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## EXTRACTION OF OIL FROM GRAPE SEEDS WITH SUPERCRITICAL CARBON DIOXIDE

### Authors

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**EXTRACTION OF OIL FROM GRAPE SEEDS WITH SUPERCRITICAL CARBON DIOXIDE**

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**KEYWORDS:** Extraction, grape seeds, supercritical carbon dioxide (SC-CO<sub>2</sub>)

**INTRODUCTION**

Grape seeds are known as a waste byproduct of juice and wine production. They contain oil between 10 and 20%, depending on grape variety, which has high amounts of unsaturated fatty acids, usually linoleic and oleic acids ranged from 53 to 78% and 16 to 31% (Bail et al., 2008; Sabir et al., 2012). Oil from grape seeds is also constituted with natural antioxidants, among others, tocopherols in the 50 to 520 mg/kg range that are responsible for health-benefits such as lowering cholesterol levels and alleviating cardiovascular diseases (Bail et al., 2008; Sabir et al., 2012). Grape seed oil is a promising lipid for human consumption due to its nutritional constituents.

The use of supercritical carbon dioxide (SC-CO<sub>2</sub>) is an effective method for oil extraction. It produces purer oil and preserves bioactive components of the oil when compared with the typical hexane extraction and refining. This is because the low critical point ( $P_c = 7.3$  MPa,  $T_c = 31^\circ\text{C}$ ) and characteristics of CO<sub>2</sub> that allows for organic solvent free extraction, making it safe and efficient for separation and fractionation of thermally sensitive oils (Agostini et al., 2012). In the present study the effects of pressure (20, 30 MPa) and temperature (30 °C, 40 °C, 50 °C) were investigated on the oil yield. The quality of SC-CO<sub>2</sub> extracted oil in term of fatty acid content was compared to that of hexane-extracted oil.

**MATERIALS AND METHODS**

**Preparation of grape seeds sample** Grape seeds of the Ribier variety were obtained from a winery in Samut Sakhon province, Thailand. The seeds were washed and oven-dried at 55°C for 4-6 h. After that they were ground using a miller and then sieved using the sieve screens between 10 and 100 mesh. The ground seed sample of particle size ranging from 0.15 to 2.0 mm was stored in a sealed plastic bag at room temperature until all extractions were carried out.

**Proximal chemical analysis** The proximate analysis of grape seeds sample was determined according to AOAC (2000). The analysis included moisture, crude protein, crude fat and ash. The moisture content was determined by drying in oven at 105°C to a constant weight. Total crude protein was determined using the Kjeldahl method. The total fat content was determined by Soxhlet extraction with petroleum ether (40 to 60 °C) for 4 h, and then oven-dried to dryness at 105°C for 1 h. Ash was determined by weighing the incinerated residue obtained at 550°C for 30 min. Total available carbohydrate was calculated as 100% minus the sum of moisture, protein, fat and ash.

**SC-CO<sub>2</sub> extraction** The SC-CO<sub>2</sub> extraction of oil was conducted using the Speed SFE instrument with a 300 ml extraction vessel (Applied Separations Inc., Allenton, PA, USA). The vessel was heated with an oven controlled by a thermostat ( $\pm 1^\circ\text{C}$ ). Liquid CO<sub>2</sub> was delivered into the vessel and pressurized to the operating value ( $\pm 10$  bar) with a high-pressure pump (Applied Separations Inc., Allenton, PA, USA). For each extraction, approximately 0.1 kg of ground grape seeds were loaded into the vessel, and packed with propylene wool. SC-CO<sub>2</sub> was left in contact with the sample for 30 min of static extraction. Then, dynamic extraction was performed with a CO<sub>2</sub> flow rate ranging about 2 L/min for 4 h. The SC-CO<sub>2</sub> with dissolved oil passed through a heated micrometering valve at 110°C, and was expanded to atmospheric pressure. The oil was collected in a pre-weighed glass vial at ambient pressure and temperature.

**Solvent extraction** Hexane extraction was also performed in a Soxhlet apparatus at 60°C for 4 h in cycles of about 30 min. Hexane was removed under vacuum in a rotary evaporator at 40°C.

