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ARTICLE

Acids, Sugars, and Sugar Alcohols in Chinese Hawthorn (*Crataegus* spp.) Fruits

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Acids, sugars, and sugar alcohols in the fruits of 22 cultivars/origins of three species of hawthorn (*Crataegus* spp.) were analyzed by gas chromatography and mass spectrometry. Citric acid (2.0−8.4 g/100 g dry mass [DM]), quinic acid (0.5−5.6 g/100 g DM), malic acid (0.3−1.1 g/100 g DM), fructose (5.5−18.4 g/100 g DM), glucose (5.3−16.6 g/100 g DM), sorbitol (3.0−15.7 g/100 g DM), and *myo*-inositol (0.1−0.3 g/100 g DM) were found in all the samples. Sucrose was present only in *C. scabrifolia* and three cultivars of *C. pinnatifida* var. *major*. *C. scabrifolia* differed from other species by its high content of quinic acid. The cultivars of *C. pinnatifida* var. *major* and *C. brettschneideri* had a higher content of total sugars and a higher sugar/acid ratio than the natural origins of *C. pinnatifida* and *C. scabrifolia* ($P < 0.05$). The hawthorn samples analyzed fell into two groups rich in sugars and acids respectively. This is the first report of the profiles of sugars and sugar alcohols and the occurrence of quinic acid in hawthorn fruits.

KEYWORDS: Acids; *Crataegus* spp.; hawthorn; sugar alcohols; sugars; sugar/acid ratio

INTRODUCTION

Hawthorn species (*Crataegus* spp.) have recently attracted increasing attention in the field of food, nutraceuticals, and medicine because of their widely reported health benefits, e.g., the reduction of the risk of cardiovascular diseases (CVDs) (*1*, *2*). Fruits and leaves of the hawthorn are rich in phenolic compounds such as flavonoids and procyanidins (*3*−*5*), which are considered the key bioactive compounds of the hawthorn offering antioxidative, free radical scavenging, anti-inflammatory, vasorelaxing, and hypolipidemic effects (*6*−*10*).

Aqueous alcohol extracts of hawthorn fruits and leaves are used as dietary supplements and herbal medicines in the United States and Europe for treating heart failure degrees I−III according to the classification of the New York Heart Association (NYHA) (*11*).

In China, the hawthorn is widely cultivated. Hawthorn fruits have long been consumed naturally and used as raw materials in the food industry and in traditional Chinese medicine (TCM) (*12*). The safety and health benefits of the fruits are strongly supported by their long history of application. Compared with those of the European hawthorn varieties, the fruits of the Chinese hawthorn are typically bigger and have a more pleasant taste. The Chinese hawthorn is commonly considered to comprise 18 species, of which *Crataegus pinnatifida* Bge. is the most important. The species and the variety Shanlihong (*Crataegus pinnatifida* Bge. var. *major* N.E.Br.) are included in the Chinese pharmacopeia (*13*). In addition, the fruits of other species such as *C. brettschneideri* (Fu hawthorn), *C. scabrifolia* (Yun'nan hawthorn), *C. hupehensis* (Hubei hawthorn), *C. kansuensis* Sarg. (Gansu hawthorn), *C. cuneata* Sieb. et Zucc. (wild hawthorn), *C. songarica* (Zhunger hawthorn), *C. wilsonii* Sarg. (Huazhong hawthorn), and *C. altaica* (loud) Lange. (A'ertai hawthorn) are also commonly used as medicinal or food materials (*14*).

The content and composition of acids, sugars, and sugar alcohols are important quality factors affecting directly the flavor and acceptability of the fruits and berries (*15*). Fruit acids promote food digestion and improve blood circulation. The total content of acids is one of the criteria used in the quality control of hawthorn fruits in TCM (*13*). Acid content may also influence the stability of phenolic compounds in the fruits (*16*). Sugar alcohols contribute to the sweetness and play an important role in the health effects of fruits and berries.

Previous studies have suggested qualitative and quantitative differences in acids and sugars among different species and origins of the hawthorn (*14*, *17*). However, no systematic investigation on the detailed composition of such compounds in the Chinese hawthorn has been conducted. This study aimed to determine the overall profile of acids, sugars, and sugar alcohols in Chinese hawthorn fruits and investigate the differences among species, varieties, and cultivars.

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MATERIALS AND METHODS

Plant Materials. Hawthorn fruits of 22 cultivars and origins were collected in China. Among these, a sample of *C. scabrifolia* was collected in Kunming, Yun'nan Province, China, September 2007. The other samples were collected in the Chinese National Fruit Germplasm Repository, Shenyang Hawthorn Garden (Shenyang, Liaoning Province, China), including 10 cultivars of *C. pinnatifida* Bge. var. *major* N.E.Br. collected in October 2007, eight cultivars of *C. brettschneideri* collected in August 2008, and three natural origins of *C. pinnatifida* Bge collected in September 2008. All the fruits were collected as optimally ripe. A total amount of 500 g of fruits was collected from 2−4 trees of each cultivar/origin, five randomly selected collection points from different sides of each tree. All the samples were sliced and dried in a cool and shady place after harvesting.

Reference Compounds. D-Fructose, D-quinic acid, and L-ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO). D-Glucose, D-sorbitol, and the internal standard D-xylose (for sugars) were purchased from Fluka Chemie AG (Buchs, Switzerland). Malic acid and the internal standard L-tartaric acid (for acids) were purchased from Merck KGaA (Darmstadt, Germany). Sucrose, citric acid, and the internal standard D-mannitol (for sugar alcohols) were purchased from J. T. Baker B.V. (Deventer, Holland). *myo*-Inositol was purchased from Alexis Co. (Lausen, Switzerland).

Reagent. Tri-Sil HTP reagent was purchased from Pierce Chemical Co. (Rockford, IL). The reagent was composed of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine (2:1:10).

Extraction of Sugars, Acids, and Sugar Alcohols. The dried, seedless hawthorn fruits were milled into a fine powder in a mortar with the aid of liquid nitrogen. A 1.0 g sample of the fruit powder was taken and transferred into a 100 mL volumetric flask with about 80 mL of Milli-Q water. The mixture was ultrasonicated for 30 min to help the dissolution of the sugars, acids, and sugar alcohols into water. After that, Milli-Q water was added to bring to the volume scale. A portion of 20 mL of the mixture was taken and centrifuged at 4420*g* for 15 min. For quantitative analysis, each sample was extracted in quadruplet, and each extract was analyzed separately.

