Separation and Character Analysis of Anthocyanins from Mulberry (*Morus alba* L.) Pomace

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Abstract

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Mulberry pomace, as a by-product of juice and wine making, was investigated as a potential source of natural anthocyanins. The results showed that anthocyanin contents in mulberry pomace from two varieties were above 250 mg/100 g, that is 74%–79% of that in mulberry whole fruit. Thus, mulberry pomace could be a potential anthocyanins source. The anthocyanins in mulberry pomace had an attractive red colour with the chroma at 5.0 and hue angle at 6.8. Five anthocyanins were identified in mulberry pomace, cyanidin-3-glucoside and cyanidin-3-rutinoside being the major anthocyanins. The method of the separation of the two anthocyanins was studied showing that both anthocyanins with purities above 98% could be well separated on Sephadex LH-20 by eluting with 10% ethanol containing 1% of acetic acid after purification with AB-8 macroporous resin. The recovery of the complete process of both anthocyanins was 57.4%. Cyanidin-3-glucoside and cyanidin-3-rutinoside had more attractive colours and strong antioxidant activities and could be used as potential food colourants and antioxidants.

Keywords*:* mulberry pomace; anthocyanins; colour; antioxidant activities; separation

Colour is usually considered as one of the major criteria in selecting one kind of food before another ones. In order to obtain the most adequate colour for each foodstuff, some additives are usually employed in their processing (Alcalde-Eon *et al*. 2004). Anthocyanins are a group of natural flavonoids responsible for the red, violet and blue colours of berries and fruits (Nyman & Kumpulainen 2001; Tian *et al*. 2006). As the common antioxidants, anthocyanins may have health benefits in the prevention of chronic and degenerative diseases including heart diseases and cancer (Wu & Prior 2005; Chen *et al*. 2006). In recent years, as increasing attention has been paid to health benefits of natural anthocyanins, the substitution of synthetic pigments by natural anthocyanins for has already become a social trend. New sources of anthocyanins with a high tinctorial power, stability, and low cost are desired as natural food colorants (Pazmiño-Durán *et a*l. 2001a). From an economic perspective, the best sources of anthocyanins are the pigment-containing by-products, for example, grape skin extracts (Pazmiño-Durán *et al*. 2001a).

Mulberry (*Morus alba* L.) is the fruit of mulberry tree belonging to the genus *Morus* of the Moreaceae family. With its high adaptability, mul-

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berry occupies a very large planting area and gives huge production in China (Yang 1998; Ercisli & Orhan 2007). Mulberry fruit has plentiful anthocyanins. These occur as cyanidin-3-glucoside and cyanidin-3-rutinoside (Suh *et al*. 2004; Chen *et al*. 2006; Lin & Tang 2007). As a traditional Chinese edible fruit, mulberry fruit is used effectively in folk medicine to treat fever, protect the liver from damage, strengthen the joints, facilitate the discharge of urine, and lower the blood pressure (Yang 1998; Jia *et al*. 1999; Chen *et al*. 2005, 2006; Bae & Suh 2007). Mulberry fruit has been consumed broadly as a health-protective fruit in recent times. However, fresh mulberry has a very short shelf life and is normally used for juice and wine production. A great deal of pomace is yielded during the process. Most of the time, mulberry pomace is discarded as a residue. Since the pomace has a very deep red colour, it could be employed as a good source of food colorant and antioxidant agents for functional foods.

Mulberry pomace is an abundant and economic resource. However, the profile and properties of the anthocyanins in mulberry pomace have never been described. In our present work, we selected two mulberry cultivars, Da-10 and Hongguo, grown on the largest cultivating areas in China, determined the anthocyanins contents in parts of the fruits, and then the anthocyanins in mulberry pomace in order to give a reference to the study and utilisation of mulberry pomace.

MATERIALS AND METHODS

Chemicals. Cyanidin-3, 5-diglucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin-3-glucoside were purchased from Extrasynthese SA (Genay, France), the purity was above 98%. Methanol, acetic acid, and acetonitrile were purchased from Fisher (Fairlawn, USA) (HPLC grade). Ultra-pure water Milli-Q (Milipore, Bedford, USA) was used. AB-8 macroporous resin was obtained from Nankai University Chemical Plant (Tianjin, China). Ethanol, hydrochloric acid, and all other reagents were obtained from Beijing Chemical Plant (Beijing, China).

