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Free Sugar and Organic Acid in the Fruit of Hawthorn (*Crataegus pinnatifida* Bunge) Selected Clones as Honey Plant in Korea

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Abstract

Hawthorn is widely distributed in Korea and has been used as herbal medicine for treating various cardiovascular disease, arteriosclerosis and hypertension in Korea. In order to select superior honey tree plant from Korea, the free sugar and organic acid in hawthorn fruits, including five Korean clones and four Chinese cultivars, were evaluated. We also compared these hawthorn fruits of five clones (selected from different area of Korea) with Chinese hawthorn cultivars. Glucose, galactose, fructose and sucrose were the major sugar components of hawthorn. In this study, we observed that sucrose, glucose and fructose content. The highest sucrose content of hawthorn fruit was 188.12g/100g in Daegeumseong cultivar. The sweetness index, which plays important role of taste, was also calculated from the content of sucrose, glucose and fructose. The contribution of each carbohydrate was calculated, based on the fact that fructose is 2.30 and sucrose 1.35 times sweeter than glucose. The highest sweetness of hawthorn fruit was 579.52 in Pocheon clone. Main organic acid detected in hawthorn fruit were citric acid, malic acid and shikimic acid. The highest citric acid and malic acid content in hawthorn fruit were 157.50g/100g (Pocheon 3) and 34.12g/100g (Daegeumseong), respectively. The results of this study would be helpful for using food and functional food products, due to the beneficial effects of free sugar and organic acid for human health such as antioxidants and anticarcinogenic properties.

Key words: Citric acid, Hawthorn fruit, Malic acid, Sucrose, Sweetness index

INTRODUCTION

The fruits of Hawthorn (*Crataegus pinnaitifida* Bunge, Rosaceae) have been used traditionally as a peptic agent in oriental medicine and recently as a local soft drink material (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. 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).

Hawthorn 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*., 2002). 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).

Phenolics, mainly flavonoids and proanthocyanidins, are known as active ingredients of hawthorn (Zhang *et al*.,

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Table 1. The operating conditions of HPLC for analysis of free sugars

Instrument	Dionex ultimate 3,000 Shodex RI-101 Detector
Column	Sugar-pak (waters, 300×6.5mm)
Solvent	Distilled water
Flow rate	0.5ml/min
Injection volume	10uL

2001). Previously, we reported 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) (Park *et al*., 2010).

Even though, there are some data on the biologically active chemical constituents such as total phenol, flavonoid and vitamin C of hawthorn fruit (Zhang *et al*., 2001), there are no studies of the free sugar and organic acid content of five clones and four Chinese hawthorn cultivars. Thus, in this study, we report the overall free sugar and organic acid content of hawthorn fruit of five clones selected from different area of Korea with Chinese four hawthorn cultivars grown in the National Institute of Forest Science, Suwon. The aim of this study was to evaluate nutrition values of hawthorn fruit, which may be useful for using fruit as food and functional food products.

MATERIALS AND METHODS

Plant materials and sample collection

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 Daekeumsung which were Chinese cultivar. *C. pinnatifida* fruits grown in the National Institute of Forest Science (Suwon, Korea) were collected in October after mature.

Free sugar content analysis

The sugars content levels (sucrose, glucose, and fructose) in the samples were analyzed by a reported LC method with some modification (Godin *et al*., 2011). A total of 2g of freeze-dried hawthorn powders was extracted with 100mL of water in a cooled ultrasonic bath (40kHz) for 60 min. Then the extract was centrifuged (15,000 rpm, 4°C, 10 min) and the supernatant was separated with membrane filter (0.45 um) and transferred to vials. Samples were analyzed on a Dionex Ultimate 300 HPLC system. A Sugar-pak (waters, 300×6.5mm) column with deionized water as the mobile phase was used for separation of the sugars. The flow rate were optimized as 0.5mL/min and a Shodex RI-101 detector was used for identification. Quantification was performed on the basis of linear calibration plots of the logarithm of peak areas versus the logarithm of concentrations. The concentrations were expressed in grams per 100g of distilled water (DW). The summary of HPLC conditions used for free sugar analysis in this study were shown in Table 1.

