ORİGİNAL ARTİCLE PHENOLİC COMPOSİTİON OF EUROPEAN CRANBERRYBUSH (VİBURNUM OPULUS L.) BERRİES AND ASTRİNGENCY REMOVAL OF İTS COMMERCİAL JUİCE

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Summary Phenolic composition of the European cranberrybush (ECB) (Viburnum opulus L.) juice was determined using high-performance liquid chromatography. The juice contained 2037 mg $kg^{)1}$ chlorogenic acid, which was 54% of total phenolics, and several other phenolics such as (+)-catechin, (**)**-epicatechin, cyanidin-3-glucoside, cyanidin-3-rutinoside and six different glucosides of quercetin. Because of its strong astringent taste, the juices were treated with various doses of two different types of activated carbons (Granucol Bi and Granucol Ge) in order to remove phenolic compounds. Results revealed that both types of activated carbons were equally effective on astringency removal ($P < 0.01$). A 20–30% reduction in total phenolics was achieved by application of 2.0–3.0 g $L^{)1}$ activated carbon which also removed unpleasant taste and odour.

Keywords Activated carbon, astringency removal, European cranberrybush, high-performance liquid chromatograph, phenolic compounds, Viburnum opulus L.

Introduction European cranberrybush (ECB) (Viburnum opulus L.) Caprifoliaceae is a fast growing, bushy shrub, to 4.5 m height and as much across (Herwig, 1986). The plant is also called crampbark, guelder rose or snowball bush (Anonymous, 2004a). The plant is native to Europe; however it is spreading everywhere, including North Asia, North Africa, and North America, but more often it is found in the central zone of the European part of
2 Russia (Anonymous, 2004b). The plant has red, ovoid
3 acidic berries, ripen in August–September, resembling cranberries and which remain through winter (Anonymous, 2004c). The berries are bitter, therefore they are seldom used as food. In Scandinavia, however, they are popular when cooked into preserves and in Canada they may substitute for cranberries. In some parts of Europe and Asia they have been fermented to make an alcoholic drink (Anonymous, 2004d). In Russia the berries are used for a number of health problems such as high blood pressure, heart troubles, coughs and colds, tuberculosis, shortness of breath, kidney and bladder affections, stomach pain, duodenal ulcers and bleedings either alone or mixed with honey. The berries supplied by commercial farms, being made into an extract and preserves for candy, fillers, pastry, marmalade (Anonymous, 2003). Astringency is a tactile sensation which is found in a variety of foods, including nuts, cranberries, persim- mons, tea, wine and soymilk (Courregelongue et al., 1999). The most important factor on mouth-feel sensations in red table wines is astringency (Gawel et al., 2001). In addition to polyphenols, major organic acids are expected to contribute to astringency (Joslyn & Goldstein, 1964; Guinard et al., 1986; Kallithraka et al., 1997). Sensory perception was primarily determined by tannin concentration. Intensities of all astringency descriptors are increased with tannin concentration (Vidal et al., 2004). Astringency is related to viscosity. As viscosity increased, astringency decreased significantly, although increased sweetness had no effect on astringency (Smith et al., 1996). There are several applications which have been reported to remove astringency successfully or change the phenolic composition in various juices, wines and beers: PVPP, activated carbon-AcC (Baron et al., 1997) and casein (Castellari et al., 1998), PVPP in cider (Siebert & Lynn, 1997), resin type-AD9205 (Schobinger et al., 1995), AcC (Artik et al., 1995) and enzymatic mash treatment time (Will et al., 2002) in apple juice; PVPP in beer (McMurrough et al., 1995); PVPP in white wine (Molina et al., 1996); laccase and adsorbent resin (Ritter & Dietrich, 1996), amberlite XAD-7 resin in orange juice (Ribeiro et al., 2002); amberlite IR-400 and naringinase enzyme

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in grapefruit juice (Mishra & Kar, 2003); AcC in peach juice (Carabasa et al., 1998); XAD-16 resin in red grapefruit juice concentrate (Lee & Kim, 2003). The objectives of this research are the determination of major phenolics in ECB fruits and description of removal of excessive phenolic content of ECB juice using AcC and producing a directly edible beverage.

