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Effects of Drying Conditions on the Antioxidant Activities and Volatile Compounds of *Chrysanthemi Flos* Flowers

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Abstract

The effects of drying conditions on the antioxidant activities, luteolin, and volatile compounds of *Chrysanthemi Flos* flowers were evaluated. The flowers were dried with hot-air or far-infrared radiation at 40°C, 50°C and 60°C, respectively, to reach 22±1% of moisture content. Each 10 g of the dried flowers were extracted with 100 mL of 95% ethanol. Increasing temperature in hot-air dried (HAD) conditions increased the antioxidant activities of the flower extracts. However, increasing temperature in far-infrared dried (FID) conditions decreased the antioxidant activities of the extracts. Luteolin, one of main flavonoids of *Chrysanthemi Flos* flowers, was present in the highest content at 60°C FID flowers with a value of 139 μg/mL. Thirteen volatile compounds including camphor and β-caryophyllene were identified in chromatograms. Higher amount of the volatiles were found at 50°C HAD and 40°C FID. The results indicated that the antioxidant activities and volatile compounds of *Chrysanthemi Flos* flowers were significantly affected by drying conditions.

Key words: antioxidant, *Chrysanthemi Flos* flowers, drying condition, volatile compounds

INTRODUCTION

Chrysanthemum (*Chrysanthemum indicum* L.) grows naturally in Korea, China and Japan. It is also called as Gamgug (sweet chrysanthemum) in Korea because of its sweet taste. In oriental traditional medicine, its flowers have been used to treat vertigo, hypertensive symptoms, and several infectious diseases such as pneumonia, colitis and stomatitis (1). Recent reports have demonstrated the medicinal action of chrysanthemum including anti-inflammatory, immunomodulatory, anti-bacteria and anti-oxidant activities (2,3). Chrysanthemum contain a variety of flavonoid compounds, essential oils, phenolics, lactones, and sesquiterpenes (4-7). Four kinds of flavonoids, luteolin, apigenin, apigenin 7-*O*-β-D-glucose, and luteolin 7-*O*-β-D-glucose, have been identified in this plant (8). Also, one of flavonoids, sesquiterpene lactones containing cumambrin A, cumambrin B, arteglasin A, and angeloyljadin, were found in chrysanthemums (4,9-11). Luteolin has been reported to have significant medicinal effects of antispasmodic, anti-inflammatory and anti-tumor activities (12-15). Various volatile constituents in essential oils of chrysanthemum are also reported to exhibit antimicrobial or anticancer activities (16-20).

Previously, crude oriental medicines were mostly prepared in a natural drying way, with the use of shade, wind or sunlight, but now they are usually dried with drying equipment that uses heat or air. Despite the development of the drying equipment, there have been very few studies of the effect of drying conditions on the quality properties of each crude drug. The dried *Chrysanthemi Flos* flowers are generally traded on the basis of external appearance, such as color and smell. In this study, we evaluated the effect of drying conditions on antioxidant activities and volatile compounds in the *Chrysanthemi Flos* flowers. Furthermore, luteolin levels were also analyzed under different drying conditions. Luteolin is one of the key bioactive compounds of the *Chrysanthemi Flos* flower.

METERIALS AND METHODS

Chemicals and plant materials

Fresh *Chrysanthemi Flos* flowers were collected from the Gyeongnam Agricultural Research and Extension Services of the Republic of Korea. Luteolin, 1,1-dinitrophenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets were purchased from Sigma Chemical Co. (St. Louis, MO, USA), superoxide dismutase (SOD) assay kit from Dojindo Molecular Technologies, Inc. (Gaithersburg,

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MD, USA), and Folin-Ciocalteu reagent from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All the other chemical reagents used were analytical grade. The water used for sample preparation and HPLC was purified with a Super Purity Water System (Purite Ltd., Oxon, England) with a resistivity of 17.5 MΩ and above.

Drying conditions and preparation of extracts

The *Chrysanthemi Flos* flowers (500 g) were dried with hot-air (170 m^3/min) dryer (inner volume 6.5 m^3, HSED-1.5, Hansung Industrial Co., Iksan, Korea) or far-infrared dryer with a far-infrared heater (output 300 W, Hakko Electric Machine Works Co., Nagoya, Japan) at 40°C, 50°C, and 60°C, respectively to reach 22±1% of moisture content, which is the most common drying condition. Moisture contents were checked by comparing with thoroughly dried flowers. Extraction was performed by soaking the *Chrysanthemi Flos* flowers (10 g dried weight) in 100 mL of 95% ethanol in a shaking incubator (100 rpm) for 24 hr at 25°C. After filtration through a Whatman No.3 filter paper (Advantec Co., Tokyo, Japan), the solvent (ethanol) was removed by evaporation to dryness under a reduced pressure. The extracts from flowers were then dissolved in 95% ethanol in a concentration of 300 μg/mL for experiments except for SOD activity determination, where the concentration was 100 μg/mL.

