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Compositional Characterization and Colorant Identification of *Omija* (*Schizandra chinensis*) Fruit Extract

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Abstract A major polyphenolic compound extracted from *omija* (*Schisandra chinensis*) fruit was structurally identified, and its composition of major nutrients was investigated as well in this study. A dominating high performance liquid chromatography (HPLC) peak of water-extracted anthocyanin represented 94.1% of total absorbable compounds at 520 nm, which was further identified with HPLC-mass spectrometry (MS). As a result, mass-to-charge ratio (*m/z*) of the predominant anthocyanin was determined to be 727, and it was identical to molecular mass of cyanidin-3-xylosylrutinoside (Cya-3-*O*-xylrut). This is the first report that colorant of *omija* is predominantly composed of Cya-3-*O*-xylrut. *Omija* fruit contained exclusively 3 types of monosaccharide such as glucose (0.68 g), galactose (0.01 g), and fructose (0.52 g) per 100 g of fruits. Several organic acids, citric (3.29 g), malic (1.4 g), acetic (0.4 g), and succinic acids (0.36 g) per 100 g of fruits, were detected by high performance anion exchange chromatography (HPAEC) analysis. During the compositional analysis of free amino acid by HPLC, it was noticed that *omija* fruit contained substantial amount (0.01 g/100 g of fruits) of γ-amino butyric acid (GABA).

Keywords: *Schisandra chinensis*, *omija*, anthocyanin, cyanidin-3-xylosylrutinoside, liquid chromatography-mass spectrometry (LC-MS)

Introduction

Schisandra chinensis is known as *omija* in Korea, of which literal meaning is berries with 5 distinctive flavor notes. This monocecius perennial liana has attractive leaves and woody stems (1). Traditionally its seeds and fruits are considered a medicinal plant and are stored as dried form. Fairly recently the effect of *omija* extract on *kimchi* fermentation was investigated to improve quality of *kimchi* (2). Bright pinkish-red color of water extract of *omija* fruits makes them attractive natural colorant source. The pigments in *omija* are highly water-soluble and thus are of great interest in alcoholic and non-alcoholic beverage-type food processing. However, this cheerful color of *omija* extract is often unstable when it is exposed to heat, light, metal ion, organic acids, oxygen, and pH change (3,4). In recent years, a diet rich in colorful fruits and vegetables is recognized as a recommended nutritional treatment for preventing various adult diseases (obesity, atherosclerosis, senescence, and cancer) (5-7). At the center of this issue is the anthocyan that is one of the flavonoids. Especially antioxidant capacity of anthocyan is derived from structurally powerful electron-donating property (8-11).

The colorful characteristics of *omija* extract are presumably endowed from certain types of anthocyan but structural identification of *omija* anthocyans has not been carefully investigated. Anthocyans which include anthocyanin (glycoside) and anthocyanidin (aglycone) are ubiquitously distributed in the plant kingdom (12) and confer pH-dependent bright attractive red, yellow, blue, and purple color (13). Six core structures of anthocyanidin have been identified from higher plants, which are cyanidin, delphinidin, petunidin, peonidin, malvidin, and pelargonidin (14). These 6 basic forms are differentiated by the degree of methoxylation and hydroxylation. Most of them exist as glycoside of which sugar moiety is substituted at 3-OH group of C ring (15).

Anthocyans have recently received great attention as natural colorant in food systems and will be substituted for synthetic ones, as a consequence of the consumer's preference toward the consumption of natural products. Thus, new natural sources of pigments such as anthocyanin, with high colorant quality and stability, and low cost, are widely investigated (16). A detrimental factor obstructing industrial applications of anthocyan-derived colorants is its low stability in general food processing environments. The conditions affecting anthocyan color stability have been reported in many previous studies (17-21), and its stability is affected by the presence of other compounds such as sugars, organic acids, etc (22). In an effort to increase color stability of anthocyan, various treatments have been designed by pH control, sugar addition, acylation, and copigmentation (23,24).

The distinct color of *omija* fruit and water-extract is speculated to be derived from *omija*-specific anthocyans. However, there is no structural identification of what types of anthocyan exist in *omija* extract and what the proportion of them is in detail. Furthermore, quantitative analysis of main components in *omija* extract was performed. Because the compositional status of *omija* extract can affect the color stability, this information would be very important in

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the following process and storage steps of *omija*. Therefore, the compositional analysis of *omija* water-extract was pursued and the characterization of colorant was achieved in this study.

Materials and Methods

Omija sample and other materials Frozen *omija* fruit samples were kindly provided by KOOKSOONDANG (Seoul, Korea), which were cultivated at Mungyeong, Korea, and harvested in 2005. The frozen samples were stored at −70°C until further analysis. Cyanidin was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC) solvents and other chemicals were reagent grade.

