Retention of Caffeic Acid Derivatives in Dried *Echinacea purpurea*

Hyun-Ock Kim, Timothy D. Durance,* Christine H. Scaman, and David D. Kitts

Food, Nutrition and Health, University of British Columbia, 6650 NW Marine Drive, Vancouver, British Columbia, Canada V6T 1Z4

Different drying methods were applied to fresh Canadian-grown *Echinacea purpurea* flowers to determine optimal drying procedures for preserving caffeic acid derivatives. Fresh flowers of *E. purpurea* were dried by freeze-drying (FD), vacuum microwave drying with full vacuum (VMD), and air-drying (AD) at 25, 40, and 70 °C. Using HPLC, chicoric acid and caftaric acid levels were quantitated in dried flowers. These acids were significantly affected by the drying method conditions used. Although significant ($p < 0.05$) loss of chicoric acid was observed when flowers were stored at high moisture, VMD flowers with a low moisture content retained the highest levels of chicoric acid and caftaric acid similar to FD flowers. Flowers that were AD at 25 °C retained about 50%, while those dried by AD at 70 °C resulted in the lowest retention of these acids. Although flowers dried by AD at 40 °C retained relatively high amounts of chicoric acid and caftaric acid, the time (55 h) required to reach optimal drying was considerably longer than that (47 min) for VMD.

Keywords: *Chicoric acid; caftaric acid; Echinacea purpurea; vacuum microwave drying; air-drying; freeze-drying*

INTRODUCTION

Echinacea is a North American native medicinal herb used traditionally for wounds, burns, snake or insect bites, colds, infections, and inflammation by indigenous Americans (Bauer and Wagner, 1991). Clinical studies have demonstrated the efficacy of *Echinacea* preparations for humans (Scaglione and Lund, 1995; Parnham, 1996; Berg et al., 1998; Brinkeborn et al., 1999; Wustenberg et al., 1999). Therefore, modern herbal medicine has adopted the use of *Echinacea* as a popular commercial herbal immunostimulant (Bauer, 1998).

It has been reported that several constituents of *Echinacea*, which include alkamides, caffeic acid derivatives (especially chicoric acid), glycoprotein, and polysaccharides, have pharmacological activity including immunostimulatory and anti-inflammatory properties (Bauer and Wagner, 1991; Bauer, 1996, 1998). Among caffeic acid derivatives, only chicoric acid has been shown to stimulate phagocyte activity in vitro and in vivo (Bauer et al., 1989). For antihyaluronidase activity, chicoric acid and caftaric acid have greater activity than cynarine, chlorogenic acid, or caffeic acid (Facino et al., 1993), whereas chicoric acid has higher free radical scavenging property than cynarine, caffeic acid, or chlorogenic acid (Facino et al., 1995). Chicoric acid also has antiviral activity (Cheminat et al., 1988) and recently has been found to inhibit HIV-1 integrase and replication and to exhibit protective effects toward HIV-infected cells (Robinson et al., 1996; King and Robinson, 1998; King et al., 1999; Lin et al., 1999).

Recent studies have concluded that the total pharmacological activity of *Echinacea* preparations depends on not a single, but the combined, activities of several plant constituents (Bauer and Wagner, 1991; Bauer, 1998). Alkamides and chicoric acid concentrations have been measured in different commercial preparations, and considerable variations in the concentration of active components, especially chicoric acid, were reported (Bauer, 1997, 1999; Wills and Stuart, 1998, 1999). This finding may be attributed to the fact that alkamides and especially chicoric acid are sensitive to degradation during postharvest handling, such as drying, storage, and processing (Wills and Stuart, 1998; Bauer, 1999). Consequently, the retained concentrations of these specific plant components are good indicators for estimating the quality of *Echinacea* preparations following postharvest processing.

The purpose of our present study was to compare the effect of different drying methods, such as freeze-drying (FD), vacuum microwave drying with full vacuum (VMD) and air-drying (AD) on the retention of caffeic acid derivatives in *Echinacea* purpurea flowers.

MATERIALS AND METHODS

Plant Materials. Freshly harvested flowers of 3-year-old *Echinacea purpurea* plants were obtained from Tuscan Farm Gardens (Langley, BC, Canada) at three periods: in the middle of August, September, and October 1999. At each time of sampling, full-bloomed flowers were picked by hand and dried on the same day as harvested.

