Original Article

Journal of Apiculture 32(4) : 375~379(2017)
DOI: 10.17519/apiculture.2017.11.32.4.375

Free Sugar and Organic Acid Contents of Pollens from *Quercus* spp. in Korea

Youngki Park*, Jae-Hee Kim and Sea-Hyun Kim

Department of Forest Genetic Resources, National Institute of Forest Science, Suwon, 16631 Korea

(Received 17 November 2017; Revised 24 November 2017; Accepted 24 November 2017)

Abstract

In this study, we analyzed free sugar and organic acid content in pollens of four different species of *Quercus* in Korea. Glucose and sucrose were the major sugar components of pollen. The highest sucrose content of pollen was 16.03g/100g in *Q. variabilis*. The sweetness index, which plays important role of taste, was also calculated from the content of sucrose and glucose. The sweetness of pollen were different with other species. The highest sweetness of pollen was 21.98 in *Q. variabilis*. Main organic acids detected in pollen were citric acid, malic acid and fumaric acid. The highest citric acid and malic acid content in pollen were 3.11g/kg (*Q. mongolica)* and 5.62g/kg (*Q. mongolica*), respectively.

Key words: Citric acid, Fumaric acid, Glucose, Malic acid, Sucrose, Sweetness index

INTRODUCTION

The consumption of natural products has increased substantially, because of the recent consumers' trends. This current concept is mentioned in recent researches, which report several bioactivities of pollens from flower direct or collected by bee (Morais *et al*., 2011; Kim *et al*., 2005). Pollen is a fine, powder-like material produced by flowering plants and it is the male reproductive cells of flowers. Generally, flower pollens contain different phytochemicals and nutrients and are rich in carotenoids, flavonoids and phytosterols. Pollen and pollen products have been successfully used for the treatment off oral desensitisation of children who have allergy (Basim *et al*., 2006).

Since the pollen obtained from flower and collected from bee has therapeutic properties such as antioxidant, anti-inflammatory, antibacterial and anticariogenic activities, it is important to know chemical constituents of pollen (Kalaycioglu *et al*., 2017). In this study, we analyzed the free sugar and organic acid content in pollens of four *Quercus* species in Korea namely *Q. acutissima, Q. mongolica, Q. serrata,* and *Q. variabilis*.

Q. acutissima is closely related to the turkey oak, classified with it in *Quercus* sect. *Quercus mongolica*, commonly known as Mongolian oak, is a species of oak native to Japan, southern Kuriles, Sakhalin, Manchuria, central and northern China, Korea, eastern Mongolia, and eastern Siberia. The species can grow to be 30 m tall. *Quercus serrata* is a deciduous oak tree reaching a height of 25m occupying elevations from 100 to 2,000m. Leaves are up to 17cm long by 9cm wide, leathery, elliptical in shape, with serrated margins. *Q. variabilis* is a medium-sized to large deciduous tree growing to 25~30m tall with a rather open crown, and thick corky bark with deep fissures and marked by sinuous ridges. The leaves are green above

*Corresponding author. E-mail: woodpark@korea.kr

and silvery below with dense short pubescence.

While there are some data on the chemical constituents such as total phenol, flavonoid and vitamin C and biological activities of pollen of *Quercus* spp. (Ghosh and Jung, 2017; Hong *et al*., 2015), there are no studies of the free sugar and organic acid content of four individual species. Thus, in this study, we report the overall free sugar and organic acid content of pollen in four *Quercus* species. We aim to increase the understanding knowledge of nutrition values of *Quercus* spp. pollen, which may be useful for using pollen as food and functional food products.

MATERIALS AND METHODS

Sample collection and preparation

Pollen of four *Quercus* spp. (QA: *Q. acutissima*, QM: *Q. mongolica*, QS: *Q. serrata*, QV: *Q. variabilis*), collected from the National Institute of Forest Science in Suwon were used in this study. A voucher specimen was deposited at the National Institute of Forest Science, Suwon, Korea.

Free sugar content analysis

The content levels of sugars (sucrose and glucose) in the samples were analysed by a modified procedure of a reported LC method (Godin *et al*., 2011). A total of 2g of freeze-dried pollen was extracted with 100 mL 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 (0.45μm) and transferred to vials. Samples were analysed on a Dionex Ultimate 300 HPLC system. A Sugar-pak (waters, 300×6.5mm) column with deionised water as the mobile phase was used for separation of the sugars. The flow rate was 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 dry weight (DW). The summary of HPLC conditions for free sugar analysis in this study was listed in Table 1.

Organic acid analysis

Approximately, 3g of freeze-dried pollen were transferred to Eppendorf tube with 5mL of distilled water. Then the extract was centrifuged (15,000 rpm, 4°C, 10 min) and the supernatant was separated with membrane filter (0.45μm) and transferred to vials. Organic acids in pollen 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, with a flow rate of 0.5mL/min, and the samples were injected into an Aminex 87H (300×10mm) column (Bio-Rad, USA). Organic acids were detected with a refractive index (RI) detector (ERC, RefractoMax, Japan).