Sample Preparation for Qualitative Analysis. After centrifugation, two samples (600 μL each) of the supernatant were filtered through a 0.45 μm membrane. A solution of reference compounds consisting of malic, citric, and quinic acids as well as glucose, fructose, sucrose, sorbitol, and *myo*-inositol was prepared (the concentration of each reference compound was 0.5 g/100 mL). An aliquot of 50 μL of the solution of the reference compounds was added to each of the filtered supernatant samples. After that, the samples were evaporated to dryness under nitrogen flow at 40 °C and dried further in a desiccator above P_2O_5 overnight.

Trimethylsilyl (TMS) derivatives of acids, sugars, and sugar alcohols of the samples were prepared by adding 600 μL of Tri-Sil HTP reagent to each sample, shaking vigorously with a Vortex (Vortex-Genie, Springfield, MA) for 5 min, and incubating at 60 °C for 30 min. The HMDS and TMCS in Tri-Sil HTP reagent reacted with the hydroxyl group of acids, sugars, and sugar alcohols producing TMS derivatives of these compounds. After silylation, the samples were left to cool at room temperature.

Sample Preparation for Quantitative Analysis. A 3.0 mL portion of the centrifuged water extract was taken, and internal standard solutions (0.5 g/100 mL) of mannitol, tartaric acid, and xylose were added (250 μL each). This was followed by thorough mixing of the sample and filtration through a 0.45 μm membrane. A 600 μL sample of the filtrate was evaporated to dryness under nitrogen flow at 40 °C and kept in a desiccator over P_2O_5 overnight. TMS derivatives of acids, sugars, and sugar alcohols were prepared as described previously.

Identification of Acids, Sugars, and Sugar Alcohols. Acids, sugars, and sugar alcohols were identified by comparing the retention times and mass spectra of the analytes with those of the reference compounds and by coanalyses of the sample and reference compounds.

TMS derivatives of the samples were first analyzed with a Hewlett-Packard 5890 series II gas chromatograph (GC, Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID) and Hewlett-Packard 7673 autosampler. The analyses were carried out with a Supelco Simplicity-1 fused silica column (30 m L × 0.25 mm i.d. × 0.25 μm d_f) (Bellefonte, PA). A sample of 1 μL was injected into a split/splitless injector. The average flow rate of the carrier gas helium was 1.4 mL/min. The temperature of the injector was 210 °C, and that of the detector 290 °C. The column temperature was programmed as 2 min at 150 °C, raised to 210 °C at a rate of 6 °C/min, before the final temperature of 275 °C at a rate of 40 °C/min, and held at 275 °C for 10 min.

TMS derivatives of the samples and the reference compounds were also analyzed using a Shimadzu QP 5000 MSD GC−MS (Kyoto, Japan). The column used was DB-1MS (30 m L × 0.25 mm i.d. × 0.25 μm d_f) (J & W Scientific, Agilent, Folsom, CA). A sample of 0.5 μL was injected manually into a split (1:24) injector. The flow rate of the carrier gas helium was 1.3 mL/min. The temperature of the injector and the column temperature program were the same as in the corresponding GC-FID analysis. The temperature of the interface was 290 °C.

Quantitative Analysis. TMS derivatives of the samples were analyzed with the same GC-FID system as used in the qualitative analysis.

The contents of acids, sugars and sugar alcohols were quantified using an internal standard method:

$$C_a = \frac{C_s A_a}{k_a A_s}$$

where k_a is the correction factor of the analyte, C_s and C_a are concentrations of the internal standard and of the analyte, respectively, and A_s and A_a are the peak areas of the internal standard and the analyte, respectively, in the chromatogram.

Tartaric acid, xylose, and mannitol were used as the internal standards to calculate the contents of acids, sugars, and sugar alcohols, respectively. Correction factors were determined with reference compounds and applied in the quantification of each component in the samples. The peaks of both xylose isomers were taken into account. The correction factors based on the detector response of the reference compounds were 0.881 for malic acid, 0.758 for citric acid, and 1.148 for quinic acid compared with tartaric acid, 0.987 for fructose, 1.373 for glucose, and 0.529 for sucrose compared with xylose, 0.993 for sorbitol, and 1.086 for *myo*-inositol compared with mannitol.

Statistical Analysis. Statistical analyses of the results were performed using SPSS 16.0.1 (SPSS Inc., Chicago, IL) and Unscrambler 9.8 (Camo Process AS, Oslo, Norway). Differences in chemical composition among the samples and species were analyzed by comparing the means using one-way analysis of variance (ANOVA) and with the Games−Howell (for the population with unequal variances) and Student−Newman−Keuls (SNK) tests (for the population with equal variances). The differences reaching a confidence level of 95% were considered significant. Principal component analysis (PCA) was used to interpret the difference between hawthorn samples based on the content of sugars, acids, and sugar alcohols.

RESULTS

Figure 1 presents two typical GC-FID chromatograms of TMS derivatives of acids, sugars, and sugar alcohols of the hawthorn samples. The chromatograms of Mopan and Shandongdajinxing represent the samples without and with sucrose, respectively. The identities and retention times of the peaks are presented in the figure captions.

Table 1 shows the results of a GC−MS analysis of TMS derivatives of acids, sugars, and sugar alcohols of Chinese hawthorn fruits. The retention times and mass spectra of the TMS derivatives of malic acid, citric acid, quinic acid, fructose, glucose, sucrose, sorbitol, and *myo*-inositol in the samples were identical with those of the reference compounds.

Malic acid, citric acid, quinic acid were the major organic acids in all the samples analyzed. In some samples, ascorbic acid was detected in trace amount. Fructose, glucose, sucrose, sorbitol, and *myo*-inositol were the major sugars and sugar alcohols found in these fruits. But sucrose was detected only in four samples, of which three belonged to *C. pinnatifida* var. *major* and one to *C. scabrifolia*.

In addition, some trace peaks representing minor components appeared in the chromatograms of some samples, but these peaks were often too small to be integrated and quantified.