**Fatty acid composition** SC-CO<sub>2</sub> and hexane extracted oils were converted into fatty acid methyl esters (FAME) according to AOAC (2000). In brief, 0.2 g of oil sample was dissolved in 10 mL of 1M methanolic sodium hydroxide, refluxed at 100 °C for 15 min, and then 12 mL of 12% BF<sub>3</sub> and 5 mL of *n*-heptane was added. After cooling, 30 mL of saturated sodium chloride was added to the mixture. The upper *n*-heptane phase was transferred into a vial and injected into a GCMS-QP2010 Ultra gas chromatograph mass spectrometer (Shimadzu, Columbia, MD, USA) equipped with a capillary column Cp-Sil 88 (100 m long, 0.25 mm i.d., 0.2 µm film thickness). The initial oven temperature was from 100°C heated to 240°C (3°C/min). Injector and detector temperatures were set at 225°C. The mass spectrometer operated at ionization energy of 70 eV with a scan range of 30-320 amu. Identification of components was carried out based on retention time and mass spectra by matching with the NIST library.

**Statistical analysis** Extractions at each condition were performed in triplicate. All analysis were done in duplicate. The mean values were calculated and subjected to analysis of variance (ANOVA) at 5% level of significance using SPSS for Window Version 12.0 (SPSS Inc., Thailand).

## RESULTS

**Proximate analysis of grape seeds** The results obtained showed the proximate composition of 8.0% moisture, 7.1% crude protein, 14.3% fat, 2.6% ash and 68.0% carbohydrate. These results are in good agreement with those reported by Elegamey et al. (2013).

**Oil extraction** Grape seed oil extracted with SC-CO<sub>2</sub> was virtually clear, light yellow-green in color, while hexane-extracted oil was yellow-brown and turbid. Yields of SC-CO<sub>2</sub> extracted oils ranged from 1.7 to 5.5%, as shown in Table 1, compared to the hexane extractable oil yield of 7.1%. The highest oil yield (5.5%), corresponding to 35.9% of the total available oil, obtained at 30 MPa, 30 °C. The oil yield can be further improved by increasing extraction time from 5.5% to 6.9% at 8 h to achieve 44.4% oil recovery. As seen the oil yield time profile in Figure 1, the oil was extracted rapidly in 4 h, then gradually with the increased extraction time until approximately 8 h. Extraction time longer than 8 h resulted in a slightly additional oil yield, representing only 1-2% of the total oil extracted. It is also observed that the SC-CO<sub>2</sub> extracted oil after 8 h was from light to dark green in color, possibly that chlorophyll was co-extracted along with the oil.

**Extracted oil composition** Grape seed oils obtained by SC-CO<sub>2</sub> under different conditions and Soxhlet extraction contained mainly palmitic (12.8 to 15.4%), stearic (6.8 to 7.7%), oleic (26.7 to 27.5%), and linoleic (46.0 to 50.4%) acids. The oils were rather poor in linolenic (0.5 to 0.6%) acid, as shown in Table 2.

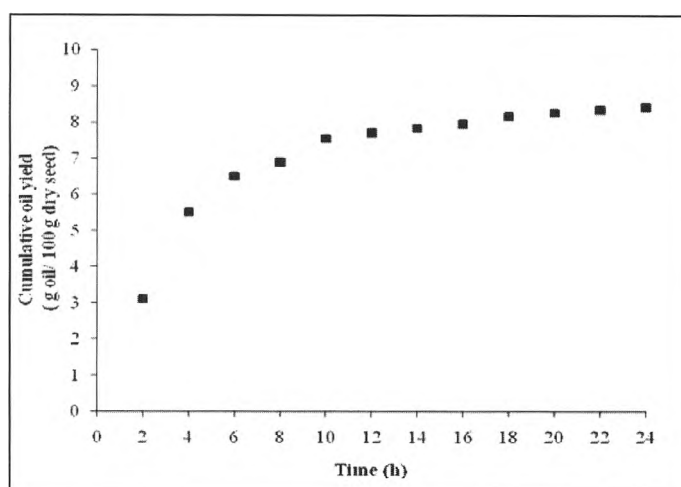
## DISCUSSION

Although oil extraction of grape seeds with the usual solvent hexane had higher oil yield, it resulted in the presence of impurities such as unsaponifiables and hexane residue in the crude oil (Gómez et al., 1996), which requires further refinement. The SC-CO<sub>2</sub> extraction method produced the purer oil. The oil yield obtained in this study was comparable to those of Gómez et al (1996) who extracted oil from Airen grape seeds with SC-CO<sub>2</sub>, using 35 MPa, 40 °C for 3 h, obtained 6.9% oil yield, about 92% yield with hexane extraction in 20 h. Comparing the SC-CO<sub>2</sub> extraction conditions, we used lower pressure and lower temperature, but longer extraction time.

