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Food Chemistry 96 (2006) 304–312
Food Chemistry
www.elsevier.com/locate/foodchem

Characteristics of *Echinacea* seed oil ☆

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Received 8 November 2004; received in revised form 15 February 2005; accepted 15 February 2005

Abstract

Seed oils from three commonly cultivated *Echinacea* species, *Echinacea Angustifolia*, *Echinacea Pallida* and *Echinacea Purpurea*, harvested in 1998 and 1999, were evaluated for physicochemical characteristics. Oil yield ranged from 13% to 23%, depending on *Echinacea* species and seed weight. UV/VIS and fluorescence spectra distinguished *Echinacea* seed oil by species. Vitamin E content of the oils ranged from 29 to 85 mg/100 g oil, with α-tocopherol constituting 83% of the total tocopherol. The oil was highly polyunsaturated and abundant in linoleic, oleic and palmitic acids, together comprising 95% of the total fatty acids. *Echinacea* seed exhibited three thermal transitions, two of which were reversing and descriptive of the species. Thermal oxidation, by differential scanning calorimetry (DSC), segregated *Echinacea* seed oil by species, based on oxidation temperature. *Echinacea* seed oil was separated, by TLC, into fractions corresponding to alkamides, ketoalkenes, echinicoside and chicoric acid, depending on species.

Keywords: *Echinacea*; *Echinacea* seed oil; Oil quality; Tocopherols; DSC; Fluorescence; TLC; Chemical and physical parameters

1. Introduction

Echinacea products are the most popular herbal immunostimulants in North America and Europe. In 1997, *Echinacea* was the top selling single botanical product (9% of the total market at $ 324 million; Marriott, 2003) and the market leader in volume sales at 10% of total medicinal herbs sales (Wills, Bone, & Morgan, 2000). Three species of *Echinacea* are used medicinally and for horticultural purposes: *Echinacea angustifolia* (DC.) Cronq.; *Echinacea purpurea* (L.) Moench; and *Echinacea pallida* (Nutt.) Cronq. (Binns, Livesay, Arnason, & Baum, 2002). *E. purpurea* is the most cultivated and widely used of the three species, due to ease of cultivation and total use of the whole plant. Pharmaceutical preparations of *Echinacea* are primarily based on extracts of the subterranean parts (root and rootstock) with high concentration and the most complex mixture of bioactive compounds, aerial parts (leaves, stems and flowerheads) and their mixture (Molgaard, Johnson, Christensen, & Cornett, 2003).

The seeds of *Echinacea* have never been investigated, and reports on the achene (small dry indehiscent fruit with one seed within the thin wall) are very limited. The achene of *E. pallida* has a remarkably low (about five times lower) content of isobutylamides compared with those of *E. angustifolia* and *E. purpurea* (Schulthess, Giger, & Bauman, 1991). The profiles of the residue from a hexane extract of *Echinacea* achene are simpler than those of the roots, containing only one major peak

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0308-8146/$ - see front matter.
doi:10.1016/j.foodchem.2005.02.037

of the tetraene alkamide **8/9** at 1.06, 0.75 and 0.08 mg/g of dry matter for *angustifolia*, *purpurea* and *pallida*, respectively (He, Lin, Bernart, & Lian, 1998). The lipophilic constituents of *Echinacea*, the alkamide in particular, have positive pharmacological benefits, responsible for the immunostimulatory properties of *Echinacea* extracts (Bauer, 2000). Antifungal activity (Merali et al., 2003) and cyclooxygenase and 5-lipoxygenase inhibitory activities (Binns et al., 2002) have also been attributed to the lipophilic constituents of *Echinacea*. Since oil is expected to be the richest source of lipophilic constituents and the seeds of *Echinacea* have not been explored, our investigation focussed on the quality of *Echinacea* seed oil. This is a continuation of our studies on complete utilization of horticultural crops for innovative uses and for the development of new products for the functional food, cosmetic, therapeutic and nutraceutical industries.

2. Materials and methods

Seeds of *Echinacea* species (*E. angustifolia*, *E. pallida* and *E. purpurea*), harvested in 1998 and 1999 from the Pacific Agri-Food Research Centre, were provided by Dr. Tom Li and seeds were also obtained from a commercial source (Richters, Goodwood, ON). Seeds from the 1998 harvest were cleaned by sifting through 4, 2.83 and 2 mm screens for 20 min, followed by air-separation in a laboratory aspirator (Cuthbert Co. Ltd., Winnipeg, MB). The length, diameter and width of 25 randomly selected seeds were measured using a Mitutoyo Digimatic caliper. All seed samples were ground in a coffee grinder. Oil from all milled samples was extracted using hexane (50 g sample in 150 ml hexane), as described by Oomah, Mazza, and Przybylski (1996), purged with nitrogen and stored at −20 °C prior to analysis. Extractions were performed in triplicate and analyzed separately.

