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Supercritical Fluid Extraction of Alkylamides from *Echinacea angustifolia*

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Echinacea has been known for its immunostimulatory activity, and its alkylamide components have been linked to such biological activity. Consequently, alkylamides in *Echinacea angustifolia* were extracted using supercritical carbon dioxide from fresh and dried roots at 45−60 °C and 34−55 MPa, and the alkylamide yield in the extracts was determined. The yield of alkylamides from fresh roots increased with temperature yet decreased with pressure, whereas the yield from air-dried roots (moisture content 8.4%) increased with both temperature and pressure. Freeze-drying of the roots to a moisture content of 4.9% did not result in any further increase in the yield compared to that of air-dried roots. Alkylamide yield of the ground dried roots extract was the highest ($p \leq 0.05$) among those from fresh, ground and unground *E. angustifolia* roots. Supercritical fluid extraction therefore shows potential for the recovery of alkylamides from dried *Echinacea* roots.

KEYWORDS: Alkylamide; *Echinacea angustifolia*; supercritical fluid extraction

INTRODUCTION

Herbal therapies have received increasing attention in both North America and Europe. Although there is not enough scientific evidence to show their efficacy, the use of such alternative medicines has been growing steadily. *Echinacea* is one of the most popular herbal plants, and it has been extensively studied in recent years. There are more than 800 *Echinacea* products on the market in Germany alone (*1*). Cosmetic products containing *Echinacea* extracts such as lip balms, shampoos, and toothpaste are also available (*2*). *Echinacea angustifolia* is one of the three species of *Echinacea* commercially available and it has a high market value (*3*, *4*). It has been used by native Americans for toothaches, headaches, snakebites and other poisonous conditions, and even for the treatment of cold and cancers (*5*).

Compounds that have been isolated from *Echinacea* include polysaccharides, caffeic acid derivatives, and lipophilic components (*6*−*8*). Alkylamides, a group of lipophilic compounds together with polar fractions, contribute to the immunostimulatory activity of *Echinacea* (*7*). Several studies have reported the analysis of alkylamides in *Echinacea* (*9*−*13*). However, more research is needed to find an effective method for the extraction of these compounds from the herb for processing purposes, as information in the literature is scarce because of the proprietary nature of the products on the market.

Alkylamides have been extracted by Soxhlet extraction or maceration using organic solvents, such as methanol (*9*, *14*), chloroform (*10*, *11*), hexane (*15*, *16*), ethanol or aqueous ethanol (*17*, *18*), and aqueous methanol (*17*). Most of these studies have been carried out using ground dried roots (air- or freeze-dried). However, the extraction of fresh roots has not been reported.

Supercritical fluid extraction (SFE) of alkylamides from *Echinacea* roots was explored by Lienert et al. (*12*) on an analytical scale. They applied the SFE conditions of 15.05 MPa and 60 °C for 30 min, followed by 30 min of dynamic extraction at a flow rate of 1 mL/min. There was no significant difference between SFE, maceration, and Soxhlet extraction methods in their study in terms of the composition of the extracts. SFE is a promising and tested technology in the food and pharmaceutical industries. More recently, its applications are expanding, especially in the "natural" products and nutraceutical areas. Carbon dioxide is the most commonly used supercritical solvent because of its moderate critical point (P_c = 7.38 MPa, T_c = 31.1 °C), and its nontoxicity and environmentally benign characteristics. Supercritical CO_2 ($SC-CO_2$) has been successfully used commercially in decaffeinating coffee and tea, and for the extraction of hops, flavors, and other natural materials (*19*−*21*). Despite the rapid developments in the applications of SFE, the supercritical extraction of alkylamides from *Echinacea* has not been reported. Therefore, the objective of this study was to examine the effect of SFE conditions on the extraction of alkylamides from fresh and dried roots of *E. angustifolia*.

MATERIALS AND METHODS

Materials. Freshly harvested three-year-old *E. angustifolia* roots were obtained within 24−48 h from the Crop Diversification Centre South, Alberta Agriculture, Food and Rural Development, Brooks, Alberta. Dichloromethane (from BDH Inc., Toronto, ON) was used to dissolve SFE extracts for further analysis. Hexadecane as an internal standard (99%) was obtained from Sigma-Aldrich Chemical Co.

