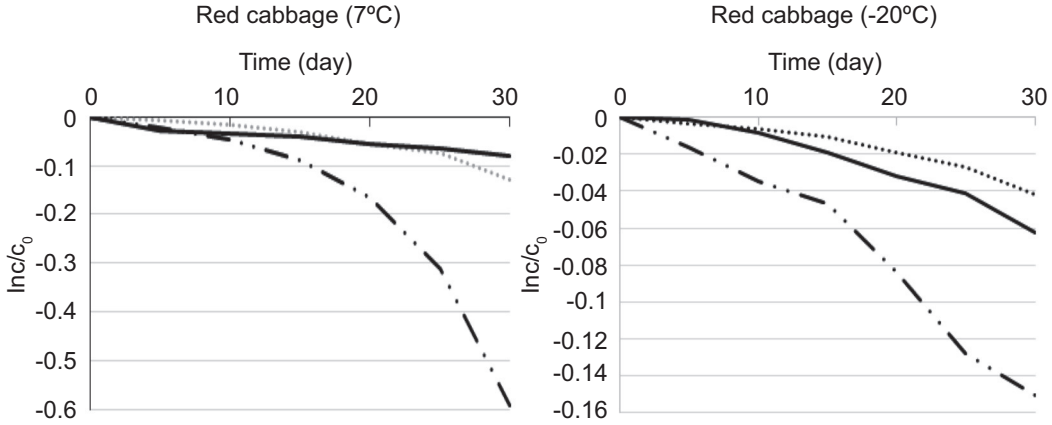

FIGURE. 2. Evolution of the concentration of monomeric anthocyanins of red cabbage with time at the different temperatures (– ·· – Water, — glycine HCl buffer and ···· citrate buffer).

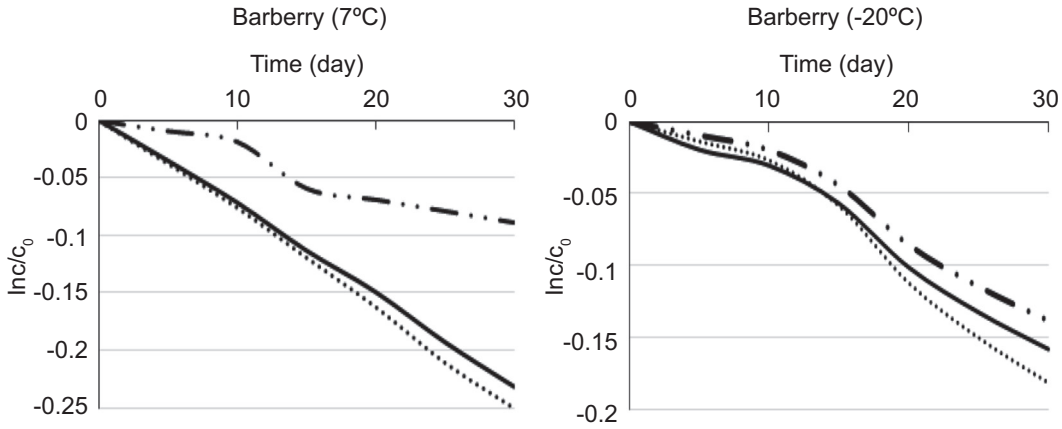

FIGURE. 3. Evolution of the concentration of monomeric anthocyanins of barberry with time at the different temperatures (– ·· – Water, — glycine HCl buffer and ···· citrate buffer).

The logarithms of anthocyanin contents of eggplant peel, red cabbage and barberry during cold storage were plotted with a regular interval of 5 days (Figures 1–3).

In each figure, the acidified solutions show the same behavior. There was no difference between buffers during 30 days of storage at 7°C but solutions containing citrate showed greater stability after the day tenth at -20° C. The similar behavior of buffers might be due to the effects of pH on the stability of anthocyanins. This is in agreement with findings reported by Kirca *et al.* [2003]. They evaluated the effect of pH on thermal stability of anthocyanins from black carrots at six different pHs (2.5–7.0) in citrate-phosphate buffer solutions and observed a significant decrease in anthocyanin stability at pHs above 5.0. In a water-based solution (pH=5.24) of eggplant peel and red cabbage, the degradation of anthocyanins during cold storage en-

TABLE 5. Thermal degradation parameters of anthocyanin during cold storage.

