EXTRACTION OF BOLDO (*Peumus boldus* M.) LEAVES WITH HOT PRESSURIZED WATER AND SUPERCRITICAL CO_2

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Boldo is a native plant from Chile that has been used in traditional medicine to treat digestive and/or hepatobiliary disorders. An attempt was made in this work to extract bioactive compounds from crushed boldo leaves using hot pressurized water (HPW) and supercritical CO_2 (SC-CO_2). Total yield after 3 h batch HPW extraction increased from 36.9% at 100 °C to 53.2% at 125 °C and then decreased as temperature was further increased to 175 °C. Boldine (polar alkaloid) yield decreased from 26.8 ppm at 100 °C to 0.7 ppm at 125 °C and was negligible at =150 °C, and nonpolar essential oil yield increased to a maximum at 110 °C and was negligible at =150 °C. Essential oils were also obtained with low-pressure SC-CO_2. Total yield after 3 h extraction (0.6-3.5%) increased with process pressure (60-150 bar) and decreased with treatment temperature (30-60 °C). Extraction of boldine, on the other hand, was performed with CO_2-ethanol mixtures at 50 °C and high pressures. Yield increased with treatment pressure (300-450 bar) and cosolvent concentration (2-10% w/w) and ranged 0.14-1.95 ppm for boldine and 1.8-4.8% for total solids following 1.5 h extraction. Boldine recovery was solubility-controlled, reaching 7.4 ppm after 7 h when using ethanol-doped (5% w/w) SC-CO_2 at 450 bar and 50 °C. Boldine solubility and yield decreased when using neat CO_2 at 600 bar and 50 °C.

INTRODUCTION

Boldo (*Peumus boldus* M.) is a Chilean endemic plant with very interesting medicinal properties, which are widely recognized in several official pharmacopoeias [1,2]. These have been related to its essential oils and aporphine alkaloids. Among the later, boldine is the main alkaloid in boldo leaves and barks. Solvent extraction may be applied to obtain plant derivatives with a high concentration of selected active principles. However, there are increasing concerns about the presence of toxic solvent residues in vegetable extracts obtained with conventional solvents, with the only exception of water, ethanol, and their mixtures. Hot pressurized water (HPW) refers to water above its normal boiling point (100 °C) but below its critical temperature (374 °C), which is kept in the liquid state by applying pressure [3,4]. The polarity of water decreases with temperature due to a breaking up of the hydrogen-bonded structure. This results in an increase in the solubility of nonpolar solutes such as essential oils as the temperature of water is increased, and a parallel decrease in the solubility of polar solutes such as alkaloids. However, high water temperatures must be used with caution, since thermal degradation of some compounds occurs at temperatures above 150 °C [4]. Supercritical fluids (SCFs) have commanded a lot of attention lately due to their special solvent properties [5]. Carbon dioxide (CO_2), in particular, is the most popular SCF in food applications due to its inertness, nontoxicity, nonflammability, and low cost [5]. Furthermore, it is used at near-critical temperatures (T_c = 31 °C), so that damage of thermally labile components is avoided. Sargenti and Lancas [6] have used SC-CO_2 for the extraction of boldo essential oils at an analytical scale. A limitation of CO_2 is that only nonpolar solutes such as essential and fatty oils are extracted at moderate pressures. Cosolvents such as ethanol, however, can be used for the extraction of polar and/or high molecular bioactive molecules [7]. The objective of this work was to extract essential oils and boldine from boldo leaves using HPW and ethanol-doped CO_2.

MATERIALS AND METHODS

Substrate. Chilean boldo bushes were sampled in March, 2002. Leaves were dried to *ca.* 4% moisture and stored in sealed polyethylene bags in a cold and dark room until analysis. Samples were crushed (d_p = 0.69 mm) prior to extraction.