Estimation of pollen taste parameters

Sweetness index in this study was calculated based on 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

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

Instrument	Dionex ultimate 3000 Shodex RI-101 Detector
Column	Sugar-pak (waters, 300×6.5mm)
Solvent	Distilled water
Flow rate	0.5mL/min
Injection volume	10μL

Table 2. Free sugar content of pollen of four *Quercus* species (g/100g)

Species	Contents of analyte (mean±SD, n=3)		Total
	Sucrose	Glucose	
Q. acutissima	14.35±0.14ns[z]	0.27±0.02c[y]	14.62±0.14ns
Q. mongolica	14.54±0.53ns	0.41±0.02a	14.96±0.54ns
Q. serrata	15.65±0.17ns	0.30±0.01bc	15.95±0.17ns
Q. variabilis	16.03±0.15ns	0.33±0.03b	16.37±0.14ns

[z]ns means not significant.
[y]Different letters indicate Duncan's multiple range test (Significant at p<0.01).

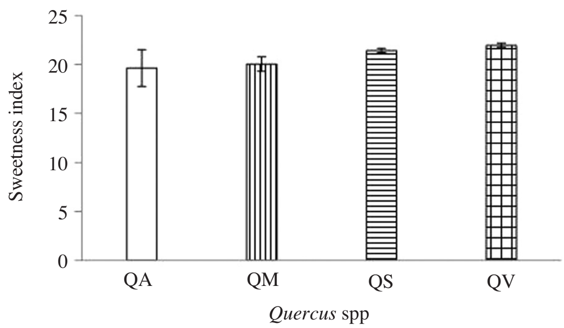

Fig. 1. Sweetness index of pollen of four *Quercus* spp. (QA: *Q. acutissima*, QM: *Q. mongolica*, QS: *Q. serrata*, QV: *Q. variabilis*).

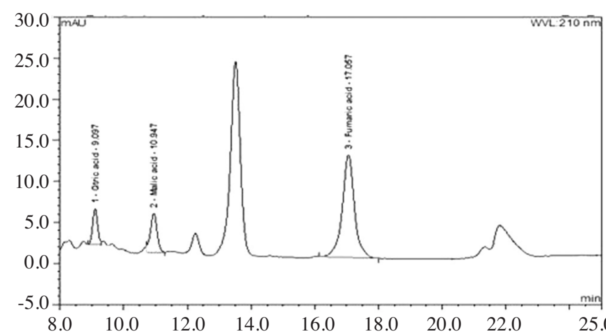

Fig. 2. Chromatogram of a pollen sample (*Q. mongolica*) for organic acid analysis by HPLC.

the results were reported as mean±standard deviation (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±SD.

RESULTS AND DISCUSSION

Free sugars analysis

The HPLC quantitative analytical data of the free sugars in pollen are listed in Table 2. Sugar composition is one of the most important parameters for food energy level. Glucose and sucrose were the major sugar components of pollen in *Quercus* spp. While fructose was detected in other pollens (Martins *et al*., 2011), there was no fructose detected in pollen of *Quercus* species in the present study. While other samples were pollen which obtained by bee collecting, we used pollen obtained directly from flower. This is the reason why we did not detected fructose from the pollen.

Glucose content are between 0.27 and 0.41g/100g, and sucrose contents are between 14.35 and 16.03g/100g. The highest sucrose content of pollen was 16.03g/100g in *Q. variabilis*.

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, increased with fruit growth and development (Fig. 1). The highest sweetness of pollen was 21.98 in *Q. variabilis* while the lowest sweetness of pollen was 19.65 in *Q. acutissima*.

Organic acid

Organic acids represent further compounds contributing to taste and flavour of pollen. Three organic acids in pollen, citric acid, malic acid, and fumaric acid, were detected by HPLC (Fig. 2). The organic acids of pollen in four *Quercus* spp. are shown in Table 3.

In this study, malic acid showed the main organic acid in pollen followed by citric acid and fumaric acid. There were different organic acids in different kinds of foods.

Table 3. Organic acid content of pollen of four *Quercus* species. (g/kg)

Species	Contents of analyte (mean±SD, n=3)			Total
	Citric acid	Malic acid	Fumaric acid	
Q. acutissima	1.91±0.14c[z]	2.04±0.06c	0.03±0.002c	3.98±0.18c
Q. mongolica	3.11±0.13a	5.62±0.02a	0.17±0.004a	8.89±0.30a
Q. serrata	2.59±0.02b	2.17±0.01bc	0.04±0.003b	4.79±0.03b
Q. variabilis	0.67±0.03d	2.30±0.05b	0.04±0.001b	3.00±0.05d

[z]Different letters indicate Duncan's multiple range test (Significant at $p<0.01$).