2.1. Analytical procedures

Spectroscopic indices, K_{232} and K_{270}, in the UV region, were determined as outlined in the Standard Methods for the Analysis of Oils, Fats and Derivatives (International Union of Pure and Applied Chemistry, IUPAC, 1985). Absorbancies at 670, 610, 560, and 535 nm, related to chlorophylls and at 475, 448, and 414 nm, related to carotenoids, of a 10% (v/v) solution of oil in hexane, were measured with a spectrophotometer (DU-640B, Beckman Instruments Inc., Fullerton, CA, USA).

Fluorescence of oil was measured with a microplate spectrofluorimeter (SPECTRAmax GEMINI XS, Molecular Devices Corp., Sunnyvale, CA). Excitation at 360 nm gave excellent results and was used as the excitation wavelength throughout the study. Emission fluorescence spectra were recorded from 390 to 750 nm. A cut-off filter, at 420 nm, was used to increase sensitivity and block light below the cut-off wavelength. The oils were filtered (0.45 μm, Gelman Science Inc., Ann Arbor, MI, USA) and a 100 μl aliquot was used for analysis.

Tocopherols in *Echinacea* seed oils were analyzed by an HPLC system (Waters 840 system, Milford, MA, USA) consisting of a pump (Model 510), an auto sampler (Model 712) and a fluorescence detector (McPherson SF-749 spectrofluorometer, Acton, MA, USA) interfaced with a personal computer. A normal phase column (4.6 × 150 mm, Primesphere 5 silica 5 μm) with guard column (4.6 × 30 mm) (Phenomenex, Torrance, CA, USA) was used with hexane/2-propanol/dimethyl propane (1000/5/1, v/v/v) as mobile phase. The system was operated isocratically at a flow rate of 1 ml/min. Separations were carried out at 25 °C (Waters TCM temperature controller) with the fluorescence detector excitation and emission wavelengths set at 297 and 325 nm, respectively. Typically, a 10-min equilibration period was used between samples, requiring about 40 min/sample. Quantitation was based on external standard method; the calibration curves ranged from 1.12 to 22.3, from 0.314 to 6.29, from 0.228 to 4.55 and from 0.047 to 0.944 μg/ml of reference compounds, α-, δ-, and β-, γ-tocopherols, respectively (Sigma Chemical Co., St. Louis, MO, USA). Prior to HPLC analysis, seed oil was diluted with hexane to obtain a concentration of about 160 g/l, filtered (0.45 μm, Gelman Science Inc., Ann Arbor, MI) and 20 μl samples were injected. Oil content of the ground *Echinacea* seed samples was determined by Soxhlet extraction with petroleum ether for 6 h. Moisture content was determined by the AOAC method (AOAC, 1984).

Thermal characteristics of *Echinacea* seed oil were measured using a modulated differential scanning calorimeter (Modulated DSC-2910, TA Instruments, New Castle, DE). A flow of nitrogen gas (100 ml/min) was used in the cell cooled by helium (150 ml/min) in a refrigerated cooling system. The instrument was calibrated for temperature and heat flow with mercury (melting point, mp = 38.83 °C, TA Instruments standard), gallium (mp = 29.76 °C, TA Instruments standard) and indium (mp = 156.6 °C, ΔH = 28.71 J/g, Aldrich Chemical Co.). Oil samples (4–5 mg) were weighed in open solid fat index (SFI) aluminium pans (T70529, TA Instruments). An empty similar pan was used as reference. The sample and reference pans were then placed inside the calorimeter and kept at 70 °C for 5 min. The temperature was lowered from 70 to −65 °C at a rate of 1 °C/min with modulation at a period of 60 s and temperature amplitude of 0.16 °C. Samples were then kept at −65 °C for 1 min, and then raised again, at the same rate, up to 70 °C. Scans were performed at 10 °C/min. For thermal oxidation, pans were

cooled to 10 °C and scanning was done by heating at 1 °C/min to 350 °C in the presence of oxygen (100 ml/min). Thermal oxidation measurements were performed in duplicate.

The oils were subjected to thin-layer chromatography (TLC) on precoated 0.20 mm silica gel 60 plates with fluorescent indicator UV_{254} (Macherey-Nagel Inc., Easton, PA), using the solvent system n-hexane/ethyl acetate (2:1, v/v) essentially as described by Bauer and Remiger (1989). Oil samples (100 μl) were mixed with ethanol (900 μl, dilution 1:10 v/v) and the mixture was allowed to separate into two phases by refrigerating it overnight. The ethanol phase (5 μl) was spotted on the TLC plate that was subsequently developed and dried at room temperature. Visualization was under short (254 nm) UV light. Anisaldehyde/sulfuric acid (anisaldehyde/ethanol/concentrated sulfuric acid/acetic acid, 5:90:5:1, v/v/v/v) was used as a spray for locating and detecting alkamides.

At least three determinations were done for all assays. Analysis of variance by the general linear models (GLM) procedure and means comparisons by Duncan's test were performed according to Statistical Analysis System (SAS Institute, 1990).