Sample	Medium	k $(h)^{-1}$ [a]		$t_{1/2}$(day)[b]		E_a (kJ mol^{-1})[c]
		7	-20	7	-20	T>0
Red cabbage	Water	0.0156(0.9775)[d]	0.0102(0.9835)	44.43	67.95	9.62
	Citrate buffer	0.0043(0.9833)	0.0026(0.9764)	161.19	266.59	10.97
	Glycine HCl buffer	0.0044(0.9835)	0.0029(0.9915)	157.53	239.01	9.09
Eggplant peel	Water	0.0479(0.9510)	0.1619(0.9447)	14.47	4.28	26.56
	Citrate buffer	0.0085(0.9639)	0.0387(0.9958)	93.66	17.91	33.06
	Glycine HCl buffer	0.0074((0.9629)	0.0236(0.9981)	81.54	29.37	25.29
Barberry	Water	0.0033(0.9485)	0.0049(0.9762)	210.04	141.45	8.60
	Citrate buffer	0.0052(0.9355)	0.0055(0.9834)	133.29	126.02	1.22
	Glycine HCl buffer	0.0067(0.9823)	0.0064(0.9768)	103.45	108.30	0.99

[a]k=reaction rate constant; [b]$t_{1/2}$=half-life of anthocyanins degradation; [C]E_a=Activation Energy; [d]Numbers in parentheses are the determination coefficients.

hanced but barberry anthocyanins appeared more stable in this situation.

Eggplant peels anthocyanins in comparison to these of other materials showed lesser stability during storage especially at -20°C, which was reflected in the k values (Table 5).

The first order reaction rate constant (k), the half-life of anthocyanins ($t_{1/2}$) and other kinetic parameters of samples are shown in Table 5. The required time for 50% degradation of red cabbages anthocyanins at -20°C is always longer than at 7°C, which is contrary to other types of anthocyanin samples. These results showed that changes of anthocyanin content were greater at the lower temperature of storage. At both temperatures, the final contents were lower than the initials. Similar results were described by Concellon *et al*. [2007] who worked with *Solanum melongena* variety of eggplant. Here, the decrease of anthocyanin content during the freezing could be carried by the antioxidant activity of anthocyanin. Anthocyanin is one of the flavonoid compounds with a stronger reducing power, and possibly plays some important role as an antioxidant. Wang & Xu [2007] have also found that a high content of anthocyanin during low temperature storage (5°C) was less affected by temperature.

Temperature dependence

The dependence of the degradation of anthocyanins on temperature was determined by calculating the activation energy (Ea) for two temperature area.

Eggplant peel solution resulted in higher Ea values during storage at both 7°C and -20°C storage (Table 5). As compared to the solution, lower Ea values were obtained by barberry anthocyanin during storage at cold temperature. Since high activation energy reactions are more sensitive to temperature changes, anthocyanins of eggplant peel are more susceptible to temperature elevation during storage.

There were differences among the Ea values for the thermal degradation of individual anthocyanins of samples at different solutions. In most cases, the Ea of citrate buffers were the highest, which indicated that anthocyanins are more stable at this condition. But the calculated Ea values of anthocyanins of barberry ranged from 8.6 to 0.99 kJ mol^{-1} in water and citrate buffer solutions, respectively. It means that the aqueous solution of barberry anthocyanin is more stable.

CONCLUSIONS

In this study, it was found that the citric acid solution was one of the best solvents for phenolic and anthocyanin extraction that showed the best scavenging activity of DPPH radical. Considering that citric acid is a weak organic acid which is a natural preservative and also used to add an acidic or sour taste to foods and drinks (E330), it has a great potential for food ingredients extraction. However, further studies on the ability of citric acid for the extraction of anthocyanin form different sources are needed.

The present study provides detailed information regarding changes in the kinetic stability of anthocyanins in red cabbage, eggplant peel and barberry at temperatures of 7°C and -20°C in different solutions. The present data shows that degradation of anthocyanins follows first-order reaction kinetics. The stability of anthocyanins depends on temperature and pH. During frozen storage and in the absence of acids anthocyanins degraded more quickly. Thus more stable natural food colorant based on anthocyanins could be achieved by using barberry and red cabbage acidified solutions. Although the acidified solution is not necessary for barberry anthocyanins.

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