Preparation of Dried Flowers. Five hundred grams of flowers were used for each drying process. The initial moisture content of *Echinacea* flowers was 85.0%. Freeze-drying (FD) at 1.6 mmHg with a chamber temperature of 20 °C and condenser temperature of − 55 °C was performed for 4 days. Using a 2450-MHz vacuum microwave drier (ENWAVE Corporation, Port Coquitlam, BC), vacuum microwave drying was performed with a power of 1 kW at full vacuum level, which was equal to an absolute pressure of 50 mmHg (VMD) for 40 and 47 min, respectively. Microwave power was measured by an IMPI 2 liter test (Buffler, 1993). The microwave drying basket was a high density polyethylene cylinder of approximately 0.26 m radius and 0.23 m length. The drying basket was rotated during the process at a rate of 6 rpm.

* To whom correspondence should be addressed (telephone 604-822-4425; fax 604-822-3959; e-mail durance@interchange.ubc.ca).

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Nitrogen gas, instead of air, was flushed into the microwave drier at a flow rate of 15 L/min for VMD. The final product temperature for VMD was 40 °C, as measured at the end of the processing cycle using an Infrared thermometer (Cole Parmer, Vernon Hills, IL). The effect of partial vacuum, which was equal to an absolute pressure of 200 mmHg (PVMD), was examined on drying time. PVMD with 1 kW power was tested for the entire drying period or for 10 min initially, and then VMD with 1 kW power was used for the rest of the drying period. A power of 2 kW with VMD was also tested for 10 min initially, and then 1 kW power with VMD was used for the rest of the drying period.

Air drying (AD) was done at 70, 40, or 25 °C. Using a Vers-a-belt dryer (Wal-Dor Industries Ltd., New Hamburg, ON), AD was conducted at 70 °C (AD/70) for 13 h. Air flow rate through 1 m^2 of belt was 0.9 m^3/s, and initial relative humidity of the air was 10%. AD at 40 °C (AD/40) was performed for 55 h by using a Berron Food Dehydrator (Berron Enterprises Ltd., Langley, BC, Canada) with an air flow rate of 0.9 m^3/s. Flowers harvested in September and AD at 25 °C (AD/25) for 1 week were obtained from Tuscan Farm Gardens (Langley, BC, Canada).

Dried flowers were ground using a Retsch Ultra centrifugal Mill with a 0.5-mm sieve and stored at −18 °C. Moisture contents of ground samples were determined by drying at 84 °C for 22 h using a vacuum oven. Water activities (a_w) of all the dried samples were measured using Aqualab (model CX-2, Decagon Devices Inc., Pullman, WA).

Extraction. Extraction of caffeic acid derivatives from dried *Echinacea* flowers was carried out by the method of Bauer and Foster (1991) with modification. Ground flower material (1.0 g) was refluxed with 50 mL of methanol for 5 h using a Goldfisch extraction apparatus. The methanol extract was dried by a rotary evaporator at 40 °C and then redissolved with 10 mL of methanol. One milliliter of methanol-dissolved solution of flower extract was applied to a Supelclean LC-18 extraction column (Supelco, 1 mL bed volume), which was previously conditioned with 2 mL of methanol, 2 mL of water, and 2 mL of methanol. The filtrate (unbound caffeic acid derivatives fraction) and the eluates were collected after washing with 1 mL of methanol. The caffeic acid derivative fraction was filtered through a syringe filter (0.45 μm) (Chromatographic Specialties Inc.). The recovery efficiency was determined by adding a known amount of pure caffeic acid standard to the samples prior to extraction and then performing the entire procedure as described above.

Standard Caffeic Acid Derivatives. L-chicoric acid (purity >99%) was kindly provided by Dr. M. G. Reinecke (Texas Christian University, Fort Worth, TX, USA). 2-0-caffeoyltartaric acid (caftaric acid) (purity 92%, HPLC) was obtained from Dr. R. Bauer (Heinrich Heine University, Dusseldorf, Germany). Caffeic acid (purity >99%) was purchased from Sigma Chemical Co. (St. Louis, MO). These caffeic acid derivatives were dissolved in methanol and various amounts of caffeic acid derivatives (2.5−10 μg) were used to make the standard curves. The linear regression coefficients (R^2) of standard curves for L-chicoric acid, caftaric acid, and caffeic acid HPLC detection were 0.9996, 0.9994, and 0.9943, respectively.