DPPH radical scavenging activity (RSA)

The DPPH RSA of the flower extracts was measured by the method of Lee et al. (21). 0.2 mL of extracts were mixed with 1 mL of 0.041 mM DPPH in ethanol for 10 min, and then the optical density (OD) of the mixture was measured at a wavelength of 517 nm by a spectrophotometer. The DPPH RSA was calculated from the following equation:

$$\% \text{ DPPH RSA} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Superoxide dismutase (SOD) activity

SOD activity was determined using SOD Assay Kit-WST. The kit consisted of four materials: water-soluble tetrazolium salts (WST) solution, enzyme solution, buffer solution, and dilution buffer. A 20 μL sample solution was added to each sample well and blank 2 in a 96-well plate, and 20 μL of double distilled water was added to blank 1 and blank 3 wells. 200 μL of dilution buffer was added to blank 2 and blank 3 wells. A 20 μL of WST working solution was added to each sample and blank 1, and mixed thoroughly. The reaction mixture was incubated at 37°C for 20 min and the optical density (OD) was measured at 450 nm by a microplate reader (Bio-Tek Instruments MQX200 μQuant, Winooski, VT). The SOD activity was calculated from the following equation:

$$\% \text{ SOD activity} = [\{(\text{Blank1 OD} - \text{Blank3 OD}) - (\text{Sample OD} - \text{Blank2 OD})\}/(\text{Blank1 OD} - \text{Blank3 OD})] \times 100$$

ABTS radical scavenging activity (RSA)

The hydrogen peroxide scavenging activity was determined by an ABTS-peroxidase medium according to the method of Muller (22). One mL of the extracts, 0.1 mL of 0.1 M potassium phosphate buffer (pH 5.0) and 20 μL of 10 mM hydrogen peroxide were mixed, and then incubated at 37°C for 5 min. After the incubation, 30 μL of 1.25 mM ABTS (pH 5.0) in 0.05 M phosphate buffer and 30 μL of peroxidase (1 U/mL) were added to the mixture, and incubated at 37°C for 10 min. The absorbance was read with an ELISA reader (Sunrise RC/TS/TS Color-TC/TW/BC/6Filter, Tecan Austria GmbH, Grödig, Austria) at 405 nm. The ABRT RSA was calculated from the following equation:

$$\% \text{ ABTS RSA} = [1 - (\text{Sample OD/Blank OD})] \times 100$$

Total phenolic content (TPC)

Total phenol contents (TPC) were determined by the method of Gutfinger (23). One mL of the extract was mixed with 1.0 mL of 2% Na_2CO_3, followed by standing for 3 min. The mixture was mixed with 0.2 mL of 50% Folin-Ciocalteu reagent, and after standing for 30 min, centrifuged at 12,000 rpm for 5 min at room temperature. The absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm and gallic acid standard curve was obtained for the calibration of TPC. TPC were expressed as gallic acid equivalents (GAE).

Analysis of luteolin

Extraction was performed by soaking the *Chrysanthemi Flos* flowers (3 g dried weight) in 50 mL of 95% methanol in a shaking incubator (100 rpm) for 24 hr at 25°C. After filtration through a Whatman No.3 filter paper (Advantec, Tokyo, Japan), the solvent (methanol) was removed by evaporation under a reduced pressure to dryness. The methanol extracts were fractionized to ethyl acetate and water. Ethyl acetate fractions were then collected and evaporated under reduced pressure to dryness. Finally, the dried ethyl acetate fractions were suspended in 20 mL of methanol (HPLC grade) and used for analysis. Luteolin was analyzed with HPLC (Shimadzu Co. Ltd., LC 20A, Kyoto, Japan) and Nova Pak C18 column (3.9×150 mm) (Waters Co., Milford, MA) in condition of UV 365 nm and the injection volume was 10 μL. The composition of mobile phase was MeOH-H_2O-acetic acid (30:70:5, v/v/v) and the flow rate was 1.0 mL/min.