Materials and methods

Chemicals and reagents

Chlorogenic acid, (+)-catechin, (−)-epicatechin were purchased from Sigma Chem. Co. (St Louis, MO, USA), cyanidin 3-glucoside and cyanidin 3-rutinoside were obtained from Extrasynthese (Genay, France), rutin (quercetin 3-rutinoside) was purchased from Wako Chem. Ind. Ltd (Osaka, Japan), quercetin 3-rhamnoside, quercetin 3-xyloside and quercetin 3-b-d-glucoside was from BioChemica-Fluka Cheme GmbH (Buchs, Switzerland). Quercetin 3-arabinoside was extracted from horse chestnut leaf (Aesculus hippocastanum L.), a major source, in our laboratory. The standards were dissolved in 80% methanol. The dilutions of standards ranged from 100 to 500 mg L^{-1} for chlorogenic acid and 10–50 mg L^{-1} for others. All reagents were of analytical or high-performance liquid chromatography (HPLC) grade. Two different types of granular activated carbon (AcC) were used as adsorbent. Granucol Bi and Granucol Ge, Erbsloh Geisenheim (1 Postf. 1240, D-6222 Geisenheim/Rh., Germany), were kindly donated by Dohler GmbH (Riedelstrasse, D.64295 Darmstadt, Germany). Besides Granucol Bi and Granucol Ge, juices were also treated with Granucol Fa, gelatine, bentonite and polyamide at the beginning of experiment. But the last four treatments were excluded after sensory analyses as they were found ineffective on astringency removal.

Determination of individual phenolics

Fruits were picked up directly from trees in Kayseri, Turkey. Seeds of the berries were manually separated and then transferred into a blender jar with 80% methanol containing 0.1% acetic acid. Berry to solvent ratio was 1:2 (w/v). The mixture was homogenised and then centrifuged at 3500 g for 5 min. The supernatant was filtered through Whatman no. 1 under vacuum and reduced to a fixed volume using nitrogen. The final extract was filtered through 0.45 μm Teflon syringe filter before injection to HPLC. The equipment (Shimadzu Class-VP HPLC system) consists of a computer-controlled system with Class-VP software and SLC-10 A VP system controller. Other accessories were a Shimadzu DGU-14A degasser, LC-10 ADVP Shimadzu pump, a CTO-10 ASVP column oven and an SPD-MIOA VP photo diode array (PDA) detector. Separation was carried out using a reversed phase ACE-5C18 (5 μm, 250 × 4.6 mm ID) column (ACE, Aberdeen, UK). The injection volume was 20 μL, flow rate 1.0 mL min^{-1} and column temperature 25 °C. The binary mobile phase consisted of 6% acetic acid in 2 mm sodium acetate (final pH 2.55, v/v solvent A) and acetonitrile (solvent B) (Tsao & Yang, 2003). Elution profile was as follows: 0–15%B in 45 min, 15–30%B in 15 min, 30–50%B in 17 min, 50–100%B in 8 min. There was a 5-min postrun at initial conditions for equilibrium of the column. The absorption spectrum of each compound was determined on line using the PDA detector. Amount of individual compounds was calculated using corresponding standards, unless noted.