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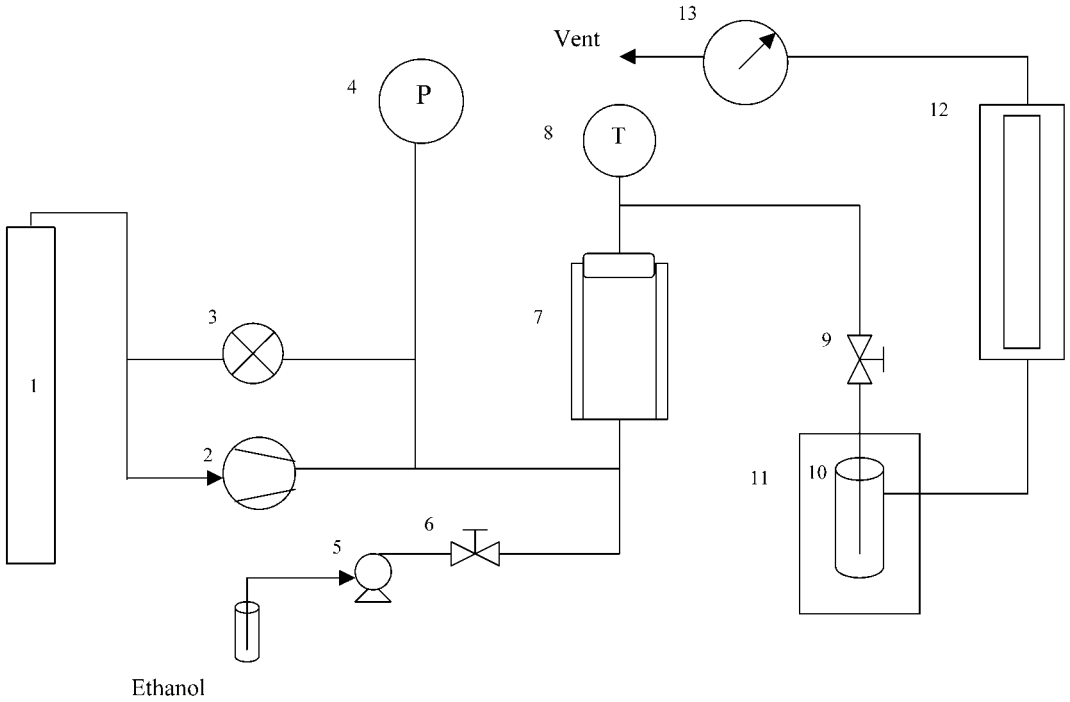

Figure 1. Schematic diagram of the supercritical fluid extraction system: 1, CO_2 cylinder; 2, compressor; 3, back-pressure regulator; 4, pressure gauge; 5, Gilson pump; 6, on/off valve; 7, extraction chamber with heater; 8, thermocouple; 9, depressurization valve; 10, extract collection tube; 11, cold bath; 12, rotameter; 13, gas meter.

(St. Louis, MO). Carbon dioxide (bone dry) used for the extraction, and helium (ultrahigh purity, 99.9999%) used as the carrier gas in the gas chromatography−mass spectrometry (GC−MS) system were obtained from Praxair Canada Inc. (Mississauga, ON). Cerulenin was used as external standard (95%) and was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

***Echinacea* Roots Processing.** *Echinacea* roots were cut into approximately 1-cm long pieces, thoroughly mixed, and vacuum packaged. Half of the sample was freeze-dried in a model FFD-42-WS drier (The Virtis Co. Inc., Gardiner, NY) for 3 d after being stored at −18 °C. The other half was dried for 7 h in a thin-layer air-dryer (Agricultural Value-Added Engineering Centre, Edmonton, AB) at 50 °C and air velocity of 1.1 m/s. The moisture contents of fresh, air-dried and freeze-dried roots were determined in duplicate according to the AOAC official method 930.15 (*22*). Dried roots were vacuum packaged in bags with a Multivac AG500 (Sepp Haggenmüller KG, Germany) and stored at −18 °C until used. Particle size distribution of ground air-dried and freeze-dried roots were determined with a portable sieve shaker (The W. S. Tyler Co. of Canada Ltd., St. Catharines, ON), using 16, 20, 40, 80, and 100 mesh (Canadian Standard) sieves.

Supercritical Fluid Extraction. Supercritical fluid extractions of *Echinacea* roots were performed using a laboratory-scale system (Newport Scientific, Inc., Jessup, MD) as shown in **Figure 1**. The original system was modified with the addition of a piston pump (Gilson 305; Gilson, Inc., Middleton, WI) to introduce an entrainer, such as ethanol, into SC−CO_2 prior to its entry into the extraction cell. The *Echinacea* sample (approximately 25 g of fresh roots or 6 g of dried roots) was loaded in a sample basket (25 cm × 27 mm i.d.) with glasswool inserted at both ends to hold the sample. Fresh roots were used as 1-cm long pieces whereas dried roots were ground before use. Dried roots were ground using an Osterizer 8 grinder (Sunbeam Co. Ltd., Canada) for a total grinding time of 37 s, allowing the metal blades to cool after every 10 s. The sample basket was then placed into the original extraction chamber. Extraction temperature was monitored within ±2 °C of the desired temperature, using a thermocouple placed at the top portion of the extraction cell, a temperature controller, and the heater around the extraction cell. A backpressure regulator was used to maintain the desired pressure.