High Performance Liquid Chromatography Analysis. Caffeic acid derivative levels of extracted flowers were determined by HPLC. Analysis equipment consisted of a Hewlett-Packard 1050 series pump control, a Macintosh computer with Dynamax HPLC Method Manager, version 1.2 (Rainin Instrument Co. Inc., Woburn, MA), and a Shimadzu SPD-EAV UV detector. Samples were analyzed using a Vydac RP-18 analytical column (250 × 4.6 mm, 5 μm) with a Brownlee RP-18 NewGuard cartridge (15 × 3.2 mm, 7 μm) fitted (Perkin-Elmer, Markham, ON).

Mobile solution A was water with 0.1% *o*-phosphoric acid (85%), and mobile solution B was acetonitrile with 0.1% *o*-phosphoric acid (85%). A linear gradient of 10% B to 40% B was developed in 30 min at a flow rate of 1.0 mL/min with UV detection at 330 nm (Bauer, 1997). Caffeic acid derivatives were identified by comparison of retention times and UV profile at 330 nm of the supplied standard caffeic acid derivatives. The concentrations of identified caffeic acid derivatives were calculated from the regression equations made from standard curves. The caffeic acid derivative levels were expressed as milligrams per gram of dry weight of flowers.

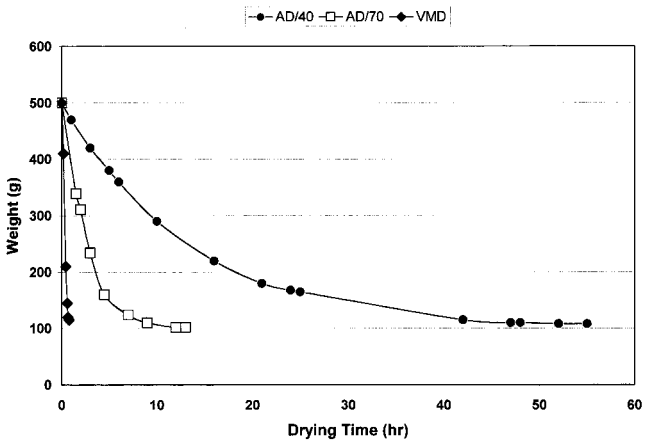

Figure 1. Dehydration of *Echinacea purpurea* flower by AD/40, AD/70, and VMD. AD/40: air-dried at 40 °C, AD/70: air-dried at 70 °C, VMD: vacuum microwave dried.

Table 1. Moisture Contents and Water Activities in Dried *Echinacea purpurea* Flowers by Different Drying Methods

drying method/temp/ harvest month[a]	moisture content (%)[b]	water activity (a_w)[b]
FD/Aug	5.0	0.310
AD/40/Aug	7.0	0.304
AD/70/Aug	4.3	0.254
FD/Sept	4.5	0.287
AD/25/Sept	6.5	0.462
AD/40/Sept	6.8	0.361
FD/Oct	4.0	0.230
VMD/Oct	6.1	0.339

[a] FD: freeze-dried, VMD: vacuum microwave dried, AD: air-dried. 25, 40, and 70 indicate the drying temperatures of 25, 40, and 70 °C. Aug, Sept, and Oct indicate the harvesting month. [b] Values are averages of duplicate determinations.

Statistical Analysis. Statistical analysis was performed using a Minitab version 12.21 program (Minitab Inc., State College, PA). One-way analysis of variance followed by Tukey test was used to compare means. Significance of difference was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Dehydration of *Echinacea purpurea* Flowers. Figure 1 shows the dehydration rate of *Echinacea* flowers using AD/40, AD/70, and VMD with 1 kW power. The final moisture content of the dried flower products was below 7.0%. Considering that it took 4 days for FD to be accomplished, VMD was found to be the fastest drying process among the different drying methods used. There was no difference in drying time (40 min) between VMD or PVMD at 1 kW power for obtaining a final moisture content of 9.3%. Also, the use of PVMD for the first 10 min and subsequent use of VMD for the remaining drying process required a similar drying time as the VMD or PVMD only. An initial use of 2 kW power for 10 min reduced the drying time by about 5 min. In this study, 1 kW power was used for VMD flowers. Table 1 shows the moisture contents and water activities in dried flowers prepared by different drying methods.