3. Results and discussion

Echinacea seeds were 4.6–5.4 (4.95 ± 0.49) mm long, 1.6–2.0 (1.79 ± 0.26) mm in diameter, 3.1–5.8 (4.6 ± 0.92) mg seed weight. Seed weight values are within the expected range reported previously (Wartidiningsih & Geneve, 1994). The commercial samples, with the exception of the *purpurea* species, had lower physical characteristics values than the harvested seeds. Moisture content of the seed was 7.2 ± 0.3%. *Echinacea* seeds averaged 18.9 ± 3.9% oil yield with *purpurea* and *pallida* seeds exhibiting the highest (~23%) and lowest (13.4–14%) yields, respectively. Oil yield from the commercial seeds was generally higher than from field-harvested seeds. The shortest and heaviest seeds (*Pallida* 98) also produced the lowest oil yield.

Crude *Echinacea* seed oil has three characteristic absorbance peaks, centred at 235, 420–435 and 670 nm (Fig. 1). The central peak at 235 nm is of maximum intensity (0.68–1.15) and corresponds to the highest absorptivity (K_{232}, Table 1). The high absorption at approximately 420, 435 and 475 nm characterized *pallida* and differentiated it from the other two species. The 420–475 nm spectra for *purpurea* and *angustifolia* can be identified by a broad shoulder, due to widening of the peaks at 420 and 435 observed in *pallida*. Colour due to chlorophyll (absorbance at 670 nm), predominated in *pallida* and *purpurea* and was very descriptive of the apparent colour of the oils. The spectrum of *Echinacea* oil showed negligible absorbance in the 310–390 nm (UVA) range; its absorbance value fell continuously as wavelength increased. High absorptivity at 232 and 270 nm, especially for *angustifolia* (4.50–4.86; 0.79–0.82), may be due to high diene conjugation. The *pallida* and *angustifolia* species exhibited the highest and lowest absorbances, respectively, in the 412–482 range, indicative of the corresponding high and low carotenoid pigments of the oil (Table 1). The *pallida* species, especially field-harvested in 1998, also exhibited the highest absorbance in the visible region at 535–670 nm, related to chlorophyll. The spectroscopic indices of the *purpurea* species were intermediate between those of *purpurea* and *angustifolia*. The green pigment, particularly chlorophyll, content was low, as indicated by absorbances (0.014–0.095) about one tenth of the value observed previously for hempseed oil (Oomah, Busson, Godfrey, & Drover, 2002).

A representative fluorescence spectrum of *Echinacea* seed oils (Fig. 2) indicates the presence of three major peaks; a broad peak with the highest intensity at

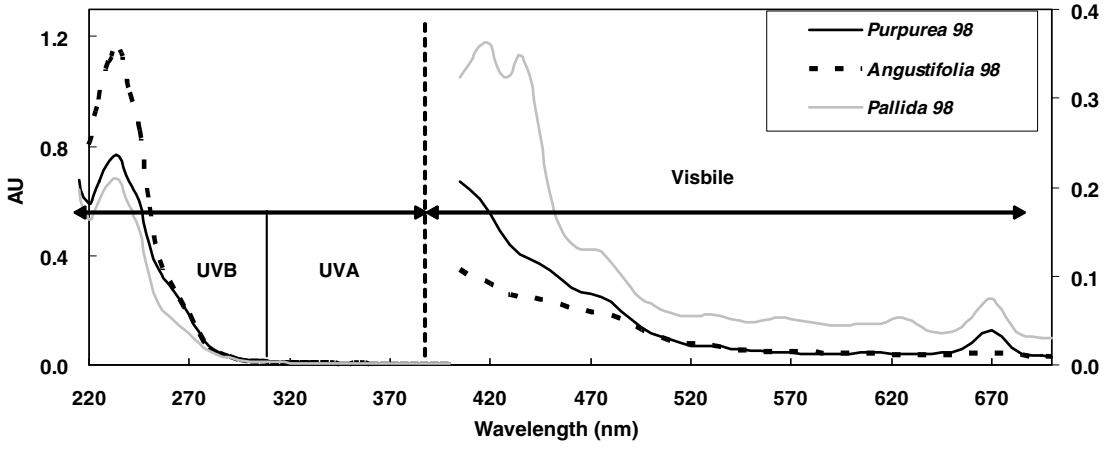

Fig. 1. Ultra violet visible spectra of oils from *Echinacea* seeds harvested in 1998, *Angustifolia* (broken line), *Pallida* (grey) and *Purpurea* (black). Figure derived from scans (λ = 200–290) of oil at 0.25 g/l; from scans (λ = 290–400) of oil at 10 g/l and from scans (λ = 400–700) of oil at 100 g/l of hexane.