The extractions were performed for 4 h in duplicate at two pressures (34 and 55 MPa) and two temperatures (45 and 60 °C). The CO_2 flow rate was maintained at an average level of 1.7 L/min (measured at ambient conditions). Alkylamide extracts were collected in two sidearmed test tubes connected in series by plastic tubing held in a refrigerated bath at −10 °C. After each collection, the test tubes were allowed to equilibrate at ambient conditions for ∼20 min, after which they were weighed, and the extract was dissolved in 10.0 mL of dichloromethane for further analysis.

At the extraction conditions of 34 MPa and 60 °C, unground air-dried roots were extracted by SC−CO_2 alone. However, for the ground air-dried roots, ethanol was added as an entrainer into SC−CO_2 at a rate of 0.21 mL/min, which corresponded to 5% (w/w) in CO_2.

Alkylamide Analysis of Extracts. Alkylamides were identified by comparison of their mass spectra patterns with those previously reported by Bauer et al. (*15*, *16*). Identification was done using a Varian Vista 6000 gas chromatograph (Varian Associates Inc., Walnut Creek, CA), equipped with a 30 m × 0.25 mm i.d., 0.25 μm film, DB 5 column (J & W Scientific, Folsom, CA), and coupled with a 7070E VG analytical mass spectrometer (V. G. Micromass Ltd., Manchester, UK). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI−TOF MS) (Bruker Analytical Systems Inc., Billerica, MA) was also used for confirmation of molecular weights of the identified alkylamides.

Analysis of extracts was carried out using a Hewlett-Packard (HP) 5890 series II gas chromatograph, equipped with a 30 m × 0.25 mm i.d., 0.25 μm film, DB 5MS column (J & W Scientific) and a HP 5971A mass selective detector (Palo Alto, CA). Helium at 0.34 MPa was used as the carrier gas. Oven temperature was programmed using HP G1034C software for the MS ChemStation (DOS series). The oven was held at 55 °C for 3 min, and the temperature was then increased at a rate of 4.5 °C/min to 230 °C and held for 5 min. The ionization voltage was 1400 eV and the mass spectra were acquired at 1.2 scan/sec. Injection volume was 5.0 or 1.0 μL depending on the concentration of the extract solution. HP G1034C software was used for integration of the peak areas.

Because pure standards of alkylamides of interest in this study were not available commercially, cerulenin (2,3-epoxy-4-oxo-7,10-dodecadienamide, $C_{12}H_{17}NO_3$, MW 223.3) was used as an external standard to determine the quantities of the alkylamides detected by GC−MS. In addition, hexadecane was used as an internal standard to normalize the peak areas among different chromatographic runs. Each batch of

Table 1. Particle Size Analysis of Ground Air-Dried and Freeze-Dried *E. angustifolia* Roots

mesh	particle size (μm)	air-dried roots (weight %)	freeze-dried roots (weight %)
16	>1190	11.6	0.3
20	850–1190	11.5	2.6
40	425–850	25.9	18.2
80	180–425	25.7	44.8
100	150–180	12.8	17.3
bottom	<150	12.5	16.8

internal standard solution (IS) was prepared by dissolving 100 μL of hexadecane in 5.0 mL of dichloromethane and was used for four successive working days. An aliquot of IS solution (100 μL) was added to the solution of each extract dissolved in 10 mL of CH_2Cl_2 and stored at 4 °C until analysis the next day.

A solution containing 0.333 mg of external standard cerulenin (STD) and 0.315 mg of internal standard hexadecane in 10 mL of CH_2Cl_2 was prepared. The solution (5.0 μL) was injected into GC−MS and retention times of 17 and 34 min were obtained for hexadecane and cerulenin, respectively. The area ratio of cerulenin with respect to hexadecane was 1:2.78. The relative response factor for conversion to weight of respective alkylamides was then calculated as IS/STD = 1:2.94 (*23*). The area count of each peak of the chromatograms was normalized to the total internal standard present in each extract solution to obtain relative quantities. The normalized quantities of the components were then obtained by dividing the relative quantities with the relative response factor based on the external standard cerulenin. Thus, the normalized quantities of identified alkylamides in each extract were reported as yield and expressed on a dry root weight basis of the original sample.

A blank SFE run conducted at 34 MPa and 45 °C resulted in some additional peaks at 31 min. In addition, GC−MS analysis of dichloromethane alone also resulted in some peaks around retention time of 54 min and later. Subsequent chromatographic analysis failed to identify these peaks; therefore, they were excluded in the normalized calculation procedure for the total extracts.

