

## Short Note

Using Supercritical Carbon Dioxide for Extraction of Western Juniper and Alaska-Cedar<sup>1)</sup>By M. Acda<sup>1</sup>, J.J. Morrell<sup>1</sup>, A. Silva<sup>1</sup>, K.L. Levien<sup>2</sup> and J. Karchesy<sup>1</sup><sup>1</sup> Department of Forest Products, Oregon State University, Corvallis, U.S.A.<sup>2</sup> Department of Chemical Engineering, Oregon State University, Corvallis, U.S.A.**Keywords:** Alaska-cedar · *Chamaecyparis nootkatensis* · Extraction · *Juniperus communis* · Supercritical carbon dioxide · Western juniper**Introduction**

The heartwood of many species contains valuable extractives, which have a variety of industrial uses. Some of these materials are by-products of the pulping process, but many others are collected by steam distillation or solvent extraction of ground wood. These processes can be energy-intensive, or, in the case of solvent extraction, pose difficulties because of flammability and release of volatile organic compounds.

The identification of alternative extraction methods for various wood species could reduce energy inputs and increase yields. One alternative method is supercritical fluid extraction. A supercritical fluid (SCF) is a fluid at or above its critical temperature and pressure. SCFs have properties that fall between those of normal liquids and gases. Like gases, SCFs have excellent diffusivities and readily move through semi-porous media such as wood. SCFs also have solvating properties that can approach those of liquid solvents. As a result, SCFs have been evaluated for extracting a wide array of flavors and other higher value materials from semi-porous media (Hubert and Vitzthum 1978; Williams 1981; Hoyer 1985; Larsen and King 1986; Moyler 1993). Although there are many potential SCFs, carbon dioxide is most often chosen because of its low cost, lack of flammability, low toxicity, and readily attainable critical temperature and pressure. Solubility in supercritical carbon dioxide can be further enhanced by the addition of small amounts of cosolvents such as methanol or ethanol (Hoyer 1985).

Although supercritical fluids have been used to remove a variety of materials from wood, there are few reports of its use for recovering extractives (Ritter and Campbell 1991; Larsen *et al.* 1992). In this report, we describe extraction trials with western juniper (*Juniperus communis*) and Alaska-cedar (*Chamaecyparis nootkatensis*). Western juniper was selected because it has invaded a significant acreage of rangeland in western North America and it has

little or no commercial timber value. Other junipers are extracted for their oils, so we proposed that a selective extraction process might make western juniper oil more attractive to industry (Adams 1987 a, b; Clark *et al.* 1990). Alaska-cedar was chosen because trials with conventional steam-distilled extractives suggest that the heartwood of this species contains a variety of possible anti-cancer compounds, as well as natural insecticides (Karchesy, unpublished).

**Experimental**

Freshly sawn western juniper and Alaska-cedar heartwood were ground to pass through a 20-mesh screen. The samples were stored at 5°C until used.

An approximately 10-g sample of one species was placed in a seamless steel vessel, 15 mm in diameter x 54 mm long, which was then placed in an ISCO 2000 Extractor (Lincoln, NE). Extractions were performed at 40°C or 60°C, for either 10 or 30 min. The ground wood was extracted with CO<sub>2</sub>, with or without a methanol cosolvent. The product fluid was sent through a solvent trap containing hexane, and its pressure was reduced to atmospheric level. The solvent trap solution was retained for later analysis.

Extraction efficiency was evaluated with supercritical CO<sub>2</sub> at 13.8 MPa at a flow rate of 10 ml min<sup>-1</sup> when the vessel was maintained at 40°C or 60°C for 10 or 30 min. In addition, the effect of 5.0% methanol as a cosolvent during extraction at 60°C for 30 min was evaluated. Each time/temperature combination was replicated three times. Following extraction, the wood samples were reequilibrated at atmospheric temperature, and weighed to determine changes as a result of treatment. The data were subjected to an analysis of variance and the means were evaluated by Tukey's HSD at  $\alpha = 0.05$ .