Analysis of volatile compounds

The dried flowers were powdered with a homogenizer (MC-811C, Novita, Seoul, Korea). And 50 mg of the powdered samples was then put into a 4-mL vial and sealed with a top-hole cap and silicone rubber septa for solid-phase micro-extraction (SPME) sampling. The vial was thermostatically heated in 60°C heat block. The SPME needle with 100-μm polydimethylsiloxane (PDMS) fiber was pushed through the septa. The fiber of SPME needle was extended into the headspace of the heated vial for 30 min and then withdrawn and removed from the vial. The needle was inserted immediately into the injection inlet of GC or GC/MS for 5 min. The gas chromatography (GC) used in the analysis of volatile compounds was Hewlett Packard 6890, equipped with a flame ionization detector coupled to Hewlett Packard Innowax (50 m×0.20 mm×0.4 μm) column and the flow rate of N_2 carrier gas was 0.5 mL/min. The column temperature increased from 70°C to 250°C at 3°C/min. The gas chromatography-mass spectrometry (GS-MS) for the identification of volatile compounds was Shimadzu GC/MS-QP 2010 and the analysis was performed in the same method as the gas chromatography. The mass spectral identification was obtained from the WILEY library 7 (Scientific Instrument Services, Inc., Ringoes, NJ).

Statistical analysis

All the measurements were triplicated, and the mean values of each treatment were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. P value of less than 0.01 was considered significant.

RESULTS AND DISCUSSION

Drying time

The time of drying to reach 22±1% of moisture contents in the *Chrysanthemi Flos* flower was 14 to 105 hr (Table 1). An increase in temperature resulted in a rapid decrease in the drying time. For example, the dry time at 40°C was 84 hr for HAD method and 105 hr for FID method, while at 60°C was 14 hr and 19 hr, respectively. The HAD using heat and air flow was a more effective way to dry than by FID.

Antioxidant activity

A series of studies have demonstrated that the water extract from chrysanthemum had a strong anti-oxidation effect (2) and the methanol extract acted as an inhibitor of xanthine oxidase (24). Some flavonoids such as luteolin, apigenin, and acacetin 7-*O*-galactopyranose, a strong antioxidant polyphenol, were also found to be abundant in chrysanthemum (10,11). For that reason, we studied the effect of drying conditions on the antioxidant activities of the *Chrysanthemi Flos* flowers.

All the antioxidant activities of the flower extracts from *Chrysanthemi Flos* were highly detected with increasing temperature in HAD condition (Table 2). DPPH RSA, ABTS RSA, and SOD activity of the extract at 40°C HAD condition were 36%, 31%, and 82%, respectively, while those at 60°C were 92%, 80%, and

Table 1. The drying time for hot-air drying (HAD) or far-infrared drying (FID) in *Chrysanthemi Flos* flowers[1)]

Drying conditions		Drying time (hr)	Moisture content (%)
HAD	40°C	84	22
	50°C	26	22
	60°C	14	21
FID	40°C	105	22
	50°C	29	23
	60°C	19	23

[1)]Drying time was measured when moisture content of the flowers was reached to 22±1%.

Table 2. Effect of hot-air drying (HAD) or far-infrared drying (FID) on antioxidant activities of the *Chrysanthemi Flos* flower extracts

Drying conditions		Antioxidant activities[1)]			
		DPPH-RSA (%)	SOD Activity (%)	ABTS-RSA (%)	TPC (μM)
HAD	40°C	36.0±1.69[2)c3)]	81.9±3.49[a]	31.4±1.02[c]	202±1.10[c]
	50°C	91.4±0.97[a]	80.7±3.71[a]	62.4±1.40[b]	200±1.10[c]
	60°C	92.0±0.55[a]	85.0±0.39[a]	80.1±12.42[a]	234±1.39[a]
FID	40°C	91.4±0.50[a]	84.3±2.22[a]	65.5±0.76[b]	208±1.85[b]
	50°C	47.8±1.90[b]	87.2±2.82[a]	8.7±5.77[d]	174±1.32[d]
	60°C	22.1±2.32[d]	74.0±0.83[b]	4.6±0.54[d]	122±1.58[e]

DPPH-RSA, DPPH radical scavenging activity; SOD, superoxide dismutase activity; ABTS-RSA, ABTS radical scavenging activity; TPC, total phenolic contents.

[1)]The extracts from flowers were then dissolved in 95% ethanol in a concentration of 300 μg/mL for experiments except for SOD activity determination, where the concentration was 100 μg/mL.

[2)]All values are mean±SD of triplicate determinations.

[3)]Different letters within a column indicate significant difference ($p \leq 0.01$), n=3.

85%, respectively. TPC of the extract at 40°C and 60°C HAD condition were 202 and 234 μM GAE, respectively. On the other hand, increasing temperature in FID condition decreased the antioxidant activities of the extracts.