Statistical Analysis. All extraction runs followed by the analysis of the extracts were carried out in duplicate. Analysis of variance of results was performed using the general linear model (GLM) procedure of SAS Statistical Software, Version 8 (*24*). The model consisted of the main effects of pressure and temperature and their interaction. Multiple comparison of means was carried out using the LSD (least significant difference) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Fresh *E. angustifolia* roots contained 75.3% (w/w) moisture. The moisture contents of air- and freeze-dried roots were 8.4% and 4.9%, respectively. Particle size analysis results of air-dried and freeze-dried roots are presented in **Table 1**. Approximately 78.9% (w/w) of freeze-dried root particles were smaller than 425 μm, whereas the same fraction made up only 51.0% of the air-dried roots. A greater proportion of the air-dried roots (25.9%) was distributed in the size range of 425 to 850 μm than that of freeze-dried roots (18.2%), and particles bigger than 850 μm were found to a larger extent (23.1%) in air-dried roots than in freeze-dried roots (2.9%).

Figure 2 shows a typical gas chromatogram of an *Echinacea* root extract obtained from SFE. Among the over 20 peaks obtained on the chromatogram, eight major alkylamides were identified (compounds **a**−**g**, **Figure 3**), appearing at 36−37, 40−41, 44−45, 46−49, 50−51, 52−53. and 53−54 min, respectively. Compound **d** is actually a mixture of tetraene alkylamides 14 and 15 reported by Bauer et al. (*16*) since they were not fully separated. Except for compound **g**, which was absent in one extract obtained from fresh roots at 55 MPa and 60 °C, all other alkylamide compounds were found in all the extracts of this study.

Means of duplicate run results of supercritical fluid extraction at each condition were reported, and there was no significant difference ($p > 0.05$) between the duplicates. The yields of individual compounds and total extracts are reported in terms of mg/g dry root basis to better facilitate a comparison among samples. Changing pressure and temperature, as well as the condition and form of samples, affected the alkylamide yield. A higher recovery could have been achieved if the extractions were continued longer.

SFE of Fresh *E. angustifolia* Roots. The yield of individual alkylamides from fresh roots ranged from 0.03 to 2.13 mg/g on a dry root basis. Alkylamides, together with other extracted components, formed yellow-brown gum-like droplets on the collection tube wall. Water was co-extracted and captured in the collection tube as well, but its amount was not quantitated. This phenomenon was observed by others during SFE of plant materials (*25*, *26*). Analysis of variance showed that both temperature and pressure had a significant effect ($p \leq 0.05$) on the yield of identified alkylamides as a whole, yet they had slightly different effects on individual components as discussed below.

Effect of Temperature. **Figure 4A** presents the effects of temperature and pressure on the normalized amounts of alkylamides in the SC−CO_2 extracts of fresh *E. angustifolia* roots. At 34 MPa, when the temperature was increased from 45 to 60 °C, the yield of all alkylamides was at least doubled, with compound **c** having the greatest increase (237%). The same temperature increase at 55 MPa caused the yield of compound **g** to decrease by 25%, whereas the rest of the compounds showed increases ranging from 75% to 188%. Compound **b** had the smallest increase, while compounds **c**, **e**, and mixture **d** almost tripled. The yield of eight identified alkylamides had different responses to an extraction temperature change at 34 MPa vs 55 MPa.

Even though the overall yield was lower at a higher pressure, individual yields of compounds **a**, **d**, and **e** showed a greater percentage (145%, 176%, and 188%, respectively) increase at 55 MPa than those obtained at 34 MPa (115%, 151%, and 169%) when the temperature was raised from 45 to 60 °C. Conversely, the yield of compound **g** was reduced at 55 MPa and 60 °C compared to that obtained at 55 MPa and 45 °C. This may be due to experimental error since its quantity was very low. Yields of all compounds, except for compound **g**, at 60 °C were significantly higher ($p \leq 0.05$) than those at 45 °C at both pressure levels.

Yield of alkylamides does not seem to be affected by their physical state as compounds **a**, **c**, and **g** are colorless oils at room temperature, whereas the rest are colorless crystals (*16*). This is due to increased vapor pressure of alkylamides with temperature so that it is easier for them to be solubilized by SC−CO_2. Furthermore, increasing temperature enhanced the diffusion rate of SC−CO_2 into the roots, leading to an increased extraction rate.

A similar temperature effect was obtained for the total extract (including unidentified compounds). At 35 MPa, extractions at 60 °C resulted in approximately 2.6 times more extract than those at 45 °C (7.71 vs 2.91 mg/g dry root, respectively). A similar increase was found at 55 MPa when temperature was increased from 45 °C to 60 °C (1.90 and 4.67 mg/g dry root, respectively). Total extract yield increased with temperature at both 34 and 55 MPa except that the rate of increase at 34 MPa was slightly higher than that at 55 MPa.