Sample preparation. Mulberry fruits (*Morus alba* L.) were harvested commercially ripe. All the materials were kept at –40°C until the analyses. Quantitative analysis of anthocyanins was carried out in the fruits of Da-10 and Hongguo cultivars, which were obtained from Mengzi, Yunnan Province, China. Mulberry fruit of 1000 g was weighed accurately and mashed (with NaF to inactivate polyphenol oxidase) (Tomás-Barberán *et al*. 2001), then centrifuged at 9000× g at 4°C for 30 minutes. The supernatant was juice and the residue was pomace. Qualitative analysis of anthocyanins was carried out in mulberry pomace. The pomace was separated from the mashed fruit after pressing in a pneumatic press for the juice and wine making. In order to reduce some impurities difficult to dissolve in ethanol and easy to concentrate, pure ethanol (if not stated below, means pure) was selected as a solvent. The mulberry pomace was extracted in darkness at 4°C for 2 h after weighing accurately, and then the slurry was centrifuged at 9000× g at 4°C for 30 minutes. The residue was extracted two times under the same conditions as for the qualitative analysis of anthocyanins, the extraction proceeding until a colourless solution was obtained for the total anthocyanins content measurement. The experiment was performed with three independent replicates.

HPLC-DAD-ESI-MS/MS analysis of anthocyanins in mulberry pomace. Chromatographic analysis of anthocyanins was performed using the Agilent 1100 series LC/MSD system (Hewlett-Packard, Palo Alto, USA). Anthocyanins were separated on the Kromasil C18 column (250 × 4.6 mm *i.d*., 5 μm) (Agilent, Palo Alto, USA) at 25°C. The mobile phase consisted of solvent A (water with 5% formic acid) and solvent B (acetonitrile with 5% (v/v) formic acid). The flow rate was 1 ml per minute. The samples were monitored at 520 nm. The eluting process was as follows: 0–4 min 10% B; 4–12 min 25% B; 12–20 min 40% B; 20–30 min 60% B; 30–40 min 100% B; 40–45 min 10% B. The MS parameters were as follows: capillary voltage 4 kV; nebuliser pressure 30 psi; dry gas temperature 350°C; dry gas flow rate 12 l/min; scan range of MS/MS from *m/z* 100 to *m/z* 1500; scan range of ion trap from *m/z* 100 to *m/z* 1500.

Quantification of anthocyanins in mulberry pomace. Cyanindin-3-glucoside and cyanidin-3-rutinoside standards were dissolved in 4 solvents, in order to select the optimum anthocyanins solvent for HPLC. The 4 solvents were: (*i*) ethanol with 0.1% (v/v) hydrochloric acid; (*ii*) pH 3.5 10% (v/v) ethanol; (*iii*) 10% (v/v) ethanol with 5% (v/v) acetic acid; (*iv*) 7% (v/v) acetonitrile with 5% (v/v) acetic acid. The limit of detection (signal/noise, 3) of cyanindin-3-glucoside and cyanidin-3-rutinoside were 0.13 mg/l and 0.21 mg/l, respectively, in

our method. Anthocyanins content in mulberry pomace was determined by external standard method, cyanidin-3-glucoside and cyanidin-3-rutinoside having been used as standards. The total anthocyanins content was calculated as the sum of the two major anthocyanins.

Colour characteristics of anthocyanins in mulberry pomace. The mulberry pomace anthocyanins solution, with pH 3.5, optical density of 0.3 nm at 520 nm, was prepared (Pazmiño-Durán *et al.* 2001a). Hunter CIE $L^*a^*b^*$ characteristics of mulberry pomace anthocyanins solution were determined using a UV-Vis spectrophotometer (UV-2450, Shimadzu, Shanghai, China). Anthocyanins solution was placed in a 0.2-cm pathlength quartz cuvette and the transmittance values at 440 nm, 530 nm, and 600 nm were measured (Ayala *et al.* 1999). The values of the L^*, a^*, and b^* were calculated according to Ayala *et al.* (1999), and the values of the chroma (c) and hue angle (h) were calculated by formulas $c = (a^{*2} + b^{*2})^{1/2}$ and $h = (\tan^{-1} a^*/b^*)$, respectively (Pazmiño-Durán *et al.* 2001a).

