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Distribution of Free Amino Acids, Flavonoids, Total Phenolics, and Antioxidative Activities of Jujube (*Ziziphus jujuba*) Fruits and Seeds Harvested from Plants Grown in Korea

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ABSTRACT: Fruit pulp and seeds from the jujube plant possess nutritional and medicinal properties. The bioactive components have been shown to vary both with cultivar and with growing conditions. Most studies report the components of varieties from China. We measured free amino acid, individual phenolic, and total phenolic content, and antioxidative activities in three jujube fruit pulp extracts from Boeun-deachu, Mechu, and Sanzoin cultivars and two seed extracts (Mechu and Sanzoin) from plants grown in Korea. In g/100 g dry weight, total free amino acid content measured by ion-exchange chromatography ranged from 5.2 to 9.8 in the pulp and from 4.0 to 5.3 in the seed. Total phenolic content measured by Folin−Ciocalteu ranged from 1.1 to 2.4 in the pulp and from 3.6 to 4.6 in the seed. Flavonoids were measured by HPLC and ranged from 0.7 to 1.8 in the pulp and from 3.2 to 4.0 in the seed. Flavonoids were identified by HPLC elution position and UV/vis and mass spectra. Fruits contained the following flavonoids: procyanidin B2, epicatechin, quercetin-3-*O*-rutinoside (Q-3-R), quercetin-3-*O*-galactoside (Q-3-G), kaempferol-glucosyl-rhamnoside (K-G-R), and two unidentified compounds. Seeds contained the following flavonoids: saponarin, spinosin, vitexin, swertish, 6‴-hydroxybenzoylspinosin (6‴-HBS), 6‴-feruloylspinosin (6‴-FS), and one unidentified substance. Dimensions and weights of the fresh fruit samples affected phenolic content. The distribution of the individual flavonoids among the different samples varied widely. Data determined by the FRAP antioxidative assay were well correlated with total phenolic content. In a departure from other studies, data from the DPPH free radical assay were not correlated with FRAP or with any of the measured compositional parameters. Because individual jujube flavonoids are reported to exhibit different health-promoting effects, knowledge of the composition and concentration of bioactive compounds of jujube products can benefit consumers.

KEYWORDS: jujube fruit, jujube seeds, free amino acids, phenolic compounds, antioxidative effects, functional food

INTRODUCTION

The Indian jujube, *Ziziphus mauritiana* Lam., (ber), and the Chinese jujube, *Z. jujuba* Mill. (common jujube), are the two major domesticated jujubes.[1] The fruit is widely cultivated from southwest Europe to China, including India and the Middle East.[2] China is the largest producer of the common jujube, at about 450,000 tons annually.[2] Jujube pulp is eaten mostly fresh, but may be dried (Chinese dates and tea) or processed into confectionary recipes in bread, cakes, compotes, and candy.[3] In addition to their food uses, jujubes have been used in many traditional medicines and have been shown to exhibit numerous health-promoting effects including antimicrobial and antiviral properties, reviewed in ref 4. Jujube seeds are especially known for their sedative effect.[5] Jujube seed flavonoids may in part contribute to this effect.[6] Jujubes are nutritious, being high in flavonoids and vitamins C, B_1, and B_2.[4] Jujubes may therefore be considered as a so-called functional food, having nutritional as well as medicinal uses. In the United States, jujube products are available at health food stores.

Besides export of dried jujube, the majority of the jujube crop is produced and consumed locally.[1] The market for fresh jujube is mostly restricted to producing regions for the following reasons: demand for fresh fruits, lack of awareness, lack of quality standards, lack of processing standards, and lack of market information systems and infrastructure.[1] FAOstat does not maintain statistics on this crop. The International Centre for Underutilized Crops in Southampton, U.K., has identified jujube as a crop with substantial growth potential.[1]

Korea is a high demand market with significant production taking place locally in Boeun County. These Korean cultivars may be distinguished from the more extensively studied Chinese varieties. Although jujube fruit is an excellent source of flavonoids, variation in levels by geographic origin is not well documented. Fruits grown under harsh conditions, e.g. arid or high-altitude, may contain more antioxidants than fruits grown under milder conditions.[7] Flavonoids may also vary significantly by variety.[8] It is of interest to analyze the composition of jujubes from Korea and compare the results to the more frequently studied Chinese jujube.

To facilitate selection of cultivars that are locally acceptable and provide optimum benefits as functional foods, the main objectives of this study were (a) to determine total phenolic and individual flavonoid content of fruits and seeds from the same plants grown in Korea; (b) to compare radical scavenging and antioxidative activities of fruit and seed extracts; and (c) to correlate total

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Table 1. Korean Jujube Fruits and Seeds Used in the Present Study[a]

variety	color	length (cm)	width (cm)	wt (g)/fruit	% water
Boeun-deachu fruit	red	4.7 ± 0.06	3.1 ± 0.10	16.7 ± 0.82	78.0
Mechu fruit	red	2.7 ± 0.26	2.5 ± 0.31	4.9 ± 0.57	86.2
Sanzoin fruit	red	1.8 ± 0.08	1.5 ± 0.09	1.9 ± 0.19	86.4
Mechu seed	dark brown	0.87 ± 0.02	0.58 ± 0.02	0.292 ± 0.010	91.9
Sanzoin seed	dark brown	0.69 ± 0.05	0.52 ± 0.04	0.221 ± 0.018	91.3

[a] Boeun-deachu seeds were not present in the fruit. Listed values are averages ± SD (n = 3).

phenolic and flavonoid content with antioxidative activities. Free amino acids were also determined because of their role in product quality during food processing.

MATERIALS AND METHODS

Materials. Quercetin-3-*O*-galactoside (Q-3-G, lot no. 1438413, 98.2%), (−)-epicatechin (lot no. 1423660, 96.9%), quercetin-3-*O*-rutinoside (Q-3-R, lot no. BCBB6172, 95.3%), tannic acid (lot no. 082K0037, ≥98%), quercetin (lot no. 113K1051, ≥98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH, cat no. D9132, lot no. 12K1944, ≥90%) and 2(3)-*t*-butyl-4-hydroxyanisole (BHA, cat no. B1253, lot no. 098K0242, ≥95%) were purchased from Sigma (St. Louis, MO). Folin−Ciocalteu phenol reagent (lot no. OF1181) was purchased from Junsei Chemical Co., LTD (Tokyo, Japan). Tripyridyltriazine (TPTZ, lot no. FHL01, ≥98%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Procyanidin B2 (Fluka #42157, lot no. BCBB3135, 90.2%) was purchased from Aldrich (Milwaukee, WI). Spinosin (lot no. 10102221, ≥90% from *Z. psinsa* Hu) was purchased from Tauto Biotech (Shanghai, P. R. China). All other reagents (analytical grade) were obtained from commercial sources. HPLC grade acetonitrile and formic acid were purchased from J.T. Baker (Phillipsburg, NJ) and Aldrich (Milwaukee, WI), respectively. The solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA) and degassed in an ultrasonic bath before use.

Sampling of Jujube Fruits. Three varieties of Korean jujube fruits were used in this experiment. Boeun-deachu (*Z. jujuba* forma hoonensis C.S.Yook) is an improved variety, widely cultivated in Korea. This variety contains no seed in the shell. Mechu (*Z. jujuba* var. intermis Rehder) is the original cultivar found in Korea. Sanzoin (*Z. jujuba* Miller) is the parent stock for commercial jujube production. The seeds of Sanzoin are commonly used in Chinese medicine. Jujube fruits were grown in a field station in Boeun-gun, Chungbuk, Korea, and were harvested on October 8−12, 2009 (optimum harvest stage for market). Fruits were collected, weighed, and measured for size as shown in Table 1.

Extraction of Amino Acids and Phenolic Compounds from Jujube Fruits and Seeds. Ten fresh, uniform-sized jujube fruits from each variety were selected for analysis. The fruits were divided into pulps and shells with a knife. The pulps were cut into 2 × 2 mm slices with a clasp knife and mixed well. A sample of the pulp mixture (6 to 8 g) was macerated in a glass mortar to which was added 80% methanol/water (20 mL). The suspension was then centrifuged at 12000*g* for 10 min at 10 °C. The residue was re-extracted three times with 80% methanol (20 mL) and centrifuged. The combined supernatants were diluted to 100 mL with 80% methanol. The shells of the varieties Mechu and Sanzoin (Boeun-deachu contains no seeds) were crushed with a pincers, and the cotyledons (seeds) were collected. The seeds were cut into 2 × 2 mm slices with a clasp knife and mixed well. The seed samples (2 to 3 g) were extracted with the same method as the pulps, and the volume was adjusted to 100 mL with 80% methanol. An aliquot (20 mL) was concentrated in a rotary evaporator at 30 °C, and the residue was dissolved in 80% ethanol (2.0 mL). The supernatant was used for analysis of amino acids, phenolic compounds, and antioxidant activities.

