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Fruit Characteristics and Variation of Phenolic Compounds in the Fruit of Hawthorn (*Crataegus pinnatifida* Bunge) Selected from Korea and Chinese Cultivars

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Abstract - In order to select superior tree from Korea, five major phenolic compounds including (-)-epicatechin(EC), chlorogenic acid (ChA), hyperoside (HP), isoquercitrin (IQ), and procyanidin B_2 (PC-B_2) in hawthorn fruit were evaluated. We also compared these hawthorn fruits of five clones with Chinese hawthorn cultivars. HPLC with a diode-array detector was used to determine the contents of the individual compounds. Hawthorn fruits of five clones (selected from different area of Korea), and four Chinese hawthorn cultivars grown in the Korea Forest Research Institute (Suwon) were utilized. With their high functional components, Jungsun is the clone including the highest contents of EC (11.26 mg/g) and PC-B_2 (24.46 mg/g). The clone of Chuncheon 15 had highest HP (0.53 mg/g) and IQ (0.41 mg/g). From the results, the clone of Jungsun and Chuncheon 15 can be evaluated to be selected breeding material for cultivar development.

Key words - *Crataegus pinnatifida* Bunge, phenolic compounds, HPLC analysis

Introduction

Hawthorn (*Crataegus pinnaitifida* Bunge) is widely distributed in Korea and has been used as herbal medicine for treating various cardiovascular disease, arteriosclerosis and hypertension in Korea (Chang *et. al.*, 2000). It is also used to improve digestion, remove retention of food, promote blood circulation and resolve blood stasis both in traditional and folk medicine (Ammon and Handel, 1981). The fruits of *C. pinnatifida* (Rosaceae) have been used traditionally as a peptic agent in Oriental medicine and recently as a local soft drink material (E.-S. Kao *et al.,* 2007). It is believed that preparations of fruits of *C. pinnatifida* improve the heart function when there are indications of declining cardiac performance, deficiency in coronary blood supply, and mild forms of arrhythmia (Popping *et al.*, 1995). The pharmacological effects of *C. pinnatifida* have mainly been attributed to their polyphenolic contents, and oligomeric procyanidins (OPCs) are abundant in hawthorn. The active constituents and the antioxidant effects of the extracts of the fruit of *C. pinnatifida* have been widely studied (Kao *et al.*, 2005). Because phenolics, mainly flavonoids and proanthocyanidins, are recognized as active ingredients of hawthorn (Zhang *et al.*, 2001), we measured the contents of five major phenolic compounds such as (-)-epicatechin (EC), procyanidin B_2 (PC-B_2), hyperoside (HP), isoquercitrin (IQ) and chlorogenic acid (ChA) for selecting superior tree used as breeding materials.

In current study, we evaluate the characteristics of fruit and measured the content of phenolic compounds in the fruit of *C. pinnatifida* selected from Korea and Chinese cultivars. We

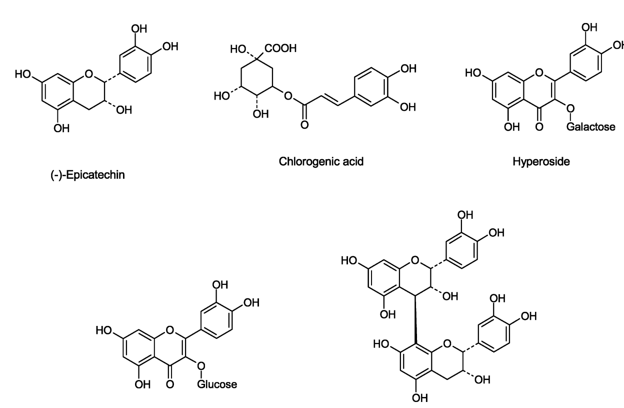

Fig. 1. Chemical structure of the major phenolics in the fruit of *C. pinnatifida.*

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also investigated the difference of the content of five major phenolics, namely EC, PC-B_2, HP, IQ and ChA (Fig. 1) in hawthorn fruits selected from Korea and Chinese hawthorn cultivar.

Materials and Methods

Plant materials

The fruits of *C. pinnatifida* used in this study were Chuncheon 1, Chuncheon 8, Chuncheon 15, Jungsun and Poechun which were selected from Korea and Maban, Changgu, Keumsung and Daekumsung which were Chinese cultivar. *C. pinnatifida* fruits grown in the Korea Forest Institute (Suwon, Korea) were collected in October after mature. Freeze dried the fruit of *C. pinnatifida* was finely ground, extracted twice with methanol (MeOH). The crude extracts of the fruit was analyzed by the modified method (Chang *et. al.*, 2006) with HPLC (Surveyor, Thermo).