Effect of Pressure. As mentioned above, the yield of alkylamides from fresh roots at 34 MPa was higher than that at

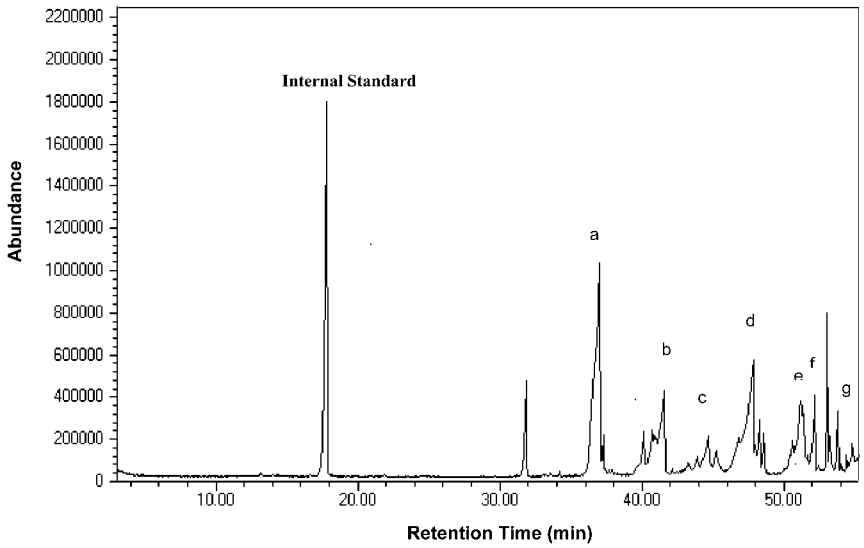

Figure 2. Typical gas chromatogram of *E. angustifolia* extracts from supercritical CO_2 extraction.

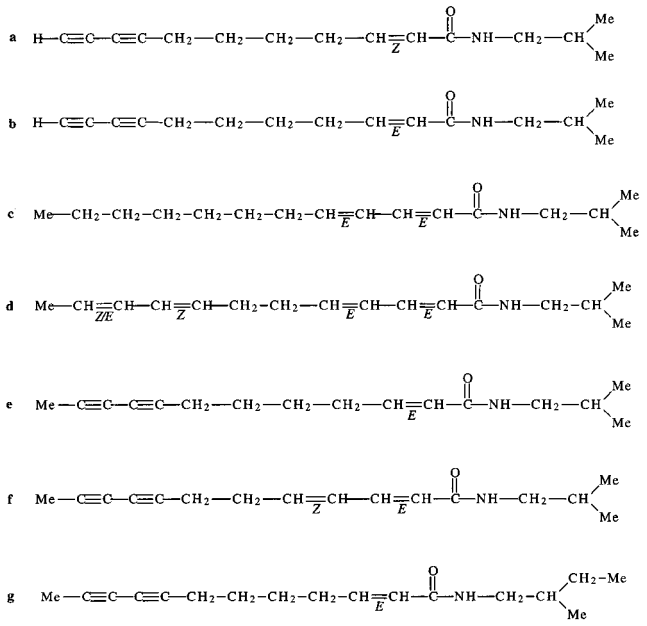

Figure 3. Chemical structures of alkylamides identified in *E. angustifolia* extracts obtained by SFE: **a**, undeca-2*Z*-en-8,10-diynoic acid isobutylamide, colorless oil; **b**, undeca-2E-en-8,10-diynoic acid isobutylamide, colorless crystals from *n*-hexane; **c**, dodeca-2E,4E-dienoic acid isobutylamide, colorless oil; **d**, mixture of dodeca-2e,4E,8Z,10Z/E-tetraenoic acid isobutylamide, mixture crystallized as needles from *n*-hexane; **e**, dodeca-2E-en-8,10-diynoic acid isobutylamide, colorless crystals from *n*-hexane; **f**, dodeca-2E,4Z-dien-8,10-diynoic acid isobutylamide, crystals from *n*-hexane; **g**, dodeca-2E-en-8,10-diynoic acid 2-methylbutylamide, colorless oil.