Analysis of Amino Acids. The analysis was carried out by ion-exchange chromatography using methods adapted from the literature.[9,10] Briefly, the supernatant (10 μL) obtained from the above extraction was used for determination of free amino acids. A Hitachi model L-8800 amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan) and a column packed with Hitachi custom ion-exchange resin 2622 (4.6 i.d. × 60 mm, particle size 5 μm) were used for the amino acids. Lithium citrate buffer and ninhydrin flow rates were 0.35 and 0.30 mL/min, respectively. The column temperature was 30−70 °C and the reaction coil temperature, 135 °C.

LC/MS Analysis of Phenolic Compounds. High-performance liquid chromatography-diode array detection (HPLC-DAD) analysis was performed on a liquid chromatography system (Agilent 1200 Series, Agilent Technologies, Santa Clara, CA). Data was acquired and processed with Analyst software (Applied Biosystems Inc., Foster City, CA).

The supernatant (20 μL) obtained from the above extraction was directly injected onto an Inertsil ODS-3v (GL Science Inc., Tokyo, Japan) 5 μm, 4.6 i.d. × 250 mm HPLC column. The mobile phase consisted of the following linear gradient: acetonitrile (A) and 0.5% formic acid (B): (A) = 5% (0−5 min), 18% (5.1−30 min), 70% (30.1−90 min), 90% (90.1−100 min), and 5% (100.1−120 min). The flow rate was 0.8 mL/min at 30 °C. Peaks were monitored at 340 nm, and UV/vis spectra were recorded.

LC/MS experiments were performed with the 3200 Q TRAP LC/MS/MS system (Applied Biosystems Inc., Foster City, CA) equipped with an HPLC system (Agilent Technologies, Santa Clara, CA) connected to a photodiode array detector. The phenolic mixture solution (20 μL) was applied on an Inertsil ODS-3v (GL Science Inc., Tokyo, Japan) 5 μm, 4.6 i.d. × 250 mm HPLC column to separate the phenolic compounds using a gradient system consisting of acetonitrile containing 0.5% formic acid at a flow rate of 0.8 mL/min. UV wavelength was set to 340 nm. The LC eluate was introduced into the mass spectrometer from 5 to 40 min. Mass (MS) and tandem mass spectrometry (MS/MS) were operated in the negative-ion mode in the mass range of m/z 160−1200. Helium was used as the collision gas for the MS/MS spectrometric procedures, followed by the isolation of ions over a selected mass window of 2 Da. MS/MS represents multiple stages of precursor ion m/z selection followed by product ion detection for successive progeny ions. Mass selection of the analyte by m/z was followed by fragmentation and analysis of the fragments. For quantification, integrated peak areas were compared to peak areas of known amounts of standard samples.

Determination of Total Phenolics and Antioxidant Activities. The supernatant (500 μL) obtained from the above extraction was placed in a 10 mL vial and then dried completely at 30 °C under reduced pressure. Each residue was weighed and then dissolved in 10% DMSO in water (10 mL). This solution was used for the determination of total phenolic content and antioxidant activities as shown below.

Determination of Total Phenolic Compounds. Total phenolic content (TP) was measured using a modified colorimetric Folin−Ciocalteu method.[11] The extract solution (1.0 mL, 0.5−1.0 mg/mL) from samples of jujube fruits and seeds was mixed with 10% Na_2CO_3 solutions (1.5 mL) and incubated at room temperature for 2 min. After addition of 50% Folin−Ciocalteu phenol reagents (500 μL) and water (7 mL), the reaction tube was further incubated for 1 h at room temperature, followed by

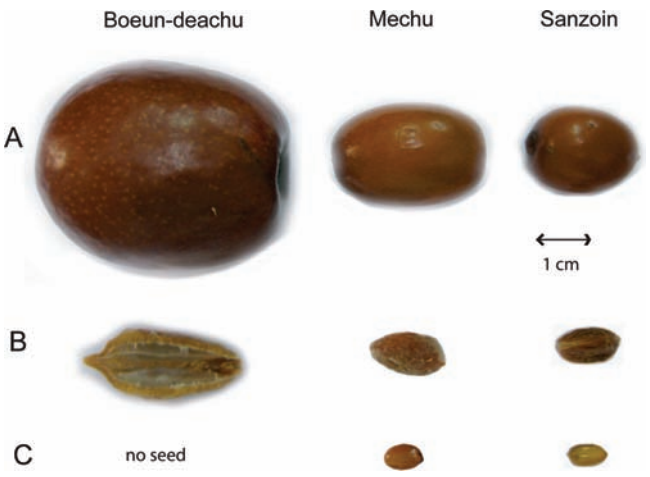

Figure 1. Photographs of whole (A), shell (B), and seed (C) of three varieties of Korea jujube fruits used in this experiment. Note: There is no seed in the shell of Boeun-deachu.

centrifugation at 5000*g* for 10 min and, finally, reading of the absorbance at 700 nm. Measurements were carried out in triplicate, and the values were based on a standard curve obtained with tannic acid. Concentrations of total phenolic compounds are expressed as tannic acid equivalents (g/100 g dry wt) of each jujube extract.

Ferric Reducing/Antioxidant Power Assay. For the determination of antioxidant capacity of jujube samples, the ferric reducing/antioxidant power (FRAP) assay was used. The assay measures the ability of antioxidants contained in the samples to reduce ferric-tripyridyltriazine (Fe^{3+} TPTZ) to the ferrous form (Fe^{2+}). The antioxidant capacity of the samples (10 μL) was measured spectrophotometrically at 593 nm.[12] The ferro and ferric ion complex with TPTZ reagent is the main product of this reaction.

DPPH Radical Scavenging Assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effects of the samples were measured by the method of Brand-Williams et al.[13] with some modifications. Different dilutions (0.8 mL, 100−1000 μg/mL) of each sample were added to 0.15 mM DPPH (0.2 mL). The commercial antioxidant BHA was used as a positive reference. After a 30 min incubation period at room temperature, the absorbance was read at 517 nm against a blank. The inhibition of the DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (1 - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} is the absorbance of the test sample. The antioxidant activity of sample was expressed as IC_{50}, defined as the concentration of sample required to inhibit the formation of DPPH radicals by 50%.

Statistical Analysis. Statistical analysis was determined with the aid of SigmaPlot 11 (Systat Software, Inc., San Jose, CA, U.S.A.). The calculated Pearson Product Moment Correlation Coefficient was used to compare results from the different assays.

RESULTS AND DISCUSSION

Dimensions of Jujube Fruits. Figure 1 shows photographs of whole fruit, shells, and seeds (Boeun-deachu contains no seed) of the three jujube fruits evaluated in this study. Table 1 lists the dimensions and weights of the three fruits and two seeds. The data show that there was a considerable difference in size between the varieties. We were interested in finding out which variety contains the highest total phenolic and flavonoid content per fruit and per unit weight.

Free Amino Acid Content of Jujube Fruits and Seeds. Free amino acids in plant foods exert a dual role in the diet. They represent a source of nitrogen and of nutritionally essential amino acids such as Lys, Met, and Thr. They can also participate in reactions to form browning products. One browning product, acrylamide, formed from free Asn and glucose during food processing is potentially toxic.[14] We and other investigators suggested that one way to mitigate the formation of acrylamide during processing of plant foods is to reduce the biosynthesis of asparagine. The total and the relative amount of free Asn affects the amount of acrylamide formed. The reducing amino acid Cys can lower formation of acrylamide.[15] Here, we describe the distribution of free amino acids in three jujube fruits and two jujube seeds listed in Table 2 in the order of elution positions. Asn, Glu, and Gln were not baseline resolved, but standard curves showed that the integration software was able to accurately calculate the values.