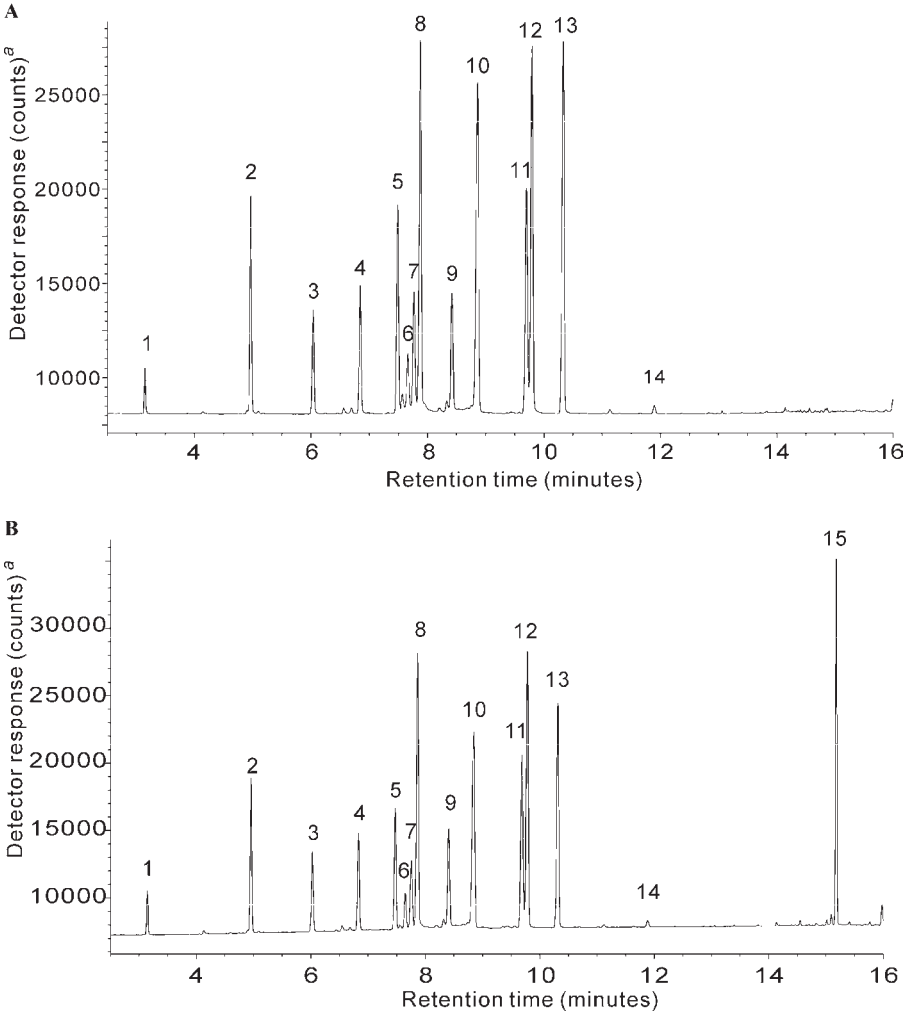

Figure 1. Gas chromatography−flame ionization detection (GC-FID) chromatograms of acids, sugars, and sugar alcohols of hawthorn fruits of Mopan (*C. pinnatifida* var. *major*) (**A**) and Shandongdajinxing (*C. pinnatifida* var. *major*) (**B**). Peaks: 1, malic acid (3.15 min); 2, tartaric acid (4.94 min); 3, xylose 1 (6.04 min); 4, xylose 2 (6.86 min); 5, citric acid (7.49 min); 6, 7, and 8, fructose 1, 2, and 3 (7.67, 7.77, and 7.88 min); 9, quinic acid (8.42 min); 10 and 13, glucose 1 and 2 (8.86 and 10.33 min); 11, mannitol (9.69 min); 12, sorbitol (9.79 min); 14, *myo*-inositol (11.89 min); 15, sucrose (15.18 min). [a]One count = 5.1×10^{-3} pA.

Table 1. Results of Analysis of TMS Derivatives of Acids, Sugars and Sugar Alcohols in Chinese Hawthorn Fruits with GC−MS

peaks	retention times (min)	ion fragments (*m/z*)
malic acid	4.85	73, 133, 147, 175, 233, 294, 309
citric acid	10.54	73, 133, 147, 183, 211, 273, 294, 309, 347, 363
quinic acid	11.75	73, 133, 147, 191, 255, 345
fructose 1	10.79	73, 103, 129, 147, 204, 217, 437
fructose 2	10.88	
fructose 3	11.12	
glucose 1	12.18	73, 103, 129, 147, 191, 204, 217
glucose 2	13.20	
sorbitol	13.02	73, 103, 117, 129, 147, 205, 217, 307, 319, 331
myo-inositol	14.26	73, 129, 147, 191, 204, 217, 265, 305, 318
sucrose	19.19	73, 103, 129, 147, 169, 217, 243, 271, 361

Acids. Table 2 summarizes the contents of fruit acids in all 22 samples analyzed in this study. The total acid content of the fruits varied from 3.1 to 11.8 g/100 g DM. High acid contents were found in two cultivars 947 (11.8 g/100 g DM) and 8321 (11.5 g/100 g DM) of *C. pinnatifida* var. *major*. The samples with low acid contents were Jiangou 2 (3.1 g/100 g DM) and Shen78201 (3.8 g/100 g DM) of *C. pinnatifida* var. *major*. A wide variation was seen (3.1 to 11.8 g/100 g DM) in the acid content among the cultivars of this variety ($P < 0.05$). The total acid content of eight cultivars of *C. brettschneideri* ranged from 5.1 to 7.4 g/100 g DM with clearly smaller variation within the species than within *C. pinnatifida* var. *major* (3.1−11.8 g/100 g DM).

Citric acid was present at levels of 2.0−8.4 g/100 g DM, the most abundant acid in practically all the hawthorn samples (55−82% of total acids) except *C. scabrifolia* (27% of total acids).

Statistical analysis showed citric acid content to be significantly lower in *C. scabrifolia* than in the other three species investigated ($P < 0.01$).

Quinic acid was the most abundant acid in the fruits of *C. scabrifolia* (5.6 g/100 g DM, 67% of total acids). In all cultivars and origins of *C. pinnatifida* var. *major* and *C. pinnatifida*, quinic acid was the second most abundant acid with contents ranging from 0.8 to 3.0 g/100 g DM. The fruits of *C. brettschneideri* contained less quinic acid (0.5−0.8 g/100 g DM accounting for 9−12% of the total acids) than those of *C. pinnatifida* var. *major* (0.8−3.0 g/100 g DM, 14−32% of total acids) and *C. scabrifolia* (5.6 g/100 g DM) ($P < 0.01$). The content of quinic acid was also lower in the natural origins of *C. pinnatifida*. The content of malic acid varied in the range of 0.3−1.1 g/100 g DM in the samples analyzed. Malic acid and quinic acid were present at roughly equal levels in the cultivars of *C. brettschneideri*.