Astringency removal experiments

European cranberrybush juice was supplied by a juice-processing factory in Kayseri, Turkey. The juice was prepared in the factory by cleaning and destalking of fruit followed by crushing, preheating to 90 °C and straining through a pulper finisher. The juice was filled into plastic bags and kept at −28 °C until analysed. Before analysis, ECB juice was thawed and centrifuged at 3500 g for 10 min to remove coarse particles. ECB juice (100 mL) was treated with AcC using a magnetic stirrer at a very low speed to avoid oxidation and vortex formation. To determine optimal treatment time, juices were mixed with AcCs for 5, 10 and 15 min at concentrations of 1 and 4 g L^{-1}. As it was observed that the effect of extended treatment time was negligible on astringency removal, 5 min of treatment time was chosen and effects of various amounts of two types of AcC were evaluated. To determine optimal doses, juices were treated with AcC varying from 0.2 to 5.0 g L^{-1}. After treatment they were centrifuged and filtered through Whatman no. 1 filter paper under vacuum. All treatments were made in replicate and assayed in duplicate. Sensory evaluation was conducted to determine acceptable concentration of the AcC-treated ECB juice samples using an untrained panel with fifteen panellists consisting of faculty staff and graduate students of the Food Engineering Department who had experience in sensorial assessment of fruits and fruit juice. Panellists were asked to taste the ECB juice samples (six samples at a time) and determine the acceptable concentration. Samples were served at room temperature and distilled water was provided for rinsing between samples.

Determination of total phenolic content

Total phenolics were determined using Folin-Cioacalteu reagent (Singleton & Rossi, 1965). Results were calcu-lated as gallic acid equivalent.

Colour measurement

Absorbance readings were made in diluted samples (1:9, juice: dw, v/v) at 515 nm (k_{max} of juice) using 10 mm quartz cells.

Statistical methods Differences among the AcC treatments were determined by analysis of variance (completely randomised design of two-way anova). Significant means were compared according to Duncan's multiple comparison test (Sokal & Rohlf, 1995). For the statistical analysis of data, a MINITAB Statistical Package Version 13.1 (Anonymous, 2000) was employed.

Results and discussion

Phenolic compounds European cranberrybush fruits contained 2037 mg kg^{-1} chlorogenic acid, which means that the majority (54%) of ECB phenolics was consisting of chlorogenic acid (Fig. 1 and Table 1). This content was extremely high when compared with other berries. Heating of ECB juice resulted in a yellow precipitation, which was identified as chlorogenic acid. Storage of the fresh juice in cold conditions for several days also resulted in similar precipitation, which was contributed to the sharp-acid flavour in juice. Chlorogenic acid content was 544 mg L^{-1} in rowanberry juice (Gil-Izquierdo & Mellenthin, 2001), 193 ± 26 mg L^{-1} in prune juice (Donovan et al., 1998), 40–430 mg kg^{-1} in apple juice (Podsedek et al., 2000) and 5.1 mg L^{-1} in canned cranberry juice (Chen et al., 2001). The result obtained from this study suggests that ECB juice is a potential substrate for food grade chlorogenic acid having a potential as good dietary source. Two of flavan 3-ols, namely, (+) catechin and (−) epicatechin were determined and (+) catechin content was 290.4 mg L^{-1}, which was much higher than the findings (8.1 mg L^{-1}) of other researchers (Chen et al., 2001). Spectra of unknown peaks at the beginning of the chromatogram indicates that there are several more flavan 3-ol monomers and dimers. ECB juice contained two anthocyanin pigments and their total amount is 82.2 mg L^{-1}; 88% of this content is cyanidin 3-glucoside and 12% of it is cyanidin 3-rutinoside. ECB juice containing 36.9 mg kg^{-1} of rutin may not be considered to be a good source of rutin, while it was 240 mg kg^{-1} in evergreen blackberries, 110 mg kg^{-1} in red raspberries and marionberries, 190 mg kg^{-1} in black raspberries (Wada & Ou, 2002). It was also reported that rutin content of prune juice was only 4 ± 1 mg kg^{-1} (Gil-Izquierdo & Mellenthin, 2001). ECB juice also contained quercetin 3-glucoside (isoquercitrin), quercetin 3-arabinoside, quercetin 3-rhamnoside (quercitrin) and a small amount of quercetin 3-xyloside.