Phenolic compounds are known to act as antioxidants not only because they are able to donate hydrogen or electrons, but also stable radical intermediates, which prevent oxidation of various food ingredients, particularly fatty acids and oils (25). Our previous studies showed that simple heat treatment of defatted sesame meal and citrus peel were effective in converting methanol insoluble phenolic compounds to methanol soluble forms (26,27). Far-infrared heating could also cleave covalently bound phenolic compounds from rice hull, while simple heat could not. In the case of *Chrysanthemi Flos* flower, simple heating (HAD method) increased antioxidant activities with increasing temperature within range of 40～60°C, however, far-infrared heating (FID method) showed the reverse effects in same temperature range. There are many kinds of phenolics in *Chrysanthemi Flos* flower, and the results indicate that phenolic compounds of the flower should present in many different bound forms. Therefore the effective processing steps for increasing antioxidant activities from different plant species may not be the same.

Luteolin content

Luteolin is one of key bioactive compounds of *Chrysanthemi Flos* flower, and plays an important role in anti-inflamation activity. The effect of drying condition on the luteolin content was determined. The luteolin content of the flower extracts was affected by both drying temperature and drying time (Table 3). Increasing temperature from 40°C to 50°C in HAD method increased the luteolin content from 103 μg/mL to 119 μg/mL, but at 60°C decreased to 95 μg/mL. However, the effect of FID method on the content of luteolin was the exact opposite of the result of HAD method. In other words, the content decreased from 99 μg/mL to 85 μg/mL according as the drying temperature of far-infrared radiation increased from 40°C to 50°C, but increased at 60°C to 139 μg/mL.

Table 3. Effect of hot-air drying (HAD) or far-infrared drying (FID) on the luteolin content of the *Chrysanthemi Flos* flower extracts

Drying conditions		Luteolin content (μg/mL)
HAD	40°C	103±0.7[1)d2)]
	50°C	119±0.7[b]
	60°C	95±0.9[e]
FID	40°C	99±0.1[c]
	50°C	85±1.7[f]
	60°C	139±1.0 [a]

[1)]All values are mean±SD of triplicate determinations.
[2)]Different letters within a column indicate significant difference ($p \leq 0.01$), n=3.

Volatile compounds

The detection and analysis of volatile compounds from the flower extracts of *Chrysanthemi Flos* was conducted by GC and GC/MS using SPME (Fig. 1). Thirteen volatile compounds were detected and used to observe the effect of drying conditions on the volatile compound. They were α-pinene, camphene, β-myrcene, 1,8-cineole, 1,3,3-trimethylcyclohex-1-ene-4-carboxaldehyde, camphor, β-elemene, β-caryophyllene, β-farnesene, borneol, germacrene D, α-farnesene, β-sesquiphellandrene, which are known to have an antimicrobial activity or anticancer activity (16,18-20). The contents of thirteen volatile compounds were calculated from the peak area of GC chromatogram, and the total content of the volatile compounds was the greatest (100%) at 50°C HAD method and 40°C FID method (Table 4). Among thirteen compounds, camphor was the main volatile components (31%) at 50°C. β-Caryophyllene having an anti-fungal and antimutagen effect was also the greatest at 50°C as 4.4% (28,29). By increasing the drying temperature in HAD method, the total content of volatile compounds did not show a set pattern, that is, the content increased from 40°C to 50°C and then decreased from 50°C to 60°C. On the contrary, as the drying temperature in-

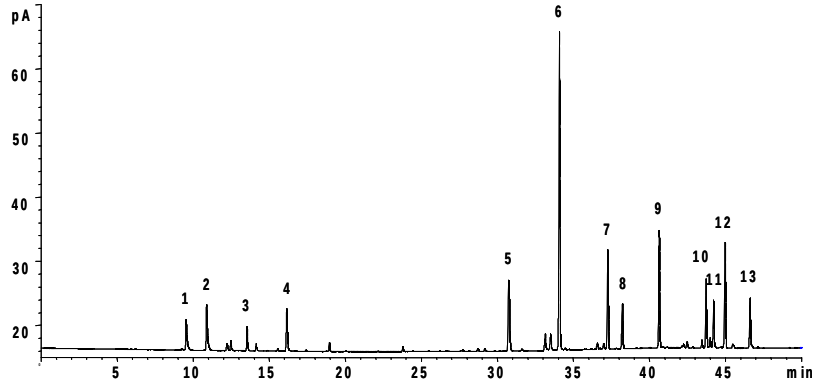

Fig. 1. Typical gas chromatography of the extract of *Chrysanthemi Flos* flowers which were dried with hot-air at 50°C. Peak: 1, α-pinene; 2, camphene; 3, β-myrcene; 4, 1,8-cineole; 5, 1,3,3-trimethylcyclohex-1-ene-4-carboxaldehyde; 6, camphor; 7, β-elemene; 8, β-caryophyllene; 9, β-farnesene; 10, borneol; 11, germacrene D; 12, α-farnesene; 13, β-sesquiphellandrene.

