Supercritical Fluid Extraction of Daphne (*Laurus nobilis* L.) Seed Oil

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ABSTRACT: *Laurus nobilis* L., commonly known as daphne tree, is an evergreen that belongs to the Lauraceae family. Daphne trees produce grape-sized shiny purplish berries having three parts: flesh, skin, and an inner kernel (single seed). This study examines supercritical CO_2 (SC-CO_2) extraction of oil from daphne seeds. The oil yield of ground seeds varied from 14 to 28% depending on the method and particle size used for oil recovery. Yields were similar for both petroleum ether and SC-CO_2 extraction. The extraction yield decreased significantly with increasing particle size. The amount of extract collected increased exponentially with increasing SC-CO_2 pressure. The highest extraction yield was obtained at the highest temperature studied, 75°C. More than 45% of the oil was lauric acid. SC-CO_2 is a viable technique to obtain high-purity *L. nobilis* L. seed oil, which is a potential ingredient for the cosmetic industry.

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KEY WORDS: Daphne, lauric acid, *Laurus nobilis* L., supercritical carbon dioxide.

Laurus nobilis L. is commonly known as bay, daphne, bay laurel, or sweet bay. Daphne trees grow wild in the coastal areas of the Mediterranean and the Black Sea. The tree is grown primarily for its leaves. Fresh or dried, they are used as a culinary herb and in the production of bay leaf essential oil. Currently, bay leaves are collected from both cultivated and wild trees in many Mediterranean countries.

The daphne tree produces small, fragrant, star-shaped flowers in late spring and early summer followed by small, round, green berries (fruits) that ripen to purplish black in the fall. These shiny, grape-sized berries consist of three parts: flesh, skin, and an inner kernel (single seed). The whole berry, flesh, skin, and kernel/seed contain 26, 38, 8, and 18% oil, respectively (1). The oil extracted from berries contains the following FA: lauric (54%), palmitic (5%), oleic (15%), and linoleic (17%) (2). Unsaponifiable components of berry oil include undecanone, α-terpinol, terpineol acetate, β-elemene, and β-sitosterol. Turkish daphne berries contain about 0.4% essential oil (1). β-Ocimene (22%), 1,8-cineole (9.5%), bicyclogermacrene (4.5%), and β-elemene (2%) are the main volatile compounds found in berries (3). According to Longo and Vasapollo (4) cyanidin 3-*O*-glucoside (41%) and cyanidin 3-*O*-rutinoside (53%) are the two major anthocyanins present in *L. nobilis* L. berries.

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Folk remedies in various countries include *L. nobilis* L. berries for the treatment of various ailments. Traditionally, aromatic berries are used as emmenagogues, which are herbs that stimulate blood flow in the pelvic area and uterus, and for treating hysteria (5). It is believed that powdered berries possess diuretic and carminative properties. Berry oil is used externally to heal furuncles, sprains, bruises, and rheumatism and as an insect repellent (5). Crushed fresh berries are consumed to treat hemorrhoids (6) and stomach ulcers (7). Afifi *et al*. (8) reported that aqueous extracts of *L. nobilis* seeds were effective in reducing ethanol-induced gastric ulcer in rats. Berry oil in the form of decoction is used to cure sore throat and muscular myalgia (6). Laurel oil is also incorporated in perfumed soaps, deodorants, hair care products for its antidandruff activity, and topical products for treating scaling (psoriasis), skin eruptions, and eczema (2). Simic *et al*. (5) have also reported antioxidant activity in crude methanol extracts of *L. nobilis* L. berries. Antioxidant activities in extracts obtained from full-fat berries were higher than that of the defatted berries.

Literature on laurel berry processing is scarce. In Turkey and some other Mediterranean countries, laurel berry fat is traditionally extracted by boiling the berries for several hours in an open drum of water using a wood fire. Then the extract is cooled and the oil layer floating over water is collected and stored. The oil is named as "tehnel" or "gar." This technique yields 10–15 % (w/w) oil. Organic solvent extraction and mechanical pressing/expression are two other techniques that can be used to recover berry oil (1).

Supercritical fluid (SCF) extraction of bay leaves has been reported (9,10). However, there has been no study on using supercritical CO_2 (SC-CO_2) to extract daphne seed oil. Furthermore, a systematic investigation of the chemical composition of *L. nobilis* L. kernel oil is lacking. Hence, the main objectives of this study are to explore the potential of the SC-CO_2 extraction technique to obtain high-quality laurel berry oil and to determine the effects of temperature, pressure, and particle size on extraction yield. The chemical compositions of SC-CO_2- and petroleum ether-extracted kernel oil were also analyzed.

MATERIALS AND METHODS

Sample preparation. Laurel berry samples were purchased at a local market in Mugla, Turkey, in 2004. After air-drying at room temperature (20–25°C), samples were crushed to free seed/kernel (endocarp) from surrounding soft tissue (pericarp).

The seeds were ground with a laboratory mill (Thomas Wiley Laboratory Mill, Model 4; Arthur H. Thomas Company, Philadelphia, PA). Ground samples were sieved using 1.25, 1.00, 0.85, 0.71, 0.60, 0.50, and 0.355 mm sieves, and fractions were stored in a refrigerator until further use.