Purification and separation of anthocyanins of mulberry pomace. Mulberry pomace extract was purified through AB-8 macroporous resin column and eluted with water followed by ethanol acidified with hydrochloric acid (0.1%). The purified extract was separated with Sephadex LH-20 column (1.6 cm × 25 cm) and the fractions were measured at λ_{max} 511 nm (the λ_{max} of the mulberry anthocyanins in 30% ethanol). To obtain the optimum separation of anthocyanins, the eluent of 1% acetic acid in different ethanol concentrations (0%, 10%, 20%, and 30%) was tested.

Antioxidant activity of anthocyanins in mulberry pomace

Antioxidant activity of scavenging hydroxyl radical. Hydroxyl radical scavenging activity was assayed by the method of Ghiselli *et al.* (1998) with some modifications. The reaction mixtures containing 1 ml of 0.1 mol/l phosphate buffer pH 7.4, 200 µl 5×10^{-3} mol/l Fe^{2+}-EDTA, 200 µl 5×10^{-3} mol/l 2-deoxyribose, 200 µl sample, and 200 µl 5×10^{-3} mol/l H_2O_2 were incubated in 37°C water bath for 1 hour. Then, 1 ml of 2.8% (w/v) trichloroacetic acid was added to the reaction mixture followed by 1 ml of 0.5% (w/v) thiobarbituric acid. The reaction mixture was boiled for 15 min and cooled, and the absorbance was measured at 532 nm. The decrease in free radical was represented as $A–A_1$, and the degree of scavenging was calculated using the following equation:

$$\text{Scavenging} = (A - A_1/A) \times 100\%$$

The SC_{50} value, defined as the amount of the antioxidant necessary to decrease the initial free radical concentration by 50%, was calculated from the concentration regression curve of the scavenging rate.

Antioxidant activity of scavenging superoxide anion radical. Superoxide radicals were generated by a modified method based on that of Jia *et al.* (1999). All solutions were made in 0.05 mol/l phosphate buffer (pH 7.8). A volume of 300 µl 0.13 mol/l methionine was added to 2 ml 0.05 mol/l phosphate buffer (pH 7.8) followed by 300 µl 0.75 mol/l blue tetrazolium and 100 µl sample. The reaction was induced by 300 µl 0.02 mol/l riboflavin and the mixture was immediately illuminated at 4000 lx in a water bath at 25°C for 20 minutes. The absorbance (A_1) was measured at 560 nm, and the scavenging activity was calculated as given in the formula above.

Antioxidant activity of scavenging DPPH radical. DPPH radicals were prepared in ethanol to the final concentration of 1.0×10^{-4} mol/l. Volumes of 200 µl samples were added to 4 ml DPPH radical solution and kept in the dark for 30 minutes. The absorbance (A_1) of the reaction mixture was measured at 517 nm against an ethanol blank, and the scavenging activity was calculated as in the formula above (Wang *et al.* 2003).

Antioxidant activities in β-carotene/linoleic acid system (β-CLAMS). The antioxidant activities of mulberry pomace in β-CLAMS were measured with a minor modification according to the report of the Lu and Foo (2000). One ml of β-carotene in chloroform (3.34 mg/ml) was added into a flask containing 40 mg linoleic acid and 400 mg Tween 20. Chloroform was removed by rotary evaporation at 40°C for 5 min and 100 ml of distilled water was slowly added to the residue under vigorous agitation to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 200 µl of the sample and the absorbance was measured at 470 nm immediately, against the blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 40°C and the absorbance measurements were made again at 15-min intervals.

RESULTS AND DISCUSSION

Contents of anthocyanins in parts of mulberry fruits

Ethanol was selected as the solvent over the whole experiment for its innocuity. The stability of anthocyanins solution was related to the solvent. Among the four solvents, 10% ethanol (pH 3.5) was the best for anthocyanins standards stability and colour. The precision and accuracy of the HPLC method were measured and proved to be good. The 7-concentrations calibration curves of cyanidin-3-glucoside and cyanidin-3-rutinoside were $Y = 36.9692x + 3.5499$ ($r = 0.9997$) and $Y = 33.8487x + 16.2923$ ($r = 0.9995$), respectively. From the HPLC spectrum of the anthocyanins from mulberry pomace (Figure 1), it is seen that peaks 1, 4, and 5 represented minor anthocyanins. The contents of the five anthocyanins were measured and the results showed that