Table 1
Quality characteristics (colour) of *Echinacea* seed oils

Quality characteristics	Field-grown in 1998			Commercial		
	Angustifolia	*Pallida*	*Purpurea*	*Angustifolia*	*Pallida*	*Purpurea*
K_{232}	4.855a	2.955d	3.265c	4.540b	2.170f	2.550e
K_{270}	0.795a	0.520b	0.785a	0.815a	0.415c	0.755a
A_{412}	0.099e	0.357b	0.195d	0.094e	0.476a	0.283c
A_{414}	0.098e	0.363b	0.190d	0.092e	0.487a	0.277c
A_{428}	0.080e	0.331b	0.142d	0.080e	0.402a	0.201c
A_{448}	0.076de	0.229b	0.111d	0.072e	0.295a	0.129c
A_{453}	0.072c	0.178b	0.102c	0.069c	0.251a	0.146b
A_{475}	0.061c	0.136b	0.078c	0.059c	0.202a	0.115b
A_{482}	0.057c	0.119b	0.069c	0.055c	0.172a	0.099b
A_{535}	0.021b	0.064a	0.022b	0.021b	0.036b	0.030b
A_{560}	0.016b	0.059a	0.016b	0.017b	0.030b	0.021b
A_{610}	0.014b	0.056a	0.016b	0.015b	0.029b	0.023b
A_{670}	0.014d	0.082ab	0.040c	0.016d	0.095a	0.072b

[a–d] Means in a row followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level.

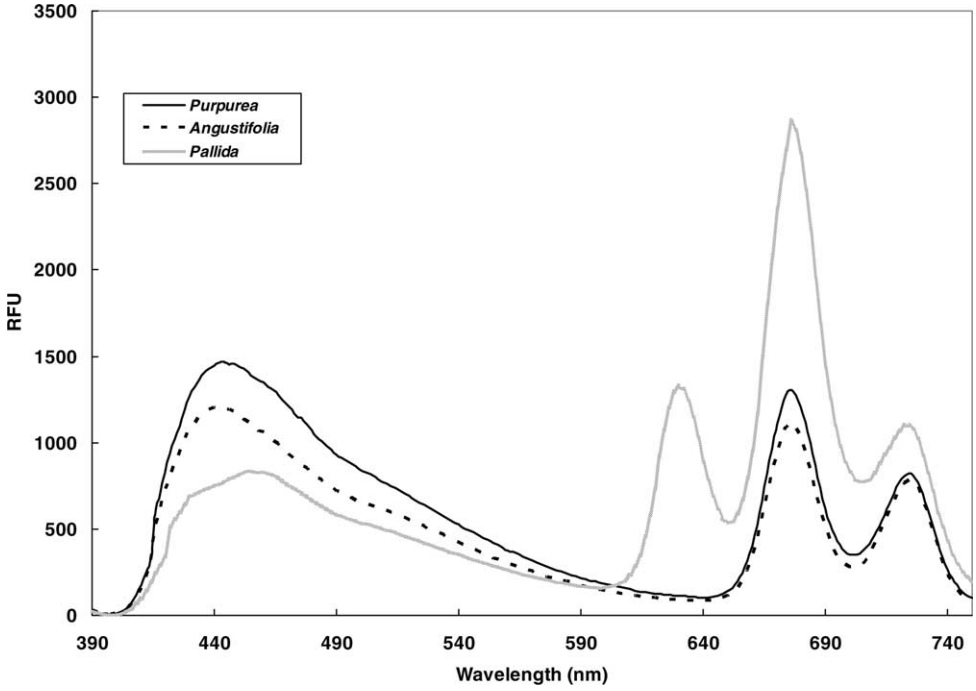

Fig. 2. Comparison of scanning fluorescence spectra of oils from *Echinacea* seeds harvested in 1998, at 360 nm excitation.

440 nm, a medium peak at 676 nm and a weak shoulder peak at 724 nm (Table 2). A fourth peak, at 630 nm, was present consistently at high intensity in *pallida*. The peaks at 440 and 680 nm, also present in vegetable oils, may be ascribed to the presence of chlorophyll in the oil according to Kyriakidis and Skarkalis (2000). The intensity of the peaks at 440 nm corresponds with the values of conjugated trienes K_{270} of 0.58, 0.85 and 0.98 for *pallida* 98, *angustifolia* 98 and *purpurea* 98, respectively. This indicates that fatty acid oxidation products may also be responsible for the fluorescence at 440 nm, as proposed by Kyriakidis and Skarkalis (2000). The peak at 630 nm may be ascribed to the presence of protochlorophyll, as proposed by Wolfbeis and Leiner (1984). The low intensity peak, with an emission at 724 nm, may be attributed to chlorophyll d, the red absorbing species typical of the far red excitation of photosystem II reduction (Mimuro, Hirayama, Uezono, Miyashita, & Miyachi, 2000; Jennings & Forti, 1975). Overlapping of spectra $\geqslant$600 nm occurred in oils from *purpurea* and *angustifolia*. The intensity of the peaks was between 18 and 1730 fluorescence arbitrary units. The highest and lowest fluores-

Table 2
Fluorescence of *Echinacea* seed oils at various emission wavelengths[a]

Sample	Emission wavelength			
	440	630	676	724
Angustifolia 98	1695a	36e	327e	90e
Angustifolia 99	529d	718a	764bc	177cd
Angustifolia commercial	1439ab	18f	51f	23f
Pallida 98	767cd	546b	563d	152d
Pallida 99	839cd	728a	732c	163d
Pallida commercial	885c	264c	852b	212b
Purpurea 98	1730a	41e	378e	104e
Purpurea 99	1634a	71d	1408a	378a
Purpurea commercial	1283b	48e	728c	195bc

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level.

cence emissions were generally observed for *purpurea* and *angustifolia* (commercial and 98), respectively.