Table 2 shows the concentrations of the individual as well as the sums of total and total essential amino acids. The levels of total essential amino acids, His, Ile, Leu, Lys, Met, Phe, Thr, and Trp are much higher in the seeds than in the fruit. Arg was higher in both the seeds and the fruit of Mechu variety than the other varieties. Cys was only found in the fruit of Sanzoin, and only in a small amount. The seeds had higher levels of Tyr than the fruit. Pro is the most abundant free amino acid in the fruits, followed by Asn. The seeds contained minimal amounts of Pro. The most prevalent amino acids in the seeds were Gln, Glu, and Asn. Asn is of interest because, as mentioned earlier, this free amino acid can contribute to formation of acrylamide. This amino acid was found in all samples in significant quantity. The amount in Mechu fruit was high; about 4 times the amount in the other samples.

In addition to genetically coded amino acids, the extracts also contained the following N-containing compounds that are either formed post-translationally from amino acids or have been independently synthesized in the plant: β-alanine (β-Ala), 4-aminobutyric acid (4Abu), carnitine (Car), citrulline (Cit), ethanolamine ($EtNH_2$), hydroxylysine (Hyl), *N*-methylhistidine (Met-His), and phosphoserine (p-Ser).

β-Ala levels were low and similar for all samples. 4Abu levels were higher in both the seed and fruit in the Mechu variety than the other varieties. Car, Hyl, and Met-His levels were low or zero in all samples. Cit was very low in the fruits of Boeun-deachu and Mechu. All the seeds contained much higher amounts than the fruits of $EtNH_2$ and p-Ser. The changes in the N-containing ingredients do not appear to follow in any apparent order or trend.

Bioactive Compounds in Jujube from Different Geographical Locations. To place our findings in proper perspective, we will first summarize reported compositional data of jujube cultivars originating from different geographical locations in chronological order. Malik et al.[16] isolated eight monomeric catechins from leaves and bark of *Z. jujuba* grown in Uzbekistan. They did not report actual concentrations of the individual phenolics. Cheng et al.[17] isolated eight flavonoid compounds from seeds of *Z. jujube* var. spinosa from plants grown in China. They did not report the concentrations of individual flavonoids in the seeds. Hudina et al.[18] determined the phenolic content of 8 cultivars harvested in China. The individual phenolic content (in mg/100 g air-dried fruit) for the different cultivars ranged as follows: chlorogenic acid (0.22−0.95); caffeic acid (0.09−0.37); Q-3-R (0.89−5.77); (±)-catechin (0.74−2.12); (−)-catechin

Table 2. Concentration of Free Amino Acids in Pulp and Seeds (Cotyledon) of Jujube Fruits[a]

	variety and plant part								
	pulp						seed		
	Boeun-deachu		Mechu		Sanzoin		Mechu		Sanzoin	
amino acid	mg/100 g dry wt	% of total	mg/100 g dry wt	% of total	mg/100 g dry wt	% of total	mg/100 g dry wt	% of total	mg/100 g dry wt	% of total
p-Ser	66.0 ± 5.2	1.26	69.7 ± 3.0	0.71	73.6 ± 5.6	1.21	83.0 ± 5.2	1.55	130.6 ± 16.9	3.27
L-Asp	63.7 ± 3.0	1.22	96.4 ± 21.5	0.99	9.0 ± 1.8	0.15	164.9 ± 19.9	3.09	122.4 ± 21.1	3.06
L-Thr	8.0 ± 0.8	0.15	114.4 ± 4.3	1.17	162.5 ± 10.3	2.66	141.0 ± 9.7	2.64	99.3 ± 5.1	2.48
L-Ser	33.8 ± 5.7	0.65	55.7 ± 3.4	0.57	50.4 ± 1.5	0.83	119.9 ± 10.5	2.24	68.7 ± 3.2	1.72
L-Asn	1213.0 ± 46.6	23.17	4101.5 ± 151.4	41.95	940.1 ± 20.5	15.39	586.3 ± 13.1	10.97	924.3 ± 29.1	23.12
L-Glu	120.0 ± 7.3	2.29	164.8 ± 12.2	1.69	145.3 ± 12.8	2.38	805.4 ± 23.7	15.07	561.5 ± 44.3	14.05
L-Gln	176.2 ± 7.8	3.37	125.9 ± 7.4	1.29	239.0 ± 14.5	3.91	1320.2 ± 64.9	24.7	521.8 ± 36.1	13.05
L-Pro	3322 ± 95.0	63.45	4350.1 ± 94.5	44.49	3989.8 ± 90.6	65.31	218.6 ± 13.8	4.09	214.4 ± 17.6	5.36
L-Gly	5.0 ± 1.3	0.1	13.8 ± 6.0	0.14	7.7 ± 1.0	0.13	122.3 ± 15.3	2.29	56.7 ± 4.3	1.42
L-Ala	23.2 ± 1.4	0.44	44.4 ± 4.3	0.46	31.5 ± 2.2	0.52	244.9 ± 25.3	4.58	228.6 ± 23.4	5.72
L-Cit	2.3 ± 0.4	0.04	2.5 ± 1.2	0.03	18.4 ± 5.1	0.30	12.5 ± 1.7	0.23	10.6 ± 2.4	0.26
L-Cys	nd	0	0.4 ± 0.1	0	4.7 ± 1.3	0.08	nd	0	nd	0
L-Val	6.5 ± 1.4	0.12	17.3 ± 1.7	0.18	24.9 ± 5.3	0.41	181.0 ± 12.6	3.39	165.1 ± 15.7	4.13
L-Met	0.3 ± 0.1	0.01	0.4 ± 0.2	0	0.4 ± 0.1	0.01	26.8 ± 8.9	0.5	12.0 ± 2.6	0.3
L-Ile	2.4 ± 0.5	0.05	4.4 ± 0.5	0.05	5.5 ± 0.8	0.09	104.0 ± 11.0	1.94	64.7 ± 7.8	1.62
L-Leu	4.1 ± 1.2	0.08	11.1 ± 1.5	0.11	9.0 ± 1.2	0.15	140.5 ± 23.5	2.63	75.5 ± 7.1	1.89
L-Tyr	1.1 ± 0.3	0.02	4.7 ± 2.4	0.05	11.2 ± 1.0	0.18	95.9 ± 15.7	1.79	61.5 ± 8.6	1.54
L-Phe	1.3 ± 0.2	0.03	2.8 ± 0.7	0.03	6.4 ± 1.0	0.10	158.8 ± 15.9	2.97	122.0 ± 19.9	3.05
β-Ala	4.4 ± 0.6	0.08	11.2 ± 4.4	0.11	6.0 ± 1.0	0.10	7.7 ± 3.0	0.14	5.3 ± 1.5	0.13
4Abu	77.5 ± 11.0	1.48	170.7 ± 5.0	1.75	60.9 ± 6.5	1.00	206.0 ± 16.3	3.86	92.0 ± 2.6	2.3
Trp	nd	0	nd	0	4.9 ± 1.2	0.08	14.8 ± 3.7	0.28	20.8 ± 3.9	0.52
$EtNH_2$	3.2 ± 0.4	0.06	7.2 ± 1.7	0.07	7.9 ± 2.0	0.13	29.9 ± 4.7	0.56	33.3 ± 2.8	0.83
Hyl	2.4 ± 0.8	0.05	2.2 ± 0.9	0.02	1.7 ± 0.5	0.03	nd	0	nd	0
L-Lys	2.5 ± 0.8	0.05	8.3 ± 1.7	0.09	5.8 ± 1.3	0.10	54.9 ± 7.7	1.03	22.4 ± 2.9	0.56
Met-His	0.7 ± 0.3	0.01	nd	0	0.0 ± 0.0	0.00	nd	0	2.0 ± 0.7	0.05
L-His	9.5 ± 4.3	0.18	18.3 ± 1.2	0.19	48.6 ± 5.5	0.30	60.4 ± 7.5	1.13	43.0 ± 3.1	1.08
L-Car	1.1 ± 0.3	0.02	0.8 ± 0.2	0.01	2.9 ± 1.8	0.05	nd	0	nd	0
L-Arg	85.6 ± 3.9	1.63	378.5 ± 4.0	3.87	240.1 ± 16.3	3.93	444.9 ± 14.7	8.32	339.0 ± 16.1	8.48
total	5236	100	9778	100	6109	100	5345	100	3997	100
total essential[b]	28.1	0.5	159.7	1.6	243.2	4.0	701.1	13.1	459.7	11.5

[a] Values are averages of duplicate determinations ± SD; amino acid abbreviations follow IUPAC standard; nd, not detected. [b] Sum of His, Ile, Leu, Lys, Met, Phe, Thr, and Trp.