Fruit characteristics

To assess the morphological variation of the hawthorn fruit selected Korean clones and Chinese hawthorn cultivars were used. Measurement of 5 quantitative characteristics (FL : Fruit Length, FW : Fruit Width, WF : Weight of Fruit, IFY : Individual Fruit Yield) were made on 9 individuals. Fruit characteristics were measured on October and analyzed by SAS System.

The HPLC Analysis

The samples were analyzed by HPLC (Thermo Separation Products (California) Inc.). The elution consisted of solvents A and B (5% and 25% acetonitrile in 25 mM sodium phosphate buffer, respectively, pH 2.4) with a flow rate of 1 ml/min. The proportion of solvent B was increased from 10% to 80% during the first 20 min, held for 10 min, and then returned to 10% in the last 5 min. The effluent was monitored at 278 nm for PC-B2 and EC, and 360 nm for ChA, HP and IQ. The studied components were quantified using the calibration curves prepared from the standards obtained. All phenolic compounds such as EC, PC-B_2, HP, IQ and ChA were purchased from Sigma Chemical Co.

Results and Discussion

Fruit characteristics of hawthorn

The measurements of 5 quantitative characteristics of 9 individuals (5 selected from Korea clones and 4 Chinese cultivars) are summarized in Table 1. Fruit length of hawthorn selected from Korea showed a range of 15.2 to 18.8 mm, while Chinese cultivars were a rage of 26.0 to 27.0 mm. The weight of hawthorn fruit selected from Korea showed a range of 2.17 to 3.84 g, while Chinese cultivars were a rage of 10.56 to 12.1 g. These results will be helpful for the understanding of morphological variation between the hawthorn clones selected from Korea and Chinese hawthorn cultivars.

In our study, principal component analyses (PCA) were

Table 1. Morphological characteristics of *C. pinnatifida* fruit

Variety	Fruit characteristics				
	FL*(mm)	FW**(mm)	FL/FW	WF***(g)	IFY****(kg)
Chuncheon 1	18.3 d	18.1 e	1.01 b	3.27 d	7.80 ab
Chuncheon 8	15.6 f	16.0 h	0.97 c	2.17 f	4.77 cde
Chuncheon 15	15.2 f	17.0 g	0.90 ef	2.30 f	3.09 ef
Jungsun	17.9 e	20.1 d	0.89 ef	3.84 c	2.44 f
Poechun	18.8 c	17.7 f	1.07 a	2.76 e	6.97 bc
Maban	26.8 a	29.5 a	0.91 e	12.00 a	9.61 a
Changgu	26.0 b	29.6 a	0.88 f	12.10 a	5.21 cde
Keumsung	26.7 a	26.9 c	0.99 c	10.56 b	4.13 def
Daekumsung	27.0 a	28.7 b	0.94 d	12.00 a	5.86 bcd

*FL : Fruit Length, **FW : Fruit Width,
***WF : Weight of Fruit, ****IFY : Individual Fruit Yield
Different letters indicate Duncan's multiple range tests (Significant at $p<0.05$)

Table 2. Eigenvalue and its contribution obtained from principal component analysis of *C. pinnatifida* fruit characteristics

Principle component	Eigenvalue	Difference	Proportion	Cumulative (%)
1	3.2009	1.8396	0.6402	64.02
2	1.3613	0.9293	0.2723	91.25
3	0.4.321	0.4274	0.0864	99.89
4	0.0047	0.0036	0.0009	99.98
5	0.0011	0.0000	0.0002	100.00

used to assess the differentiation among 9 individuals (Table 2). The PCA analyses were performed using 5 quantitative characteristics. The first and second principal components accounted for 64.02% and 91.25% of the total variance, respectively. As a results of cluster analysis, we can observed that fruit morphological differences between the hawthorn clones selected from Korea and Chinese hawthorn cultivars were founds (Fig. 2).

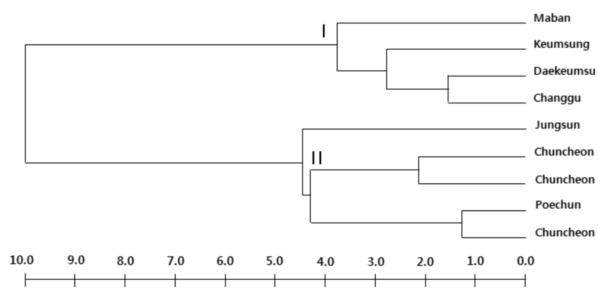

Fig. 2. Cluster dendrograms of *C. pinnatifida* based on fruit characteristics.