The major tocopherol in *Echinacea* seed oil was the α-isomer, at 83 ± 6% of the total tocopherol, while the β-isomer constituted only 17 ± 6% of the total. Tocopherol contents of oil differed significantly ($P < 0.05$) among species (Table 3), with *angustifolia* and *pallida* having the highest and lowest tocopherol contents, respectively. γ-Tocopherol was present only in *angustifolia*. The oil from commercial seed had between 12% and 23% higher levels of α-tocopherol than those obtained from field harvested seeds. *Echinacea* seed oil is a rich source of β-tocopherol, second only to wheat germ oil and its tocopherol content was within the range reported for sunflower oil (Padley, Gunstone, & Harwood, 1994). The biologically active vitamin E content relative to that of α-tocopherol, calculated by using the formula proposed by McLaughlin and Weihrauch (1979), ranged from 29.3 to 85 mg/100 g of oil for *pallida* 98 sample and the commercial *angustifolia*, respectively. Vitamin E content of *Echinacea* seed oil was similar to those of peanut oil, olive oil and soybean oil (Ensminger, Ensminger, Konlande, & Robson, 1993). The ratio of the tocopherol isomers (α/β) in *Echinacea* seed oil ranged from 2.7 to 9.8, depending on the species.

Table 3
Tocopherol contents of *Echinacea* seed oils (mg/100 g)[a]

	Tocopherol				Vitamin E
	α	β	γ	Total	
Angustifolia 98	70.2b	11.9b	1.2a	83.3b	75.1b
Angustifolia commercial	78.3a	16.5a	1.7a	95.7a	85.0a
Pallida 98	25.6e	9.4c	nd	35.0e	29.3e
Pallida commercial	31.4d	9.2c	nd	40.7d	35.1d
Purpurea 98	35.2d	3.6e	nd	38.8de	36.6d
Purpurea commercial	42.3c	4.7d	nd	47.0c	44.2c

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level; nd, not detected.

Table 4
Fatty acid composition of *Echinacea* seed oil

Sample	Composition (mass%)							
	16:0	16:1	18:0	18:1	18:2	18:3 ($n-6$)	20:0	22:0
Angustifolia 99	7.0	0.3	2.8	7.8	80.8	0.2	0.1	0.4
Pallida 99	6.7	0.2	2.5	9.3	79.3	0.2	0.2	0.4
Purpurea 99	7.0	0.2	2.9	12.2	75.6	0.2	0.2	0.6
Purpurea 98	8.0	0.2	2.7	21.4	66.5	0.2	0.2	0.5

The most abundant fatty acids of *Echinacea* seed oil were linoleic, oleic, and palmitic acids, which together comprised 95% of the total fatty acid (Table 4). The fatty acid profile of *Echinacea* seed oil was similar to that of safflower oil (Christie, 2003). The negligible amounts of linolenic acid (0.2%) permit the use of the oil, especially where autoxidation may be a concern, without being subjected to hydrogenation. The polyunsaturates of the oils amounted to 76% of the total fatty acids, while the monounsaturated and saturated fatty acids amounted to 13% and 11%, respectively. Hence, the ratio of polyunsaturates to monounsaturates to saturate was 76:13:11, similar to hempseed oil at 78:11:11 (Oomah et al., 2002). The oil was characterized by a high polyunsaturated/saturated (P/S) ratio of 7.2, similar to those of walnuts (6.5–7.8) (Ruggeri, Cappelloni, Gambelli, Nicoli, & Carnovale, 1998). A high ratio of P/S is regarded favourably in the reduction of serum cholesterol and atherosclerosis and prevention of heart diseases (Rudel, Kelly, Sawyer, Shah, & Wilso, 1998). The iodine number was estimated to be 142 (134–147) which is within the range (135–150) reported for high linoleic safflower oil (Padley et al., 1994). An increase in oleic acid, with a concomitant decrease in linoleic acid (by about10%), was observed between *purpurea* 1999 and 1998, indicative of seasonal variation in fatty acid synthesis.

Echinacea seed oil exhibited at least three distinct thermal structural transitions between −46 and −7 °C. Two reversing transitions (−46 to −34 °C) and (−17 to −14 °C), indicative of crystalline melting, were observed, corresponding to the α and β-polymorphic forms, respectively (Fig. 3). A minor transition occurred at −6 °C. The reversing component of the heat flow was highly sensitive to *Echinacea* species (Table 5). For example, the first endothermic peak (the α-form) of *purpurea* 1999 seed oil occurred at significantly higher temperature and heat flow than those of *pallida* and *angustifolia*. Further increases in transition temperature and heat flow were observed as an expression of differences in crop year (*purpurea* 1999 and 1998). In the non-reversing component curves, to which kinetic events such as crystallization, crystal perfection and reorganization are ascribed, one exothermic and an endothermic peak were observed in the −45 to −14 °C region (Fig. 4). The exothermic peak (−45 to −35 °C with high