Extraction of water and alcohol soluble components in *omija* fruit Frozen *omija* fruits (200 g) were soaked without stirring in 1 L of distilled water at 4°C for 24 hr. The residual *omija* fruits were removed by vacuum filtration and the filtrate was either used for analysis of free sugars, organic acids, and amino acids or further treated by Sep-pak® Plus C_{18} cartridge (Waters Corp., Milford, MA, USA) for colorant analysis. The Sep-pak® Plus C_{18} cartridge was pre-activated by methanol, and the filtrate was injected into it using a 10-mL syringe. After washing the cartridge with 0.01% methanol, the adsorbed colorants were eluted with 0.01%(v/v) HCl-methanol. Finally the *omija* extracts were concentrated using a speed-vacuum dryer (SPD1010 Intergrated SpeedVac System; Thermo Savant, Millford, MA, USA).

Analysis of free sugars by high performance anion-exchange chromatography (HPAEC) and pulsed amperometric detector (PAD) The water extract of *omija* fruits was diluted by 40 times and injected to a HPAEC system after filtration with a 0.45-μm membrane filter. A Dionex HPAEC system (DX 300; Dionex Corp., Sunnyvale, CA, USA) combining with a PAD was connected with an analytical Carbopac PA-1 column (Dionex, 4Φ×250 mm) for this analysis (25). The system had an injection volume of 10 μL, and the sample was separated at a flow rate of 1.0 mL/min. The sample was isocratically eluted with 18 mM NaOH, and 200 mM NaOH was used as a washing eluent. D(-)Fructose, D(+)glucose, and D(+)galactose were purchased from Sigma-Aldrich and used for establishing standard curves.

Analysis of organic acids by HPAEC and electrochemical detector (ECD) The water extract of *omija* fruits was diluted by 50 times and injected to a HPAEC system after filtration with a 0.45 mm membrane filter. A Dionex HPAEC system (DX 600; Dionex) combining with an ECD and an anion micromembrane suppressor AMMS-ICE II was connected with an analytical IonPac ICE-AS6 column (9Φ×250 mm) for this analysis (26,27). The system had an injection volume of 20 μL, and the sample was separated at a flow rate of 1.0 mL/min. The sample was isocratically eluted with 0.4 mM heptafluorobutyric acid, and 5.0 mM tetrabutylammonium hydroxide was used as a washing eluent. Citric acid, D(+)malic acid, succinic acid, and acetic acid were purchased from Sigma-Aldrich and

Table 1. Instrumentation and operating condition for HPLC analysis of *omija* colorant

	HPLC-PDA	LC-MS
HPLC system	LC-10AD VP (Shimadzu, Japan)	Agilent 1100 series (USA)
Column	Xterra™ (4.6 cm×250 mm, Waters, Ireland)	
Eluents	A: 0.1% Trifluoroacetic acid (TFA) B: Acetonitrile:H$_2$O in 0.1% TFA=1:1 (v/v)	

Elution mode (Gradient condition)	Elution time (min)	% Eluent B
	0	20
	18	33
	30	40
	40	40
	42	20
	50	20

Flow rate	0.8 mL/min	0.6 mL/min
UV detector	SPD-M10A VP Photo-diode array detector (Shimadzu, Japan)	Agilent 1200 series multiple wavelength detector and Agilent 110 series MSD (USA)
Detection range	190-800 nm	520 nm and m/z=100-1,500

used for establishing standard curves.

Analysis of amino acid and γ-aminobutyric acid (GABA) by HPLC Amino acids in 50 μL of *omija* extract were mixed with 250 μL of 100 mM borate buffer (pH 10) and derivatized with ortho-phthalaldehyde (C$_8$H$_6$O$_2$; OPA) reagent. The derivatized amino acids were analyzed by HPLC method. The filtered sample (20 μL) using 0.45 μm syringe filter was injected into a HPLC system (Summit® HPLC System; Dionex Corp.) equipped with a ultraviolet (UV) detector (detection wavelength=338 nm). A Nova-pak C_{18} analytical column (Waters Corp.) was used to separate amino acids at a flow rate of 1 mL/min. Potassium phosphate buffer (60 mM; pH 6.65) was prepared as eluent A, and eluent B consisted of eluent A:methanol:2-propanol:acetonitrile=46:18:18:18.

Structural identification of anthocyanin: HPLC-photo-diode array (PDA) and -mass spectrometry (MS) Fifty mL of *omija* colorants eluted from Sep-pak™ Plus C_{18} cartridge was concentrated to the final volume of 10 mL using a speed-vacuum drier. The HPLC elution conditions and operating parameters are summarized in Table 1. Mass spectra of baseline-separated major peak were obtained by using an Agilent 1100 series LC/MSD (Agilent Technologies, Palo Alto, CA, USA) using a positive electrospray mode. Sample solution was delivered to the electrospray source at a flow rate of 600 μL/min and the injection volume was 10 μL. The eluent used was 50%(v/v) of acetonitrile in deionized water with 0.1% TFA. The fragmentor voltage varied from 75 to 200 V, and the capillary voltage was maintained at 4 kV. Source temperature was maintained at 350°C. The drying gas flow in the source chamber was 11.5 L/min, and the detection scanning range was m/z 100 to 1,500.