The hexane extracts were analyzed using a Shimadzu (Kyoto, Japan) GC17A gas chromatograph (GC) with a flame ionization detector. A 0.1 ml aliquot of oil extract was dissolved in 4.9 ml hexane; then 1 ml of this sample was injected into the GC using a solvent flush method (1 ml hexane followed by 2 ml air; then 1 ml sample). Reference steam-distilled juniper and Alaska-cedar oils were prepared by adding 100 ml of oil to 9.9 ml hexane in a volumetric flask; then 250 ml of this solution was removed and added to 250 ml hexane. One microliter of this resulting mixture was injected for analysis.

Conditions on the GC were: split injection system (1:50 ratio); carrier gas, He, flow rate 30 ml min<sup>-1</sup>; hydrogen flow, 50 ml min<sup>-1</sup>. A J & W Scientific (Folsom, CA) DBTM-5 [(5% phenyl)methyl-

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polysiloxane] column, 25 m x 0.25  $\mu$ m i.d. (0.25  $\mu$ m film thickness of liquid phase), was used.

Temperature programming was held at 100°C for 1 min., then increased to 150°C at 5°C min<sup>-1</sup>, to 220°C at 3°C min<sup>-1</sup>, and finally to 240°C at 5°C min<sup>-1</sup>. Holding time at 240°C was 2 min. Total analysis time for each injection was 40.3 min. The injector and detector temperatures were 250°C.

## Results and Discussion

### Extraction efficiency

As is shown in Table 1, whether for 10 or 30 min periods, the efficiency of SCF extraction from western juniper was significantly greater at 60°C than at 40°C. Alaska-cedar showed similar results. At a given temperature, the longer extraction time also increased efficiency; this difference is significant for 60°C juniper extractions. Extraction levels were invariably higher for Alaska-cedar than for juniper at a given time/temperature. These species differences are consistent with those of conventional steam distillation, which typically produces yields between 1.5% and 4.0% (wt/wt) (Adams 1987a; Karchesy, unpublished). The increases in extraction efficiency with higher temperatures are similar to findings by Hoyer (1985) and Williams (1981), which showed that solubilities of many compounds in supercritical carbon dioxide increase at higher temperatures. The inconsistent effects of extraction time on weight loss may imply that most of the extraction occurs rapidly. As a result, prolonged extraction does not markedly improve efficiency.

The addition of a small amount of methanol as a cosolvent produced various effects on extraction. Extraction levels from Alaska-cedar were only about half the levels for the comparable non-cosolvent conditions, whereas juniper samples actually gained weight. In both instances, residual methanol appears to have affected the method. Methanol is generally a highly effective cosolvent that increases the solubility of a variety of compounds. Residual methanol would mask this expected enhanced extraction efficiency. Although oven-drying at a higher temperature would drive off this cosolvent, we were

**Table 1.** Average weight loss of western juniper and Alaska-cedar after extraction with supercritical CO<sub>2</sub> at 13.8 Mpa and 10 ml min<sup>-1</sup> flow rate

Treatment Time/temperature	Wood weight loss (%) <sup>a</sup>	
	Western juniper	Alaska-cedar
10 min		
40°C	3.44 (0.24) B	6.99 (2.34) A
60°C	5.82 (0.13) C	10.34 (0.32) BC
30 min		
40°C	4.67 (0.16) BC	9.09 (0.35) AB
60°C	7.47 (0.47) D	13.27 (0.51) C
60°C with 5% methanol	-1.23 (1.14) A <sup>b</sup>	7.08 (1.10) AB

<sup>a</sup> Each value is the average of three replicates. Values for a given wood species followed by the same letter(s) do not differ significantly (Tukey's HSD,  $\alpha=0.05$ ). Values in parentheses are standard deviations. <sup>b</sup> These samples gained weight during treatment.

concerned that this process would also drive off volatile residual wood extractives, thereby artificially inflating the extraction efficiency.

### Extract characterization

In addition to the relative quantities of extractives removed from the two wood species, the comparable components present in each extract were of interest. An SCF offers a unique prospect for selective extraction by varying combinations of treatment time and temperature to alter solubility. Careful selection of such conditions could make possible simultaneous extraction and separation of various wood components.