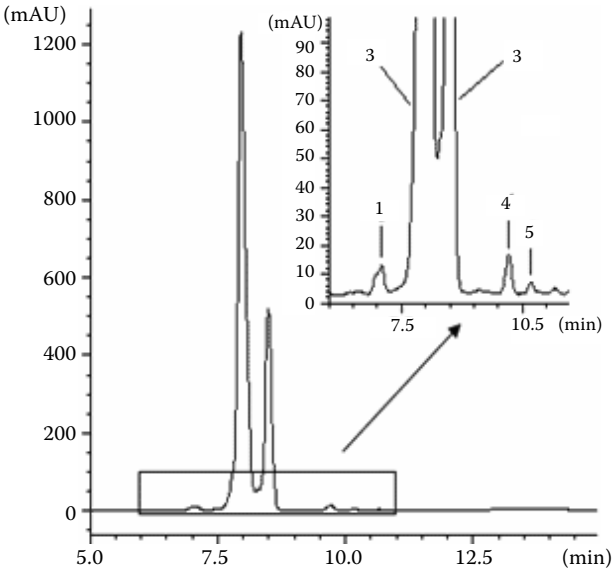

Figure 1. HPLC chromatograms of the mulberry pomace extract at 520 nm

the proportion of the two major anthocyanins was over 98% of the five anthocyanins detected. The minor anthocyanins were ignored in the anthocyanins analysis described below. The anthocyanins contents in different parts of the Da-10 and Hongguo mulberry fruit are presented in Table 1. There were no significant differences in anthocyanins content between the two cultivars of mulberry. The mulberry pomace contained a high content of anthocyanins (257.8–285.5 mg/100 g fresh mulberry). This was considered to be a high level as compared with red cabbage (25 mg/100 g), already commercially available as a food colour extract (Timberlake & Henry 1988), and some natural pigments sources reported in the literature, such as *Oxalis triangularis* (195 mg/100 g leaves), banana bracts (250 mg/100 g dry weight), coffee husks (19.2 mg/100 g fresh husks), and some types of berries, such as blackberries and cranberries, with 83–326 g and 60–200 mg/100 g berries, respectively (Pazmiño-Durán *et al.* 2001a,b; Prata & Oliveira 2007). The anthocyanins contents in mulberry pomace were 74–79% of those in mulberry whole fruits of the two cultivars, which indicated that most of the anthocyanins in mulberry fruit were present in the mulberry pomace. Therefore, mulberry pomace as a by-product in the mulberry juice and wine processing is a potential and economical source of natural anthocyanins.

The colour characteristics of anthocyanins in mulberry pomace

As shown in Table 2, the water solution of the ethanol extract of mulberry pomace, with the absorbance of 0.3 nm at 520 nm and pH 3.5, exhibited an attractive red hue and Hunter CIE colour characteristics $L^* = 97.0$, $a^* = +5.0$, and $b^* = +0.6$. The chroma was 5.0 and the hue angle was 6.8.

Table 1 Anthocyanin contents in juice and pomace of two mulberry cultivars

Parts	Da-10		Hongguo	
	juice	pomace	juice	pomace
Each part (mg/100g FW)	91.2 ± 3.4[b]	257.8 ± 26.3[a]	75.0 ± 2.3[b]	285.5 ± 27.5[a]
Whole fruit (mg/100g FW)	349.1 ± 15.2[a]		360.5 ± 14.2[a]	
Percentage	26.1 ± 1.2[b]	73.9 ± 5.4[a]	20.8 ± 0.8[b]	79.2 ± 3.7[a]

Values are expressed as means ± SD of three replicates; FW – fresh weight

Table 2. The colour characteristics of the mulberry pomace extract, separated anthocyanins and their standards

	L^*	a^*	b^*	c	h
Extract	97.0 ± 0.0[a]	5.0 ± 0.0[a]	0.6 ± 0.0[a]	5.0 ± 0.0[a]	6.8 ± 0.4[c]
Cy 3-glu	97.3 ± 0.0[c]	5.4 ± 0.1[b]	−0.4 ± 0.1[b]	5.5 ± 0.1[b]	−3.8 ± 1.2[a]
Cy 3-rut	97.4 ± 0.0[d]	5.9 ± 0.1[c]	−0.2 ± 0.1[b]	5.9 ± 0.1[c]	−2.2 ± 0.6[b]
Cy 3-glu-std	97.1 ± 0.0[b]	5.5 ± 0.1[b]	−0.4 ± 0.1[b]	5.6 ± 0.1[b]	−3.5 ± 0.7[a]
Cy 3-rut-std	97.3 ± 0.0[c]	5.8 ± 0.1[c]	−0.2 ± 0.1[b]	5.9 ± 0.1[c]	−2.4 ± 0.4[b]

cy – cyanidin; glu – glucoside; rut – rutinoside; std – standards; each value is expressed as mean ± SD (n = 3); means with different letter within a line are significantly different ($P < 0.05$)