Results and Discussion

Compositional analysis of free sugars, organic acids, and amino acids in water extract of *omija* fruit *Omija* extract was prepared by soaking the fruits (200 g) in 1.0 L of distilled water at 4°C for 24 hr. In order to determine the amount of major components (free sugars, organic acids, and amino acids) in the extract, the HPAEC and HPLC methods were applied in this study. In HPAEC analysis using a PA-1 analytical column, it was clearly identified that 3 monosaccharides existed in the water extract of *omija* fruit. It contained 0.68 g of glucose, 0.52 g of fructose, and 0.01 g of galactose per 100 g (fresh weight) of *omija* fruit. The extractable amount of glucose was 1.3 times higher than that of fructose, which was fairly agreeable to other study (28). No traceable amount of sucrose was detected while previous studies reported small but detectable amount of sucrose in the extract (29,30). Another peculiar finding in this study was that less than 1% but identifiable amount of galactose exist in *omija* fruit extract.

The pH of *omija* extract was determined to be 2.99 under our extraction condition, which was fairly low and were mainly attributed to the considerable amount of endogenous organic acids. In HPAEC analysis using an IonPac ICE-AS6 analytical column, 4 types of organic acids were identified and quantified. Citric (3.29 g), malic (1.40 g), acetic (0.4 g), and succinic acids (0.36 g) per 100 g (fresh weight) of *omija* fruit extract were observed in this quantification analysis. Tartaric acid was not observed in this study while other study reported the existence of tartaric acid in *omija* fruit (31). Oxalic acid is widely distributed in edible plants as either soluble or insoluble salt form (32). In this study there exists no detectable amount of oxalic acid in *omija* extract. Insoluble salt form, which is mainly calcium oxalate, may remain in the fruit tissue and may not freely diffuse out during water extraction. There was inconsistency in the reported amount

Table 2. Released amount of free amino acids in water extract of *omija* fruit

No.	Type of amino acid	Amount mg/100 g, fresh weight basis
1	Aspartic acid	1.74
2	Glutamic acid	4.58
3	Asparagine	0.88
4	Serine	0.65
5	Histidine+Glutamine	14.48
6	Arginine	4.82
7	Glycine	0.20
8	Alanine	0.57
9	Threonine	1.43
10	γ-Aminobutyric acid (GABA)	11.17
11	Tryptophan	2.80
12	Phenylalanine	0.62
13	Isoleucine	0.49
14	Leucine	0.28
15	Lysine	0.75

of oxalic acid in *omija*, which could be attributed to the applied instrumental analysis methods, the developmental stage of growing *omija*, and storage condition of harvested one.

In the extractable amino acids from *omija* fruit, total 14 or 15 amino acids plus GABA were detected and quantified by the HPLC analysis. It was known that 3 amino acids (cysteine, proline, and tyrosine) could not be analyzed using this OPA-derivatization method. Even if this fact was considered, still 2 amino acids, methionine and valine, were not observed in the water extract of *omija* fruit from this HPLC analysis. The quantified amounts of individual amino acids extracted from *omija* fruit are presented in
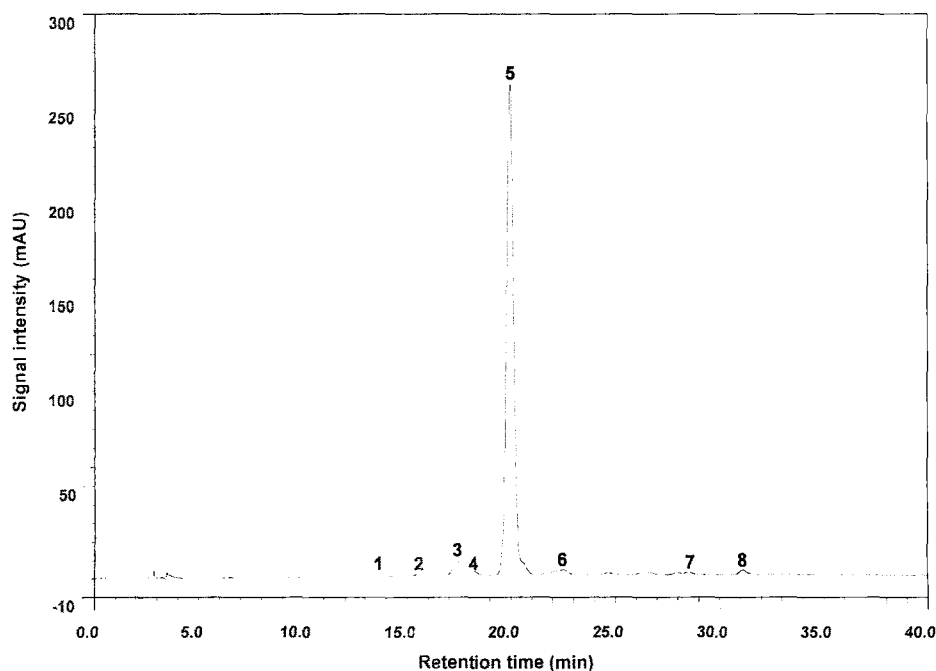