Table 2. Contents (Mean ± SD) of Acids in Chinese Hawthorn Fruits[a]

cultivars/origins	N	acid content (g/100 g DM)			
		malic	citric	quinic	total
		C. pinnatifida var. *major*			
947	4	0.70 ± 0.05 fgh	8.38 ± 0.45 k	2.68 ± 0.16 gh	11.76 ± 0.64 m
8321	4	0.86 ± 0.03 j	7.93 ± 0.39 j	2.73 ± 0.18 gh	11.51 ± 0.30 m
Dajinxing	4	0.68 ± 0.01 fg	4.62 ± 0.10 de	0.85 ± 0.06 cd	6.16 ± 0.10 ef
Huixiandahong	4	0.95 ± 0.07 k	5.36 ± 0.35 fg	2.99 ± 0.12 i	9.29 ± 0.22 kl
Jiangou 2	4	0.35 ± 0.01 a	1.97 ± 0.02 a	0.80 ± 0.05 bcd	3.12 ± 0.05 a
Mopan	4	0.85 ± 0.07 j	6.08 ± 0.15 h	2.61 ± 0.24 g	9.54 ± 0.32 l
Qiujinxing	4	0.60 ± 0.04 de	3.79 ± 0.46 c	0.81 ± 0.03 bcd	5.20 ± 0.46 cd
Shandongdajinxing	4	1.10 ± 0.02 l	4.95 ± 0.18 ef	2.87 ± 0.15 hi	8.92 ± 0.29 k
Shen78201	4	0.56 ± 0.01 cd	2.16 ± 0.06 a	1.04 ± 0.13 de	3.77 ± 0.18 b
Zizhenzhu	4	0.59 ± 0.02 de	3.14 ± 0.33 b	1.31 ± 0.06 f	5.04 ± 0.25 c
C. pinnatifida var. *major* combined	40	0.72 ± 0.21 A	4.84 ± 2.12 B	1.87 ± 0.94 B	7.43 ± 3.05 AB
		C. brettschneideri			
Caihong	4	0.76 ± 0.06 hi	3.75 ± 0.23 c	0.57 ± 0.09 abc	5.08 ± 0.35 c
Hongroushanlihong	4	0.49 ± 0.01 b	5.04 ± 0.14 ef	0.77 ± 0.04 abcd	6.30 ± 0.16 fg
Hongroushanzha	4	0.81 ± 0.01 ij	4.41 ± 0.10 d	0.52 ± 0.05 ab	5.74 ± 0.06 de
Jifu 1	4	0.76 ± 0.01 hi	3.97 ± 0.07 c	0.49 ± 0.07 a	5.22 ± 0.05 cd
Jifu 3	4	0.71 ± 0.01 fgh	3.82 ± 0.07 c	0.53 ± 0.07 ab	5.06 ± 0.04 c
Xinghong 2	4	0.52 ± 0.02 bc	4.62 ± 0.17 de	0.52 ± 0.03 ab	5.66 ± 0.14 cde
Zuofu 1	4	0.73 ± 0.03 gh	4.90 ± 0.11 def	0.76 ± 0.08 abcd	6.39 ± 0.20 fg
Zuofu 2	4	0.67 ± 0.07 fg	5.98 ± 0.65 h	0.75 ± 0.06 abcd	7.40 ± 0.77 i
C. brettschneideri combined	32	0.68 ± 0.11 A	4.56 ± 0.75 B	0.61 ± 0.13 A	5.86 ± 0.81 A
		C. pinnatifida			
Shanzha 1	4	0.65 ± 0.05 ef	7.17 ± 0.14 i	1.20 ± 0.16 ef	9.02 ± 0.17 kl
Shanzha 2	4	0.32 ± 0.00 a	5.54 ± 0.05 g	0.94 ± 0.00 d	6.80 ± 0.05 fgh
Shanzha 3	4	1.12 ± 0.01 l	4.63 ± 0.09 de	1.38 ± 0.03 f	7.13 ± 0.05 hi
C. pinnatifida combined	12	0.70 ± 0.34 A	5.78 ± 1.10 B	1.17 ± 0.21 A	7.65 ± 1.03 AB
		C. scabrifolia			
Yun′nan shanzha	4	0.55 ± 0.03 bcd A	2.29 ± 0.06 a A	5.64 ± 0.39 j C	8.48 ± 0.46 j B

[a] Values (mean ± SD) without common letters within a column are significantly different ($P < 0.05$). Significant differences are marked with different letters a−m between cultivars/origins and A−C between species.

Sugars. The contents of sugars in the samples analyzed are presented in **Table 3**. The total sugar content including sugar alcohols ranged from 18.5 to 44.5 g/100 g DM. All three natural origins of *C. pinnatifida* had lower total sugar contents (18.5−27.1 g/100 g DM) than *C. scabrifolia* and cultivars of *C. brettschneideri* and *C. pinnatifida* var. *major* (28.2−44.5 g/100 g DM, $P < 0.01$).

Fructose and glucose existed in abundance in all 22 samples analyzed. The content of fructose varied from 5.5 to 18.4 g/100 g DM (16−45% of total sugars) and that of glucose from 5.3 to 16.6 g/100 g DM (14−40% of total sugars). The fructose content was often slightly higher than that of glucose.

Sucrose was found in four samples only. The highest content, 23.8 g/100 g DM, was in the cultivar 8321 of *C. pinnatifida* var. *major*, accounting for 54% of the total sugars. The situation was quite similar in Huixiandahong, a cultivar of *C. pinnatifida* var. *major*, with a sucrose content of 21.4 g/100 g DM (53% of total sugars). In addition, sucrose was found in Shandongdajinxing of the *C. pinnatifida* var. *major* (11.0 g/100 g DM) and in *C. scabrifolia* (15.2 g/100 g DM). In three sucrose containing cultivars of *C. pinnatifida* var. *major*, the contents of glucose (5.5−8.7 g/100 g DM) and fructose (6.8−10.0 g/100 g DM) were lower compared with other samples of the same species (glucose, 11.1−16.6 g/100 g DM; fructose, 10.7−18.4 g/100 g DM) ($P < 0.01$).

Sugar Alcohols. Sorbitol and *myo*-inositol were detected in all the hawthorn samples analyzed. The mass spectra of sorbitol and *myo*-inositol are presented in **Figure 2**. Comparing the mass spectra and retention times of the sample peaks with those of the reference compounds verified the identification. The ions in the mass spectrum of sorbitol were ions at m/z 103, 205, and 307 obtained by α-cleavage, while the strong ions at m/z 319 and 217 were obtained by secondary cleavage of trimethylsilanol. The main ions of *myo*-inositol were at m/z 318, 305, 217, 204, 191, 147, and 73, typical for TMS derivatives of 6-carbon cyclitols (*18*).