Profile of anthocyanins in mulberry pomace

Figure 1 shows that there are more than five anthocyanins in mulberry pomace extract. The major anthocyanins in mulberry fruit were peak 2 and peak 3. Peak 2, 3, and 4 were identified as cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin 3-glucoside respectively, according to their HPLC retention times, UV-Vis spectra, and MS/MS fragmentation patterns compared with the standards. As demonstrated by Giusti *et al*. (1999), some structural properties of anthocyanins can be inferred from the spectral data. $Abs_{440}/Abs_{\lambda_{max}}$ ratio values calculated for each anthocyanin, ranging from 33% to 54%, indicated that all the three anthocyanins were substituted at the C-3 position of the flavylium ring (Giusti *et al*. 1999). Peak 1 produced molecular ion at *m/z* 611 in MS and fragment *m/z* 287 in MS/MS (due to the loss of sophorose – 324 amu) (Table 3), which was identical to the fragment pattern of cyanidin 3-sophoroside (Giusti *et al*. 1999; Wu & Prior 2005). Therefore, peak 1 was identified as cyanidin 3-sophoroside. Peak 5 showed a spectrum with obvious shoulder peaks at about 440 nm and an absorption maximum at about 504 nm, which is the typical pelargonidin-anthocyanidin-type skeleton (Hong & Wrolstad 1990; Longo & Vasapollo 2006). Moreover, peak 5 presented the molecular ion at *m/z* 579 in MS and two fragments, *m/z* 433 (due to the loss of one rhamnose moiety) and *m/z* 271 (due to the loss of one rutinose moiety) (Table 3), in MS/MS spectra. Therefore, peak 5 was pelargonidin 3-rutinoside (Lopes-da-Silva *et al*. 2002; Wu *et al*. 2005; Longo & Vasapollo 2006). The profile of the anthocyanins in mulberry pomace was similar to that in the mulberry fruit reported by Dugo *et al*. (2001).

The separation of anthocyanins in mulberry pomace

The anthocyanins purified by the AB-8 macroporous resin were separated on Sephadex LH-20 column. The elution curve in Figure 2a shows that the two big peaks (fraction I, II) were separated on the base line when eluted with 1% acetic acid and 10% ethanol with 1% acetic acid. The latter was a better eluting solvent because of its shorter elution time. The result of LC analysis indicated, on comparison with the standards, that fraction I

Table 3. Chromatographic and spectral characteristics of the anthocyanin standards and anthocyanins in mulberry pomace

Peak No.	T_R (min)	λ_{max}(nm)	$A_{440}/A_{\lambda_{max}}$ (%)	M^+ *m/z*	$(M^+ - X)$ *m/z*	Compound
1	7.06	518	54.4	611.4	287	cy 3-soph
2	8.00	516	35.9	449	287	cy 3-glu
3	8.38	518	33.1	595	449, 287	cy 3-rut
4	9.65	440^{sh}, 502	51.0	433	271	pg 3-glu
5	10.10	440^{sh}, 504	49.5	579	433, 271	pg 3-rut

[sh]shoulder peak; cy – cyanidin; soph – sophoroside; glu – glucoside; rut – rutinoside; pg – pelargonidin

