Original Article

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Evaluation of Total Polyphenol, Flavonoid and Vitamin Content from the Crushed Pollens of Acorn and Actinidia

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Abstract

Bee pollen is rich in various nutrients. Bee pollens of acorn (*Quercus acutissima*) and actinidia (*Actinidia arguta*) are the most collected in Korea. But stiff pollen wall hinders dissolution of polysaccharides and lowers extraction efficiency. Thus, we evaluated total polyphenol, total flavonoid and vitamin content from the pollen grains crushed by pulverization and freeze-drying method, and pollen extracts obtained by aqueous and ethanolic extraction, respectively. The total polyphenol contents were higher in acorn pollens than in actinidia pollens, in ethanol extracts than in H_2O extracts, and highest in freeze_ dried pollen followed by pulverized pollen and pollen load. The same pattern was found in total flavonoid contents. Vitamin B2 contents were very low, but B3 were high regardless of crushing. Vitamin C was only recovered from pollen loads, but negligible in pulverized or freeze-dried pollens.

Key words: Acorn, Actinidia, Flavonoid, Freeze-dry, Polyphenol, Vitamin

INTRODUCTION

Bee pollen is the pollen ball that has been packed by worker honeybees into pellets. Bee pollen added honey and glandular secretions is stored in brood cells (Bogdanov, 2014).

Bee pollen collected from various flowers is the only natural source of proteins, lipids, minerals, vitamins and amino acids for brood rearing and bee growth and development (Human and Nicolson, 2006; Campos *et al*., 1997). Recent research demonstrates that bee pollen possesses therapeutic benefits like promoting antitumor effects, scavenging free radicals, enhancing the immune function to mention a few (Bogdanov, 2014; Kroyer and Hegedus, 2001), and a good nutritional supplement with the beneficial effect for health (Bogdanov, 2014). Also pollen has high contents of polyphenolic compounds. Recently, many researchers have been focusing on flavonoids and phenolic acids that possess antibacterial, antiinflammatory, anticarcinogenic, immunemodulatory and antioxidant activities (Dini, 2011; Fang *et al*., 2008; Li *et al*., 2009; Zhang *et al*., 2006). This pollen grain wall usually has an outer layer (exine) made of sporopollenin in combination with an inner layer (intine) which is made up largely of cellulose. Sporopollenin elaborated in both surface sculpture and internal structure greatly resists decay and digestion or chemical degradation. The intine compo-sed primarily of cellulose and pectin also resists decay and digestion, and forms the final barrier to the nutrient-rich cytoplasm (Kress *et al*., 1978; Lee, 1986). Thus, any animal or insect absorbs only some cytoplasmic nutrients (Roulston and Cane, 2000; Blackmore *et al*., 2010). The

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research for extracting the cytoplasmic nutrients from pollen grains has been actively carried out recently. Rupture techniques of pollen cell wall were reported using planetary mill (Han *et al*., 2004; Kim and Son, 1990) or using supercritical carbon dioxide (Xu *et al*., 2009). Also, extracting nutrients from pollen grains were achieved by treating them with cell-wall-degrading chemical enzymes such as bee larva gut enzyme (Kim, 1989) and hexane or protease (Lee *et al*., 1997; Choi and Jeong, 2004).

In this study, we evaluated total polyphenol, total flavonoid and vitamin content from acorn and actinidia pollen grains crushed by pulverizing and freeze-drying method, and pollen extracts obtained by aqueous extraction or ethanolic extraction.

MATERIALS AND METHODS

Bee pollen samples

Acorn and actinidia pollen grains were collected from the National Academy of Agricultural Science (NAAS) apiary located in Wanju province of Korea during April to June 2014. The selected beehives were equipped with bottom-fitted pollen traps. The collected bee pollens were stored at −20°C in freezer for analysis.