Fig. 1. HPLC separation profile of *omija* colorant extract at 520 nm.
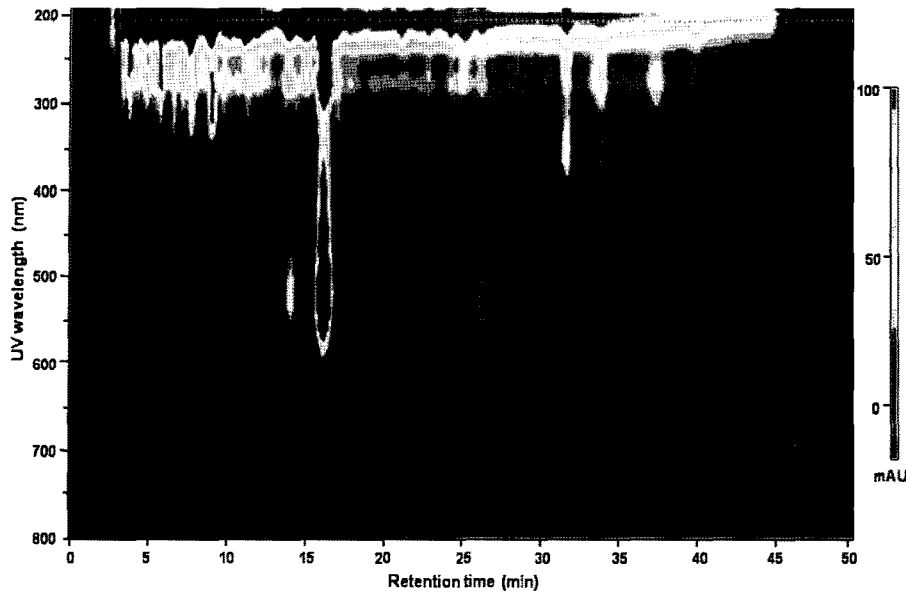
Fig. 2a. PDA detection diagram of *omija* colorant extract.
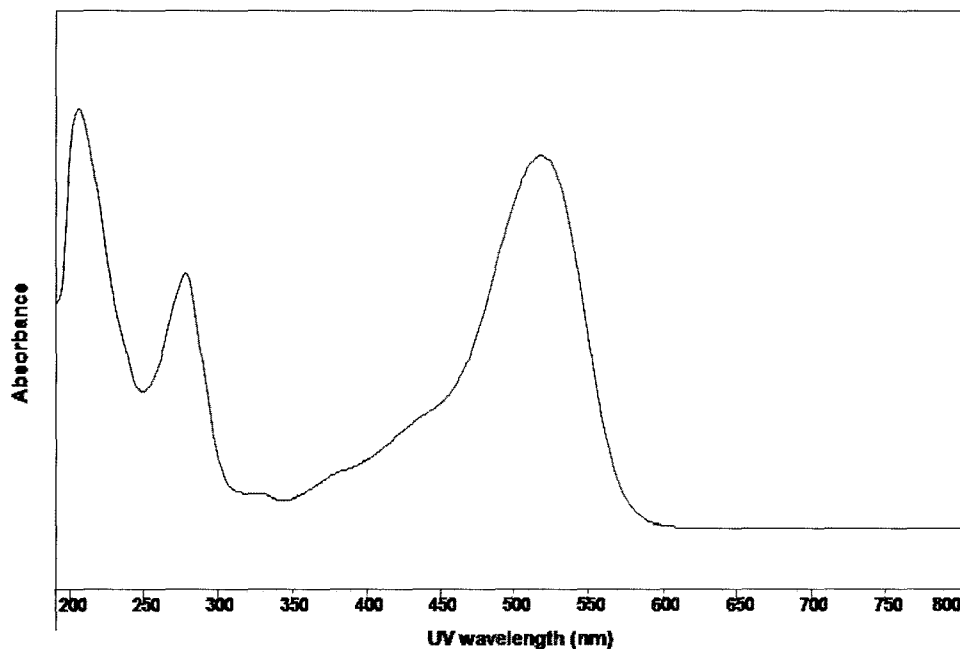

Fig. 2b. Full UV-Vis wavelength scan (190-800 nm) of the dominating component in *omija* colorant extract.