**Table 1** Grape seed oil yield<sup>1</sup> and recovery<sup>2</sup> obtained with SC-CO<sub>2</sub> extraction under different conditions at CO<sub>2</sub> flow rate about 2 L/min

Pressure (MPa)	Temperature (°C)	Oil yield <sup>1</sup>	Recovery <sup>2</sup> (% of total oil)
20	30	2.5 <sup>b</sup>	16.4 <sup>b</sup>
20	40	2.3 <sup>b</sup>	15.2 <sup>b</sup>
20	50	1.7 <sup>a</sup>	11.3 <sup>a</sup>
30	30	5.5 <sup>d</sup>	35.9 <sup>d</sup>
30	40	5.0 <sup>c</sup>	32.5 <sup>c</sup>
30	50	5.1 <sup>c</sup>	32.9 <sup>c</sup>

<sup>1,2</sup>Oil yield obtained after 4 h (g oil/100 g seed, dry matter basis) is mean of duplicate; values with the same superscript letter in a column are not significantly different ( $p > 0.05$ ).

**Figure 1** Cumulative oil yield from grape seeds of the Ribier variety using SC-CO<sub>2</sub> at 30 MPa, 30 °C and CO<sub>2</sub> flow rate about 2 L/min. Each point represents the mean of triplicate extractions.**Table 2** Fatty acid composition of grape seed oils obtained with SC-CO<sub>2</sub> under different conditions at CO<sub>2</sub> flow rate about 2 L/min and Soxhlet extraction

Extraction method	Fatty acid (% of total fatty acids)				
	C16:0	C18:0	C18:1	C18:2	C18:3
SC-CO <sub>2</sub> , 20 MPa/ 30 °C	14.3	7.4	27.3	47.7	0.5
SC-CO <sub>2</sub> , 20 MPa/ 40 °C	15.3	7.4	27.5	46.1	0.6
SC-CO <sub>2</sub> , 20 MPa/ 50 °C	15.4	7.5	27.7	46.0	0.6
SC-CO <sub>2</sub> , 30 MPa/ 30 °C	13.1	6.8	26.7	50.4	0.5
SC-CO <sub>2</sub> , 30 MPa/ 40 °C	13.5	6.9	27.1	49.7	0.5
SC-CO <sub>2</sub> , 30 MPa/ 50 °C	13.7	7.1	27.3	48.9	0.5
Soxhlet	12.8	7.7	26.7	49.4	0.5

Values are the mean of duplicate analyses

The pressure was the most important factor. The oil yield increased ( $p < 0.05$ ) by about 2-3 folds when the pressure increased from 20 to 30 MPa at lower temperature (30 °C). This may be explained by the increase of CO<sub>2</sub> density with pressure, resulting in a higher oil recovery. However, results of extraction temperature differed from those of extraction pressure. The decreased oil yield with an increase in temperature at constant pressure was not statistically significant. This indicates that the CO<sub>2</sub> density did

not change drastically when the temperature was increased from 30 to 50 °C. The analyzed fatty acid composition showed high amount of unsaturated fatty acids (about 75%), indicating a good nutritional oil. The values obtained in the present study were comparable to the results by Elegamey et al. (2013), although wide variability in fatty acid profiles was observed with different grape varieties (Elegamey et al., 2013; Sabir et al., 2012). The fatty acid profile of SC-CO<sub>2</sub> extracted oils varied slightly with pressure and temperature, and was comparable to that of hexane-extracted oil.

#### CONCLUSION

SC-CO<sub>2</sub> extraction was used to produce oil from grape seeds and compared to Soxhlet extraction using hexane. SC-CO<sub>2</sub> extraction at 30MPa, 30 °C gave the highest oil yield at 5.5% for 4 h initial extraction. The SC-CO<sub>2</sub> extracted oil recovery obtained at 30MPa, 30 °C and 8 h was 44.4% of the total available oil. The SC-CO<sub>2</sub> extracted and hexane-extracted oils had similar fatty acid profiles. In both cases the content of unsaturated fatty acids in the oil was about 75%, mainly linoleic and oleic acids. Additional characterization of tocopherols and phytosterols in grape seed oil obtained by SC-CO<sub>2</sub> extraction is being investigated.

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