Extraction. Boldo samples (10 g) were treated with 100 cm^3 hot water in a stirred 200 cm^3 high-pressure autoclave (Berghof GmbH Labortechnik, Germany). The autoclave was heated to the desired temperature (100-175 °C) for 3 h extraction, and then cooled down in a water bath to below 80 °C. Extract samples were kept in a refrigerator (4 °C) until analysis.

Boldo samples (3 g) were treated with CO_2 in 10 cm^3-vessels in a Spe-ed SFE unit (Applied Separations, Allentown, PA). The effect of extraction pressure (60-150 bar), temperature (30-60 °C), and solvent ratio (0.91-2.72 g CO_2/g/min) on total yield and essential oil yield was evaluated in 3-h low-pressure experiments where one variable was changed at a time. Subsequently, the effect of extraction pressure (300 and 450 bar) and cosolvent concentration (2-10% ethanol) on total and boldine yield was evaluated in 1.5-h experiments at 50 °C using a factorial experimental design. Additional 7 h–kinetic experiments were performed at 50 °C using 1.51 g/g/min SC-CO_2 at 90 bar or 600 bar, or 1.51 g/g/min ethanol-doped (5%) SC-CO_2 at 450 bar. Extract samples were collected in pre-weighed glass vials which were kept in a cold trap. Ethanol, if used, was removed by rotary evaporation prior to further analysis.

Analysis. Boldo essential oils were obtained by steam distillation of 20 g-samples in a Karlsruher apparatus. The essential oils were recovered in an *n*-pentane layer and then analyzed by GC-FID and GC-MS. Total hexane- and methanol extracts from boldo were obtained by Soxhlet extraction of 5 g-samples. The methanol extract was further analyzed by HPLC to determine boldine content.

Essential oil composition was assessed by GC-MS and GC-FID using the protocol of Sargenti and Lancas [6]. HPW extracts were evaporated to dryness and dissolved in *n*-hexane. Low-pressure SC-CO_2 extracts were also dissolved in *n*-hexane prior to analysis. The essential oil content in these samples was calculated by comparing the peak areas of the corresponding GC-FID chromatograms with those of the steam distillate. A retention time of 48.2 min was selected as the limit between the essential oils and higher molecular weight (*e.g.*, waxes, fatty acids) components.

Boldine content in extract samples was determined using the isocratic HPLC method of del Valle *et al.* [2]. HPW extracts were purified by liquid-liquid extraction with chloroform and the organic phases were evaporated to dryness. These purified extracts, SC-CO_2 extracts and methanol extract were diluted in HPLC grade water prior to analysis.

RESULTS AND DISCUSSION

Extraction with hot pressurized water. Figure 1 shows the results of a parametric study to determine the effect of extraction temperature on the yield and selectivity of HPW extraction for boldo. Total yield increased with rising temperature up to 125 °C and decreased as temperature was further increased. Boldine yield was maximal at 100 °C, and HPW extracts obtained at temperatures above 150 °C did not contain boldine. This was due to the decrease in water polarity and thus, boldine solubility, as temperature increases above 100 °C. Yield of essential oils increased up to a temperature of 110 °C and was lower at higher temperatures. This was probably due to the long contact times between the solvent and the substrate in our experiments, which may have caused partial destruction of essential oil components [4]. These results are preliminary in that careful removal of oxygen from water, and a continuous flow of HPW through the extraction vessel are recommended to minimize thermal and oxidative damage of extract samples. They show promise, however, in that they confirm the possibility of changing the selectivity of the extraction for different plant constituents with moderate changes in temperature [3,4].

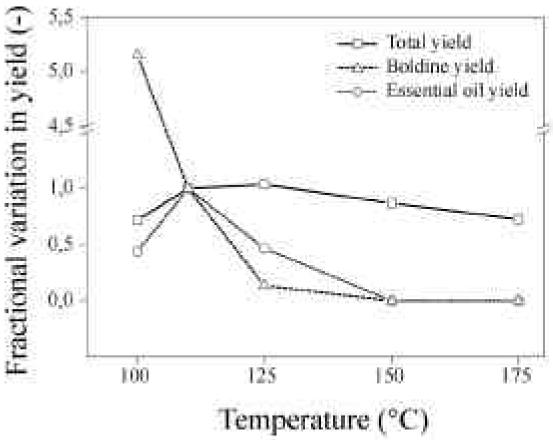

Figure 1. Total, essential oil, and boldine yield with HPW as a function of extraction temperature.