Pollen wall rupture

Pollen cell wall was crushed by low-temperature ultrafine pulverizer (HKP-02, Korea Energy Technology, Seoul Korea) and freeze dryer (Freeze dryer, FD5508, Ilshin Lab Co. Ltd, Korea). Pollen grains were ground to a high-speed rotation speed at 80,000rpm by pulverizer which was equipped with a filter of 120 mesh and set at the temperature of −20°C to control the temperature increase of the sample. Pollen grains were frozen to −80°C in the rapid refrigeration (Deep Freezer, DF 9010, Ilshin Lab Co. Ltd, Korea), and then dried in a freeze dryer freeze dryer to −45°C

Pollen extracts preparation

4g of crushed pollens in 20ml of ethanol or distilled water (DW) was shaken in a water bath for 6 hours and centrifuged for 10 min at 10,000rpm. The extracted solution was filtered by Whatman filter paper No. 2 (Whatman, UK).

Total polyphenol content (TPC)

The total polyphenol contents in the pollen extract was estimated according to the Folin-Ciocâlteu method described by Blois (1958), with minor modifications. 0.5ml of the pollen extract was mixed with 1ml of 80% ethanol, 5ml of distilled water and 5ml of 0.2 N Folin-Ciocâlteu reagent. After agitating at room temperature for 5 min, 1ml of 5% sodium carbonate solution (Na_2CO_3) was added to mixtures and kept in the dark for 1 hour. The absorbance was read at 725 nm in a spectrophotometer. The total quantities of polyphenols in pollen samples were determined from the calibration curve using gallic acid (GA) standard solutions. Total phenol content was expressed as mg GAE/g pollen (mg gallic acid equivalents per gram pollen).

Total flavonoid content (TFC)

The total flavonoid content in the pollen extract was determined in accordance with the method described by Moreno *et al*. (2000). 25μl of the pollen extract was mixed with 8μl of 5% sodium nitrite ($NaNO_2$) and incubated at room temperature for 5 min. 15μl of 10% aluminium chloride ($AlCl_3$) was added to mixtures and agitated for 6 min and then neutralized with 50μl of 1M sodium hydroxide (NaOH). Absorbance was read at 510 nm in a spectrophotometer. The total quantity of flavonoid was calculated as quercetin equivalents per g pollen (mg QE/g pollen).

Vitamin

Vitamin content of acorn and actinidia pollen grains was determined following the standard method described by Korean Food Standards Codex (2008). The vitamins were measured by Nanospace SI-2 HPLC system (Shiseido, Japan) with a fluorescence (FL) detection for vitamin B2 (Riboflavin) or photodiode array PDA) detector for vitamin B3 (niacin) and vitamin C.

RESULTS AND DISCUSSION

Total polyphenol contents

Total polyphenol content of pollen extracts of acorn and actinidia was obtained using the standard curve of gallic acid (R2=0.99). Fig. 1 and 2 show that the total polyphenol contents varied with the extract solvents or plants. The total polyphenol content was found to be highest in freeze-dried pollen, followed by pulverized pollen and pollen grain in actinidia pollen. The total polyphenol content from actinidia pollen was increased 10% in pulverized pollen and 27.4% in freeze-dried pollen than pollen grain, respectively. We obtained similar result from acorn pollen. The total polyphenol content from freeze-dried or pulverized acorn pollen was increased 28.7% than pollen grain. Also the ethanol pollen extracts showed higher levels of total polyphenol content than water extracts in both acorn and actinidia pollens in this study, which are comparable to the results of other research. The total polyphenol content from freeze-dried pollen extracted with ethanol was 2-fold higher than that from acorn and actinidia pollen extracted with water. The freeze-dried acorn pollen extracted with ethanol showed the highest values of total polyphenol in comparison to the uncrushed acorn pollen or actinidia pollen. The total polyphenol content from freeze-dried or pulverized acorn pollen was higher than reported by Hong *et al*. (2014), who measured the total phenolic compound from acorn pollen treated with medicinal mushrooms.

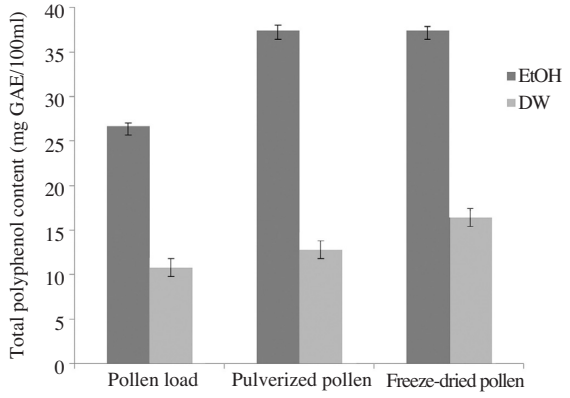

Fig. 1. Total polyphenol content of acorn pollen crushed by ultrafine pulverizer and freeze-dryer.