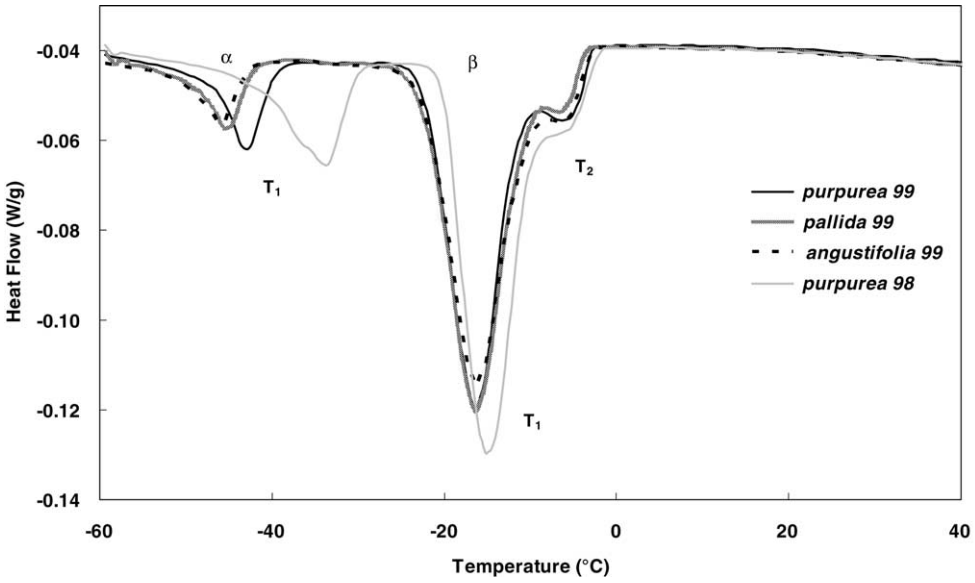

Fig. 3. Modulated differential scanning calorimetry (MDSC) reversing component of *Echinacea* seed oils.

Table 5
Thermal characteristics of *Echinacea* seed oil

Sample	Reversing heat flow				Non-reversing heat flow			
	T_∞	ΔH_α	T_β	ΔH_β	T_∞	ΔH_α	T_β	ΔH_β
Angustifolia 99	−45.7d	−4.1a	−16.4b	−36.4a	−45.1c	15.4c	−16.3b	−34.4c
Pallida 99	−45.2c	−4.5a	−16.4b	−37.2a	−44.3bc	15.8c	−16.3b	−33.3c
Purpurea 99	−43.0b	−6.2b	−16.3b	−35.0a	−43.0b	18.7b	−16.3b	−30.2b
Purpurea 98	−33.7a	−9.1c	−15.0a	−36.4a	−35.3a	24.0a	−14.0a	−22.7a

Means in a column followed by the same letter are not significantly different by Duncan's multiple range tests at the 5% level.

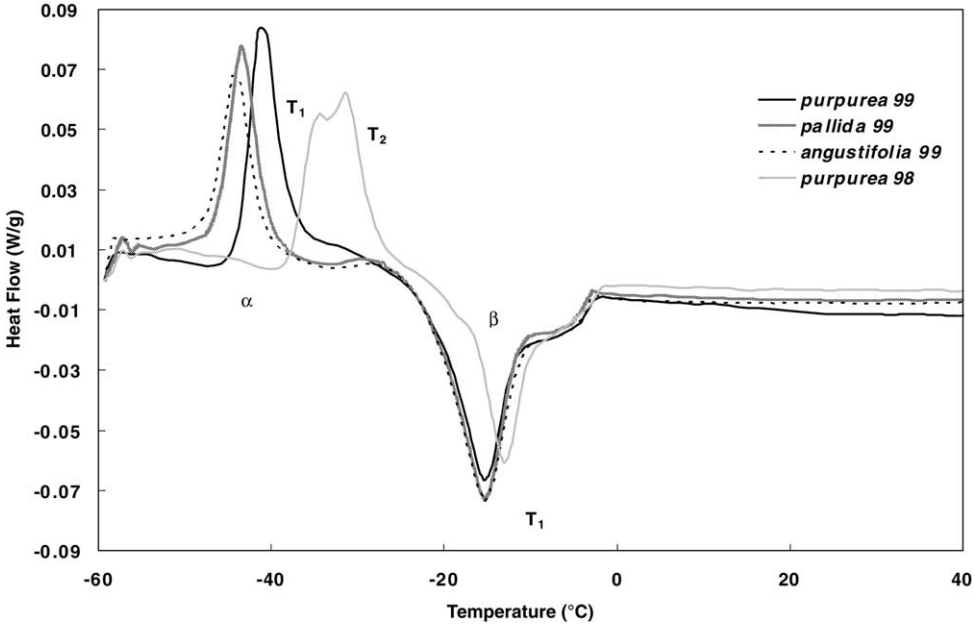

Fig. 4. Modulated differential scanning calorimetry (MDSC) non-reversing component of *Echinacea* seed oils.