The contents of sorbitol and *myo*-inositol in hawthorn fruits are listed in **Table 3**. Sorbitol was the major sugar alcohol in all 22 samples. The content varied widely between 3.0 and 15.7 g/100 g DM (10−40% of total sugars) with smaller variation seen within the species but significant difference between species ($P < 0.01$). *C. brettschneideri* had the highest sorbitol content among all the species (10.6−15.7 g/100 g DM), whereas *C. scabrifolia* had the lowest (3.0 g/100 g DM). No statistically significant difference was found between the natural origins of *C. pinnatifida* and cultivars of *C. pinnatifida* var. *major* ($P > 0.05$).

myo-Inositol existed in all the samples with contents varying from 0.1 to 0.3 g/100 g DM.

Sugar/Acid Ratio. The sugar/acid (S/A) ratio varied widely from 2.4 to 13.2 among the cultivars of *C. pinnatifida* var. *major*. Less variation (4.1−8.8) was seen within the species *C. brettschneideri*. The S/A ratios in the fruits of *C. scabrifolia* (3.7) and the natural origins of *C. pinnatifida* (2.5−3.8) were low primarily because of the low sugar content.

Table 3. Contents (Mean ± SD) of Sugars, Sugar Alcohols, and the Sugar/Acid Ratio in Chinese Hawthorn Fruits[a]

origins/cultivars	N	sugar and sugar alcohol content (g/100 g DM)						
		fructose	glucose	sucrose	sorbitol	*myo*-inositol	total sugars[b]	sugar/acid
				C. pinnatifida var. *major*				
947	4	10.74 ± 0.23 e	11.06 ± 0.31 f		6.24 ± 0.23 b	0.12 ± 0.00 cd	28.17 ± 0.56 c	2.40 ± 0.09 a
8321	4	7.19 ± 0.09 b	6.22 ± 0.08 b	23.77 ± 0.52 d	6.43 ± 0.05 b	0.14 ± 0.00 ef	43.75 ± 0.61 i	3.80 ± 0.10 de
Dajinxing	4	17.32 ± 0.42 l	15.96 ± 0.16 k		8.82 ± 0.17 d	0.16 ± 0.00 h	42.26 ± 0.69 fghi	6.87 ± 0.10 j
Huixiandahong	4	6.81 ± 0.12 b	5.51 ± 0.03 a	21.40 ± 0.73 c	6.55 ± 0.01 b	0.13 ± 0.00 def	40.40 ± 0.73 fg	4.35 ± 0.10 g
Jiangou 2	4	18.44 ± 0.11 m	12.89 ± 0.13 ij		9.79 ± 0.07 e	0.08 ± 0.01 b	41.20 ± 0.31 fgh	13.19 ± 0.20 p
Mopan	4	11.85 ± 0.41 f	11.60 ± 0.21 fg		6.56 ± 0.11 b	0.14 ± 0.00 def	30.13 ± 0.69 d	3.16 ± 0.06 c
Qiujinxing	4	16.26 ± 1.45 k	15.94 ± 0.67 k		7.81 ± 0.33 c	0.21 ± 0.01 j	40.21 ± 2.44 fg	7.75 ± 0.29 kl
Shandongdajinxing	4	10.05 ± 0.06 de	8.73 ± 0.29 d	10.95 ± 0.57 a	6.18 ± 0.23 b	0.14 ± 0.01 fg	36.06 ± 1.09 e	4.04 ± 0.03 ef
Shen78201	4	18.00 ± 0.38 lm	16.64 ± 0.38 l		8.90 ± 0.09 d	0.12 ± 0.00 c	43.66 ± 0.83 i	11.61 ± 0.36 o
Zizhenzhu	4	17.32 ± 0.15 l	12.18 ± 0.23 gh		9.43 ± 0.17 d	0.07 ± 0.00 a	38.99 ± 0.53 f	7.76 ± 0.47 kl
Crataegus pinnatifida var. *major* combined	40	13.40 ± 4.41 B	11.67 ± 3.79 B	5.61 ± 9.21 A	7.67 ± 1.40 B	0.13 ± 0.04 A	38.48 ± 5.29 C	6.49 ± 3.52 B
				C. brettschneideri				
Caihong	4	15.12 ± 1.16 j	13.58 ± 1.08 j		15.67 ± 0.53 j	0.15 ± 0.01 gh	44.53 ± 2.78 i	8.76 ± 0.13 n
Hongroushanlihong	4	12.63 ± 0.39 g	11.05 ± 0.39 f		13.33 ± 0.28 fg	0.12 ± 0.01 c	37.13 ± 1.06 e	5.90 ± 0.05 h
Hongroushanzha	4	13.38 ± 0.06 gh	12.21 ± 0.06 gh		13.56 ± 0.06 gh	0.15 ± 0.00 h	39.31 ± 0.04 f	6.84 ± 0.07 j
Jifu 1	4	14.70 ± 0.08 ij	13.35 ± 0.05 j		14.75 ± 0.17 i	0.14 ± 0.00 def	42.94 ± 0.24 hi	8.22 ± 0.05 m
Jifu 3	4	13.63 ± 0.46 h	12.58 ± 0.25 hi		13.99 ± 0.15 h	0.13 ± 0.00 def	40.33 ± 0.79 fg	7.98 ± 0.18 l
Xinghong 2	4	14.03 ± 0.15 hi	13.31 ± 0.26 j		15.10 ± 0.32 i	0.13 ± 0.00 def	42.58 ± 0.72 ghi	7.53 ± 0.25 k
Zuofu 1	4	13.34 ± 0.15 gh	11.95 ± 0.19 gh		15.08 ± 0.70 i	0.15 ± 0.01 gh	40.52 ± 0.98 fg	6.34 ± 0.05 i
Zuofu 2	4	10.57 ± 0.90 e	9.53 ± 0.79 e		10.60 ± 1.06 e	0.13 ± 0.01 def	30.84 ± 2.77 d	4.17 ± 0.06 fg
C. brettschneideri combined	32	13.43 ± 1.42 B	12.20 ± 1.38 B		14.01 ± 1.59 C	0.14 ± 0.01 A	39.77 ± 4.29 C	6.97 ± 1.42 B
				C. pinnatifida				
Shanzha 1	4	8.10 ± 0.06 c	7.43 ± 0.09 c		7.30 ± 0.18 c	0.13 ± 0.00 de	22.96 ± 0.21 b	2.55 ± 0.07 ab
Shanzha 2	4	5.53 ± 0.10 a	5.33 ± 0.03 a		7.37 ± 0.08 c	0.28 ± 0.00 l	18.51 ± 0.21 a	2.72 ± 0.03 b
Shanzha 3	4	9.67 ± 0.17 d	8.85 ± 0.08 d		8.40 ± 0.18 d	0.19 ± 0.00 i	27.11 ± 0.43 c	3.80 ± 0.08 de
C. pinnatifida combined	12	7.76 ± 1.78 A	7.20 ± 1.51 A		7.69 ± 0.54 B	0.20 ± 0.06 B	22.86 ± 3.68 A	3.02 ± 0.58 A
				C. scabrifolia				
Yun'nan shanzha	4	7.04 ± 0.28 b A	5.78 ± 0.23 ab A	15.19 ± 0.67 b B	3.01 ± 0.07 a A	0.22 ± 0.01 k B	31.25 ± 1.26 d B	3.69 ± 0.07 d A