Table 4. Effect of hot-air drying (HAD) or far-infrared drying (FID) methods on volatile compounds of the *Chrysanthemi Flos* flower extracts

	Peak area (%)					
	HAD			FID		
	40°C	50°C	60°C	40°C	50°C	60°C
α-Pinene	2.7±0.06[1)c2)]	3.1±0.06[b]	2.8±0.18[c]	3.5±0.05[a]	2.5±0.00[c]	1.8±0.15[d]
Camphene	3.7±0.04[b]	4.9±0.06[a]	2.7±0.76[cd]	3.4±0.03[bc]	2.4±0.02[d]	1.5±0.11[e]
β-Myrcene	2.3±0.07[a]	1.9±0.01[c]	1.8±0.08[c]	2.1±0.03[b]	1.6±0.02[d]	1.1±0.09[e]
1,8-Cineole	1.9±0.06[c]	4.3±0.04[a]	2.3±0.10[b]	2.5±0.03[b]	2.3±0.03[b]	1.5±0.07[d]
1,3,3-Trimethylcyclohex-1-ene-4-carboxaldehyde	4.5±0.25[e]	7.3±0.15[c]	10.0±0.27[a]	9.3±0.17[b]	7.1±0.10[c]	5.9±0.21[d]
Camphor	29.8±0.39[b]	31.0±0.38[a]	16.9±0.48[d]	18.1±0.15[c]	18.8±0.01[c]	13.0±0.42[e]
β-Elemene	5.9±0.35[d]	9.5±0.40[a]	6.7±0.15[c]	7.9±0.11[b]	7.3±0.38[bc]	7.4±0.21[bc]
β-Caryophyllene	2.5±0.12[e]	4.4±0.07[a]	3.3±0.01[c]	3.7±0.02[b]	3.4±0.11[c]	3.1±0.02[d]
β-Farnesene	6.5±0.32[c]	9.9±0.10[b]	9.3±0.11[b]	11.1±0.11[a]	9.7±0.49[b]	9.6±0.22[b]
Borneol	3.0±0.25[c]	5.6±0.05[b]	5.8±0.05[ab]	6.2±0.05[a]	6.0±0.32[ab]	5.9±0.13[ab]
Germacrene D	3.6±0.30[c]	4.4±0.08[b]	6.7±0.07[a]	6.7±0.15[a]	6.9±0.27[a]	6.7±0.13[a]
α-Farnesene	7.1±0.50[c]	8.8±0.17[b]	10.8±0.11[a]	10.4±0.15[a]	10.7±0.51[a]	10.5±0.35[a]
β-Sesquiphellandrene	5.7±0.35[c]	4.9±0.10[d]	7.7±0.06[b]	9.4±0.06[a]	8.2±0.41[b]	9.2±0.30[a]
Total	79.2±2.39[d]	100.0±1.00[a]	86.8±1.16[c]	94.3±0.61[b]	86.9±2.52[c]	77.2±0.76[e]

[1)]All values are mean±SD of triplicate determinations.
[2)]Different letters within a row indicate significant difference ($p \leq 0.01$), n=3.

creased in FID method, the total content of volatile compounds decreased significantly ($p<0.01$). The content of the extracts at 40°C FID method was 94.3%, while at 60°C FID method was 77.2%. Far-infrared rays are defined as electromagnetic waves having a wavelength of longer than 4 μm but shorter than microwaves ($\lambda>0.1$ cm). FIR rays transfer heat to the center of materials evenly, while simple hot air heats from the surface to center of materials. Therefore, FID method might significantly affect the content of volatile compared to the HAD method.

In conclusion, drying conditions of *Chrysanthemi Flos* flower significantly affect the antioxidant activities, luteolin, and volatile compounds of 95% ethanol extract of flowers. For example, increasing temperature in HAD conditions increased the antioxidant activities, while increasing temperature in FID conditions decreased the antioxidant activities of the extracts. Luteolin was present in the highest content at 60°C FID flowers, and higher amount of the volatiles were found at 50°C HAD and 40°C FID. These results support that the drying condition should be primarily considered when the *Chrysanthemi Flos* flowers are dried for a medicinal resource.

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