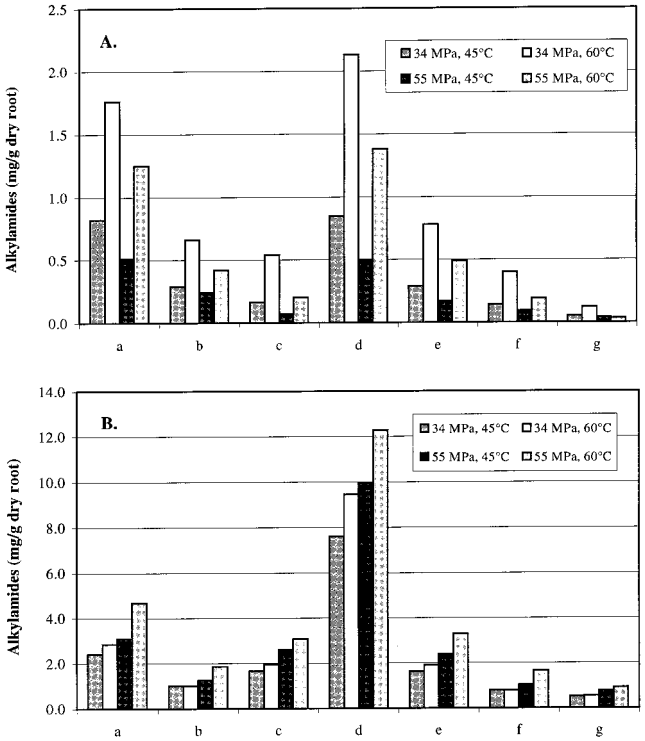

Figure 4. A. Temperature and pressure effects on the normalized yields of alkylamides (compounds **a**–**g**) in supercritical CO_2 extracts of fresh *E. angustifolia* roots. B. Temperature and pressure effects on the normalized yields of alkylamides (compounds **a**–**g**) in supercritical CO_2 extracts of air-dried *E. angustifolia* roots.

55 MPa. At 45 °C, a decrease in pressure from 55 to 34 MPa led to an increase in the yield of eight alkylamides ranging from 21% to as high as 128%. The percentage increase in yield was almost doubled when extractions were carried out at 60 °C, where the increase ranged from 41% to 170% with a drop in pressure from 55 to 34 MPa. Therefore, the pressure effect on the extraction of alkylamides from fresh roots was enhanced at higher extraction temperatures. Increasing pressure led to an increase in $SC-CO_2$ density, which should correspond to a higher yield of alkylamides. However, there is a simultaneous increase in the solubility of water in $SC-CO_2$, which also acts as a barrier to the extraction of lipophilic alkylamides from the cell structure of the roots (*27*).

Further examination of the change in the yield of individual compounds with a drop in pressure revealed that compounds **a**, **d**, and **e** had lower increases in yield at 60 °C (41%, 54%, and 59% compared to 61%, 70% and 71% at 45 °C, respectively). Except for the yield of alkylamides **a**, **b**, and **g**, the yields of the remaining compounds at 55 MPa were significantly lower ($p \leq 0.05$) than those at 34 MPa. The temperature−pressure interaction effect on the yield of individual compounds and total extracts was not significant ($p > 0.05$).

At 34 MPa and 60 °C, a higher extract yield was obtained from fresh roots. This was true for total extracts as well as for the eight major compounds identified. Total extracts were reduced by at least 53%, from 7.71 to 4.67 mg/g dry root at 60 °C and 2.91 to 1.90 mg/g dry root at 45 °C, when the pressure was increased from 34 to 55 MPa. Fresh *E. angustifolia* roots resulted in enhanced yield at lower pressure and higher temperature (i.e., 34 MPa and 60 °C). Quantitation of the co-extracted water from fresh roots at different SFE conditions needs to be studied further.

SFE of Dried *E. angustifolia* Roots. It was observed that extracts from fresh roots dissolved in dichloromethane were light yellow compared to bright yellow for those of dried roots. This indicated that additional pigments were extracted from dried samples. Unlike alkylamide yields from fresh roots, yields from dried roots were at a much higher level, ranging from 0.52 to 12.28 mg/g dry root. **Figure 4B** presents the temperature and pressure effects on the normalized yield of alkylamides in SC−CO_2 extracts of air-dried *E. angustifolia* roots. Analysis of variance proved that temperature and pressure had significant effects ($p \leq 0.05$) on alkylamide yields from dried roots as discussed below.

Effect of Temperature. Temperature had a similar effect on the yield of alkylamides and total extracts from dried roots as those from fresh roots. At 34 MPa, the yield of compounds **b**, **f**, and **g** had a slight change, whereas the remaining five compounds showed 18−24% increase when temperature was changed from 45 °C to 60 °C. With an increase in pressure to 55 MPa, there was a yield increase for every compound ranging from 19% to 62%, and compound **f** showed the greatest increase. Compounds **c** and **d** had the same yield increase at both pressure conditions due to temperature change. The yield of alkylamide **e** was significantly higher ($p \leq 0.05$) at 60 °C compared to that at 45 °C.

Stuart and Wills (*18*) studied the temperature effect during the aqueous ethanol extraction of alkylamides from dried *E. purpurea*. They found the optimal extraction of alkylamides to be at 20 °C with 60% recovery from the raw material compared to 35% at 60 °C, which indicated a decrease in alkylamides recovery with elevated extraction temperature. Investigation of the extraction residue further indicated that there was considerable degradation of alkylamides during the aqueous ethanol extraction process (*18*).