[a] Values (means ± SD) without common letters within a column are significantly different ($P < 0.05$). Significant differences are marked with different letters a−p between cultivars/origins and A−C between species. [b] Total sugars includes sugars and sugar alcohols.

Principal Component Analysis. The PCA biplot was applied to illustrate the compositional characteristics of different hawthorn samples. The result is shown in **Figure 3**. The first three principal components (PCs) explained 81% of the variance of the data. The closer the hawthorn species/cultivars/origins lie on the plot, the more similar they are in the composition of sugars, acids, and sugar alcohols. On the other hand, a sample located distant from the others may have significantly different compositional characteristics.

Chemical components located close together on a PCA biplot often correlate positively with each other. Fructose and glucose lie close to each other, indicating a positive correlation between the two sugars in hawthorn fruits, whereas sucrose lies on the opposite diagonal corner, indicating a negative correlation with fructose and glucose (**Figure 3A**). The correlation between fructose and glucose has also been reported in other fruits (*19*, *20*). A species/cultivar/origin in the vicinity of a component is usually rich in this specific component.

PC1 (53%) separates the acid-rich samples from those with a higher sugar content. The samples lying on the right part of the biplot are relatively abundant in acids, whereas the ones on the left part are characterized by high contents of sugars. PC2 (15%) explains the differences in the contents of individual compounds among the samples. The sucrose-containing samples were clearly separated from the nonsucrose samples.

PC3 (13%) separates the samples according to the abundance of *myo*-inositol in the fruit. The samples containing higher levels of *myo*-inositol such as Yun'nan shanzha (*C. scabrifolia*) and Shanzha 2 of *C. pinnatifida* are separated from the others.

The cultivars of *C. brettschneideri* are closely positioned in all the PCA biplots, suggesting close similarities in the contents and profiles of acids, sugars, and sugar alcohols. In contrast, the cultivars of *C. pinnatifida* var. *major* fell into two separate clusters, indicating much bigger compositional variations among the cultivars within the variety. The cultivars 947, 8321, Huixiandahong, Mopan, and Shandongdajinxing were richer in acids, whereas Dajinxing, Jiangou 2, Shen78201, and Zizhenzhu were more abundant in sugars.

DISCUSSION

The fruits of different hawthorn species and origins are known to vary widely in their contents of acids. The content of titratable acids was reported to be 1.5−4.5% in fruits of *C. pinnatifida*, 2.1% in *C. cuneata* and *C. hupehensis*, 1.67−2.75% in *C. scabrifolia*, 1.16−2.70% in *C. brettschneideri*, and 0.56% in *C. altaica* (*14*). Gao et al. investigated the fruit acids in eight Chinese hawthorn species and varieties. The acid content ranged from 1.72 to 5.86% DM. Tartaric acid and succinic acid instead of malic acid were found in the fruits of *C. pinnatifida* var. *major*, *C. scabrifolia*, *C. kansuensis*, *C. hupehensis*, and *C. cuneata*. In the fruits of