(0.48−513). Similar results are reported by San and Yildirim[19] for Turkish jujube cultivars and by Wang et al.[20] for Chinese cultivars, who concluded that most phenolic compounds with antioxidative activity in different tissues of jujube are present as the glycoside, insoluble-bound forms. Xue et al.[21] determined the total phenolic content of peel and pulp of three Chinese jujube cultivars. They found that the total phenolic content in the peel was 5−6 times higher than in the pulp and that the antioxidative values determined by FRAP and TEAC methods of the peel correlated to the total phenolic content. Bai et al.[22] isolated three flavonoids from Chinese *Z. jujuba* seeds by high-speed counter-current chromatography. They did not report levels of these compounds in the seeds. Pawlowska et al.[23] reported the following quantitative amounts (in mg/100 g dry wt) of flavonoids from jujube fruit grown in Italy: kaemferol-3-*O*-robinobioside (68); kaempferol-3-*O*-rutinoside (48); quercetin- 3-*O*-robinobioside (746); Q-3-R (814); quercetin-3-*O*-α-L-arabnosyl-(1→2)-α-rhamnoside (407); quercetin-3-*O*-β-D-xylosyl-(1→2)-α-rhamnoside (61). These results show that the Italian fruit had a high content of flavonoids. Zhang et al.[24] found that the peel of different jujube species has a high antioxidant capacity that is associated with the high phenolic content, and Sun et al.[7] used TOPSIS (technique for order performance by similarity to ideal solution) to predict factors that affect antioxidative potential of jujube fruit. The correlation analysis showed that fruit originating in Nigeria, Gansu, and Shaanbei grown in semiarid regions had the highest activity. Finally, Niu et al.[25] identified a number of flavonoids from seeds of Chinese *Z. spinosa*. They did not report on the levels of the individual flavonoids in the seed.

The cited studies show the wide variation in composition among jujube cultivars (species) from different geographic locations and that few studies reported actual amounts of

bioactive compounds present in the fruit and seeds. One objective of the present study was to investigate the composition as well as levels of individual compounds of both fruit and seeds from the same Korean plant grown under known environmental conditions.

Identification of Flavonoids in Pulp of Korean Jujube Fruits. Jujube extracts were analyzed by HPLC in conjunction with DAD and MS detectors. Based on chromatographic retention times (t_R), UV/vis spectra, and MS and MS/MS data of authentic standard, peaks 1, 2, 4, 5 were identified as procyanidin B2, epicatechin, Q-3-R, and Q-3-G, respectively (Table 3). The structures of these compounds are shown in Figure 2A.

Peak 6 showed a $[M - H]^-$ ion of m/z = 593.7 with retention time at 43.04 min. The fragment ion of m/z = 285.1 (kaempferol moiety) in MS/MS was produced by loss of hexose-rhamnose. From these results and other studies,[10,26] peak 6 was identified as kaempferol-glucosyl-rhamnoside (KGR). Figure 3A shows the unique spectra (MS and UV) for each of the peaks.

Pawlowska et al.[23] found 6 quercetin and kaempferol derivatives in *Z. jujube* harvested in Italy, but only one derivative was common to our jujube fruits, Q-3-R. The others differed from the flavonoids in our sample mostly by the substituted sugar(s). San and Yildirim[19] found predominantly catechin and Q-3-R in Turkish *Z. jujuba* Miller, but also epicatechin and the phenolic compounds caffeic acid, ferulic acid, *p*-hydroxybenzoic acid, and chlorogenic acid. Hudina et al.[18] found epicatechin and Q-3-R in a number of Chinese varieties, along with catechin, caffeic acid, and chlorogenic acid. It seems most jujube fruits have the compound Q-3-R in common.

Identification of Flavonoids in Jujube Seeds. Jujube seed extracts were also analyzed by HPLC in conjunction with DAD and MS detection. Table 4 shows the indentifying characteristics of the peaks from jujube seeds. The structures of the identified compounds are shown in Figure 2B.

Peak 1 showed a $[M - H]^-$ ion of m/z = 593.2 with retention time at 38.46 min. The fragment ion of m/z = 413.3 in MS/MS was produced by loss of a glucose unit ($[M - H]^-$ m/z = 593.2 − 413.3 = 179.9), and also m/z = 293.2 was produced by loss of 120 Da (4-ethynylphenol, m/z = 413.3 − 293.2 = 120.1). These and related observations[17,27] confirmed assignment of peak 1 to saponarin.

Peak 2 showed a $[M - H]^-$ m/z 607.1 with retention time at 39.52 min on HPLC. In the MS/MS spectrum of m/z = 607.1, the fragment ion at m/z = 427.2 had the most abundant intensity and corresponded to the loss of glucose from the protonated molecule. The predominant product ion at $[M - H]^-$ m/z = 307.6 in MS/MS was produced by successive loss of a fragment of 120 Da (4-ethynylphenol, m/z = 427.2 − 307.6), resulting from the cleavage of the flavonoid skeleton at position C-3 of spinosin. Standard spinosin showed the same t_R, UV, MS, and MS/MS as those of peak 2. These and related observations[17,25] confirmed the assignment of peak 2 to spinosin.

Peak 3 showed a $[M - H]^-$ m/z 431.3 with retention time at 40.73 min on HPLC. The fragment ions of m/z = 341.2 and 311.2 in MS/MS were produced by loss of a $C_3H_6O_3$ $[M - H - 90]^-$ and a $C_4H_8O_4$ $[M - H - 120]^-$, which are consistent with the characteristic ions of a C-glycosidic flavonoid. Based on these results and related observations,[28,29] peak 3 was identified as vitexin.

Peak 4 eluting at 41.08 min on HPLC created a $[M - H]^-$ at m/z = 445.2. This compound produced an ion of m/z = 325.2 in MS/MS, which resulted from the neutral loss of 4-ethynylphenol (120 Da). It was easily identified as swertish derived from

Table 3. Flavonoid Compounds in the Pulp Extracts from Three Varieties of Jujube Fruits

	1[a]	2	3	4	5	6	7	
t_R (min)	29.8	32.96	36.47	40.29	41.94	43.04	46.47	
UV/vis max (nm)	280, 242	280, 242	354, 254	354, 256	354, 254	346, 266, 248	334, 278, 248	
$[M - H]^-$ (m/z)	577.1	289.3	741.7	609.2	463.6	593.7	716.8	
MS/MS fragments	451.1, 425.2, 407.2	136		301.2	300.1	285.1	698.4, 641.4, 615.4	
identification	procyanidin B2	epicatechin	unidentified substance	quercetin-3-*O* rutinoside	quercetin-3-*O* galactoside	kaempferol-glucosyl-(1→2)rhamnoside	unidentified substance	
abbreviation	PCDB	EP	UIS-I	Q-3-R	Q-3-G	KGR	UIS-II	total
concn in pulp[b]								
Boeun-deachu	30.7 ± 2.0	261.0 ± 4.9	43.1 ± 2.9	295.5 ± 7.9	4.7 ± 0.3	18.7 ± 0.6	36.7 ± 0.3	690.4
Mechu	28.0 ± 0.9	258.8 ± 7.6	46.4 ± 5.2	707.5 ± 7.9	1.4 ± 0.3	32.6 ± 3.8	146.7 ± 10.8	1223
Sanzoin	14.0 ± 1.5	352.7 ± 7.5	138.1 ± 9.0	1147 ± 11	15.7 ± 1.7	31.3 ± 5.8	95.1 ± 5.1	1794

[a] Peak number on HPLC/elution order. [b] Listed values are average (mg/100g of dry wt) ± SD (n = 3). The values of UIS-I, KGR and UIS-II are expressed as Q-3-R content.