Determination of Caffeic Acid Derivatives. A preliminary experiment was conducted to examine the

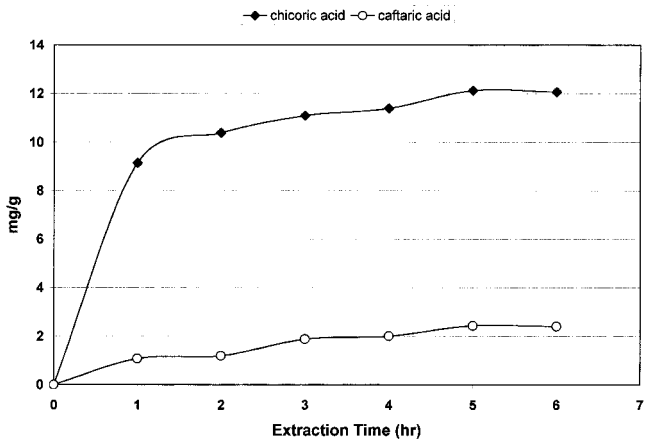

Figure 2. Effect of extraction time on the concentrations of chicoric acid and caftaric acid in *Echinacea purpurea* flowers. Averages of duplicate determinations. Differences between duplicates were less than 7%.

effect of extraction time on the concentrations of chicoric acid and caftaric acid extracted from dried flowers using a Goldfisch extraction apparatus. A 5-h extraction time was found to be sufficient for the extraction of caffeic acid derivatives (Figure 2). When a known amount of caffeic acid was added to samples before extraction and the whole extraction and filtration procedures were conducted, a percent recovery of 101 ± 5% (n = 6) for caffeic acid was obtained. There was no difference in the levels of caffeic acid derivatives measured in samples, whether or not a Supelclean LC-18 column was used. However, use of the Supelclean LC-18 column reduced the contamination of the HPLC analytical column.

Caffeic Acid Derivatives in Dried *Echinacea purpurea* Flowers. Figure 3A shows the HPLC profile of a standard caffeic acid derivatives mixture using a linear gradient elution (10−40% acetonitrile in water with 0.1% *o*-phosphoric acid, 85%). Caftaric acid (2-0-caffeoyltartaric acid) was eluted first, with caffeic acid following and chicoric acid (2,3-0-dicaffeoyltartaric acid) eluting last, thus reflecting the relative order of polarity of these three specific caffeic acid derivatives.

A typical HPLC profile of caffeic acid derivatives in VMD flowers harvested in September is shown in Figure 3B. Although there were about seven peaks shown in each HPLC profile, only caftaric acid and chicoric acid were identified by comparison of retention times and UV profiles at 330 nm. Chicoric acid was the predominant plant phenolic in all the dried *Echinacea* flowers with concentrations ranging from 63 to 75% of the relative peak areas measured for caffeic acid derivatives. This result supports the previous finding by Wills and Stuart (1999), who reported that chicoric acid constituted 63 and 67% of the relative peak area for aerial sections. In contrast, caftaric acid content was only 8−18% of the relative peak area measured. Caffeic acid could not be quantified in all the dried flowers using this HPLC method. However, when standard caffeic acid was added to flower samples before the extraction procedure, recovery of the added caffeic acid was made according to the HPLC profile.

Effect of Drying Methods on the Levels of Chicoric Acid. Although flowers were harvested at different times, there was no significant difference ($p < 0.05$) in the chicoric acid concentrations when they were harvested at full bloom (Table 2). However, there was a decreasing trend for the chicoric acid concentrations

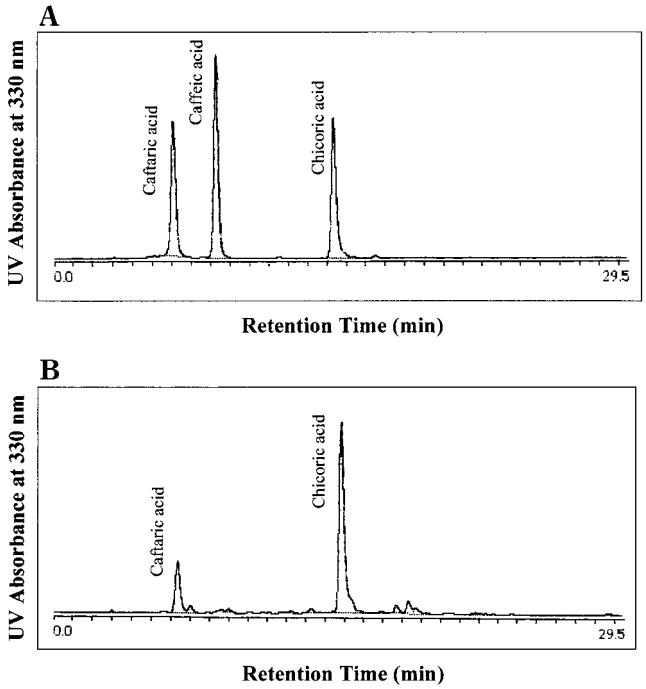

Figure 3. (Panel A) HPLC profile of standard caffeic acid derivatives. Separation parameters: mobile solution (A) water + 0.1% *o*-phosphoric acid, 85%; (B) acetonitrile + 0.1% *o*-phosphoric acid, 85%; linear gradient 10−40% B in 30 min; flow rate 1.0 mL/min. (Panel B) HPLC analysis of caffeic acid derivatives in a vacuum microwave dried flowers of *Echinacea purpurea*. Flowers were harvested in September. Separation parameters were same as for standard caffeic acid derivatives.