Table 2. The amount of histidine plus glutamine was the greatest among the detectable amino acids but further analysis should be achieved for identifying which one is the major amino acid between them. There were a couple of reports that GABA was detected in grape berries by either HPLC or ^1H NMR but the authors did not provide the quantified amount of GABA (33). Total extractable amount of free amino acids were determined to be 45.47 mg per 100 g of *omija* fruit, among which the GABA consisted of 24.6% (11.17 mg per 100 g) of total free amino acids. This result suggests that *omija* fruit could be one of excellent dietary sources of GABA.

Identification of *omija*'s anthocyans by HPLC-PDA analysis Among the identifiable 8 peaks in the chromatogram at 520 nm, during the preliminary test, only 1 dominating peak was considered for further analysis because this major peak 5 at 19.96 min accounted for 94.1% and other 7 peaks only explain less than 6% of total colorants (Fig. 1). In order to identify the number of anthocyan types in *omija* extract, PDA detection method was adopted. Once individual colorant was separated by an HPLC system, each peak was fully scanned from 190 to 800 nm of wavelength. As shown in Fig. 2a, the peak at the elution time of 16.24 min was dominating in the visible wavelength range, and the maximum absorption of the major peak was observed at 518 nm (Fig. 2b). A few of other minor peaks were shown but barely identifiable on the PDA diagram (Fig. 2a).
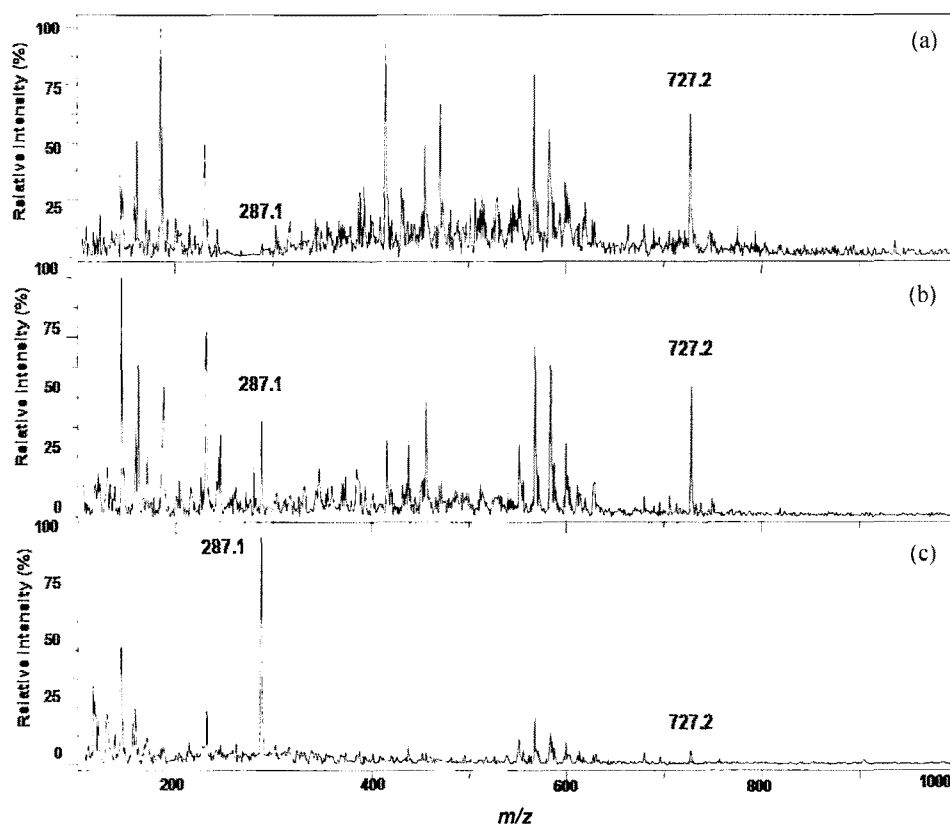
Fig. 3. ESI mass spectra of *omija* colorant extract. The MS fragmentor voltages are (a) 75, (b) 125, and (c) 180 eV.

Identification of *omija*'s major colorant by HPLC-MS analysis When we directly analyzed the *omija* extract by mass spectrometry without HPLC separation to obtain structural information of the major colorant by analyzing the fragmentation pattern, the variation of the mass spectra with applied fragmentor voltages was observed (Fig. 3). Based on the fragmentation pattern and mass-per-charge ratio of precursor ion, the major anthocyan was tentatively assigned as cyanidine-3-*O*-xylosylrutinoside (Cya-3-*O*-xylrut) in this study. Relative intensity of the Cya-3-*O*-xylrut (*m/z* 727) decreased with the increase of the fragmentor voltage whereas the *m/z* 287 assigned to cyanidin is generated and its relative intensity increased as shown in Fig. 3. The positively charged aglycone, cyanidin (*m/z* 287), was formed by loss of trisaccharide sugar moiety (xylosylrutinoside) from the Cya-3-*O*-xylrut (Fig. 4). In the analysis of anthocyanins, it was reported that the precursor ions detected were typically [M]⁺ and [M-sugar]⁺ (34). From our experimental result, it was concluded that even without chromatographic separation some principal components of the extract mixture could be identified with LC/MS by regulating the fragmentor voltage and comparing the mass spectra.