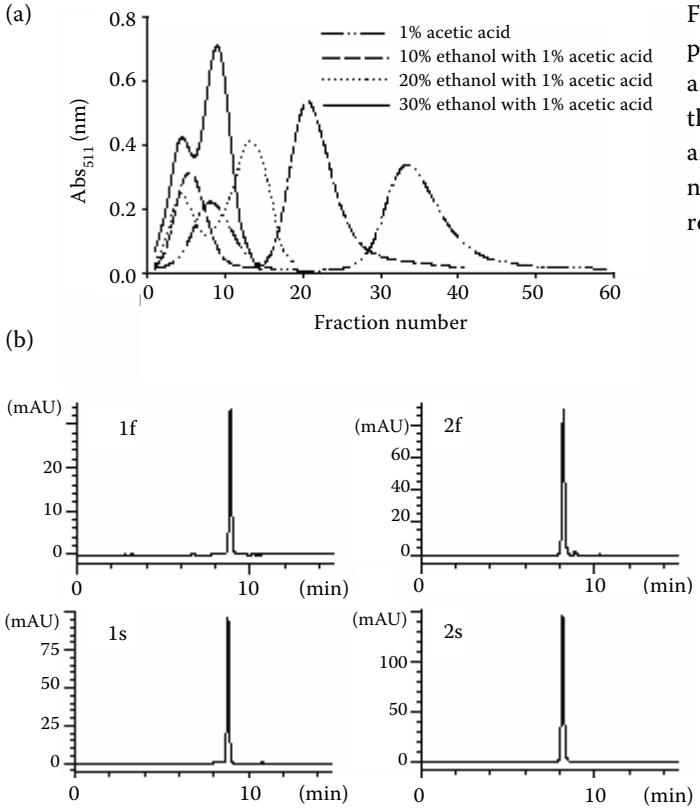

Figure 2. Elution curve of the purified mulberry pomace extract separated by Sephadex LH-20 (a) and HPLC chromatograms of the two fractions and the anthocyanin standards at 520 nm (b) (1f, 2f, 1s and 2s were fraction I, fraction II, cyanidin-3-rutinoside standard and cyanidin-3-glucoside standard, respectively)

was cyanidin-3-rutinoside and fraction II was cyanidin-3-glucoside (Figure 2b). The purity of both anthocyanins was above 98%, according to their peak areas at 520 nm.

The recoveries of the extraction, purification, and separation procedures were measured in order to develop an economical industrial process of cyanidin-3-glucoside and cyanidin-3-rutinoside production from mulberry pomace. The recoveries of the extraction, purification, and separation procedures are presented in Table 4. The results showed that the recoveries of extraction, purification, and separation procedures were 95.0%, 91.0%, and 73.4%, respectively, and the recovery of the whole mulberry pomace anthocyanins utilisation process in our research was 57.4%. One of the indices of the evaluation of a natural anthocyanins source is the efficiency of its utility. The recovery of 57.4% indicated that the method of the mulberry pomace utilisation to gain cyanidin-3-glucoside and cyanidin-3-rutinoside in our experiment is effective, and that the use of mulberry pomace for anthocyanins production is of a hich value. Thus, the anthocyanins present in mulberry pomace were extracted by ethanol, purified on AB-8 macroporous resin, and separated on Sephadex LH-20. This process yielded cyanidin-3-glucoside and cyanidin-3-rutinoside with purity above 98% and recovery of 57.4%. However, in order to improve the recovery and reduce the cost even more, the process of extraction, purification, and separation needs a further research.

Table 4. The recovery of the process of extraction, purification and separation of the anthocyanins

Treatment process	Recovery (%)		
	cy 3-glu	cy 3-rut	total
Extraction	95.4	94.1	95.0
Purification	83.5	79.3	91.0
Separation	70.0	82.3	73.4
Complete process	55.8	61.4	57.4

cy – cyanidin; glu – glucoside; rut – rutinoside

Colour characteristics of the anthocyanins separated from mulberry pomace and their standards

Water solutions of cyanidin-3-glucoside and cyanidin-3-rutinoside separated from mulberry pomace and their standards, with absorbance of

Table 5. Free radical-scavenging activity (SC_{50}) of the cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace

	Scavenging ability on HO·	Scavenging ability on O_2^{-}·	Scavenging ability on DPPH
Cy 3-glu (μg/ml)	41.8 ± 7.2	32.3 ± 0.4	55.6 ± 0.5
Cy 3-rut (μg/ml)	92.2 ± 2.0	59.4 ± 3.7	86.6 ± 2.3
Trolox (μg/ml)	–	1989.0 ± 8.1	83.3 ± 2.2

SC_{50} values – amount of the mulberry pomace or the Trolox at which the free radical scavenging rate was 50%; SC_{50} value was obtained by interpolation from the regression analysis; each value is expressed as mean ± SD (n = 3)

0.3 nm at 520 nm and pH 3.5, were measured. The results showed that the colour characteristics of the cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace revealed no significant difference compared to their standards (except the L^* value) (Table 2). Both the cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace had an attractive colour as their standards.