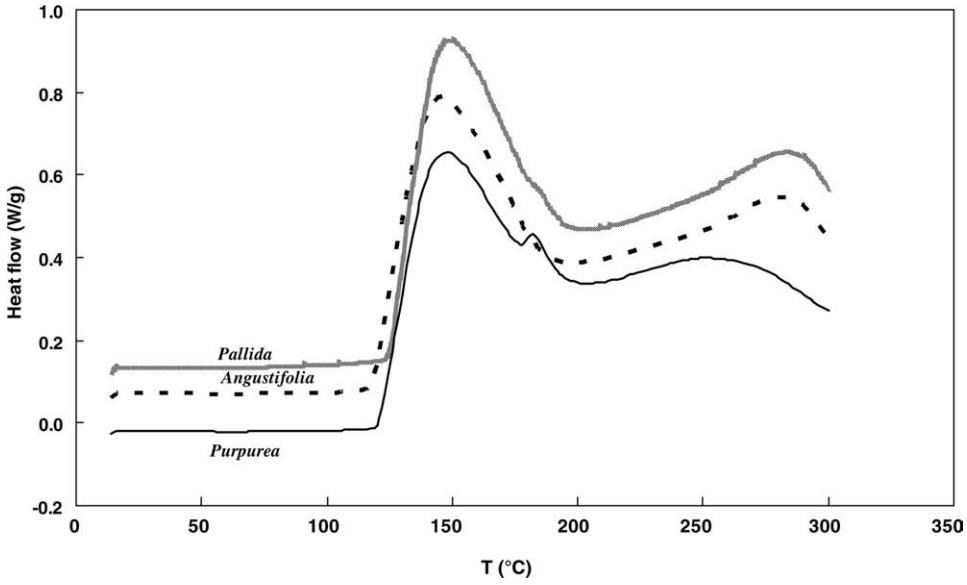

Fig. 5. Differential scanning calorimety (DSC) of the thermo-oxidation profiles of *Echinacea* seed oils.

activation energy of 15–24 J/g) suggests kinetic stability with high entropy, implying a first-order transition. The second endothermic peak, in the −16 to −13 °C region, was assigned to the β-crystallization form. The values of enthalpies, ΔH_α and ΔH_β, of the seed oil, changed with *Echinacea* species (Table 5). The ΔH_α values were similar for both reversing and nonreversing heat flow. The ΔH_β values for the nonreversing heat flow for *purpurea* species were significantly lower than those of *pallida* and *angustifolia*. The transition temperatures for both the reversing and nonreversing heat flow were similar.

Echinacea seed oil exhibited two maxima on the DSC oxidation curves, indicating that thermoxidation can be characterized by at least two step exothermic effects (Fig. 5). These peaks could be considered as an indication of the level of cross-linking. The oil from the *purpurea* species showed an additional peak at ≈182 °C. Oxidation of *Echinacea* seed oil started at 116–132 °C, slightly below the temperatures reported for edible oils (130–180 °C) and peaked at 146–153°C, depending on species and sample types (Fig. 5; Table 6). The *pallida* 98 and 99 oils had the lowest and highest onset, oxidation and peak1 temperatures, respectively. Significant differences were observed in oxidation and peak1 temperatures among species (*angustifolia*, *pallida* and *purpurea*) ($P < 0.005$ and 0.03, respectively) among types (1998, 1999 and commercial) ($P < 0.0001$ and 0.0002, respectively) and their interactions (species × type) at $P < 0.0003$ and 0.0001, respectively. The second peak at ca ≈ 182 °C was present only in the oil of commercial *purpurea* 98, indicating instability at this particular temperature. The third peak at 273–285 °C indicates the inability for oxygen uptake, resulting in complete thermal polymerization. *Echinacea* seed oil showed no significant differences in the temperature of the third peak; an observation similar to that reported previously for hempseed oil (Oomah et al., 2002).

Table 6
Thermoxidation temperatures (°C) of *Echinacea* seed oils[a]

Sample	Onset	Oxidation temperature	Peak 1	Peak 2	Peak 3
Angustifolia 98	116.2ab	122.8b	147.4cd	nd	280.8
Angustifolia 99	126.1a	130.2a	151.1ab	nd	274.1
Pallida 98	111.9b	116.4c	145.0e	nd	275.4
Pallida 99	123.8a	132.4a	152.6a	nd	273.1
Pallida commercial	120.2ab	123.4b	148.3cd	nd	275.4
Purpurea 98	117.9ab	120.8bc	147.9cd	182.9a	275.6
Purpurea 99	119.4ab	120.0bc	146.2de	nd	276.6
Purpurea commercial	114.5ab	124.9b	149.2bc	181.6a	284.5

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level; nd, not detected.