After HPLC separation of major anthocyan, it was further characterized with on-line MS system. Figure 5 shows the mass spectrum of the major peak in the chromatogram and a clear molecular mass ion of *m/z* 727.2, which is corresponding to that of Cya-3-*O*-xylrut. Recently, the positive ion ESI-MS of acidic methanol extract of black raspberry was shown to have a base peak at *m/z* 727, corresponding to M⁺ of Cya-3-*O*-xylrut and an aglycone fragment at *m/z* 287 (35). Further sophisticated structural

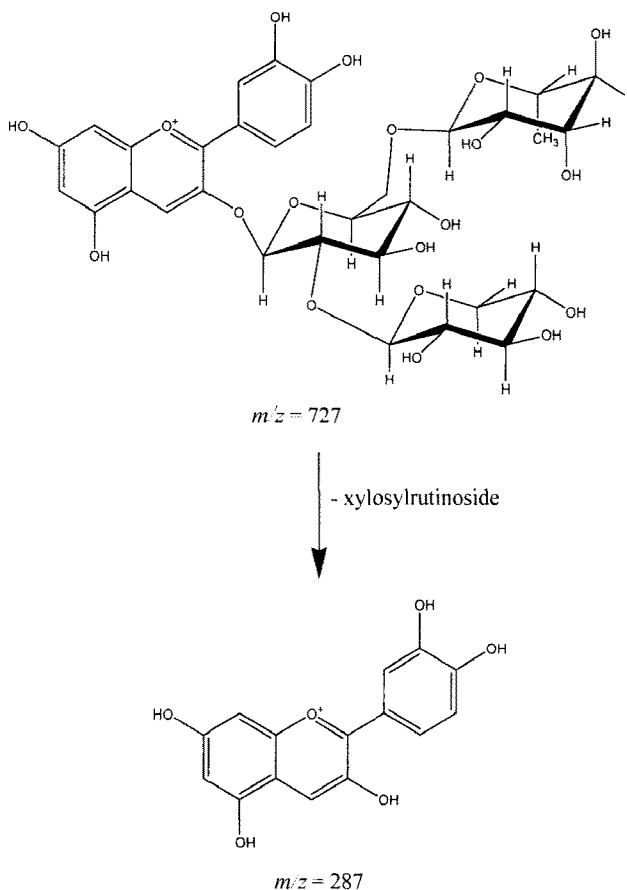

m/z = 727

- xylosylrutinoside

m/z = 287

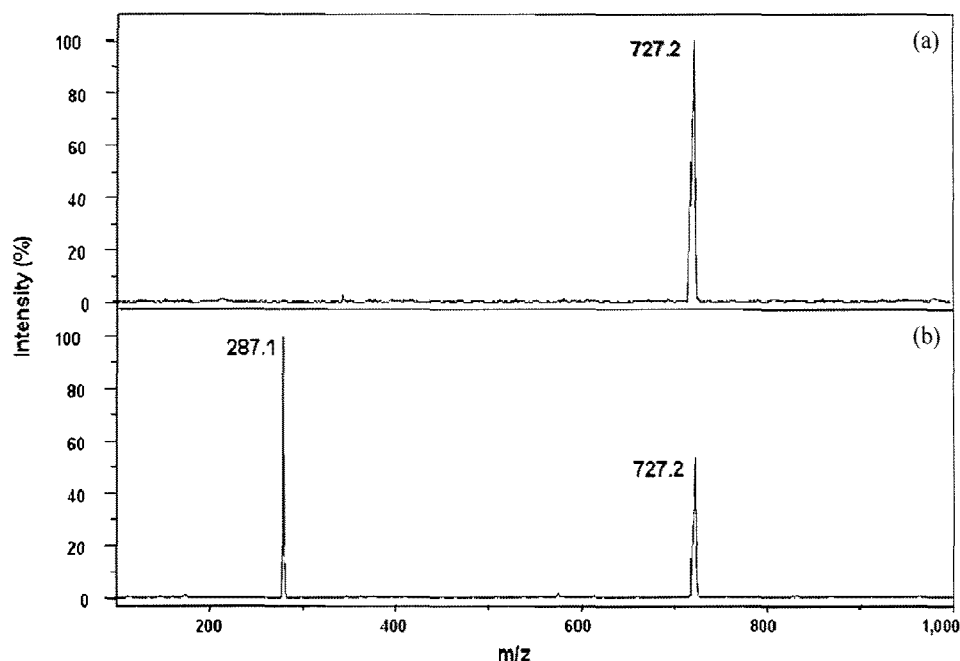Fig. 4. Fragmentation mechanism of cyanidin-3-*O*-xylosylrutinoside (Cya-3-*O*-xylrut).