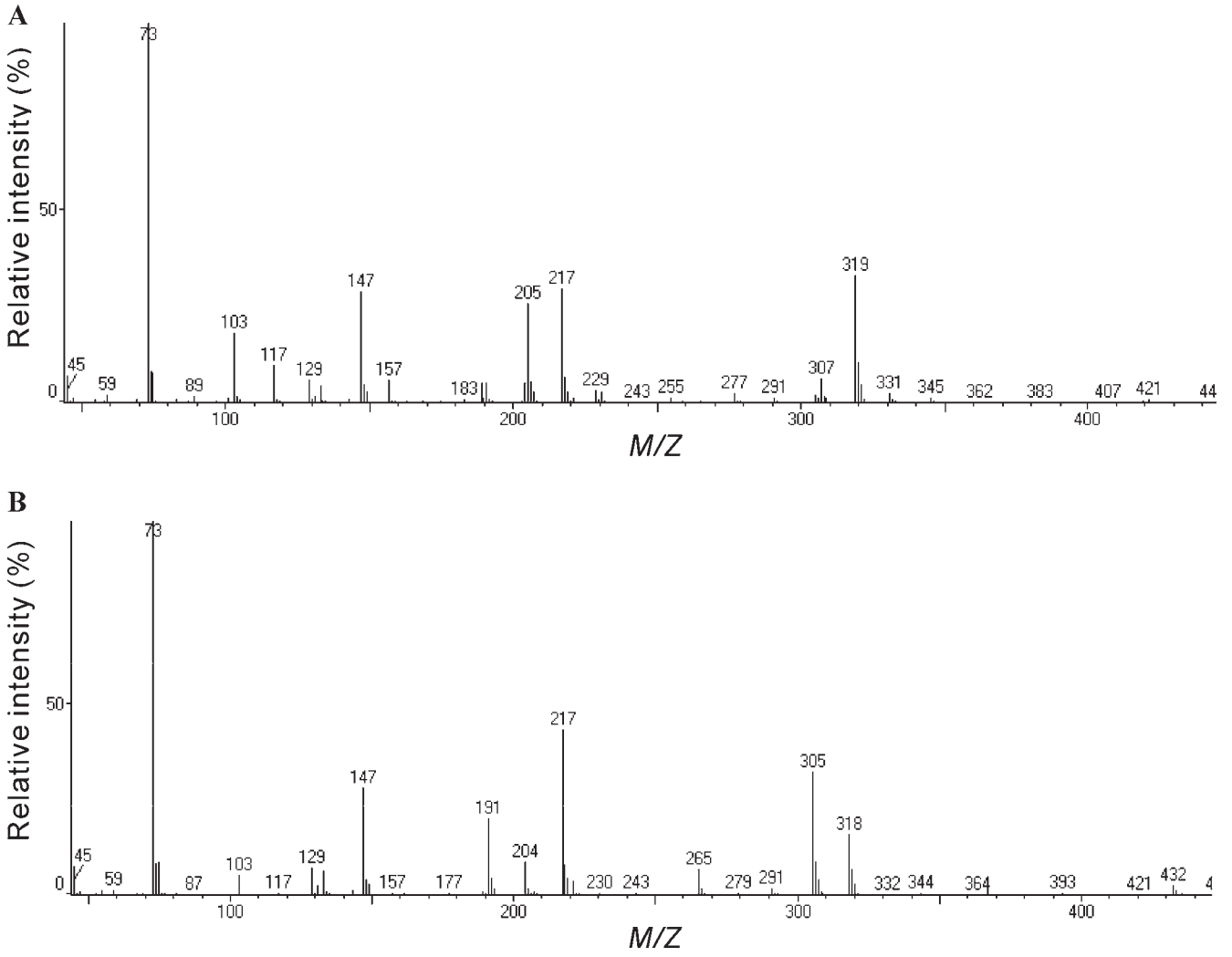

Figure 2. Mass spectra of (**A**) sorbitol and (**B**) *myo*-inositol detected with GC−MS, EI, 70 eV.

C. pinnatifida, tartaric acid was found but not malic acid and succinic acid (*17*). In our study, malic acid and quinic acid existed in all the samples analyzed, whereas tartaric acid and succinic acids were undetected. This is also the first report of the occurrence of quinic acid in hawthorn fruits.

The total content of acids is an important quality factor for hawthorn fruits when they are used in TCM. In TCM, hawthorn fruits are used for stimulating food digestion, improving blood circulation, and reducing the risk of hypertension and hyperlipidemia (*12*, *13*). The organic acids in hawthorn fruits are considered among the key bioactive compounds contributing to those functions. The Chinese pharmacopeia sets a minimal acid content of 4% dry weight for hawthorn fruits for application in TCM (*13*). In this study, the total acid contents of two cultivars, Jiangou 2 and Shen78201 of *C. pinnatifida*. var. *major*, was found lower than the standard. But most of the samples are qualified (**Table 2**).

Sucrose was only detected in 3 cultivars (8321, Huixiandahong and Shangdongdajinxing) of *C. pinnatifida* var. *major* and origin of *C. scabrifolia*. Sucrose represents the major transport metabolite form of photosynthetically assimilated carbon in plants, and its metabolism plays a key role, particularly in sink tissues such as fruits. The key enzymes of sucrose metabolism are sucrose phosphate synthase (SPS), sucrose synthase (SUS), and invertases. SPS and SUS catalyze the synthesis of sucrose, whereas invertases catalyze the irreversible hydrolysis of sucrose to glucose and fructose. All these enzymes are known to play a major role in sucrose partitioning for energy purposes in plant cells. An increase in SUS activity was associated with sucrose accumulation in the fruits of the peach, citrus and muskmelon (*21*, *22*). The clear on/off situation of sucrose in the fruits of cultivars within *C. pinnatifida* var. *major* indicates significant differences in sucrose metabolism probably because of the introduction of a different genotype to some of the cultivars during breeding. Cultivars containing sucrose may have a closer genotypic relationship compared with the nonsucrose cultivars/species. However, this should be verified by further genetic, metabolic, and enzymatic studies (*23*).

Sugar alcohols are cyclic or acyclic polyols playing key metabolic roles in various higher plants, e.g. mannitol in the plants of the Oleaceae family, sorbitol in Rosaceae and dulcitol in Celastraceae (*24*). Some sugar alcohols are used as sweeteners, humectants, and texturizing agents in the food industry. Recent investigations have revealed that sugar alcohols have numerous beneficial properties to human health such as regulating the glycemia of diabetic patients (*25*). However, this is the first report on the presence of sugar alcohols in Chinese hawthorn fruits.

Sorbitol is commonly found in fruits of the Rosaceae family. The level of sorbitol has been reported in the apple (0.2−0.75% fresh weight, 1.50−9.43% of total sugars), pear (1.21−2.83% fresh weight, 9.70−23.6% of total sugars), plum (6.02−23.4% dry weight, 6.61−22.9% of total sugars), prune (9.40−13.9% fresh weight, 19.0−28.3% of total sugars), peach (0.03−0.47% fresh weight, 0.55−5.50% of total sugars), and apricot (0.05−0.46%