Antioxidant activity of cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace

The scavenging free radical activities of the anthocyanins separated from mulberry pomace assayed herein were summarised in Table 5. In order to make the scavenging activities of the mulberry easier to understand, the standard antioxidant, Trolox, was adopted as the positive antioxidant and the results were normalised by computing the antioxidant concentration at which the scavenging rate was 50% and expressed as SC_{50}. The antioxidant properties were inversely correlated with the SC_{50} values. According to the SC_{50} values (Table 5), the scavenging free radical abilities of anthocyanins were different with different free radicals index adopted. The scavenging superoxide anion radical activity of the cyanidin-3-glucoside and cyanidin-3-rutinoside were 61 and 33 times that of Trolox. The DPPH radical scavenging activities of cyanidin-3-glucoside and cyanidin-3-rutinoside were similar to that of the Trolox. The SC_{50} of cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace on hydroxyl radicals were 41.8 μg/ml and 92.2 μg/ml respectively, which meant strong antioxidant activities. Figure 3 shows the decrease in the absorbance of β-carotene in the presence of cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace with the couple oxidation of β-carotene and linoleic acid. From the results shown in Figure 3, though cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry had lower antioxidant activities than Trolox at a concentration of 0.2 mg/ml, both of the two anthocyanins exhibited a good antioxidant activity in the linoleic acid peroxidation system. Therefore, it can be concluded from our experiments that cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace had high antioxidant activities.

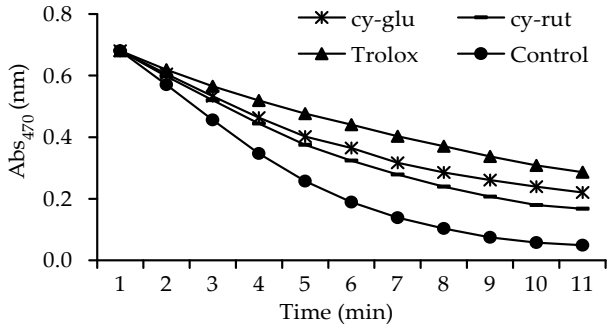

Figure 3. Antioxidant activity of the cyanidin-3-glucoside and cyanidin-3-rutinoside separated from mulberry pomace and that of Trolox at 0.2 mg/ml in β-carotene/linoleic acid system

CONCLUSION

Mulberry pomace possesses a very deep red colour. This paper compared the contents of anthocyanins in mulberry juice and pomace and ascertained that the mulberry pomace had high contents of anthocyanins (cv. Da-10 257.8 mg/100 g; cv. Hongguo 285.5 mg/100 g). The results of the colour characteristics measurement indicated that the anthocyanins present in mulberry pomace had attractive colours. Qualitative analysis of the anthocyanins showed the presence of five anthocyanins (cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargoni-

din-3-glucoside and pelargonidin-3-rutinoside) in mulberry pomace. Cyanidin-3-glucoside and cyanidin-3-rutinoside (above 98% of the total anthocyanins content) were the major anthocyanins, which was similar to the anthocyanins profile in mulberry fruit. In order to find an effective way to explore the anthocyanins in mulberry, the separation method of cyanidin-3-glucoside and cyanidin-3-rutinoside was studied. It was found that the anthocyanins in mulberry pomace could be separated well on Sephadex LH-20 and eluted with 10% ethanol with 1% acetic acid, the purity of both anthocyanins being above 98%. The recovery achieved over the whole extraction, purification, and separation process was 57.4%. The colour chroma and the hue angles of cyanidin-3-glucoside and cyanidin-3-rutinoside were similar to their commercial standards. Very important are the scavenging superoxide anion activities of cyanidin-3-glucoside and cyanidin-3-rutinoside from mulberry pomace, which were far in excess of the standard antioxidant, Trolox. The scavenging hydroxyl radical, DPPH radical activity, and antioxidant activity in β-carotene/linoleic acid system of the two anthocyanins from mulberry pomace were also strong. Thus, mulberry pomace could be an economical source of cyanidin-3-glucoside and cyanidin-3-rutinoside, both having a good colour and a strong antioxidant activity.

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