

Supercritical Carbon Dioxide Extraction of Cedarwood Oil: a Study of Extraction Parameters and Oil Characteristics†

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The extraction of cedarwood oil (CWO) using supercritical carbon dioxide (SC-CO₂) has been investigated with respect to the effects of extraction temperature and pressure, length of extraction, and age of cedarwood chips. Steam distilled and SC-CO₂ derived CWOs were compared by gas chromatography and sensory evaluation. The extraction of CWO increased with extraction temperature, except at the lowest pressure utilised. The highest percentage contribution of thujopsene to the SC-CO₂ derived CWO occurred with the combination of 1500 psi and 70°C or 100°C. Essentially all of the CWO was extracted from the wood matrix in the first 10 min, however, complete extraction of water required ca. 25 min. The amount of CWO extracted decreased with increasing age of the cedarwood chips. This decrease was greatest for the more volatile hydrocarbon components, thujopsene and cedrene. The mean weight percentage yields of CWO for steam distillation and SC-CO₂ extraction were 1.3 and 4.4%, respectively. An experienced analytical sensory panel selected the SC-CO₂ derived CWO as being more similar to the original cedarwood chips than the steam distilled CWO. Volatile collections performed on SC-CO₂ extracted, steam distilled and unextracted cedarwood chips indicated that the SC-CO₂ extracted chips released almost no volatiles, whereas the unextracted chips released a higher amount of volatiles. The steam distilled cedarwood chips released an intermediate level of volatiles. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Supercritical fluid extraction; carbon dioxide; cedarwood oil; cedrol; thujopsene; *Juniperus virginiana*.

INTRODUCTION

Cedarwood oil (CWO; CAS no. 8000-27-9) is obtained by steam distillation in the USA from *Juniperus virginiana* L., (Cupressaceae, Virginia CWO) and *J. ashei* Buch. (Texas CWO; Adams, 1987). However, steam distillation has several limitations (Tim Cannon, Cross Timbers Forestry, personal communication) in that it removes only about 50% of the oil from the wood, and the high temperature of the steam and the presence of oxygen causes decomposition of some of the oil components, producing oil having an off odour.

The potential benefits of supercritical fluid extraction (SFE) over steam distillation for obtaining essential oils have been described (Moyler *et al.*, 1992). The low viscosity and high diffusivity of supercritical carbon dioxide (SC-CO₂) can result in higher extraction efficiencies, and SC-CO₂ is easily removed from the extract when the mixture is depressurised, leaving an extract uncontaminated by any solvent residue. In

addition, extractions performed using SC-CO₂ can avoid the elevated temperatures used in steam distillations, and SC-CO₂ protects the substrate from oxygen, resulting in fewer decomposition products and a higher quality oil (Pickett *et al.*, 1975).

Although Hawthorne *et al.* (1988) coupled SFE with GC and MS to extract and identify cedrene and cedrol in CWO, the use of SFE for CWO extraction has otherwise not been investigated. The purpose of this study was to investigate the use of SC-CO₂ for the extraction of CWO, including an investigation of pertinent extraction parameters, as well as an analysis of the characteristics of the extracted oils, including a comparison with steam distilled CWO.

EXPERIMENTAL

Source of cedarwood chips. Cedarwood chips used in this study were prepared from a kiln-dried cedar board purchased from a local lumber mill. A power wood planer was used to produce the chips which were immediately packaged in zipper-lock plastic bags, then wrapped in aluminium foil and stored at -70°C until required for extraction experiments.