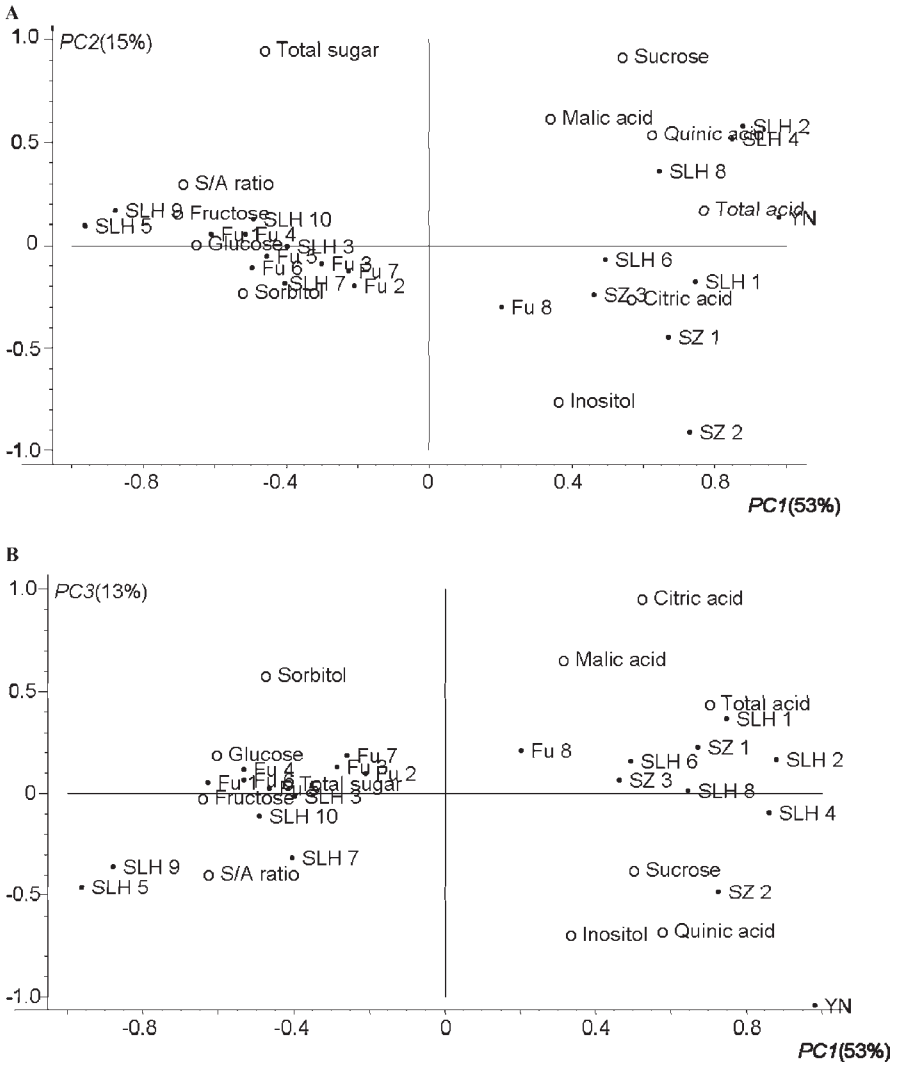

Figure 3. PCA biplot of acids, sugars, and sugar alcohols in 22 Chinese hawthorn fruit samples. SLH, *C. pinnatifida* var. *major*; SLH 1, 947; SLH 2, 8321; SLH 3, Dajinxing; SLH 4, Huixiandahong; SLH 5, Jiangou 2; SLH 6, Mopan; SLH 7, Qiujinxing; SLH 8, Shandongdajinxing; SLH 9, Shen78201; SLH 10, Zizhenzhu. Fu, *C. brettschneideri*; Fu 1, Caihong; Fu 2, Hongroushanlihong; Fu 3, Hongroushanzha; Fu 4, Jifu 1; Fu 5, Jifu 3; Fu 6, Xinghong 2; Fu 7, Zuofu 1; Fu 8, Zuofu 2. SZ, *C. pinnatifida* Bge.; SZ 1, Shanzha 1; SZ 2, Shanzha 2; SZ 3, Shanzha 3. YN (*C. scabrifolia*), Yun'nan shanzha.

fresh weight, 0.74−6.05% of total sugars) (*26*). Compared with the situation in most of these fruits, sorbitol accounted for a higher proportion of the total sugar in the Chinese hawthorn fruits.

The sorbitol content correlated with the ripeness stage of the fruits. Sorbitol dehydrogenase is the predominant enzyme influencing the sorbitol content in mature fruits. The activity of the enzyme is sensitive to the influence of the growth season and temperature (*24*). The cultivars/species of the hawthorn maturating in the early autumn tended to contain higher levels of sorbitol than those ripening later. *C. brettschneideri* mature around late August and early September. The levels of sorbitol in these fruits were 10.6−15.7 g/100 g DM. *C. pinnatifida* var. *major* and *C. pinnatifida* typically reach the stage of optimal ripeness in late September to October, and the sorbitol contents in the fruits are typically 6.2−9.8 g/100 g DM. *C. scabrifolia* is the only species growing in southern China. The low sorbitol content of the fruit may be related to the high temperature of the region (*24*).

Sorbitol is known to be a prebiotic compound for several species of *Lactobacillus* and is also a preferred carbon source for human intestinal *Bifidobacteria* (*27*). Other studies have shown that sorbitol may reduce body fat and the level of toxic ammonia (*28*). Thus, sorbitol in the hawthorn may be an important bioactive compound that has so far been ignored.

myo-Inositol has been indicated to have some beneficial effects on human health such as the regulation of insulin sensitivity, amelioration of diabetes-induced vascular dysfunction and alleviation of metabolic syndrome (*29*). Thus, the levels of *myo*-inositol should be considered when screening hawthorn fruit for application in health products such as food supplements and functional foods.

The S/A ratio is crucial for determining the sensory properties and acceptability of berries and fruits (*15*, *19*). The total sugar content and S/A ratio both correlate positively with sweetness and fruity flavor. Compared with sugar content, the S/A ratio has been shown to be a stronger indicator of the sweetness of berries (*15*, *19*). Two cultivars of *C. pinnatifida* var. *major*, Jiangou 2 and Shen78201, had exceptionally high S/A ratios. Therefore, we expect good sensory properties of these fruits. In most cultivars of *C. brettschneideri* and *C. pinnatifida* var. *major* the S/A ratio was 6−9. These cultivars may also have good sensory profiles. All 3 origins of *C. pinnatifida* had quite low S/A ratio and total sugar contents, suggesting some less pleasant taste of the fruits.

Sugar composition also affects the taste of fruits. For example, sucrose and fructose are known to be sweeter than glucose and

sorbitol. The relative sweetness of fructose, glucose, and sorbitol is 1.2−1.5, 0.5−0.8, and 0.5 compared with sucrose (*30*). Cultivars with high sucrose contents, e.g., 8321 and Huixiandahong, may possess high sweetness. Although *C. scabrifolia* contained sucrose, the total sugar content and the S/A ratio were both quite low. The fruits of *C. scabrifolia* may have a strong sour taste.

The PCA biplot can assist prediction of the food quality and sensory properties of hawthorn fruits. The samples with high S/A ratios lie close to the "S/A ratio" in the PCA biplot, suggesting sweet and fruity taste of the fruits.

This research investigated the contents and profiles of acids, sugars, and sugar alcohols in 18 cultivars of *C. pinnatifida* var. *major* and *C. brettschneideri* commonly cultivated in China and four samples of the species *C. pinnatifida* and *C scabrifolia* of natural origin. The compositional differences among the samples may be used as chemotaxonomic information distinguishing the hawthorn species from one another. *C. scabrifolia* contained a significantly higher level of quinic acid and a lower level of sorbitol than the rest of the samples studied ($P < 0.01$). The content of sorbitol in *C. brettschneideri* was typically higher compared with those in *C. pinnatifida* var. *major*, *C. scabrifolia* and *C. pinnatifida* ($P < 0.01$). The samples of *C. pinnatifida* var. *major* differed from *C. pinnatifida* by the high sugar content ($P < 0.01$). The hawthorn samples analyzed fell into two groups rich in sugars and acids respectively. This is the first report on the profile of sugars and sugar alcohols and the occurrence of quinic acid in hawthorn fruits. The results provide valuable information for distinguishing hawthorn fruits of different origins and evaluating the quality of hawthorn fruits for industrial application.

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