Organic acid analysis

The freeze-dried hawthorn powders of 3g were transferred to Eppendorf tube with 5 ml of distilled water. Then the extract was centrifuged (15,000 rpm, 4°C, 10 min) and the supernatant was separated with membrane filter (0.45 um) and transferred to vials. Organic acids in hawthorn fruit were analyzed by a Dionex Ultimate 300 HPLC system. The chromatographic separation used for organic acid detection employed 0.01 N H_2SO_4 as the mobile phase, Aminex 87H (300×10mm) column (Bio-Rad, USA) with a flow rate of 0.5ml/min, samples organic acids were detected with a RI detector (ERC, RefractoMax, Japan).

Estimation of hawthorn taste parameters

Sweetness index in this study was calculated based on

Table 2. Free sugar content of the fruit of hawthorn clones and Chinese cultivars

Variety	Contents of analyte (mean±SD, n=3), g/100g				
	Sucrose	Glucose	Fructose	Mannitol	Sorbitol
Chuncheon 1	12.57±0.35c*	125.89±3.91d	114.69±3.91d	1.13±0.02cd	101.53±4.06c
Chuncheon 8	26.47±1.21b	79.86±3.14f	73.91±3.37f	1.58±0.25a	98.38±5.00c
Chuncheon 15	3.26±0.12d	111.18±1.80e	104.94±1.67e	1.51±0.13ab	118.04±3.20b
Jungsun	0.0±0.0e	151.28±5.71b	159.57±5.86b	1.47±0.06ab	128.83±4.73a
Poechun	0.0±0.0e	179.34±6.02a	173.99±5.75a	0.94±0.19d	120.19±3.48b
Maban	0.0±0.0e	110.35±9.14e	114.53±9.60d	0.87±0.05d	54.63±4.59e
Changgu	0.0±0.0e	134.70±2.97c	140.61±3.15c	1.02±0.02d	65.76±1.26d
Keumsung	0.42±0.04e	117.08±1.56e	122.22±1.42d	1.29±0.03bc	51.75±0.98e
Daekeumsung	188.12±4.29a	48.90±1.46g	49.04±0.75g	0.90±0.02d	51.30±1.40e

*Different letters in the same column indicate significant difference.

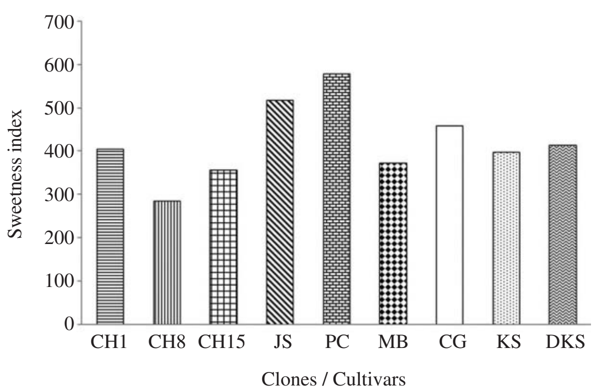

Fig. 1. Sweetness index of the fruit of hawthorn selected clones and Chinese cultivars. (CH1: Chuncheon 1, CH8: Chuncheon 8, CH15: Chuncheon 15, JS: Jungsun, PC: Poechun, MB: Maban, CG: Changgu, KS: Keumsung, DKS: Daekeumsung).

the amount and sweetness properties of individual carbohydrate in fruit (Keutgen and Pawelzik, 2007). The contribution of each carbohydrate was calculated, based on the fact that fructose is 2.30 and sucrose 1.35 times sweeter than glucose. Accordingly, sweetness index=1.0 (glucose) + 2.30 (fructose) +1.35 (sucrose).

Statistical analysis

All experiments were performed at least three times and the results were reported as mean±SD. Data were statistically evaluated by one-way ANOVA analysis and Duncan's multiple range test. Results of tables and figure were presented as mean±standard deviation.

RESULTS AND DISCUSSION

Free sugars analysis of hawthorn fruit

Generally, the sugar composition is one of the most important parameters for food energy level. In Table 2, we listed the HPLC quantitative analytical data of the free sugars in the hawthorn fruit. The fruits of five different hawthorn clones selected from Korea and four Chinese cultivars were used in this study. Glucose, sorbitol and sucrose were the major sugar components of the hawthorn fruit.