Effect of Pressure. Pressure had a different effect on the yield of alkylamides and total extracts from dried roots compared to the results of fresh roots. At 45 °C, increasing extraction pressure from 34 to 55 MPa resulted in a yield increase (24−56%) for all eight compounds with compound **c** having the highest percentage increase. At 60 °C, the alkylamide yields increased 30−112% when the pressure was increased from 34 to 55 MPa. Compounds **c** and **d** had a similar increase at the two temperatures due to an increase in extraction pressure. Yields of three compounds (**a**, **e**, and **g**) were significantly increased ($p \leq 0.05$) when the extraction pressure was changed from 34 to 55 MPa at 60 °C.

Figure 5 shows a comparison of the yield of total extracts obtained from SC−CO_2 extraction of fresh and air-dried roots at the different conditions used in this study. At 55 MPa and 45 °C, the most unfavorable extraction condition for fresh roots, there was over a 10-fold increase of total extract from the air-dried roots. These results indicate that it is much easier to extract alkylamides and other components from dried roots using SC−CO_2.

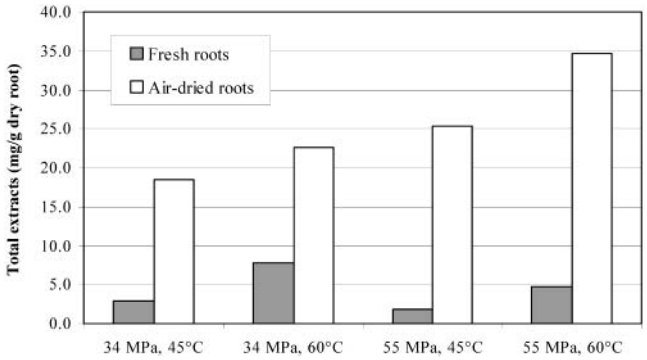

Figure 5. Yield of total extracts from fresh and air-dried *E. angustifolia* roots obtained by supercritical CO_2 extraction at different conditions.

SFE of Air-Dried Roots Using Ethanol as an Entrainer. To enhance the solvent power of SC−CO_2 and increase its polarity, ethanol was injected and mixed into the flow of CO_2 before it entered the extraction cell. Ethanol and water are the best "natural" entrainers for food-grade products (*28*).

SC−CO_2 extraction using ethanol (5%, w/w) as an entrainer was conducted at 34 MPa and 60 °C on the air-dried roots. The yield of alkylamides ranged from 0.54 to 8.43 mg/g dry root. Except for compounds **b**, **c**, and **e**, the yields of other alkylamides were reduced when compared to the yields obtained from the same roots without ethanol addition. Overall, there was no significant difference ($p > 0.05$) between the yields of alkylamides obtained with or without ethanol addition at the level studied.

Comparison of Ground and Unground Air-Dried Roots. The alkylamide yields obtained from unground air-dried roots (**Figure 6A**) were substantially lower than those from ground roots, and even less than those from fresh roots at 34 MPa and 45 °C. At 34 MPa and 60 °C, the yield of eight major alkylamides from unground air-dried roots were in the range 0.09−0.78 mg/g dry root, and the total extract yield was 2.09 mg/g dry root. Compared to yields from ground air-dried roots, those from unground roots were significantly lower ($p \leq 0.05$) for every individual compound in the extract.

As expected, the physical structure of the solid matrix had a significant effect on the extraction yields. The woody surface of the fresh roots and the cell structure shrunk after drying such that the roots became crumpled. This phenomenon is referred to as "case hardening" and is common in air-drying of biological materials. This change in the physical structure of the roots made it harder for SC−CO_2 to penetrate into. However, grinding greatly increased the surface area of the roots in contact with the SC−CO_2 and led to an increase in alkylamide yield. Such factors may contribute to the low yield of alkylamides from unground air-dried roots.

Comparison of Fresh and Dried Roots. It is apparent from the above discussions that the best SFE conditions depend on the sample moisture level, considering 34 MPa and 60 °C was best for fresh roots and 55 MPa and 60 °C was optimal for dried roots. SFE of freeze-dried roots was conducted at the best extraction conditions found for air-dried roots because its moisture content (4.9%) was comparable to that of air-dried samples (8.4%). **Figure 6B** gives a comparison of the yields of eight identified alkylamides from fresh roots, air-dried roots, and freeze-dried roots at their best respective supercritical extraction conditions.