Removal of co-extracted water from CWO extracts. In order to remove water which is invariably co-extracted with the CWO, ca. 0.5 g of anhydrous sodium sulphate,

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† Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

Table 1. SFE of cedarwood: effects of temperature and pressure on yield and oil composition

Temperature (°C)	Pressure (psi)	CO ₂ Density (g/mL)	Mean weight percentage collected		Oil composition (%)	
			Total	CWO	Thujopsene	Cedrol
40	1500	0.66	5.1	3.0	16.2	42.3
	2750	0.84	6.3	3.9	16.6	42.2
	4000	0.91	6.4	3.7	16.0	41.2
	6000	0.97	6.3	3.6	16.1	41.3
	8000	1.02	6.4	3.6	15.6	40.0
	10000	1.05	6.2	3.5	16.2	41.4
70	1500	0.27	4.5	1.0	19.7	36.6
	2750	0.63	8.4	4.1	15.1	41.4
	4000	0.77	8.3	4.3	16.1	40.6
	6000	0.87	8.9	4.3	16.1	40.0
	8000	0.93	8.8	4.3	16.0	39.9
	10000	0.97	7.5	4.0	15.9	40.5
100	1500	0.20	6.9	1.0	20.4	35.3
	2750	0.44	10.3	4.2	14.8	41.8
	4000	0.63	10.3	4.4	16.0	39.6
	6000	0.77	10.4	4.6	16.0	40.3
	8000	0.85	10.2	4.4	15.5	39.8
	10000	0.89	9.8	4.3	15.7	40.7

1 mL of water saturated with sodium sulphate and 2 mL diethyl ether were added to the SFE collection vial and mixed thoroughly. The ether layer containing the CWO was removed and transferred to a weighed vial. The collection vial was re-extracted twice with 2 mL diethyl ether and the combined ether extracts were concentrated under a gentle stream of nitrogen until there was no detectable weight loss with further drying. The weight of the CWO was then determined and the percentage CWO extracted was calculated based on the original sample weight.

Chemical analyses. Solutions of CWO in hexane (ca. 200 ng/ μ L) were analysed by GC to determine the percentage contribution of individual components (Coleman and Lawrence, 1997). CWO extracts were analysed by 0.5 min split-delay splitless injection onto a Hewlett-Packard (HP; Santa Clarita, CA, USA) 5890 Series II GC equipped with a flame ionisation detector and an SP-2380 column (60 m \times 0.25 mm i.d.; 0.20 μ m film thickness; Supelco, Bellefonte, PA, USA) using helium as the carrier gas at a linear flow velocity of 18 cm/s. The temperature program was 60°C for 1 min, rising by 5°C/min to 250°C. The injector and detector temperatures were 235 and 250°C, respectively. Injections were made using an HP model 7683 auto-injector and sample volumes were 1 μ L. The chromatographic data were acquired using an HP Vectra VL2 computer and ChemStation software.

Electron impact MS were obtained using an HP model 5971 mass selective detector using an ionisation potential of 70 eV. Sample introduction was through an HP 5890 GC with a Supelco SP-2340 column operating under the conditions described above.

Effect of SC-CO₂ pressure and temperature on CWO extraction. SFE was conducted with an ISCO Model 3560 SFE (ISCO Corporation, Lincoln, NE, USA). The sample (ca. 2.4 g) was weighed to the nearest 0.0001 g and added to the extraction cell between glass-fibre filter disks (18 mm dia.) on the top and bottom of the cell. Eighteen extraction conditions were evaluated, consist-

ing of all combinations of three extraction temperatures (40, 70 and 100°C) and six extraction pressures (1500, 2750, 4000, 6000, 8000 and 100000 psi); (Table 1). The extraction sequence was 1 min static extraction followed by a 25 min dynamic extraction using SFE/SFC-grade CO₂ (Air Products and Chemicals, Allentown, PA, USA) at 2 mL/min. The variable restrictor was heated to 80°C and extracts were collected in 20 mL pre-cooled (0°C) and pressurised vials. Each extraction condition was replicated twice.

Effect of length of extraction. Twelve extraction times, varying from 5 to 60 min in 5 min increments, were compared: the extraction temperature was 100°C, the pressure was 4000 psi, and the restrictor was 100°C. The cedarwood chips were weighed after extraction by SC-CO₂ to determine the weight loss through extraction. The cells were allowed to cool to room temperature for ca. 1 h to allow the dissipation of CO₂ from the chips. Each extraction time was replicated twice.