Extraction with supercritical CO_2. Figure 2 shows the results of the parametric study to determine the effects of extraction pressure, temperature, and solvent ratio on the yield and selectivity for boldo essential oils of 3 h-experiments with low-pressure SC-CO_2. The reference experiment was carried out using 1.51 g/g/min CO_2 at 90 bar and 50 °C. Percent recovery was >95% in all experiments. The total yield as well as the yield of essential oils increased as a result of an increase in pressure from 60 to 150 bar (Fig. 2A). Maximal yield was *ca.* 140% higher at 150 bar than 90 bar. As pressure increases at a constant temperature, the density of near-critical CO_2 and its associated solvent power increase pronouncedly [5]. Total yield decreased slightly as a result of an increase in solvent temperature from 30 to 60 °C, and was 36% higher at 30 °C than 50 °C (Fig. 2A). Density-decreasing effects predominate over vapor pressure-increasing effects on the actual solubility of boldo components, as it commonly occurs at near-critical pressure conditions, where SC-CO_2 is highly compressible [5]. There was a very limited effect of solvent ratio on total yield (Fig. 2A). As the solvent ratio increases, the superficial velocity of SC-CO_2 increases, and the external resistance to mass transfer decreases. In our experiments the maximal total yield was 36% higher using 2.12 than 1.51 g/g/min CO_2 at 50 °C and 90 bar. However, it is important to point out that the superficial velocity of the solvent in the extraction vessel depends not only on the solvent ratio but also on the solvent density, with the latter being a complex function of the extraction pressure and temperature. Thus, pressure and temperature effects in Figure 2 are compounded with solvent ratio effects.

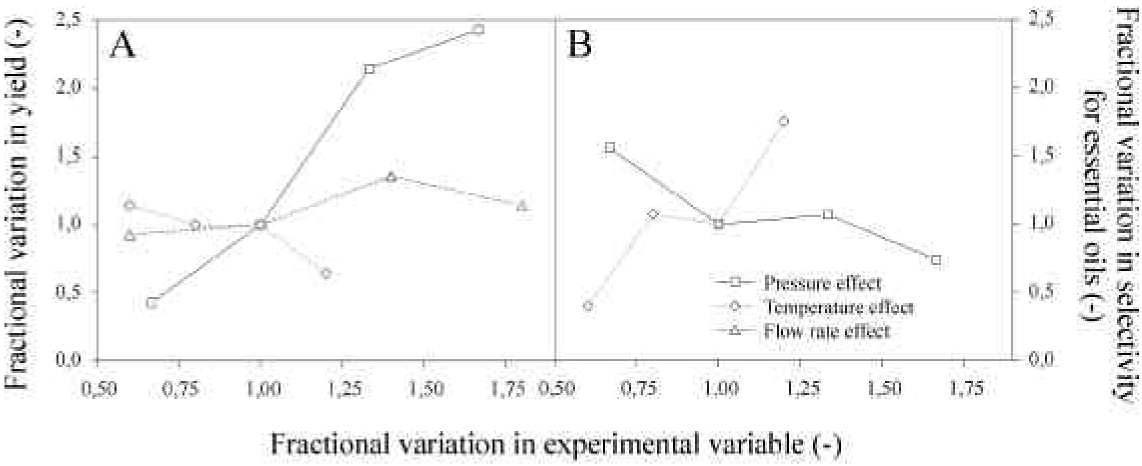

Figure 2. Sensibility analysis for low-pressure SC-CO_2 extraction experiments. **(A)** Total yield. **(B)** Selectivity for extraction of essential oils.