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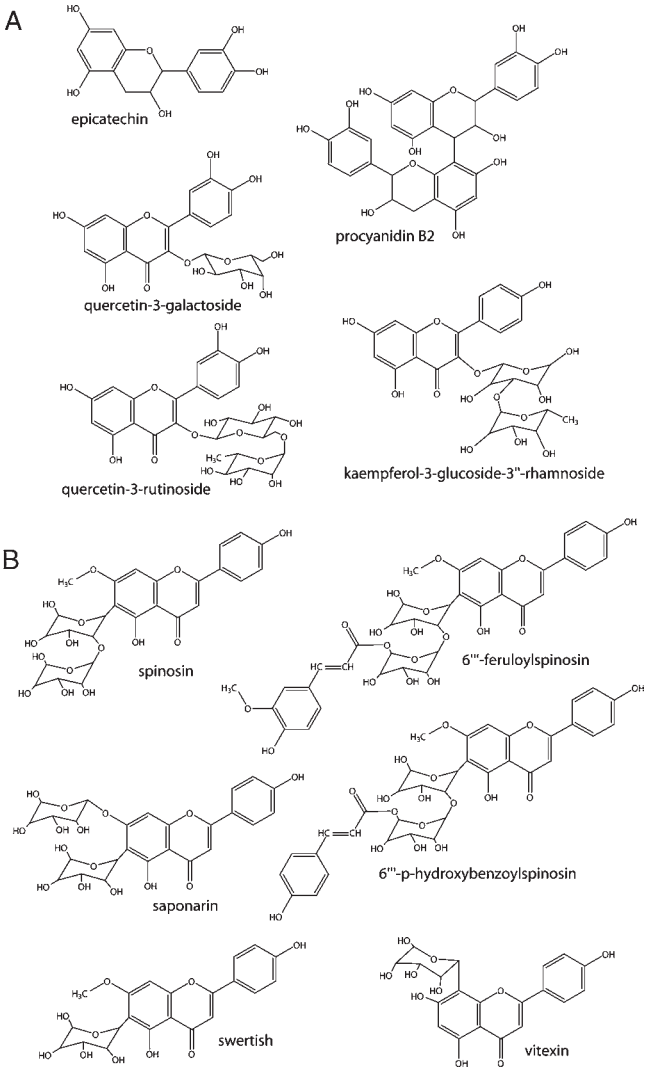

Figure 2. (A) Structure of flavonoids in pulp of jujube fruits evaluated in the present study. (B) Structure of flavonoids in seeds of jujube fruits evaluated in this experiment.

spinosin ($[M - H]^-$ m/z = 607.1) by natural loss of a glucosyl unit (162 Da). These MS/MS data and related findings[17,27] confirmed the assigned structure to swertish.

Peak 5 with retention time at 42.86 min on HPLC, showed a $[M - H]^-$ ion of m/z = 727.2. The MS/MS spectrum of this compound with one acyl group ($[M - H]^-$ at m/z = 727.2 − 607.3 = 119.9) showed fragmentations similar to those of spinosin (m/z = 607.3) without the acyl group (120 Da). These results and related observations[25] confirmed assignment of peak 5 to 6‴-hydroxybenzoylspinosin (6‴-HBS).

Peak 6 with retention time at 44.59 min on HPLC, showed a $[M - H]^-$ ion of m/z = 783.6. In the MS/MS spectrum, the fragment ion of m/z = 607.2 was generated by natural loss of a feruloyl group ($[M - H]^-$ m/z = 783.6 − 607.2 = 176.4). It may also degrade into a fragment with $[M - H]^-$ m/z = 427.7 by loss of a feruloyl group and a glucose unit ($[M - H]^-$ m/z = 607.2 − 427.7 = 179.5). These and related observations[25,27] confirmed assignment of peak 6 to 6‴-feruloylspinosin (6‴-FS). Figure 3B also shows that each compound formed a unique mass fragment pattern.

Table 4. Flavonoid Compounds in the Seed Extracts from Two Varieties of Jujube Fruits

	1[a]	2	3	4	5	6	7	
t_R (min)	38.46	39.52	40.73	41.08	42.86	44.59	47.93	
UV/vis max (nm)	336, 270, 242	338, 270, 234	334, 270, 242	332, 272, 248	334, 270, 242	330, 272, 240	328, 274, 246	
$[M - H]^-$ (m/z)	593.2	607.1	431.3	445.2	727.2	783.6	403.9	
MS/MS fragments	413.3, 293.2	427.2, 307.6	341.2, 311.2, 283.3	325.2, 297.4, 282.1	607.3, 427.1, 239.2	607.2, 427.7	235.3, 217.2, 193.2, 149.0	
identification	saponarin (1→2)	spinosin (2→3)	vitexin (4→5)	swertish (1→2)	6‴-hydroxybenzoylspinosin (3)	6‴-feruloylspinosin (1→3)	unidentified substance	
abbreviation	SPN	SPNS	VTX	SWT	6‴-HBS	6‴-FS	UIS	total
concn of flavonoids[b]								
Mechu	170.4 ± 12.2	1303.8 ± 30.6	134.0 ± 6.9	10.9 ± 1.4	84.6 ± 7.2	1237.3 ± 41.5	256.9 ± 12.0	3197.9
Sanzoin	133.0 ± 10.5	2237.7 ± 69.0	44.7 ± 6.1	14.8 ± 1.0	54.0 ± 5.4	1242.9 ± 87.7	271.1 ± 11.8	3998.2

[a] Peak number on HPLC. [b] Listed values are average (mg/100 g of dry wt) ± SD (n = 3). SPN, VTX, STW, 6‴-HBS, 6‴-FS and UIS are expressed as SPNS content.

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Table 5. Total Phenol and Antioxidant Activity (FRAP Value) of Pulp and Seed Extracts of Jujube Fruit

variety	TP (g/100 g dry wt)	FRAP value (mol Fe^{2+} /100 g dry wt)	DPPH scavenging effect (%) IC_{50} (μg/mL)
Boeun-deachu (pulp)	1.13 ± 0.12	3.2 ± 0.8	343.8
Mechu (pulp)	1.93 ± 0.11	9.3 ± 1.1	115.6
Sanzoin (pulp)	2.36 ± 0.07	9.0 ± 0.6	145.8
Mechu (seed)	3.61 ± 0.14	9.9 ± 3.0	310.2
Sanzoin (seed)	4.62 ± 0.12	13.2 ± 5.0	195.4
standard BHA			3.95

Bai et al.[22] also found spinosin and 6‴-FS in *Z. jujube* Mill. var. spinosa Hu ex H.F. Chou (sour jujube) and another flavonoid, 6‴-sinapolylspinosin, which was not present in the Korean cultivar. Similarly, Cheng et al.[17] found swertish, 6‴-FS, and spinosin in the sour jujube seed. They also found the following compounds we did not find: puerarin; apigenin-6-*C*-β-D-glucopyranoside; 6‴-feruloylisospinosin; isospinosin; and isovitexin-2″-*O*-β-D-glucopyranoside, as well as rotamers for some of the compounds. Bao et al.[30] found isovitexin-2″-*O*-β-D-glucopyranoside, 6‴-FS, and spinosin at comparable levels. Nui et al.[25] separated 19 flavonoids by HPLC from sour jujube seed, with spinosin and 6‴-FS among the most abundant. Other abundant flavonoids were isovitexin, 6‴-*p*-hydroxybenzoylspinosin, and 6‴-*p*-coumaroylspinosin. It appears that 6‴-FS and spinosin are present in all seed samples tested.

In an effort to understand the metabolism of these flavonoids, Bao et al.[27] measured blood levels of the flavonoids and their metabolites after consumption of sour jujube in rats. The parent compound 6‴-FS was metabolized to spinosin and swertish among other compounds. Spinosin appears to be both used directly *in vivo* and metabolized further into other compounds.

Flavonoid Content of Jujube Fruit Pulp. Table 3 lists the content of individual flavonoids in extracts of the three jujube pulps separated by HPLC. Individual flavonoids in the pulp of the three jujubes were quantitated by UV absorbance. Figure 2A illustrates the assigned structures of the flavonoids based on the LC−PDA, MS, MS/MS, and UV data illustrated in Figure 3A and summarized in Table 3.

The individual flavonoid content of the three fruits we analyzed ranged as follows: Boeun-deachu, Q-3-R > epicatechin > UIS-I > UIS-II > procyanidin > K-G-R > Q-3-G (lowest amount); Mechu, Q-3-R > epicatechin > UIS-II > UIS-I > K-G-R > procyanidin > Q-3-G; and Sanzoin, Q-3-R > epicatechin > UIS-I > UIS-II > K-G-R > Q-3-G > procyanidin. There appear to be only minor variations in the observed relative concentrations of individual flavonoids among the three jujube fruits. Q-3-R was the most predominant flavonoid in all three varieties, although it was much higher in Sanzoin than the others. Epicatechin was the second most prominent, being higher in the Boeun-deachu variety than in the others. The two unidentified flavonoids were present at levels between 5 and 12% of the total. The other flavonoids each accounted for less than 5% of the total.