Effect of age of cedarwood chips. This experiment was designed to test the hypothesis that the more volatile components of CWO were lost between chipping and extraction. Approximately 20 g of freshly prepared cedarwood chips were separated from a large batch and ca. 5 g were immediately isolated and sealed in zipper-lock plastic bags, enclosed in aluminium foil and stored at -70°C. The remaining chips were placed in a 190 mm dia. \times 100 mm deep Pyrex[®] dish and placed on a bench in the laboratory at ambient temperature to age. Samples (5 g) were removed from the Pyrex dish, packaged and stored at -70°C after 1, 2 and 3 weeks on the lab bench. Sub-samples (2 g) from each of the four treatments (i.e. 0-, 1-, 2- and 3-week-old chips) were extracted (100°C; 4000 psi; 1 min static followed by 25 min dynamic extraction) and analysed using the conditions described above. Two replicate samples of each age of chip were extracted and analysed.

Comparison to steam distilled CWO. The cedarwood chips were also extracted by steam distillation to compare

the two extraction methods for efficiency as well as the chemical characteristics of the resultant oils. For this comparison, a somewhat larger scale SFE was performed using a Spe-ed SFE unit (Applied Separations, Allentown, PA, USA). Cedarwood chips (ca. 12 g) were weighed and placed in a 50 mL extraction cell and extracted with SC-CO₂ using a 10 min static hold followed by a 60 min dynamic extraction at 1 L/min (expanded gas) flow. The extraction and restrictor temperatures were both 100°C and the extraction pressure was 4000 psi. For this experiment, pentane was used to extract/separate the CWO from the water in the collection vial.

Steam distillation was performed using an improved version of a modified Nielsen-Kryger steam distillation apparatus (Ace Glass, Vineland, NJ, USA) employing the method described by Veith and Kiwus (1977). Here, cedarwood chips (ca. 25 g) were placed in 500 mL round-bottomed flask with 200 mL deionised water; pentane (10 mL) was added to the condenser to trap distilled CWO and the condenser was cooled to 10°C. The flask was heated to provide gentle boiling and the distillation was carried out for ca. 2 h: the condenser was then rinsed with deionised water and the pentane containing the CWO removed. The condenser was rinsed twice with 5 mL pentane and the combined pentane extracts were concentrated under a gentle stream of nitrogen. There were three replications of each extraction method.

The SC-CO₂ derived and steam distilled CWOs were analysed by GC. In addition, the odours of the two CWOs were compared for similarity to the original cedarwood chips. A 10 member experienced analytical sensory panel evaluated the odours of the CWO samples in comparison with that of the original cedarwood chips. In a paired comparison test, panellists were given 1 g of cedar chips in a closed 250 mL wide-mouth jar identified as the cedar control. They were then given the extracts (0.2 g on filter paper in closed 250 mL wide-mouth jars) in randomised order and asked to select the one that was closest to the odour of the control. The complete test was repeated twice. The number selecting the SC-CO₂ extract was compared to the null hypothesis of no preference for either extract using a *t*-test.

Collection of volatiles from chips. The cedarwood chips extracted with SC-CO₂ had almost no detectable odour, while the steam distilled cedarwood chips retained much of the original cedar odour. Therefore, volatile collections were performed on SC-CO₂ extracted chips, steam distilled chips, as well as unextracted cedarwood chips to quantify the effectiveness of SFE and steam distillation for removal of CWO from cedarwood chips. Using the volatile collection system described by Eller *et al.* (1994), CWO volatiles were trapped on Super Q polymeric adsorbent (Alltech Associates, Deerfield, IL, USA). Approximately 0.5 g of unextracted and SC-CO₂ extracted chips were placed in the sample tubes, while ca. 0.7 g of the wet steam distilled chips were used (equivalent to ca. 0.5 g dry chips). The cedarwood chips were placed in the centre of the sample tube and collections were made on four consecutive days. Trapped volatiles were extracted from the Super Q adsorbent with 500 µL of hexane. An aliquot (10 µL) of a 25.0 µg/µL solution of docosane (C₂₂) was added to the extract as an internal standard to quantify the amount of collected volatiles. The extracts were subsequently analysed by

GC, and the weights of the individual components, as well as the total collected volatiles, were calculated on the basis of their peak integrations relative to the 250 µg docosane internal standard added. Release rates were normalised to µg/h g dry weight of sample.