Glucose contents are between 48.90 and 179.34g/100g, and sucrose contents are between 188.12 and 0.00g/100g, respectively. The highest sucrose content of hawthorn fruit was 188.12g/100g in Daekeumsung cultivar.

Because the relationship between fructose and glucose content was about 1:1, which is typical for berry fruit, (Keutgen and Pawelzik, 2007) and the same results were observed in this study. It is well known that fruit sweetness depends not only on the content of each sugar, but also on the ratios of the main individual sugars (Zheng *et al.*, 2016). It is also found that the sweetness index, which plays important role of taste in hawthorn fruit (Fig. 1).

The highest sweetness of hawthorn fruit was 579.52 in Poecheon clone while the lowest sweetness of hawthorn fruit was 285.59 in Chuncheon 8 clone.

Organic acids analysis of hawthorn fruit

Hawthorn fruits have long been used in traditional

Table 3. Organic acid content of the fruit of hawthorn clones and Chinese cultivars

Variety	Contents of analyte (mean±SD, n=3), g/100g		
	Citric acid	Malic acid	Shikimic acid
Chuncheon 1	65.41±1.96e*	11.81±0.40e	0.22±0.0g
Chuncheon 8	63.95±3.01e	16.41±1.54cd	0.32±0.03f
Chuncheon 15	98.87±2.36d	14.67±0.41d	0.61±0.02b
Jungsun	36.84±1.33f	18.22±2.17c	0.67±0.03a
Poechun	157.50±4.60a	29.41±2.55b	0.47±0.01de
Maban	95.26±8.07d	29.12±2.81b	0.47±0.05de
Changgu	131.42±3.65b	32.44±0.65a	0.54±0.00c
Keumsung	107.59±2.20c	27.01±0.56b	0.50±0.01d
Daekeumsung	111.49±2.00c	34.12±1.22a	0.46±0.00e

*Different letters in the same column indicate significant difference.

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.

Organic acids represent further compounds contributing to taste and flavour of hawthorn fruit. Three major organic acids in hawthorn fruit, citric acid, malic acid, and shikimic acid, were detected by HPLC. The organic acids of the fruit of hawthorn clones and Chinese cultivars are shown in Table 3. The results showed that citric acid showed was the main organic acid in hawthorn fruit and followed by malic acid and shikimic acid. While, Albertini *et al*. (2006) reported that citric acid dominated in lemons, limes and orange, the malic acid was the main organic acid in hawthorn fruit. Citric acid content in Poechun clone fruit was the highest as 157.50g/100g. Citric acid can be used for proper mineral supplementation of food and as a flavoring ingredient. It is also reported that citric acid ensures proper functioning of the kidneys and prevents the formation of kidney stones. Recently, it is found that organic acids have been used in food preservation and as a new generation of growth promotors instead of antibiotics (Kim and Rhee, 2015). Shikimic acid was the third-most common organic acid, and occupied 0.67~0.22g/100g according to clones and cultivars. A similar trend was reported for strawberry and jujube fruit (Keutgen and Pawelzik, 2007; Park and Kim, 2016). Malic acid contents in Daekeumsung cultivar was the highest as 34.12g/100g.

In this study, we analyzed the free sugars and organic acid using HPLC. To our knowledge, this is the first study investigating the content of free sugars and organic acids in the fruit of five different hawthorn clones selected from Korea and four Chinese hawthorn cultivars. From the results of this study we found that the glucose sorbitol and sucrose were the major sugar components of the hawthorn fruit. The sweetness index was also calculated from the content of free sugars. The highest sweetness of hawthorn fruit was 579.52 in Pocheon clone while the lowest sweetness of hawthorn fruit was 285.59 in Chuncheon 8 clone.

Three major organic acids in hawthorn fruit were citric acid, malic acid, and shikimic acid. The highest citric acid and malic acid content in hawthorn fruit were 157.50g/100g (Poechun clone) and 34.12g/100g (Daekeumsung cultivar), respectively. The results of this study would be helpful for using food and functional food products, due to the beneficial effects of free sugar and organic acid for human health such as antioxidants and anticarcinogenic properties.

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