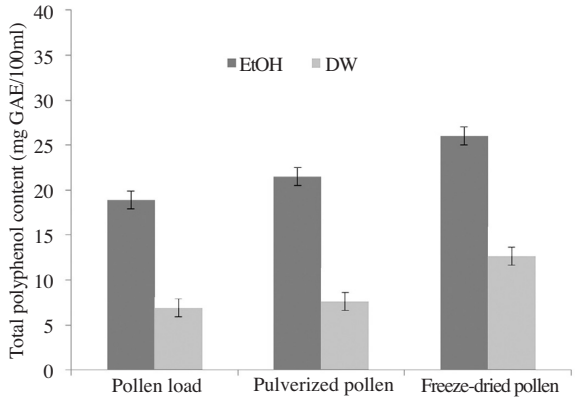

Fig. 2. Total polyphenol content of actinidia pollen by ultrafine pulverizer and freeze-dryer.

Total flavonoid content

Using the standard curve generated by quercetin (R2=0.9806), the total flavonoid content from acorn and actinidia pollen extracts are presented in Table 1 and 2. The freeze-dried acorn pollen extracted with ethanol showed the highest values of 26.7±0.47mg, followed by pulverized pollen and pollen grain in total flavonoid content. The total flavonoid content from acorn pollen was increased 17.7% in pulverized pollen and 27.4% in freeze-dried pollen compared to pollen grain, respectively. Similar results were obtained from actinidia pollen. Also higher total flavonoid levels were detected in ethanol extract than in water extract from both acorn and actinidia pollen. The total flavonoid content from freeze-dried acorn pollen extracted with ethanol was 6.8 times higher than that from pollen extracted with water. There are no reports of the flavonoid content in acorn and actinidia pollen.

Vitamin

The content of vitamins B2, B3 and C in acorn and actinidia pollens is shown in Tables 3 and 4. Data shows that the amount of vitamin C was the highest value of 98.9mg/100g, followed by vitamin B3 and B2 in acorn and actinidia pollen grains. The content of vitamin C was largely decreased in acorn pollen after pulverizing or freeze-drying, whereas that of vitamin B3 was slightly decreased. The vitamins B2 and B3 content in actinidia pollen was slightly increased after pulverizing or freeze-drying, but that of vitamin C was largely decreased as acorn pollen. The vitamin B2 concentration of acorn pollen

Table 1. Total flavonoid contents of acorn pollen

Extractant	Total flavonoid content (mg CE/ml)		
	Pollen load	Pulverized pollen	Freeze-dried pollen
EtOH	8.99±0.60	23.49±0.91	26.71±0.46
DW	0.36±0.024	3.65±0.59	3.914±0.49

Table 2. Total flavonoid contents of actinidia pollen

Extractant	Total flavonoid content (mg CE/ml)		
	Pollen load	Pulverized pollen	Freeze-dried pollen
EtOH	4.70±0.37	8.61±0.62	11.00±0.28
DW	0.62±0.01	0.32±0.12	0.24±0.03

Table 3. Vitamin contents of acorn pollen

Pollen	Vitamin content (mg/100g)		
	B2 (riboflavin)	B3 (niacin)	C
Pollen load	1.2	48.2	98.9
Pulverized pollen	1.8	32.4	0.6
Freeze-dried pollen	1.9	30.5	0.7

Table 4. Vitamin contents of actinidia pollen

Pollen	Vitamin content (mg/100g)		
	B2 (riboflavin)	B3 (niacin)	C
Pollen load	1.0	21.0	48.44
Pulverized pollen	1.7	25.5	0.4
Freeze-dried pollen	2.5	36.0	5.6

grain found in the present study was slightly higher than that reported by Lee *et al*. (1997). The vitamin B2 concentrations of acorn and actinidia pollen grains obtained was slightly higher than that of pine pollen grain reported by Lee *et al*. (1997), but lower than reported from Han *et al*. (2004).

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