Chemical standards. A commercial sample of CWO was purchased from Aldrich (Milwaukee, WI, USA): (–)- α -cedrene (CAS no. 469-61-4), (+)- β -cedrene (CAS no. 546-28-1), (–)-thujopsene (CAS no. 470-40-6), (+)-cuparene (CAS no. 16982-00-6), and (+)-cedrol (CAS no. 77-53-2) were purchased from Fluka (Milwaukee, WI, USA).

RESULTS AND DISCUSSION

Effect of pressure and temperature

The total amount of material collected increased with higher extraction temperatures and, to a lesser degree, with higher extraction pressures (Table 1). With the exception of the combination of 1500 psi and 70 or 100°C, the amount of CWO also increased with temperature: with the combination specified, however, very low yields of CWO were obtained, presumably as a result of the very low density of CO₂ under these conditions (0.27 and 0.20 g/mL, respectively). Reverchon *et al.* (1995a) noted that, because sesquiterpene hydrocarbons and oxygenated sesquiterpenes are readily soluble in SC-CO₂, high densities are not required for their extraction. Our results support this conclusion in that some of the highest yields were not obtained at the highest densities. For example, the combination of 100°C and 2750 psi (density of 0.44 g/mL) gave a relatively high CWO yield of 4.2%.

The amount of CWO reported to be present in *J. virginiana* varies widely from 0.97–1.41% (Guenther, 1948), 2% (Hayward and Seymour, 1948), 3.2% (Adams, 1987) to 3.5% (Runeberg, 1960). In our extraction temperature/pressure experiments, the highest yield observed was 4.6% (higher than any of the previous reports), suggesting that SC-CO₂ is a very effective method for the extraction of CWO from cedarwood chips.

Although CWO is a mixture of over 30 compounds, six components account for ca. 80% of CWO (Heide *et al.*, 1988; Adams, 1991). These major components and their percentage contributions to CWO are: α -cedrene (27.2%), β -cedrene (7.7%), thujopsene (27.6%), cuparene (6.3%), cedrol (15.8%), and widdrol (1.0%) (Adams, 1987). All of these components were found in SC-CO₂ extracts and, with the exception of widdrol (CAS no. 6892-80-4), were identified by matching the GC retention times of standards as well as by GC-MS. Widdrol was identified by GC-MS library match only. The component ratios for the SC-CO₂ extracts (Table 1) differed slightly from previous reports, but this may be due to the fact that the cedarwood chips used in the present work came from a kiln-dried board from which some of the more volatile compounds had been lost.

Although it was hoped the various temperature/pressure combinations would yield CWOs with very different component ratios allowing for a simple enrichment of the most valuable components, only moderate