HPLC analysis of Phenolic compounds

Five phenolic compounds, (-)-epicatechin (EC), Procyanidin B_2 (PC-B_2), hyperoside (HP), isoquercitrin (IQ) and Chlorogenic acid (ChA) from the samples were identified by comparing their retention times with those of authentic standard. Fig. 3 shows the HPLC chromatograms of the five authentic standards and phenolic compounds in the hawthorn fruit (Chuncheon 8). Calibration curves were generated by plotting peak area (Y) versus the concentrations (X, mg/mL) of standard solutions. Results, expressed as the values of the correlation coefficient (R^2), were also listed in Table 3. Good linearity was achieved in the investigated ranges for all the analytes.

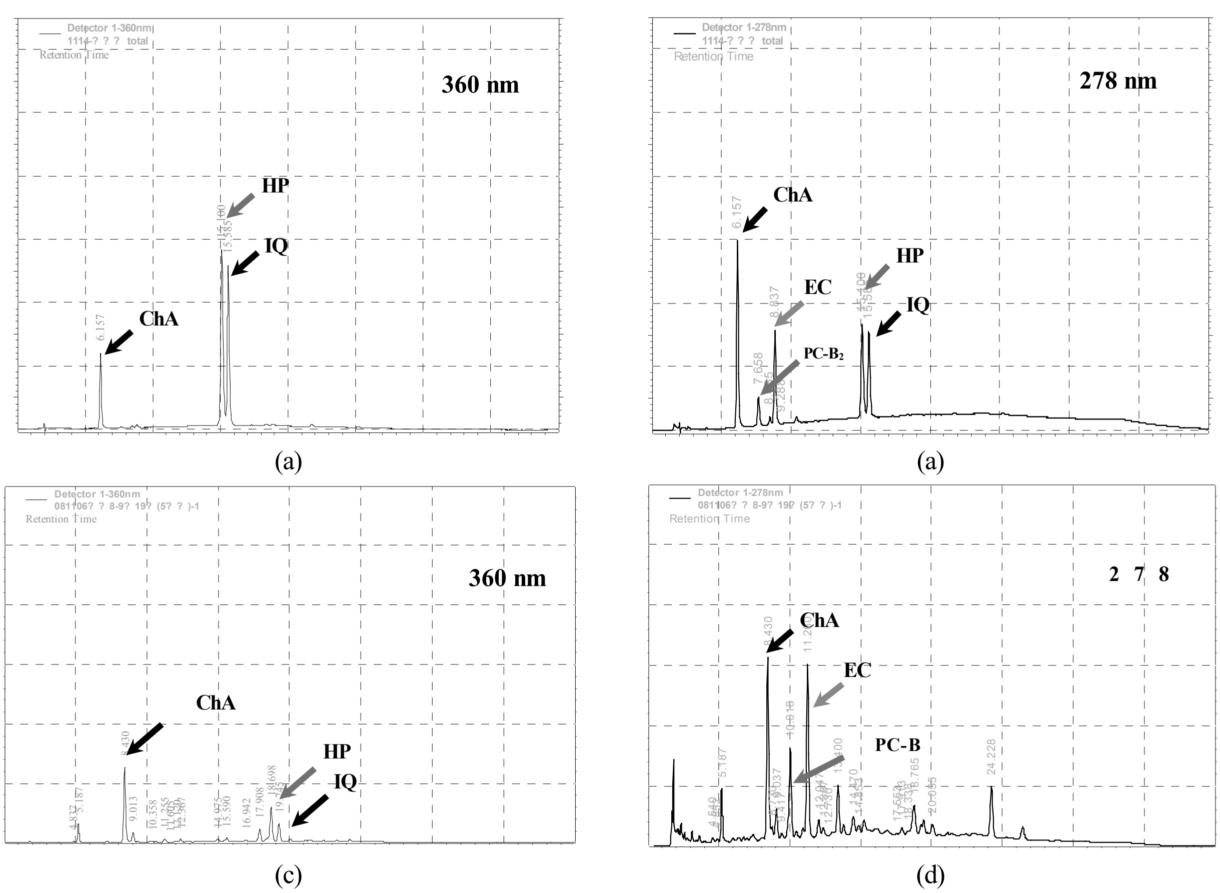

Fig. 3. HPLC chromatograms of five standard phenolic compounds (a and b) and phenolic compounds in the hawthorn fruit (Chuncheon 8) (c and d).