Figure 2B shows the results of a parametric study for the effects of the extraction pressure and temperature on the selectivity of 3 h-experiments with 1.51 g/g/min low-pressure SC-CO_2. In our experiments, selectivity was estimated as the proportion of essential oil components in extract samples, and it is as important in the performance of low-pressure SC-CO_2 experiments as the extraction yield. Selectivity decreased as pressure increased from 60 to 150 bar, being 56% higher at 60 bar than 90 bar. It also increased with extraction temperature, being 75% higher at 60 °C than 50 °C. These trends are opposite to those observed with the yield of essential oils, and reflect the well known fact that any increase in process yield is usually associated with a decrease in process selectivity.

Figure 3 shows the results of a parametric study to determine the effects of extraction pressure, and polar cosolvent addition on total yield and boldine yield in 1.5-h high-pressure experiments with 1.51 g/g/min SC-CO_2 at 50 °C. Yields were positively affected by an increase in pressure from 300 to 450 bar, and an increase in cosolvent concentration from 2 to 10% (w/w) ethanol. Linearity between yield and ethanol concentration was observed at both pressures and for both total yield and boldine yield. When low levels of cosolvent were added (2% ethanol), there was a large positive effect of increasing extraction pressure from 300 to 450 bar, which resulted in an increase of 64% in total yield and 430% in boldine yield. This is possibly related to the increase in density and solvent power of SC-CO_2. Corresponding percent increases in yield for CO_2 with 10% ethanol were only 10% for soluble solids and 14% for boldine, indicating that the solvent power of SC-CO_2 is less important under these conditions. The cosolvent effect, that is, the relative increase in solubility at a given pressure and temperature that comes about when a SCF is modified with

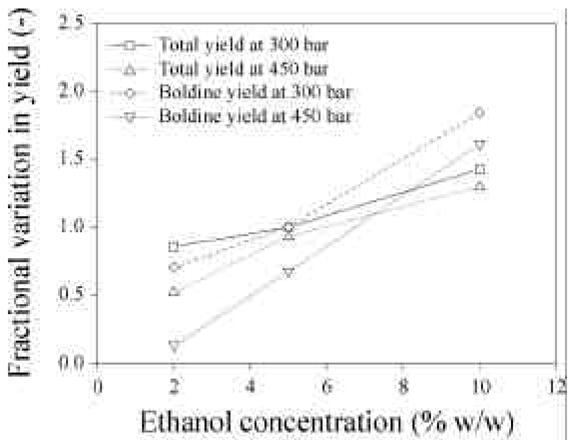

Figure 3. Total and boldine yield as a function of extraction pressure and co-solvent concentration in high-pressure SC-CO_2 extraction experiments.

cosolvent, generally decreased as pressure increased in the 80-200 bar range for the solubility of several solutes in doped methane at 50 °C with different cosolvents [8]. Ekart *et al.* [8] also observed that the cosolvent effect was fairly linear with respect to the concentration of ethanol for selected solutes. Since a portion of the cosolvent effect is due to the increase in density of the solvent mixture relative to the pure SCF at the same pressure, and the relative increase in density decreases as the pressure increases, the portion of the cosolvent effect that is due to the increase in density becomes progressively smaller. However, since the yield of boldine depends more pronouncedly on pressure and cosolvent concentration than that of other extract components, it may be concluded that ethanol interacts preferentially with boldine.