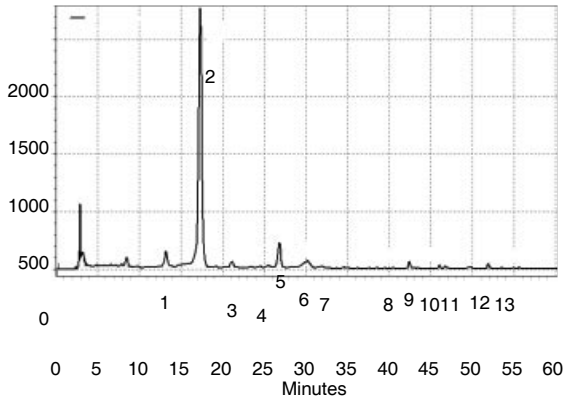

Figure 1 HPLC chromatogram of phenolic constituents of European cranberrybush (Viburnum opulus L.) fruits at 280 nm. See Table 1 for peak identities.

Astringency removal To determine the effect of treatment time on astringency removal, 1 and 4 g L^{-1} concentrations of two AcC types were applied for 5, 10 and 15 min (Fig. 2). Results obtained with anova statistical analysis, treatments of AcC had significant effect on astringency removal ($P < 0.01$), but Duncan's test revealed that the treatment time had no effect ($P < 0.01$). Table 2 shows the effect of various concentrations of AcC types on astringency removal after 5-min treatment time. anova showed that both types of AcCs as well as their concentrations as indicated on the HPLC chromatograms were effective on phenolics removal ($P < 0.01$) (Table 2). Effects of both types of AcCs on astringency removal were similar at the level of $P < 0.01$ and a positive correlation value of $r = 0.944$ was obtained. These data were verified with a negative correlation value of $r = -0.960$ and $r = -0.952$ for Bi and Ge types of AcCs, respectively. A comparison of results by Duncan's multiple comparison test is given in Table 2. According to this test, increased amounts of AcC yielded increased astringency removal. A high correlation was found between reduction in total phenolics and reduction in colour ($P < 0.01$). Correlation coefficients were found as $r = 0.979$ and $r = 0.972$ for Granucol Bi and Granucol Ge, respectively. Analysis of anova indicated that AcC doses had significant effect on colour decrease ($P < 0.01$). Correlation coefficients was found as $r = 0.978$ and $r = 0.973$ for Granucol Bi and Granucol Ge, respectively. Duncan's test also indicated the relationship between colour reduction and AcC concentration (Table 2). This colour reduction can be considered to be acceptable for sensorial aspect. Even with the highest dose of AcC applied to ECB juice, absorbance at 515 nm was still above 0.26 in 1:9 diluted

Table 1 Phenolic compounds in ECB fruits

Peak no.	Compound	Content (mg kg^{-1})	Retention time (min)	k_{max} (nm)
1	(+)-Catechin	290.4	13.25	237.8, 276.6
2	Chlorogenic acid	2037.0	17.32	323.5
3	Procyanidin[a]	82.8	21.16	237.8, 278.4
4	(−)-Epicatechin	26.9	24.55	237.8, 276.6
5	Hydroxybenzoic acid derivative[b]	184.0	26.81	240.5, 313.6
6	Cyanidin 3-glucoside	72.3	30.16	514.8
7	Cyanidin 3-rutinoside	9.9	31.85	518.0
8	Quercetin 3-xyloside	3.4	39.46	254, 352.4
9	Quercetin 3-rutinoside	36.9	42.03	254.0, 352.4
10	Quercetin glucoside[c]	52.1	42.44	254.0, 353.3
11	Quercetin 3-glucoside	26.1	46.71	254.0, 352.4
12	Quercetin 3-arabinoside	41.6	51.89	254.0, 352.4
13	Quercetin 3-rhamnoside	10.1	53.50	254.0, 350.0

[a,b,c]Tentatively identified according to peak spectrums obtained by PDA detector. Concentrations of this three peaks are calculated as (+)-catechin, chlorogenic acid and quercetin-3-glucoside, respectively.