GC analysis of western juniper revealed that varying extraction conditions resulted in an array of compounds in the extracts (Table 2). In general, extracts from the shorter extraction times tended to have relatively few of the compounds that had been found in the steam-distilled extracts. For example, only 3 of the 10 peaks present in the steam distillate were found in the samples extracted for 10 min at 40°C. Extraction at 60°C for this same period produced an extract devoid of peaks corresponding to the steam distillate. Extraction for 30 min at either temperature produced chromatograms that resembled those of the steam distillate. An additional peak at 28.35 min present in many chromatograms of only the SCF extracts suggests that more substantial extraction of higher molecular weight compounds was occurring. Extraction with methanol as a cosolvent produced the same peaks found in the steam distillate, indicating that solubility of some components in pure carbon dioxide might be lower.

The significance of missing compounds in the various extracts depends on their ultimate usefulness. The first 5 peaks in these extracts were identified as  $\alpha$ -cedrene,  $\beta$ -cedrene, thujopsene, (+)-cedrol, and cedren-9-ol, respectively, by comparison with prepared standards. These elements are of interest as many have antimicrobial and acaricidal properties (Karchesy, unpublished). Although these components are absent from most of the pure supercritical carbon dioxide extracts, the addition of methanol results in recovery of all five fractions. Therefore, methanol or another cosolvent appears to be essential for this process.

Extracts of Alaska-cedar also varied somewhat between steam-distilled and supercritical fluid treatments (Table 3). Again, prolonged extraction increased the number of peaks present at 40°C, but did not improve recovery at 60°C. Whereas 7 out of 10 peaks present in the steam-distilled sample also were detected in the sample extracted with SCF for 30 minutes at 40°C, only 3 of 10 peaks were detected in extracts at 60°C. The reasons for this decrease in recovery are unclear. The addition of methanol as a cosolvent again resulted in extracts with peaks similar to those found with steam distillates. Until these fractions can be identified and their usefulness evaluated, it cannot be determined whether the absence of certain peaks in some extraction methods is critical.

**Table 2.** Frequency of selected gas chromatograph (GC) peaks of western juniper extracted with supercritical CO<sub>2</sub> or steam

Treatment <sup>a</sup>	GC retention times (min)										
	14.2	14.4	14.6	20.0	21.4	21.8	22.8	22.9	23.5	26.2	28.35
10 min											
40°C	-	-	-	+++ <sup>b</sup>	-	-	-	+	+	-	+
60°C	-	-	-	-	-	-	-	-	-	-	-
30 min											
40°C	-	-	-	+++	+	+	-	+++	+++	+++	+++
60°C	-	-	-	-	+	+	+	+	+	+	++
60°C with 5% methanol	+	+	+++	+++	++	++	++	+++	+++	++	+++
360 min, steam distillate	+	+	+	+	+	+	+	+	+	+	++

<sup>a</sup> n = 3 for all treatments except steam extract, where n = 1. <sup>b</sup> represents number of samples in which a peak is detected; + = detected once, ++ = detected twice, +++ = detected three times.

**Table 3.** Frequency of selected gas chromatograph (GC) peaks of Alaska-cedar extracted with supercritical CO<sub>2</sub> or steam

Treatment <sup>a</sup>	GC retention times (min)									
	8.1	8.3	10.2	10.8	16.4	17.1	23.0	23.5	25.2	26.3
10 min										
40°C	-	-	+ <sup>b</sup>	++	-	-	-	+	+	+
60°C	-	-	-	++	-	-	-	++	+	++
30 min										
40°C	-	-	+	+++	-	+	+	+++	+++	+++
60°C	-	-	-	+	-	-	-	+	-	+
60°C with 5% methanol	+	+	+++	+++	++	++	+++	+++	+++	+++
360 min, steam distillate	+	+	+	+	+	+	+	+	+	+

<sup>a</sup> n = 3 for all treatments except steam extract, where n = 1. <sup>b</sup> represents number of samples in which a peak is detected; + = detected once, ++ = detected twice, +++ = detected three times.

## Conclusions

Supercritical carbon dioxide was capable of removing substantial amounts of extractives from both western juniper and Alaska-cedar. However, the variety of compounds in these extracts differed from those found with conventional steam extracts. The addition of methanol as a cosolvent resulted in extracted components which were more similar to those found in the steam-distilled extract. The recovery of residual methanol from the chips would likely be an important aspect of this process.

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