Figure 4 shows the effect of extraction time on the integral yield of soluble solids, essential oils (low-pressure extractions), and boldine (high-pressure extractions) from boldo leaves using 1.51 g/g/min pure and ethanol-doped SC-CO_2 at 50 °C. The conditions selected for the extraction of essential oils (90 bar and 50 °C) represent a compromise between yield and selectivity (Fig. 1), and have been recommended for the extraction of volatiles from several herbs and spices [9]. Essential oils were extracted faster than other components at 90 bar and 50 °C; thus the selectivity of the process decreases with extraction time due to incorporation of less soluble components. Extraction kinetics of soluble solids was similar when using high-pressure SC-CO_2 with either no added cosolvent (600 bar) or 5% ethanol (450 bar). In both cases the substrate did not appear to be fully extracted even following 7 h extraction, which suggested the existence of a low-solubility fraction. This low-solubility fraction may correspond to high-molecular weight, polar components such as aporphine alkaloids. Figure 4 strongly suggests that boldine extraction is solubility-controlled, and that solubility is much higher with ethanol-doped SC-CO_2 at 450 bar than neat SC-CO_2 at 600 bar. In both cases 30-45 min treatment were required to initiate boldine extraction.

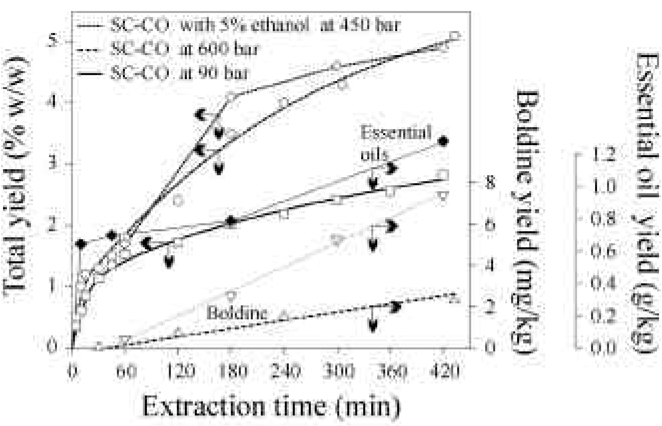

Figure 4. Total, essential oil, and boldine yield as a function of extraction time and other conditions (temperature = 50 °C, solvent ratio = 1.51 g/g/min).

Comparison between boldo extracts obtained with different solvents. Table 1 summarizes the yield and composition of selected boldo extracts that were obtained in these studies. Boldo samples contained 5.1% hexane-soluble solutes, 36.6% methanol-soluble solutes, 0.4% essential oils, and 59.7 ppm boldine. The essential oil content of boldo leaves is presumably larger than measured in this work [2,10]. The chemical make up of the steam distillate, on the other hand, was similar to the one reported by Miraldi *et al.* [10]. Boldine content of boldo leaves is of the same order of magnitude as reported by others [1,2]. On the other hand, more than 7 h extraction may be required to obtain most essential oils in boldo leaves with low-pressure SC-CO_2 (unless there was a recovery problem with the most volatile components of the extract), as well as to fully extract boldine with high-pressure SC-CO_2, even when using 5% ethanol as a cosolvent. Essential oils were not quantified in high-pressure SC-CO_2 extracts, whereas boldine contents were not determined in low-pressure SC-CO_2 extracts due to the extremely low solubility that was expected under these conditions. On the other hand, it would be expected a quantitative recovery of essential oils in high-pressure SC-CO_2 experiments. HPW at 110 °C fully extracted all essential oils in boldo leaf samples. Finally, 1.4 times as much boldine was extracted in 7 h ethanol-doped high-pressure SC-CO_2 than in 3 h with HPW.

Table 1. Total yield, essential oil yield and boldine yield from crushed boldo leaves as a function of extraction solvent and treatment conditions.

Extract	Yield		
	Total (% w/w)	Essential oil (g/kg)	Boldine (mg/kg)
Steam distillate	0.4	4.00	n.d.
Hexane extract	5.1	n.d.	n.d.
Methanol extract	36.6	n.d.	59.7
HPW at 110 °C	51.4	4.02	5.2
SC-CO_2 at 90 bar	2.8	1.28	n.d.
SC-CO_2 at 600 bar	5.1	n.d.	2.3
SC-CO_2 with 5% (w/w) ethanol at 450 bar	4.9	n.d.	7.4

n.d. indicates a non determined yield.

Acknowledgements

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