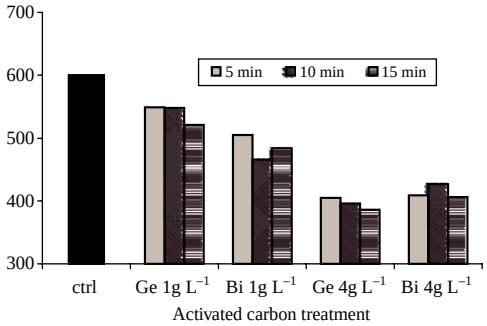

Figure 2 Effect of the AcC type, concentration and treatment time on phenolics removal.

sample which would come around 2.5 absorbance unit, meaning that juice was still having a strong colour.

Conclusions This work indicated that ECB is one of the major sources of chlorogenic acid. Although we have the standards of gallic, caffeic, p-coumaric, ferulic, sinapic, gentisic and benzoic acids, which were identified in cranberry juice (Chen et al., 2001), these compounds have not been detected in ECB juice. This indicates that phenolic composition of ECB juice is quite different
than that of cranberry juice. Sensory studies showed that a 20–30% reduction in total phenolics yielded a

Table 2 Effects of the AcC type and concentration dose on total phenolics reduction and colour

AcC concentration (g L^{-1})	Total phenolics (mg 100 mL^{-1})		Colour at 515 nm[a]	
	Granucol Bi	Granucol Ge	Granucol Bi	Granucol Ge
Control	590.3 ± 3.69 a	590.2 ± 3.69 a	0.61 ± 0.01 a	0.61 ± 0.01 a
0.2	570.1 ± 8.97 ab	547.7 ± 0.53 b	0.56 ± 0.01 b	0.57 ± 0.02 ab
0.4	563.0 ± 15.82 ab	544.4 ± 8.44 b	0.54 ± 0.01 c	0.56 ± 0.002 b
0.6	547.4 ± 844 bc	537.7 ± 7.38 b	0.54 ± 0.0004 c	0.52 ± 0.04 bc
0.8	556.7 ± 1.58 b	533.6 ± 10.02 b	0.55 ± 0.002 c	0.52 ± 0.002 bc
1.0	528.7 ± 3.16 c	528.7 ± 7.38 b	0.50 ± 0.004 d	0.50 ± 0.02 cd
2.0	467.2 ± 0.53 de	493.3 ± 18.46 c	0.45 ± 0.01 e	0.43 ± 0.02 e
2.5	472.4 ± 7.91 d	489.2 ± 9.49 c	0.43 ± 0.005 e	0.45 ± 0.01 de
3.0	466.4 ± 8.97 de	475.8 ± 6.33 c	0.38 ± 0.001 f	0.41 ± 0.01 e
3.5	412.0 ± 7.91 f	406.4 ± 3.16 d	0.34 ± 0.002 g	0.35 ± 0.1 f
4.0	443.0 ± 12.66 e	389.2 ± 15.82 d	0.35 ± 0.002 g	0.33 ± 0.002 f
5.0	391.8 ± 1.58 f	382.9 ± 2.64 d	0.28 ± 0.003 h	0.26 ± 0.002 g

Different letters in same columns indicates the difference between two means is statistically significant ($P < 0.01$) for each treatment.
[a]Diluted samples (1:9, juice: dw, v/v).

juice with a good consumption quality. Before AcC treatment, drinking of the juice was quite hard and a yellow precipitation was forming in bottle during storage. Application of 2.0–3.0 g L^{-1} AcC inhibited this formation, yielding mild drinking properties and the juice could be consumed without further dilution or addition of sugar. AcC application also removed the unpleasant odour probably caused by the presence of valeric acid (Anonymous, 2004a). Direct injection of de- watered sample to GC-MS also verified that the juice had valeric acid.

Chlorogenic acid caused an increase in colour intensity (hyperchromic effect) interfering with anthocyanins (Mazza & Brouillard, 1990), therefore the juice kept its strong red colour even after highest dose of AcC treatment.

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