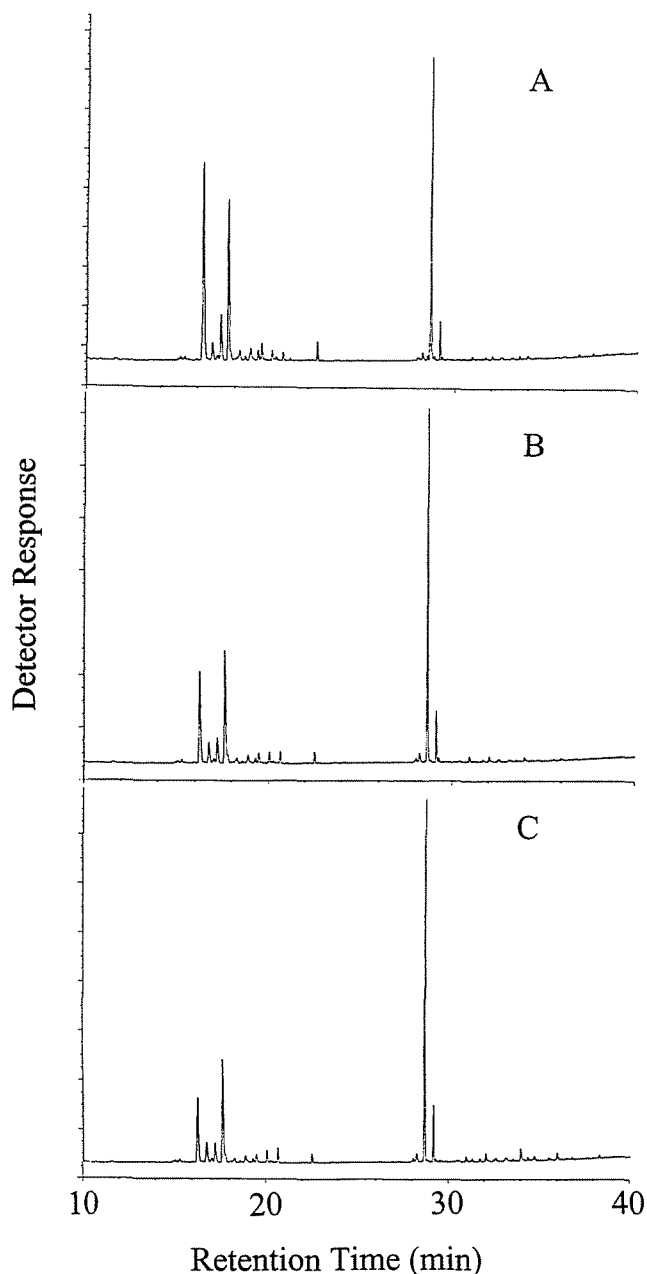


Figure 1. Characteristic gas chromatograms of (A) commercial steam distilled, (B) laboratory steam distilled, and (C) SC-CO₂ extracted cedarwood oils. (For chromatographic protocol see the Experimental section.)

differences were observed. However, it may be possible to deterpenate (i.e. remove non-odorous compounds from) CWO using SFE in combination with silica, as has been described for sweet orange and lemon essential oils (Dugo *et al.*, 1995).

Effect of length of extraction

The length of extraction had little effect on CWO composition; the content of thujopsene varied from a minimum of 15.2% to a maximum of 16.2%, whilst that of cedrol varied only from 40.9% to 42.2%. The total percentage extracted increased from ca. 6.8% at 5 min to ca. 11% at 25 min where the amount extracted levelled off. Similarly, the total percentage collected increased from ca. 5.6% at 5 min to ca. 10% at 25 min where the amount collected levelled off. The difference in the