Fig. 5. ESI mass spectra of the major anthocyan in *omija* colorant extract. The MS fragmentor voltage are (a) 75 and (b) 150 eV.

analysis should be performed to identify the exact chemical structure of this tentatively assigned Cya-3-*O*-xylrut from *omija* extract.

Omija's anthocyan profile consisting exclusively of Cya-3-*O*-xylrut has not been reported from any other plant materials. Thus, *omija* could be very unique source of highly pure Cya-3-*O*-xylrut. According to very few obtainable literature, Cya-3-*O*-xylrut was one of major anthocyanins in red current (*Ribus rubrum*) (28), and it was only minor component in blue berry (*Vaccinium padifolium*) (36). Fairly recently, it was also reported that black raspberry (*Rubus occidentalis*) contained substantial amount of Cya-3-*O*-xylrut (35). These are only 3 reported plant materials containing Cya-3-*O*-xylrut until now. Thus, it seems that Cya-3-*O*-xylrut is relatively rare in nature as pointed out by Cabrita *et al.* (36).

The profiles of the anthocyans present in fruit and vegetables can be used as fingerprints through which the authenticity of raw materials, products, juices and extracts may be assessed as described by Montoro *et al.* (34). This distinguishable peak pattern of *omija* extract under visible light absorption detection can provide a strong screening tool of adulteration in the processed *omija* foods. This identified Cya-3-*O*-xylrut in *omija* fruit is responsible for the bright reddish pink color of *omija* extract at proper pH range controlled by endogenous organic acids. Based on this structural characterization of *omija* colorant, further study will be pursued to improve color stability of *omija* extract and to increase applicability into food processing.

References

1. Hancke JL, Burgos RA, Ahumada F. *Schisandra chinensis* (Turcz.) Baill. Fitoterapia 70: 451-471 (1999)

2. Moon SW, Kim BK, Jang MS. Effects of *omija* (*Schizandra chinensis* Baillon) extract on the physicochemical properties of *nabakkimchi* during fermentation. Food Sci. Biotechnol. 15: 564-571 (2006)

3. Haglind C, Tengblad J. Effects of caffeine containing energy drinks. Scand. J. Nutr. 43: 169-175 (1994)

4. Tompsett T. Herbal attraction. Soft Drinks Int. 23: 27 (1999)

5. Nomura M, Kakachiyama M, Hida T, Ohtaki Y, Sudo K, Aizawa T, Aburada M, Miyamoto KZ. Gomisin A, a lignan component of *Scizandrora* fruits, inhibits development of preneoplastic lesions in rat liver by 3'-methyl-1,4-dimethylamino-azobenzene. Cancer Lett. 76: 11-18 (1994)

6. Ohtaki Y, Hida T, Hiramatsu K, Kanitani M, Ohshima T, Nomura M, Wakita H, Aburada M, Miyamoto KI. Deoxycholoic acid as an endogenous risk factor for hepatocarcinogenesis and effect of gomisin A, a lignan component of *Schizandra* fruits. Anticancer Res. 16: 751-755 (1996)

7. Rhyu MR, Kim EY, Yoon BK, Lee YJ, Chen SN. Aqueous extracts of *Schizandra chinensis* fruit cause endothelium-dependent and -independent relaxation of isolated rat thoracic aorta. Phytomedicine 13: 651-657 (2006)

8. Tamura H, Yamaganci A. Antioxidative activity of monoacylated anthocyanins isolated from muscat bailey a grape. J. Agr. Food Chem. 42: 1612-1615 (1994)

9. Wang H, Nair M, Strasburg G, Chang Y, Booren A, Gray J, DeWitt DL. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J. Nat. Prod. 62: 294-296 (1999)

10. Wang S, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J. Agr. Food Chem. 75: 5677-5684 (2000)

11. Tsuda T, Watanabe M, Ohshima K, Norinobu S, Sang WC, Kawakishi S, Osawa T. Antioxidative activity of the anthocyanin pigments cyaniding 3-*O*-β-D-glucoside and cyanidin. J. Agr. Food Chem. 42: 2407-2410 (1994)

12. Cooke D, Steward WP, Gescher AJ, Marczyro T. Anthocyans from fruits and vegetables - Does bright colour signal cancer chemopreventive activity? Eur. J. Cancer 41: 1931-1940 (2005)

13. Lapidot T, Harel S, Akiri B, Granit R, Kanner J. pH-dependent forms of red wine anthocyanins as antioxidants. J. Agr. Food Chem. 47: 67-70 (1999)