Thin-layer chromatography separated *Echinacea* seed oils into 14 fractions, eight of which were visible under short UV irradiation (Fig. 6; Table 7). The patterns were similar for the three *Echinacea* species, except for the absence of four spots ($R_f = 0.16$, 0.52, 0.83 and 0.91) in *angustifolia* and two spots ($R_f = 0.24$ and 0.38) in *purpurea*. The R_f values of spots 3 and 6 (0.31 and 0.52) corresponded closely with compounds 4 and 5 ($R_f = 0.33$ and 0.49), respectively described by Bauer and Remiger (1989), based on the characteristic violet colour reaction with anisaldehyde/sulphuric acid spray. These compounds (spots 3 and 6) may therefore be considered to be alkamides of 2,4-dienamide structures, as reported by Bauer and Remiger (1989). The spot (5) with $R_f = 0.47$ matched compounds 8 + 9 of Bauer and Remiger with similar $R_f = (0.48)$, indicating the possible

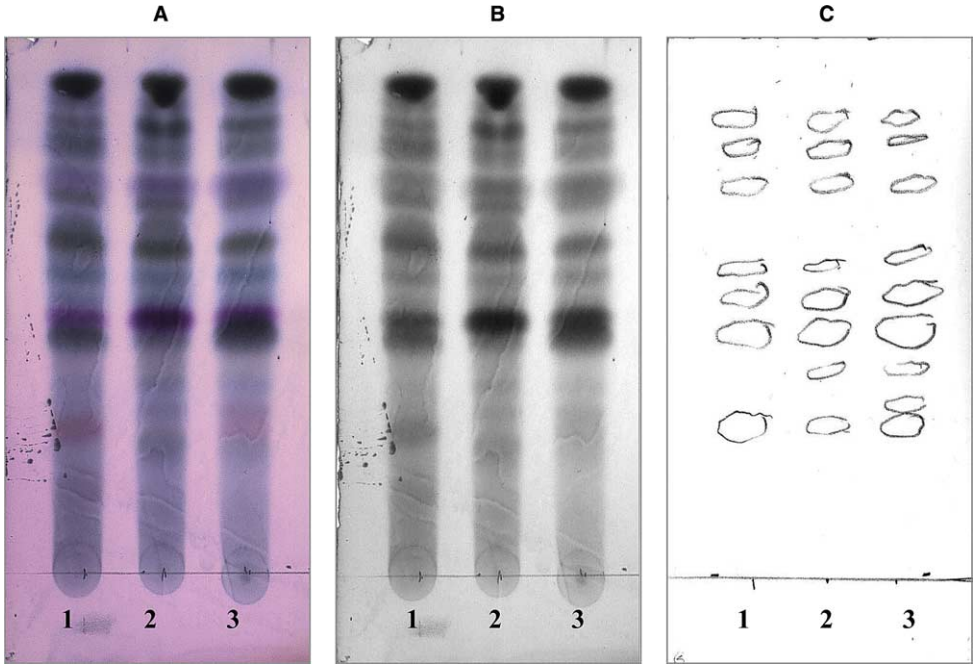

Fig. 6. Thin-layer chromatographic (TLC) separation of *Echinacea* seed oils at 1:5 (v/v) dilution in *n*-hexane/ethyl acetate (2:1, v/v). Detection was by anisaldehyde/sulfuric acid for slides A and B and UV at 254 nm for slide C. Lanes 1, 2 and 3 denote oils of *E. purpurea*, *pallida* and *angustifolia*, respectively.

Table 7
TLC of *Echinacea* seed (1998 harvest) oils

Spot	R_f	Color spot	*Purpurea*	*Pallida*	*Angustifolia*
1	0.16	Gray	+[a]	+	–
2	0.24	Light green	–[b]	+	+
3	0.31	Violet	+	+	+
4	0.38	Gray	–	+	+
5	0.47	Brown	+	+	+
6	0.52	Violet	+	+	–
7	0.55	Gray	+	+	+
8	0.60	Gray	+	+	+
9	0.66	Brown	+	+	+
10	0.74	Gray	+	+	+
11	0.78	Violet	+	+	+
12	0.83	Gray-green	+	+	–
13	0.91	Violet	+	+	–
14	0.95	Brown	+	+	+

[a,b] Presence and absence of this particular spot, respectively.

presence of the major *Echinacea* alkamide, dodeca-2, 4,8,10-tetraenoic acid isobutylamide. Spots 9–12 with R_f values between 0.66 and 0.83 had no correspondence with those of the alkamides reported by Bauer and Remiger (1989). The R_f values of spot 13 (0.91) was equivalent to the ketoalkenes and alkynes group of compounds 22–25 present only in the roots of the *pallida* species (Bauer & Remiger, 1989). Spots 4 and 14 ($R_f = 0.38$ and 0.95) probably reflect the presence of echinacoside and cichoric acid ($R_f = 0.37$ and 0.95), respectively (Bodinet & Freudenstein, 1999). Both cichoric acid and echinacoside have been demonstrated to dose-dependently protect the free radical-induced degradation of Type III collagen by a reactive scavenging effect, suggesting that they could help prevent UV-B damage to skin (Facino et al., 1995).

The oils sampled on the tongue elicited sensations characteristic of alkylamide, described as tingling, numbness, enhanced cooling on inspiration of air, a buzzing type of irritation (Bryant, Mezine, & Epple, 1999). The thermal, extraordinarily sharp tingling feeling produced by the oil is a desirable sensation in many oral-care products and some food items. Hence, *Echinacea* seed oil may find use for imparting sensory impact and/or adding freshness to oral-, hair- or skin-care products, and other products for human consumption. The presumed presence of alkylamide may potentially enable the seed oil to act as a repelling agent against rodents and birds.

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