Table 2. Effects of Drying Methods on the Levels of Chicoric Acid and Caftaric Acid in Dried *Echinacea purpurea* Flowers[a]

drying method/temp/ harvest period	chicoric acid	caftaric acid
FD/Aug	13.03 ± 0.52[a]	2.56 ± 0.07[a]
AD/40/Aug	10.98 ± 0.76[b,c]	2.68 ± 0.20[a]
AD/70/Aug	2.54 ± 0.47[e]	0.61 ± 0.13[d]
FD/Sept	12.13 ± 0.98[a,b]	2.51 ± 0.15[a]
AD/25/Sept	6.59 ± 0.67[d]	1.26 ± 0.25[c]
AD/40/Sept	9.95 ± 1.89[c]	2.14 ± 0.76[a,b]
FD/Oct	12.05 ± 0.51[a,b]	1.65 ± 0.22[b,c]
VMD/Oct	11.20 ± 0.52[b,c]	1.76 ± 0.11[b]

[a] Means ± standard deviation (n = 6); mg/g, dry weight. FD: freeze-dried, VMD: vacuum microwave dried, AD: air-dried. 25, 40 and 70 indicate the drying temperatures of 25 °C, 40 °C and 70 °C. Aug, Sept, and Oct indicate the harvesting period. Different letters in the same columns indicate a significant difference ($p <$ 0.05).

(13.03−12.05 mg/g, dry wt) for FD flower samples when harvested later in the season. Wills and Stuart (1999) reported that 12.9 ± 4.5 mg chicoric acid/g of dry wt was present in dried aerial parts of *Echinacea purpurea* grown in eastern Australia. For *E. purpurea* grown in Germany, Becker and Hsieh (1985) reported a chicoric acid content of 13 mg/g in the flower and leaf, while Bauer et al. (1988) reported ranges of 13−30 mg/g in the flower.

VMD for 40 min was performed for *Echinacea* flower samples harvested in September until the moisture content of the dried products reached 9.3%. This moisture was somewhat higher than other dried samples. The measurement of chicoric acid content after storage and grinding revealed that the chicoric acid content of the VMD/Sept (high moisture) sample was approximately 80% of that in the corresponding FD/Sept sample

Table 3. Effect of Moisture Content on the Retention of Chicoric Acid and Caftaric Acid by VMD Flowers of *Echinacea purpurea*

	moisture (%)	water activity (a_w)	chicoric acid[a] (mg/g)	caftaric acid[a] (mg/g)
VMD low moisture[b]	6.1	0.339	11.20 (93%)	1.76 (106%)
VMD high moisture[c]	9.3	0.568	9.83 (81%)	2.38 (95%)

[a] Mean values for chicoric acid and caftaric acid ($n = 6$) were expressed mg/g, dry weight. Percent ratio of chicoric acid and caftaric acid to the amounts present in corresponding FD samples harvested in October and September, respectively (values in parentheses). [b] Flower samples were harvested in October and vacuum microwave dried for 47 min. [c] Flower samples were harvested in September and vacuum microwave dried for 40 min.

(Table 3). However, for the *Echinacea* flower samples harvested in October, the VMD process applied for 47 min (VMD/Oct) resulted in a final dried product with a moisture content of 6.1%. The chicoric acid content of the VMD/Oct (low moisture) sample was not different ($p < 0.05$) from that of the FD/Oct sample. FD or VMD allowed the highest retention of chicoric acid among the drying methods used. This result implies that the low moisture content of the dried product was very important for preserving the chicoric acid during storage. Higher moisture contents obtained with the VMD/Sept sample may have contributed to degradation of chicoric acid during storage.