Guo et al.[8] found that the content of Q-3-R was distinguishing between two varieties collected from 28 regions in China. Although this flavonoid is common to most jujubes, the levels were more dependent on variety than growing conditions, although harsh growing conditions were found to increase flavonoid levels.[7] The level of Q-3-R in our samples was similar to those found in the Italian jujube,[23] but nearly 2 orders of magnitude higher than in samples from Turkey[19] and three orders higher than in sun-dried jujube from China.[18] Zhang et al.[24] compared one freshly eaten to two sun-dried varieties and found total flavonoids in the sun-dried samples were nearly one-half the levels in the fresh sample.

Total Flavonoid Content. The last column in Table 3 shows that the total flavonoid content including the two unknown compounds (in mg/100 g dry wt) ranged as follows: Boeun-deachu, 690.4; Mechu, 1,223; Sanzoin, 1,794. The results show that the total flavonoid content per unit weight of the smallest (Sanzoin) is 2.6 times greater than of the largest fruit (Boeun-deachu).

Based on the fruit wt data in Table 1 and the data per gram in Table 3, it is also instructive to calculate the total flavonoid content per unit fruit. Boeun-deachu contains 25.4 mg of total flavonoids/fruit, Mechu 8.3 mg/fruit, and Sanzoin 4.6 mg/fruit. Thus, the flavonoid content of the largest fruit is three times greater than that of the intermediate sized fruit. The corresponding ratio for the largest to the smallest fruit is 5.5. These results show that the smallest fruit has the highest flavonoid content per unit weight and the lowest content per unit fruit.

The level of total flavonoids was somewhat lower than that found in Italian fruits measured by HPLC[23] and 2−4× higher than that found in Chinese fruits measured by a colorimetric method.[24] As with Q-3-R, total flavonoids were much lower in the studies by Hudina et al.[18] and San and Yildirim.[19]

Flavonoid Content of Jujube Seeds. Table 4 lists the content of individual flavonoids in extracts of the two jujube seeds separated by HPLC. Figure 2B illustrates the assigned structures of the flavonoids based on the LC−PDA, MS, MS/MS, and UV data shown in Figure 3B and Table 4. Spinosin was the most predominant flavonoid, followed by 6‴-FS. In agreement, Bao et al.[27] found that spinosin and 6‴-FS accounted for >60% of the flavonoid extract from sour jujube seed.

Sanzoin was higher in spinosin than in Mechu, accounting for most of the difference in total flavonoids. Mechu was higher in the other flavonoids than was Sanzoin, except for swertish, which was present at very low levels in both varieties. The unidentified compound contributed 7−8% to the total. The other minor flavonoids contributed <5% to the total flavonoids.

These results show that, except for spinosin, the distribution of all compounds in the two seeds was similar. In a survey of 25 geographically diverse samples of sour jujube within China, spinosin was usually the most abundant flavonoid, followed by isovitexin-2″-*O*-β-glucopyranside and then by 6‴-FS.[20] However, the authors note that there was much variability among these compounds in the samples. The levels in this study were about 10× less than in our samples. Zhang et al.[24] measured total flavonoids by a colorimetric method and also found levels about 10× less than in our samples.

Total Phenolic Content of Jujube Fruits and Seeds. Table 5 lists the total phenolic content of the five jujube samples. The values (in g/100 g dry wt) of each jujube extract range from 1.13 (Boeun-deachu