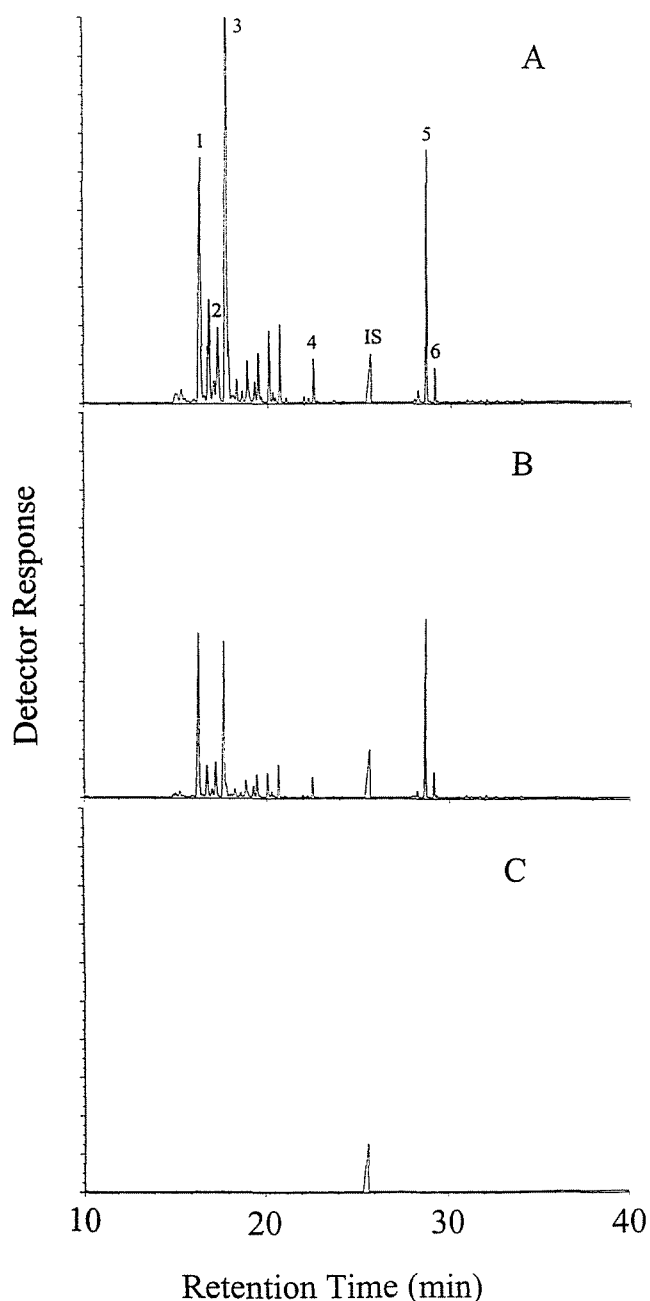


Figure 2. Gas chromatograms of volatile collections of (A) unextracted, (B) steam distilled, and (C) SC-CO₂-extracted cedarwood chips. Key to peak identity: 1, α -cedrene; 2, β -cedrene; 3, thujopsene; 4, cuparene; 5, cedrol; 6, widdrol; IS, internal standard (C₂₂; 250 μ g). (For chromatographic protocol see the Experimental section.)

amount extracted and that collected represents material lost during the collection. Subsequent experimentation indicated that cooling the restrictor temperature from the 100°C used in these experiments to 80°C significantly increased the amount of total material collected, as well as increasing slightly the amount of CWO from 4.0 to 4.2%. Interestingly, the amount of CWO collected did not vary greatly with length of extraction; there was a slight increase in CWO yield from ca. 3.7% at 5 min to ca. 4% at 10 min, where CWO yield levelled off. Apparently, the complete extraction of water requires 25 min. Although the co-extraction of water with CWO by SC-CO₂ precludes the direct gravimetric determination of CWO yield, the effective removal of water from cedarwood by

Table 2. Gas chromatographic analyses of daily volatile collections of cedarwood chips: SC-CO₂ extracted, steam distilled, and unextracted chips

Chip sample analysed	Collection day	Release Rate ($\mu\text{g/h g}$)	Composition of volatiles (%)	
			Thujopsene	Cedrol
SC-CO ₂ extracted	1	7.5	39.4	4.5
	2	2.0	21.5	15.2
	3	1.4	13.7	24.3
	4	0.4	nd ^a	52.5
Steam distilled	1	124.9	25.3	4.7
	2	84.0	20.7	15.5
	3	22.1	4.8	54.0
	4	11.6	2.7	59.0
Unextracted	1	530.9	39.0	4.1
	2	504.0	33.2	9.2
	3	186.9	17.6	26.7
	4	74.6	4.8	49.4

^a nd = none detected.

SC-CO₂ suggests that SC-CO₂ may have some utility for water content determination. Water content is very important to wood processors; however, simple oven drying of cedarwood lacks a means for compensating for the accompanying loss of volatile oils (Payne *et al.*, 1998; Smith, 1992). Currently, water content in eastern red cedar is determined by using a large amount of toluene (i.e. 200 mL) for the co-distillation of water with volatile oils (American Society for Testing Materials, 1992). SC-CO₂ extraction could be an alternative to the toluene distillation method for determining both CWO and water content of cedarwood, thereby avoiding the use of hazardous toluene.