Although citric acid dominated in lemons, limes and orange, the malic acid was the main organic acid in pollen (Albertini *et al*., 2006). Like pollen, malic acid was the most common in ripe apple, sweet cherry and blackberry (Zhoa *et al*., 2015). Organic acid are intermediates in the tricarboxylic acid (TCA) cycle, one of the respiratory pathways.

Citric acid content in pollen of *Q. mongolica* was the highest as 3.11g/kg. Fumaric acid were the third-most common organic acids, and occupied 0.03~0.17g/kg according to species. 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).

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 pollen of four different *Quercus* species. From the results of this study we found that the glucose and sucrose were the major sugar components of pollen. The sweetness index was also calculated from the content of free sugars. The highest sweetness of pollen was 21.98 in *Q. variabilis*. Organic acid in pollen of *Quercus* spp. were mainly composed of citric acid, malic acid and fumaric acid. The highest citric acid and malic acid content in pollen were 3.11g/kg (*Q. mongolica*) and 5.62g/kg (*Q. mongolica*), respectively. The results would be helpful for using food and functional food products for pollen, due to the beneficial effects of free sugar and organic acid for human health such as antioxidants and anticarcinogenic properties.

LITERATURE CITED

Albertini, M.V., E. Carcouet, O. Pailly, C. Gambotti, F. Luro and L. Berti. 2006. Changes in organic acids and sugars during early stages of development of acidic and acidless citrus fruit. J. of Agri. & Food Chem. 54: 8335-8339.

Ashoor, S.H. and J.M. Knox. 1982. Determination of organic acids in foods by high-performance liquid chromatography. J. of Chromatography 299: 288-292.

Basim, E., H. Basim and M. Ozcan. 2006. Antibacterial activities of Turkish pollen and propolis extracs against plant bacterial phathogens. J. of Food Eng. 77: 992-996.

Godin, B., R. Agneessens, P.A. Gerin and J. Delcarte. 2011. Composition of structural carbohydrates in biomass: Precision of a liquid chromatography method using a neutral detergent extraction and a charged aerosol detector. Talanta 85: 2014-2026.

Ghosh, S. and C. Jung. 2017. Nutritional value of bee-collected pollens of hardy kiwi, *Actinidia arguta* (Actinidiaceae) and oak, Quercus sp. (Fagaceae). J. of Asia-Pacific Entomol. 20: 245-251.

Hong, I. P., S. O. Woo, S. M. Han, S. G. Kim, H. R. Jang, M. Y. Lee, Y. S. Choi, H. K. Kim and M. L. Lee. 2015. Evaluation of total phenol, flavonoid and vitamin content from the crushed pollens of acorn and Actinidia. J. of Apiculture 30: 225-229.

Ghosh, S. and C. Jung. 2017. Nutritional value of bee-collected pollens of hardy kiwi, *Actinidia arguta* (Actinidiaceae) and oak, Quercus sp. (Fagaceae). J. of Asia-Pacific Entomol. 20: 245-251.

Kalaycioglu, Z., H. Kaygusuz, S. Doker, S. Kolayli and F. B. Erim. 2017. Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. LWT-Food Sci. and Techn. 84: 402-408.

Keutgen, A. and E. Pawelzik. 2007. Modification of taste-relevant compounds in strawberry fruit under NaCl salinity. Food Chem. 105: 1487-1494.

Kim, S. A. and M. S. Rhee. 2015. Synergistic antimicrobial activity of caprylic acid in combination with citric acid against both *Escherichia coli* O157:H7 and indigenous microflora in carrot juice. Food Microbiol. 49: 166-172.

Kim, S. J., K. S. Youn and H. S. Park. 2005. Antioxidant effect of pine, oak, and lily pollen extracts. Koran J. Food Sci. Technol. 37: 833-837.

Martins, M. C. T., M. A. Morgano, E. Vicente, S. R. Baggio and D. B. Rodrigu- ez-Amaya. 2011. Physicochemical composition of bee pollen from eleven Brazilian states. J. of Apicultural Sci. 55: 107-116.

Morais, M., L. Moreira, X. Feas and L. M. Estevinho. 2011. Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. Food & Chemical Toxi. 49: 1096-1101.

Zhao, J., H. Li, W. Xi, W. An, L. Niu, Y. Cao, H. Wang, Y. Wang and Y. Yin. 2015. Changes in sugars and organic acids in wolfberry (*Lycium barbarum* L.) fruit during development and maturation. Food Chem. 173: 718-724.

Zheng, H., Q. Zhang, J. Quan, Q. Zheng and W. Xi. 2016. Determination of sugars organic acids, aroma components, and carotenoids in grapefruit pulps. Food Chem. 205: 112-121.