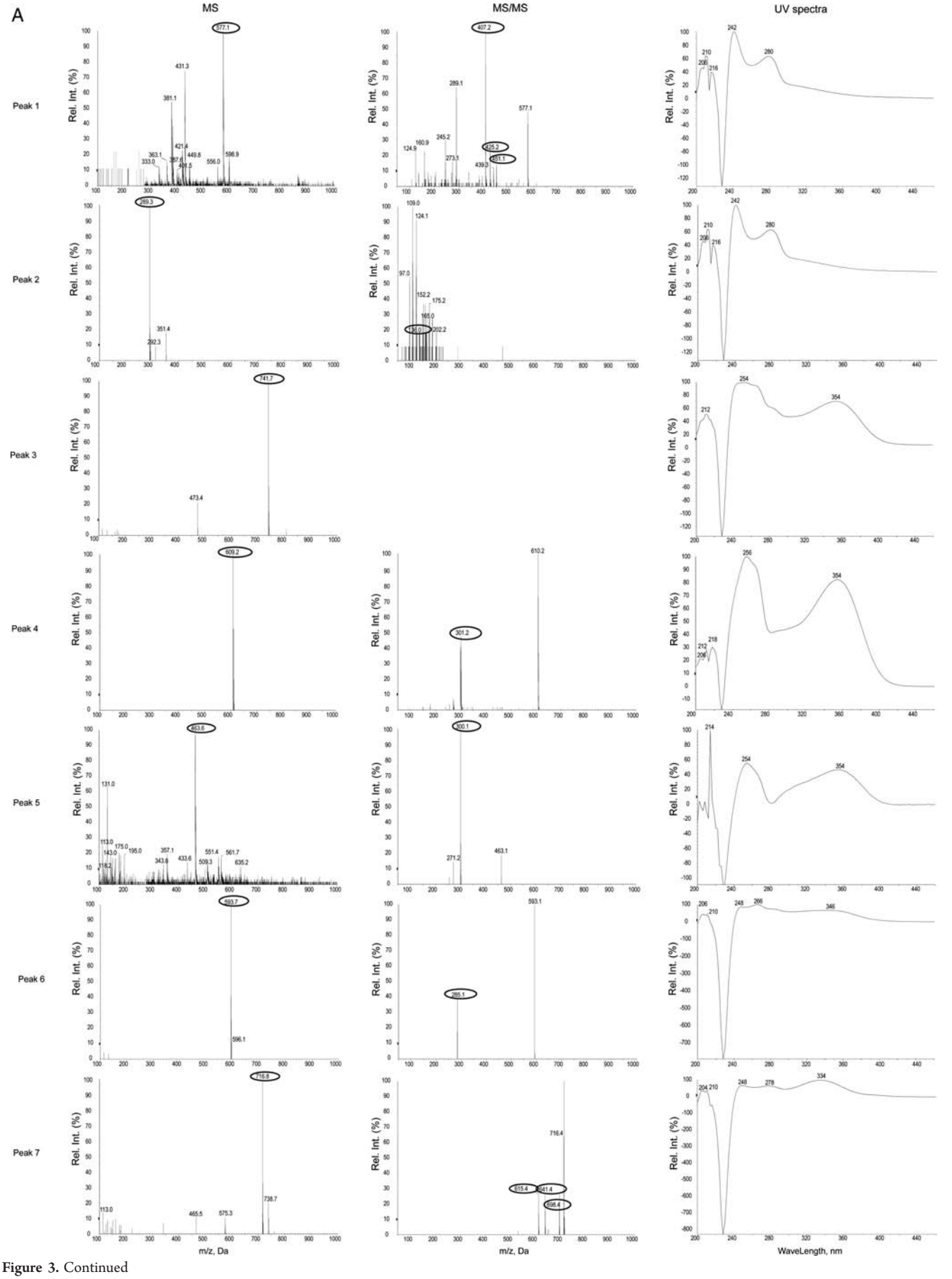

Figure 3. Continued

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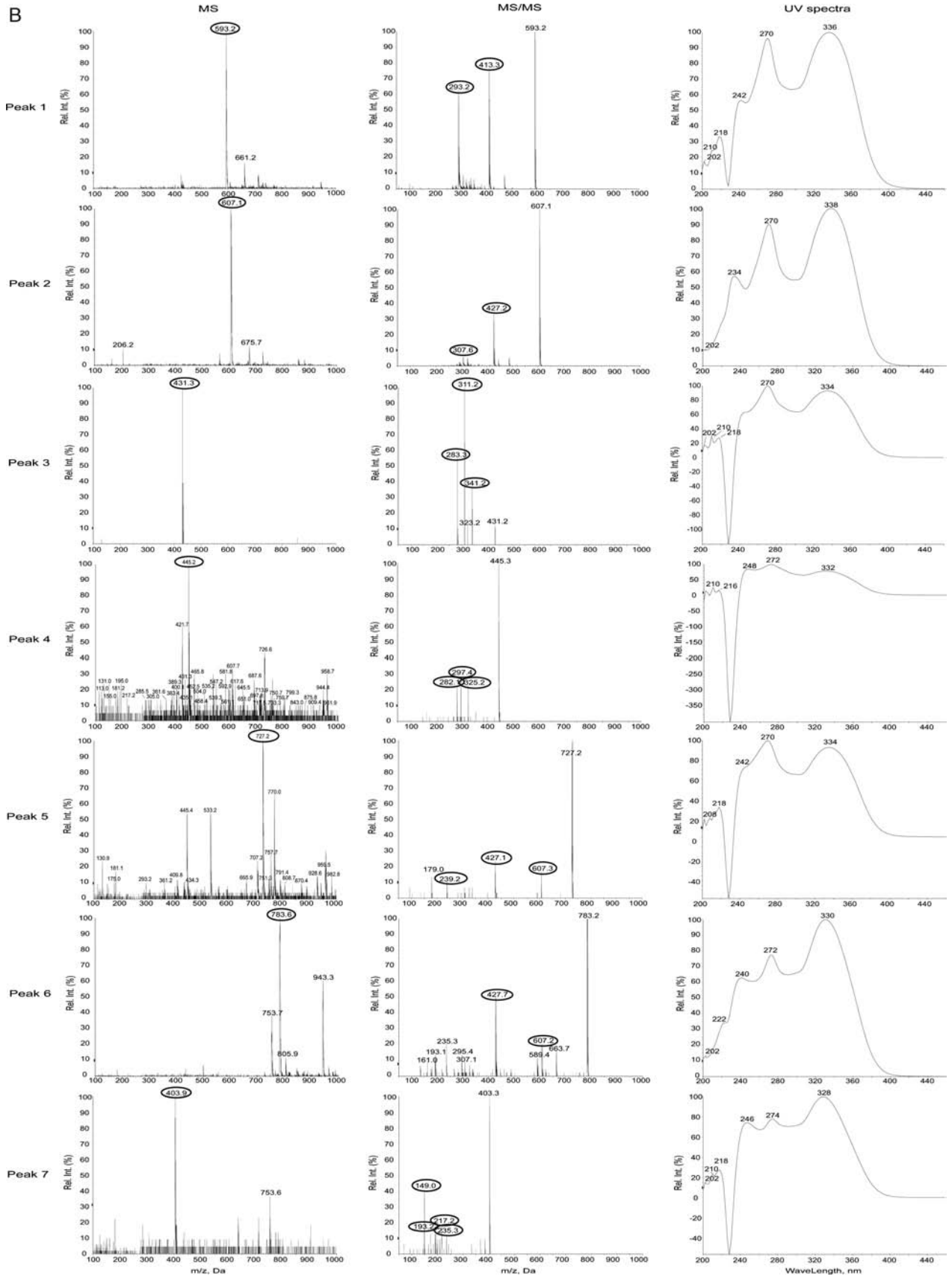

Figure 3. (A) Identification of HPLC/LC of peaks 1−7 of pulp extracts by UV−vis and mass spectra (MS and MS/MS) collected from the HPLC column. (B) Identification of HPLC/LC of peaks 1−7 of seed extracts by UV−vis and mass spectra (MS and MS/MS) collected from the HPLC column.

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fruit) to 4.62 (Sanzoin seeds) or about a 4.1-fold variation from lowest to highest value. Total phenolic content of the two seeds (3.61 and 4.62) is higher than in the three fruits (1.13, 1.93, and 2.36).

Total phenolic content of our samples was similar to what is reported by other investigators. Sun et al.[7] found an average of about 0.9 ± 0.2 in the pulp of diversely collected sour jujubes. Xue et al.[21] found levels of 4−5 in three commercial Chinese cultivars. Zhang et al.[24] found 0.8 in fresh pulp, but about 25% less in the sun-dried jujube. The corresponding seeds had 0.4 in the fresh sample and 30−45% less in the sun-dried sample. Wang et al.[20] found 3.9 and 3.1 in the flesh and seed, respectively. In contrast to our results, these last two studies had lower levels in the seed than in the pulp.

Antioxidative Activity. Reactive oxygen species (ROS) have been postulated to contribute to cause chronic diseases, especially cancer and arteriosclerosis, through oxidative damage of enzymes and tissues. Phenolic compounds can reduce ROS levels by trapping and dissipating reactive free electrons (free radicals).[31,32] Antioxidative activity is generally measured by two distinct methods: direct determination of oxidative damage and indirect determination of levels of known reactive species.[32]

We determined the antioxidative potential of jujube fruit and seeds by one direct (FRAP) method and one indirect (DDPH) method. FRAP monitors the reduction of a ferric ion complex to the ferrous form.[5] Addition of antioxidants reduces production of the oxidation products and the resultant color of the solution. Table 5 lists the FRAP values for three fruits and two seeds. These range (in mol of Fe^{2+}/100 g dry wt) from 3.2 (lowest antioxidative effect) for Boeun-deachu fruit to 13.2 (highest effect) for Sanzoin seeds or a 4.1-fold variation from highest to lowest activity.

The DPPH method is a convenient and rapid indirect assay for screening plant samples for radical scavenging activity known to be involved with oxidation.[31] As antioxidants react with the stable, highly colored free radical DPPH, the absorbance of the solution decreases. Table 5 lists data for the antiradical activities of the test substances as well as values for the reference commercial antioxidant BHA. The calculated IC_{50} values (in μg/mL) range from 115.6 (highest activity) for Mechu fruit to 343.8 for Boeun-deachu fruit (lowest activity) or about a 2.97-fold variation from lowest to highest activities. The data also show that the BHA IC_{50} value of 3.95 is much lower (higher activity) than the corresponding values for the jujube samples.

Statistical Correlation of Antioxidative Effects to Phenolic Content. Total flavonoids by HPLC are well correlated with Folin−Ciocalteu total phenolics (Pearson moment correlation coefficient = 0.996, p = 0.000293). This indicates that the flavonoids measured by HPLC may be responsible for the most of the phenolics in the samples. However, FRAP is correlated significantly with Folin−Ciocalteu phenolics (0.888, p = 0.0445) but not significantly with total HPLC flavonoids (0.847, p = 0.0700). FRAP was significantly correlated with levels of the individual flavonoid, K-G-R (0.999, p = 0.0290), but not with the other flavonoids. DPPH was not correlated with total phenolic content or with FRAP.

These results differ from those reported by other researchers. Xue et al.[21] found that DPPH, FRAP, and Trolox equivalent antioxidant capacity (TEAC) gave similar results that were highly correlated with Folin−Ciocalteu total phenolics. Zhang et al.[24] found that DPPH and FRAP values were highly correlated with total phenolics by Folin−Ciocalteu and total flavonoids by a colorimetric method. The values were not correlated with total anthocyanin or ascorbic acid content.

Dietary Significance. To our knowledge, this is the first report on the free amino acid content of jujube fruits and seeds. Both fruits and seeds contained high amounts of free amino acids and other N-containing compounds. The content of Asn and Pro in the fruit but not in the seed samples was exceptionally high. Free amino acid content of plant foods is assuming an important role in food safety because, as mentioned above, Asn is the major precursor of acrylamide and Cys inhibits its formation during food processing (baking, frying).

Jujube fruits contained five known and two unknown flavonoids. Seeds contained six known and one unknown flavonoids. As in other studies,[22,27] spinosin and 6‴-FS were two of the most prevalent flavonoids in the seeds. Structures of fruit flavonoids differed from those of seed flavonoids. Total flavonoid contents of the two seed samples on a dry weight basis were two to four times higher than of the three fruit samples. Flavonoid content/g is lower in the large-sized jujube fruits than in the small-sized ones. The distribution of the individual flavonoids among the different samples also varied widely.

Statistical analysis showed that results from the antioxidative FRAP assay were well correlated with total phenolic content measured by Folin−Ciocalteu reagent and also with the level of one individual phenolic compound measured by HPLC, K-G-R. By contrast, results from the DPPH assay were not correlated with any of the measured compositional data.

Our findings emphasize the substantial variation in individual and total phenolic and free amino acid content found among jujube fruits and seeds. The data suggest that consumers have a choice in selecting varieties with a high content of phenolic and flavonoid compounds and high antioxidative effects. Because individual jujube flavonoids are reported to exhibit different health-promoting effects, knowledge of both composition and concentrations of bioactive compounds of jujube products can benefit consumers.