Effect of age of cedarwood chips

Unexpectedly, the weight of the total collected material increased with the age of the chips. The total percentages collected for chips aged 0, 1, 2 or 3 weeks were 5.9%, 6.0%, 6.5% and 7.5%, respectively. However, the yield of dry CWO decreased with increased chip age (i.e. 2.6%, 2.0%, 1.9% and 1.7%, respectively). The increase in total collected material with chip age is a result of the cedarwood chips water being adsorbed from the air and subsequently being co-extracted with the CWO. The relative percentage of thujopsene decreased (i.e. 16.7%, 10.0%, 3.6%, 1.4%, respectively) as chip age increased, while that of cedrol increased (i.e. 38.6%, 55.8%, 70.0% and 74.9%, respectively). This indicates that the more volatile hydrocarbons, such as thujopsene and cedrene, are lost more quickly from the chips than the alcohol, cedrol. This suggests that chips should be extracted as soon as possible after chipping to prevent the loss of the volatile components.

Comparison to steam distilled CWO

The SC-CO₂ extracts were slightly darker in colour than those obtained from the steam distillation probably because the SC-CO₂ extracted some higher molecular weight compounds not co-distilled with water (Moyler, 1984). The mean percentage yields of CWO for steam distillation and SC-CO₂ extraction were 1.3% and 4.4%, respectively. Although our steam distillation apparatus may not adequately represent the efficiency of commercial

distillation methods, the 4.4% SC-CO₂ CWO yield was higher than any yields previously reported for steam distillation methods. Therefore, we conclude that SC-CO₂ is at least as efficient, if not more so, than steam distillation for extraction of CWO from cedarwood chips.

In our test of the sensory attributes of the steam distilled and SC-CO₂ cedarwood oils, the panel members selected the SC-CO₂ CWO as being more similar in odour to the original cedarwood chips in 16 of 18 judgements, which is statistically significant ($p < 0.05$). SC-CO₂ extracts are often characterised as typical of or having greater resemblance to the raw material when compared with steam distilled material (Gopalakrishnan *et al.*, 1990; Sinha *et al.*, 1992; Reverchon and Senatore, 1992; Reverchon *et al.*, 1995b). This finding is further confirmation that SFE avoids the degradation of labile compounds and the hydrosolubilisation of some compounds (Reverchon, 1997).

The gas chromatograms of the two laboratory produced CWOs (SC-CO₂ and steam distilled) are virtually identical and both are very similar to the commercial steam distilled CWO (Fig. 1). Heide *et al.* (1988) identified 37 compounds in CWO and reported that the compounds responsible for the characteristic cedar aroma constituted only ca. 0.2% of the oil. The most abundant components of CWO (i.e. cedrene, thujopsene and cedrol) have only very weak odours, without the specific cedar aroma (Heide *et al.*, 1988). Therefore, it is unlikely that there would be any obvious differences between the chromatograms of SFE and steam distilled CWOs, even though the two types of CWO differ significantly in their characteristic odours.

Collection of volatiles from chips

The GC chromatograms of the volatile collections after day 1 for the unextracted, steam distilled and SC-CO₂ extracted cedarwood chips are shown in Fig. 2. The chromatograms indicate that the SC-CO₂ extracted chips release almost no volatiles, while the unextracted chips release high amounts of CWO volatiles. The steam distilled cedarwood chips are intermediate in their release of CWO volatiles, presumably due to the incomplete extraction of CWO by steam distillation. The release rate data for the three different chips are shown in Table 2.

The unextracted chips were nearly two orders of magnitude higher in their release of total volatiles than the SC-CO₂ extracted chips and ca. four times higher than the steam distilled chips. These results clearly demonstrate that SC-CO₂ is very effective at extracting CWO from cedarwood chips. For all three types of chips, the release rates of total volatiles decreased as the collection experiment proceeded, as did the relative amount of thujopsene, while the relative amount of cedrol increased. It is interesting to note that the SC-CO₂ extract from chips aged 0 weeks contained 16.7% thujopsene, while the volatiles collected on day 1 from unextracted chips contained 39.0% thujopsene. Cedrol, on the other hand, constituted 38.6% of the SC-CO₂ extract from chips aged 0 weeks, but only 4.1% of the volatiles collected on day 1. The results of both experiments

indicate that the hydrocarbon, thujopsene, is more volatile and lost more quickly than the alcohol, cedrol.

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