14. Escribano-Bailón MT, Santos-Buelga C, Rivas-Gonzalo JC. Anthocyanins in cereals. J. Chromatogr. A 1054: 129-141 (2004)

15. Zhang Y, Vareed SK, Nair MG. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in an elderly population. Life Sci. 76: 1465-1472 (2005)

16. Longo L, Vasapollo G. Extraction and identification of anthocyanins from *Smilax asperax* L. berries. Food Chem. 94: 226-231 (2006)

17. Kirca A, Özkan M, Cemeroôlu B. Effects of temperature, solid content, and pH on the stability of black carrot anthocyanins. Food Chem. 101: 2001-2008 (2007)

18. Bordignon LMT, Gauche D, Gris EF, Falcão LD. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. Food Sci. Technol. 40: 594-599 (2007)

19. Janna OA, Khairul AK, Maziah M. Anthocyanin stability studies in *Tibouchina semidecandra* L. Food Chem. 101: 1640-1646 (2007)

20. Mazza G, Brouillard R. Recent developments in the stabilization of anthocyanins in food products. Food Chem. 25: 207-225 (1987)

21. Markakis P. Stability of anthocyanin in food. pp. 163-180. In: Anthocyanin as Food Colors. Academic Press, New York, NY, USA (1982)

22. Jackmann RL, Yada RY, Tung MA, Speer RA. Anthocyanins as good colorants a review. J. Food Biochem. 11: 201-247 (1987)

23. Eiro MJ, Heinonen M. Anthocyanin color behavior and stability during storage. J. Agr. Food Chem. 50: 7461-7466 (2002)

24. Anthony JC, Sarabia V, Keenleyside W, Maclachlan PR, Whitfield C. The compositional analysis of bacterial extracellular polysaccharides by high performance anion-exchange chromatography. Anal. Biochem. 199: 67-84 (1991)

25. Fischer K, Kotalik J, Kettrup A. Chromatographic properties of the ion-exclusion column IonPac ICE-AS6 and its application in environmental analysis. Part II: Application in environmental analysis. J. Chromatogr. Sci. 38: 409-415 (2000)

26. Beighton D, Brailsford SR, Gilbert SC, Clark DT, Rao S, Wilkins JC, Tarelli E, Homer KA. Intra-oral acid production associated with eating whole or pulped raw fruits. Caries Res. 38: 341-349 (2004)

27. Lee JS, Lee SW. A study on the compositions of free sugars, lipids, and nonvolatile organic acids in parts of *omija*. Korean J. Diet. Culture 4: 177-179 (1989)

28. Goiffon JP, Mouly PP, Gaydou EM. Anthocyanin pigment determination in red fruit juices, concentrated juices, and syrups using liquid chromatography. Anal. Chim. Acta 382: 39-50 (1999)

29. Jeong PH, Kim YS, Shin DW. Changes of physicochemical characteristics of *Schizandra chinensis* during post harvest ripening of various temperatures. Korean J. Food Sci. Technol. 38: 469-474 (2006)

30. Kim YM, Kim DH, Yum CA. Changes in flavor component of *omija, Schizandra chinensis* Baillon, with various extraction times. Korean J. Soc. Food. Sci. 7: 27-34 (1991)

31. Hikino H, Kiso Y. Natural products for liver diseases. pp. 53-72. In: Economic and Medicinal Plant Research. Wagner H, Fransworth N (eds). Academic Press, London, UK (1988)

32. Judprasong K, Charoenkiatkul S, Sungpuag P, Vasanachitt K, Nakjamanong Y. Total and soluble oxalate contents in Thai vegetables, cereal grains, and legume seeds and their changes after cooking. J. Food Compos. Anal. 19: 340-347 (2006)

33. Pereira GE, Gaudillere JP, Pieri P, Hilbert G, Maucourt M, Deborde D, Moing A, Rolin D. Microclimate influence on mineral and metabolic profiles of grape berries. J. Agr. Food Chem. 54: 6765-6775 (2006)

34. Montoro P, Tuberoso CIG, Perrone A, Piacente S, Cabras P, Pizza C. Characterization by liquid chromatography-electrospray tandem mass spectrometry of anthocyanins in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. J. Chromatogr. A 1112: 232-240 (2006)

35. Hecht SS, Huang D, Stoner GD, Li J, Kenney PMJ, Sturla SJ, Carmella SG. Identification of cyanidin glycosides as constituents of freeze-dried black raspberries which inhibit anti-benzopyrene -7,8-diol-9,10-epoxide induced NFκB and AP-1 activity. Carcinogenesis 27: 1617-1626 (2006)

36. Cabrita L, Frøystein NÅ, Andersen ØM. Anthocyanin trisaccharides in blue berries of *Vaccinium padifolium*. Food Chem. 69: 33-36 (2000)