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REFERENCES

(1) Azam-Ali, S.; Bonkoungou, E.; Bowe, C.; deKock, C.; Godara, A.; Williams, J. T. *Fruits for the Future 2 (Revised ed.): Ber and Other Jujubes*; International Centre for Underutilised Crops: Southampton, U.K., 2006; p 289.

(2) Outlaw, W. H., Jr.; Zhang, S.; Riddle, K. A.; Womble, A. K.; Anderson, L. C.; Outlaw, W. M.; Outlaw, N. N.; Outlaw, E. C.; Thistle, A. B. The jujube (*Ziziphus jujuba* Mill.), a multipurpose plant. *Econ. Bot.* **2002**, *56*, 198–200.

(3) Krška, B.; Mishra, S. Sensory evaluation of different products of *Ziziphus jujuba* Mill. *Acta Hortic.* **2009**, *840*, 557–562.

(4) Mahajan, R. T.; Chopda, M. Z. Phyto-pharmacology of *Ziziphus jujuba* mill - A plant review. *Pharmacogn. Rev.* **2009**, *3*, 320–329.

(5) Koetter, U.; Barrett, M.; Lacher, S.; Abdelrahman, A.; Dolnick, D. Interactions of Magnolia and Ziziphus extracts with selected central nervous system receptors. *J. Ethnopharmacol.* **2009**, *124*, 421–425.

(6) Jiang, J.-G.; Huang, X.-J.; Chen, J.; Lin, Q.-S. Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen *Ziziphus jujube*. *Nat. Prod. Res.* **2007**, *21*, 310–320.

(7) Sun, Y.-F.; Liang, Z.-S.; Shan, C.-J.; Viernstein, H.; Unger, F. Comprehensive evaluation of natural antioxidants and antioxidant potentials in *Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou* fruits based on geographical origin by TOPSIS method. *Food Chem.* **2011**, *124*, 1612–1619.

(8) Guo, S.; Duan, J. A.; Tang, Y.; Qian, D.; Zhu, Z.; Qian, Y.; Shang, E.; Su, S. UHPLC-TOFMS coupled with chemometric method as a powerful technique for rapid exploring of differentiating components between two *Ziziphus* species. *J. Sep. Sci.* **2011**, *34*, 659–666.

(9) Allard, P.; Cowell, L. D.; Zytkovicz, T. H.; Korson, M. S.; Ampola, M. G. Determination of phenylalanine and tyrosine in dried blood specimens by ion-exchange chromatography using the Hitachi L-8800 analyzer. *Clin. Biochem.* **2004**, *37*, 857–862.

(10) Choi, S.-H.; Lee, S.-H.; Kim, H.-J.; Lee, I.-S.; Nobuyuki, K.; Levin, C. E.; Friedman, M. Changes in free amino acid, phenolic, chlorophyll, carotenoid, and glycoalkaloid contents in tomatoes during 11 stages of growth and inhibition of cervical and lung human cancer cells by green tomato extracts. *J. Agric. Food Chem.* **2010**, *58*, 7547–7556.

(11) Chew, Y.-L.; Goh, J.-K.; Lim, Y.-Y. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. *Food Chem.* **2009**, *116*, 13–18.

(12) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.

(13) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25–30.

(14) Friedman, M.; Levin, C. E. Review of methods for the reduction of dietary content and toxicity of acrylamide. *J. Agric. Food Chem.* **2008**, *56*, 6113–6140.

(15) Rayburn, J. R.; Friedman, M. L-cysteine, N-acetyl-L-cysteine, and glutathione protect xenopus laevis embryos against acrylamide-induced malformations and mortality in the frog embryo teratogenesis assay. *J. Agric. Food Chem.* **2010**, *58*, 11172–11178.

(16) Malik, A.; Kuliev, Z. A.; Akhmedov, Y. A.; Vdovin, A. D.; Abdullaev, N. D. Proanthocyanidins of *Ziziphus jujuba*. *Chem. Nat. Compd.* **1997**, *33*, 165–173.

(17) Cheng, G.; Bai, Y.; Zhao, Y.; Tao, J.; Liu, Y.; Tu, G.; Ma, L.; Liao, N.; Xu, X. Flavonoids from *Ziziphus jujuba* Mill var. Spinosa. *Tetrahedron* **2000**, *56*, 8915–8920.

(18) Hudina, M.; Liu, M.; Veberic, R.; Stampar, F.; Colaric, M. Phenolic compounds in the fruit of different varieties of Chinese jujube (*Ziziphus jujuba* Mill.). *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 305–308.

(19) San, B.; Yildirim, A. N. Phenolic, alpha-tocopherol, beta-carotene and fatty acid composition of four promising jujube (*Ziziphus jujuba* Miller) selections. *J. Food Compos. Anal.* **2010**, *23*, 706–710.

(20) Wang, B.-N.; Liu, H. F.; Zheng, J. B.; Fan, M. T.; Cao, W. Distribution of phenolic acids in different tissues of jujube and their antioxidant activity. *J. Agric. Food Chem.* **2011**, *59*, 1288–1292.

(21) Xue, Z.; Feng, W.; Cao, J.; Cao, D.; Jiang, W. Antioxidant activity and total phenolic contents in peel and pulp of Chinese jujube (*Ziziphus jujuba* mill) fruits. *J. Food Biochem.* **2009**, *33*, 613–629.

(22) Bai, H.-L.; Wang, J.; Liu, C.-M.; Li, L. Isolation and purification of flavonoids from *Ziziphus jujuba* by High-Speed Counter-Current Chromatography. *J. Chin. Chem. Soc.* **2010**, *57*, 1071–1076.

(23) Pawlowska, A. M.; Camangi, F.; Bader, A.; Braca, A. Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits. *Food Chem.* **2009**, *112*, 858–862.

(24) Zhang, H.; Jiang, L.; Ye, S.; Ye, Y.; Ren, F. Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (*Ziziphus jujuba* Mill.) from China. *Food Chem. Toxicol.* **2010**, *48*, 1461–1465.

(25) Niu, C.-Y.; Wu, C.-S.; Sheng, Y.-X.; Zhang, J.-L. Identification and characterization of flavonoids from semen *Zizyphi spinosae* by high-performance liquid chromatography/linear ion trap FTICR hybrid mass spectrometry. *J. Asian Nat. Prod. Res.* **2010**, *12*, 300–312.

(26) Moco, S.; Bino, R. J.; Vorst, O.; Verhoeven, H. A.; De Groot, J.; Van Beek, T. A.; Vervoort, J.; Ric De Vos, C. H. A liquid chromatography-mass spectrometry-based metabolome database for tomato. *Plant Physiol.* **2006**, *141*, 1205–1218.

(27) Bao, K.-D.; Li, P.; Qi, L.-W.; Li, H.-J.; Yi, L.; Wang, W.; Wang, Y.-Q. Characterization of flavonoid metabolites in rat plasma, urine, and feces after oral administration of semen *Ziziphi spinosae* extract by HPLC-diode-array detection (DAD) and Ion-Trap mass spectrometry (ms^n). *Chem. Pharm. Bull. (Tokyo)* **2009**, *57*, 144–148.

(28) Li, X.; Xiong, Z.; Ying, X.; Cui, L.; Zhu, W.; Li, F. A rapid ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometric method for the qualitative and quantitative analysis of the constituents of the flower of *Trollius ledibouri* Reichb. *Anal. Chim. Acta* **2006**, *580*, 170–180.

(29) Grayer, R. J.; Kite, G. C.; Abou-Zaid, M.; Archer, L. J. The application of atmospheric pressure chemical ionisation liquid chromatography-mass spectrometry in the chemotaxonomic study of flavonoids: Characterisation of flavonoids from *Ocimum gratissimum* var. gratissimum. *Phytochem. Anal.* **2000**, *11*, 257–267.

(30) Bao, K.-D.; Li, P.; Li, H.-J.; Qi, L.-W. Simultaneous determination of flavonoids and saponins in Semen *Ziziphi spinosae* (Suanzaoren) by high performance liquid chromatography with evaporative light scattering detection. *Chin. J. Nat. Med.* **2009**, *7*, 47–53.

(31) Koleva, I. I.; Van Beek, T. A.; Linssen, J. P. H.; De Groot, A.; Evstatieva, L. N. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem. Anal.* **2002**, *13*, 8–17.

(32) Roginski, V.; Lissi, E. A. Review of methods to determine chain-braking antioxidant activity in food. *Food Chem.* **2005**, *92*, 235–254.