AD at 40 °C for samples harvested in August and September (AD/40/Aug and AD/40/Sept) resulted in 82−84% of the chicoric acid content present in the corresponding FD/Aug or FD/Sept samples. The relative chicoric acid content present in the AD/25/Sept sample was approximately half (54%) that of the FD/Sept sample. The AD/70/Aug sample contained only 20% of chicoric acid measured in the FD/Aug sample and represented the lowest retention of this phytochemical in all dried samples. Of the AD treatments, AD at 40 °C was found to be the best AD treatment for retaining chicoric acid. This result supported the findings that chicoric acid is readily degraded during handling and drying processes (Natural Factors Products Ltd., 1998; Bauer, 1999; Wills and Stuart, 1998, 1999).

Effect of Drying Methods on the Level of Caftaric Acid. Although FD/Aug and FD/Sept samples contained the same concentration of caftaric acid, this caftaric acid was reduced in the FD/Oct sample and was significantly ($p < 0.05$) lower than FD samples harvested earlier in the year (Table 2). The VMD/Sept (high moisture) sample or the VMD/Oct (low moisture) sample contained the same level of caftaric acid as counterpart FD/Sept or FD/Oct samples (Table 3). AD/40 samples had the same caftaric acid content as FD samples. Similar to the result with chicoric acid, the AD/25/Sept sample contained about 50% of the caftaric acid present in the FD/Sept sample. The AD/70/Aug sample had the lowest amount of caftaric acid (about 24% of the FD/Aug sample) among all the dried flowers measured.

The present results indicate that a long drying time (e.g., 1 week), high drying temperature (e.g., 70 °C), or high moisture content (9.3%) of the dried product is detrimental for preserving both the chicoric acid and the caftaric acid in stored *Echinacea purpurea* flowers. Although AD/40 produced dried flowers with relatively high contents of both chicoric acid (82−84%) and caftaric acid (>85%), the drying time was excessively long (55 h) to reduce the moisture content to a required level (6.8−7.0%) for the dried product. In contrast, VMD took only 47 min to obtain a final moisture content of 6.1% for the dried product. VMD processing also produced dried flowers with the same high content of both chicoric and caftaric acids that were found in FD flowers. Figure 4 shows representative *Echinacea purpurea* flowers processed by different drying methods. VMD flowers retained natural color similar to FD flower and did not exhibit the enzymatic browning observed with the AD samples. This study therefore shows that the VMD method is a superior procedure to conventional air-drying for dehydrating *Echinacea purpurea* flower while adequately retaining caffeic acid derivative contents.

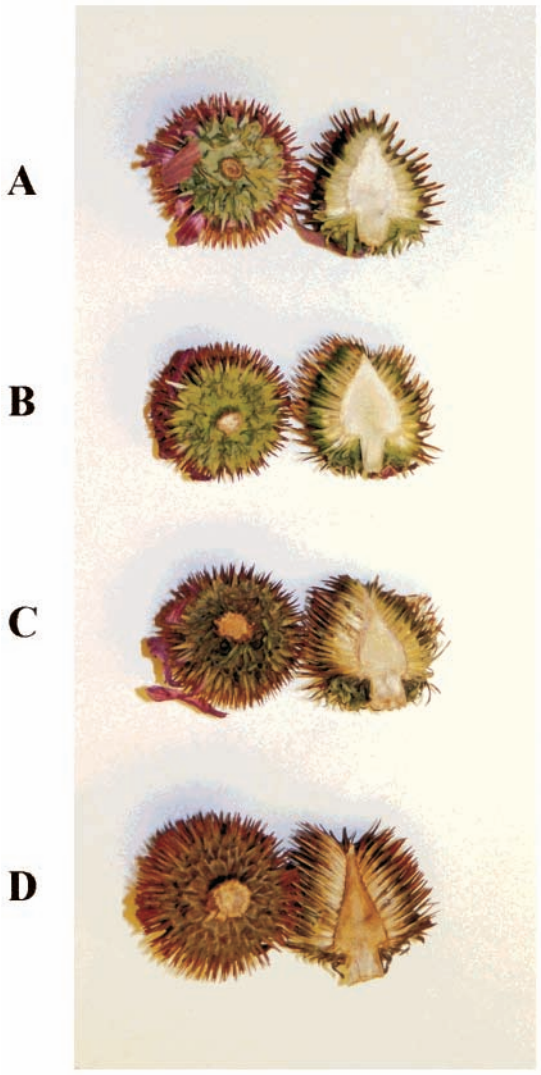

Figure 4. Representative *Echinacea purpurea* flowers prepared by different drying methods. (A) freeze-dried for 4 days, (B) vacuum microwave dried for 47 min, (C) air-dried at 40 °C for 55 h, (D) air-dried at 70 °C for 13 h.

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