Table 3. Calibration curves of 5 phenolic compounds in *C. pinnatifida* fruit

standard	equation of standard curve	correlation coefficient(R^2)
ChA	y = 25420.502 x + 941787.00	0.994
EC	y = 31413.323 x + 926554.813	0.995
PC-B_2	y = 11300.690 x + 924132.355	0.996
HP	y = 104878.234 x + 232144.338	0.998
IQ	y = 114732.906 x - 716648.006	0.995

Table 4. Variation of the major active phenolics, (-)-epicatechin (EC), Procyanidin B_2 (PC-B_2), hyperoside (HP), isoquercitrin (IQ) and Chlorogenic acid (ChA), present in *C. pinnatifida* fruit

Variety	Content (mg/g)				
	EC	PC-B_2	HP	IQ	ChA
Chuncheon 1	6.20 ± 0.23*	13.49 ± 1.15	0.42 ± 0.05	0.36 ± 0.01	2.85 ± 0.15
Chuncheon 8	5.86 ± 0.13	13.03 ± 0.82	0.48 ± 0.01	0.37 ± 0.01	3.18 ± 0.08
Chuncheon 15	6.32 ± 0.15	14.02 ± 0.51	0.53 ± 0.02	0.41 ± 0.01	1.47 ± 0.10
Jungsun	11.26 ± 0.69	24.46 ± 0.29	0.38 ± 0.00	0.39 ± 0.00	0.53 ± 0.03
Poechun	1.72 ± 0.01	6.24 ± 0.29	0.08 ± 0.01	0.19 ± 0.00	2.12 ± 0.04
Maban	12.23 ± 0.28	28.56 ± 0.50	0.25 ± 0.01	0.32 ± 0.00	0.77 ± 0.02
Changgu	11.18 ± 0.56	29.52 ± 1.29	0.28 ± 0.01	0.32 ± 0.01	0.85 ± 0.07
Keumsung	10.33 ± 0.94	25.00 ± 0.17	0.15 ± 0.01	0.31 ± 0.01	1.70 ± 0.04
Daekumsung	12.77 ± 0.17	27.76 ± 0.23	0.20 ± 0.01	0.35 ± 0.01	1.66 ± 0.06

*Data are expressed as mean ± SD (n=3).

Variation of phenolic compounds from hawthorn fruit

The contents of five phenolic compounds measured in this study were analyzed using HPLC method. The variation in the contents of phenolic compounds from the fruit of hawthorn is presented in Table 4. The EC content in Korean hawthorn and Chinese hawthorn fruit ranged from 1.72 to 12.77 mg/g, and the highest concentration was observed from daekumsung, while the lowest concentration was detected from chuncheon 8. It is reported that the (-)-epicatechin has protective effect on cytotoxicity and inhibits cell membrane damage (Valls-Belles *et al*., 2004). The PC-B_2 content and HP content ranged from 6.24 to 28.56 mg/g, and from 0.08 to 0.53 mg/g, respectively. It is also observed that IQ and ChA concentrations were in the range of 0.19 - 0.41 mg/g and 0.53 - 3.18 mg/g, respectively. In general, PC-B_2 has anti- and pro-oxidant effects (Sakano *et al.,* 2005) and HP has protective effects on PC12 cells against cytotoxicity (Liu *et al.*, 2005). It is also reported that IQ and ChA has anti-inflammatory activity (Rogerio *et al*., 2007) and protective effects against gamma-radiation-induced damage (Abraham *et al*., 1993), respectively.

Hawthorn fruits have long been used in traditional Korean, Chinese and European medicine, and are widely consumed as food, in the form of juice, drink, jam and canned fruit. Due to the significant amounts of various organic acids, including caffeic acid, malic acid and tartaric acid, contained in the hawthorn fruits (Gao *et al.*, 1995), the components in hawthorn preparations, such as hawthorn drink, might exist in acidic condition. Since chlorogenic acid content is an important factor in making hawthorn drink, it is more useful to use hawthorn clones selected from Korea than those of Chinese cultivars.

In conclusion, Jungsun is the clone including the highest contents of EC (11.26 mg/g) and PC-B_2 (24.46 mg/g). The clone of Chuncheon 15 had highest HP (0.53 mg/g) and IQ (0.41 mg/g). The clone of Chuncheon 8 had the highest content of ChA (3.18 mg/g). Due to their high functional components, the clone of Jungsun and Chuncheon 15 can be evaluated as the most proper breeding materials. We also compared the content of phenolic compounds in Korean clones with those in Chinese cultivar. Besides, the content of EC and PC-B_2, the contents of other compounds in Korean clones were higher than those of Chinese cultivars.

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