It was clear that extracts of dried roots contained more alkylamides than those of fresh roots. Freeze-dried root extracts seemed to have slightly more alkylamides than those of air-

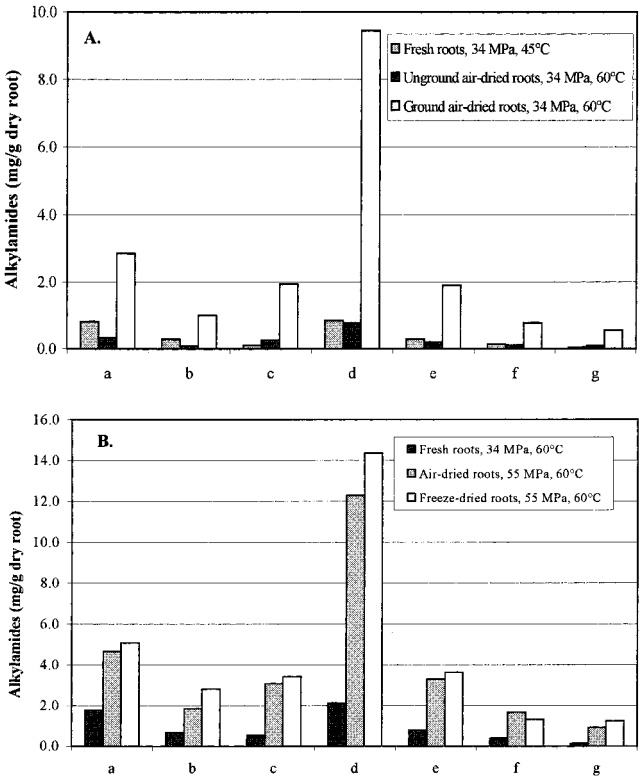

Figure 6. A. Alkylamide (compounds **a**–**g**) yields from fresh, ground and unground, *E. angustifolia* roots obtained by supercritical CO_2 extraction at different conditions. B. Alkylamide (compounds **a**–**g**) yields from fresh, air-dried, and freeze-dried *E. angustifolia* roots obtained by supercritical CO_2 extraction at their optimal extraction conditions.

dried roots for most of the identified compounds. Analysis of variance showed that the alkylamide yields from fresh roots were significantly ($p \leq 0.01$) lower than those from dried roots; whereas air- and freeze-dried roots yielded similar amounts of extracts ($p > 0.05$). This is consistent with the Atlantic mackerel study done by Dunford et al. (*27*) in which they found that the oil extract yields from mackerel containing 10.2% and 3.8% moisture were similar (2.5−2.7 g). Snyder et al. (*25*) also had a similar finding on SC−CO_2 extraction of soybeans at moisture levels of 3% and 12%.

When fresh root extracts were compared to those of air-dried roots at their best respective extraction conditions, there was a 166−658% increase in the yield of eight individual alkylamides and 350% increase in the yield of total extract, indicating that it is much easier to extract alkylamides from dry roots than from fresh roots. Yields of compounds **c**, **d**, and **g** increased by at least four times.

Water is soluble in SC−CO_2 to a very limited extent (*29*). In the case of fresh roots, water may be acting as an entrainer in the extraction process. Unfortunately, even though water helps the roots maintain their structure, it dissolves in SC−CO_2 and may reduce the solubility of alkylamides, due to their lipophilic nature. Furthermore, fresh roots were in 1-cm pieces, not possessing sufficient surface area for the SC−CO_2 to penetrate into the matrix, thereby not facilitating efficient extraction. On the other hand, powdered dried roots were the ideal matrix for SFE (*30*). Grinding produced a fine powder with good permeability, enabling contact between SC−CO_2 and roots.

Bauer et al. (*16*) found that compound mixture **d** is the major constituents of alkylamides in *E. angustifolia* at a level of about 0.009−0.151% (*10*). The yield of mixture **d** using SFE was within the range of 0.50−14.34 mg/g dry root. In both fresh and dried samples, the compound mixture **d** had the highest yield among the eight identified alkylamides. The five principle compounds **d**, **a**, **e**, **c**, and **b** in the SC−CO_2 corresponded to, and were consistent with, the top five compounds extracted using hexane (**14** and **15**, **2**, **10**, **1** and **3**) by Bauer et al. (*16*). It seems that alkylamide **e** is more efficiently extracted by SFE than by *n*-hexane extraction.

In summary, alkylamides have been successfully extracted using SC−CO_2. Temperature and pressure of SFE, as well as moisture content of the roots, had significant effects on the yield of alkylamides and total extracts. Alkylamides were better extracted at 34 MPa and 60 °C from fresh roots, whereas dried roots resulted in much higher alkylamide yield at 55 MPa and 60 °C. Grinding significantly increased the yield of alkylamides from dried roots compared to that from unground dried roots. Ethanol addition as an entrainer in SFE did not enhance the alkylamide yields from air-dried roots. There were slightly more alkylamides obtained from freeze-dried roots but those yields were not significantly different from those of air-dried roots.

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