SC-CO_2 extraction. SC-CO_2 extraction of the samples was conducted by using an SFX 220 (Isco, Inc., Lincoln, NE) extraction system. About 2 g of sample was placed on a glass wool bed that was placed at the bottom of a 10-mL extraction cell. A second layer of glass wool was placed on top of the sample to keep it in place. The extraction cell was pressurized with CO_2 (Research Grade, min. purity 99.998%; Matheson Tri-Gas, Houston, TX). When the system reached the desired temperature and pressure, extract collection was started. Extract-laden CO_2 was expanded through a heated restrictor maintained at 80°C during all the experiments (1.5 mL/min CO_2 flow rate at 34 MPa and 80°C extractor and restrictor temperature). The extract was collected in vials cooled by two Microban ICE-PAKs (Fisher Scientific, Pittsburgh, PA). Extract fractions were collected by pausing the dynamic extraction after 2 mL of CO_2/fraction had passed through the feedstock in the extractor. The pressure of the system was maintained and CO_2 flow was stopped while changing the collection vials for each fraction. The extraction yield was determined gravimetrically by measuring the weights of the collection vials before and after extract collection. Extraction of the feed material was continued until no measurable amount of extract was collected. Each run lasted at least 1 h. The effect of temperature on extraction yield was examined at 35, 45, 55, 65, and 75°C. The pressure of the system was maintained at 68 MPa for these experiments. The pressure effect was studied at 14, 20, 27, 41, 54, and 68 MPa and 75°C. The effect of particle size on extraction efficiency was examined in the following particle size range: 0.17, 0.42, 0.55, 0.65, 0.78, 0.92, and 1.12 mm at 68 MPa and 75°C.

Analytical methods. Moisture/volatile content of the ground seeds was determined by using a Computrac Max 2000 moisture analyzer (Arizona Instrument LLC, Tempe, AZ). The oil content of the samples was determined according to AOAC Method 960.39 (11). Approximately 1 g of sample (particle size 0.17 mm) was placed in a Soxtec extraction unit (Model 1043; Tecator, Hoganas, Sweden), and oil was extracted using petroleum ether. The oil content was determined gravimetrically.

Analytical separations of TAG, FFA, and free phytosterols and their FA esters in oil samples were achieved by using an HPLC method developed by Moreau *et al*. (12). A LiChrosorb Diol, 5 μm, 100 × 3.0 mm (Chrompack Inc., Raritan, NJ) column was used for the analysis. The mobile phase consisted of the following: A, hexane/acetic acid (1000:1); B, 2-propanol. The solvent gradient system was 100% A for 8 min, 100% A to 99% A/1% B in 2 min, hold for 20 min, from 99% A to 100% A in 1 min, and hold for 29 min, resulting in 60 min total analysis time. The mobile phase flow rate was 0.5 mL/min. Oil samples were dissolved in hexane. All the solvents used in this study were HPLC grade and filtered by using a GH Polypro (47 mm, 0.45 μm) hydrophilic polypropylene membrane filter (Pal Life Sciences, Ann Arbor, MI) before use in HPLC analysis.

FA compositions of the oil samples were analyzed by an HP 6890 Series GC system coupled with a 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA). Methylation of the FA was carried out according to the AOAC official method 965.49 (11). FA standards were purchased from Supelco (Supelco 37-component FAME mix; Supelco, Bellefonte, PA). A Supelco 18508-04A Equity 5 capillary column with 30 m × 0.25 mm × 0.5 μm film thickness was used. Oven temperature was programmed from 80 to 250°C at 4°C/min. Helium was used as carrier gas at a 1.0 mL/min flow rate. The inlet temperature was 300°C. GC–MS operating temperatures were as follows: MS transfer line 280°C, ion source 230°C, and MS quadrupole 150°C. The ionization energy was 70 eV. The scan range and rate were 100–600 amu and 2 scans/s, respectively. The samples (3 μL) were injected into the GC–MS by using an autosampler (HP 7683; HP Company, Wilmington, DE). The split ratio was 1:10. The data collection and analysis were managed by using an HP Chemstation (Enhanced Chemstation G1701DA Version D.00.00.38; Agilent Technologies). The FA compositions of the samples were identified by direct comparison of their chromatographic retention times and the mass spectra with those of the authentic compounds. The peaks were also confirmed with National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health Mass Spectral Library (Version 2.0).

Statistical analysis. All extraction runs and analyses were carried out in duplicate and in randomized order with the mean values being reported. ANOVA of the results was performed using General Linear Model of SAS (Software version 8.1; SAS Institute Inc., Cary, NC). Multiple comparisons of the various means was carried out by LSD (Least Significant Difference) test at $P = 0.05$.

RESULTS AND DISCUSSION

Moisture/volatile content of ground daphne seeds was 7.5% (w/w) prior to extraction. Oil yields varied from 14 to 28% depending on the method and particle size used for oil recovery. Similar data were reported in the literature for the oil content of daphne fruit (1). Particle size had a significant effect on the daphne seed extraction yields for both SC-CO_2 and Soxtec extraction techniques (Fig. 1). The extraction yield decreased with increasing particle size. Maximal yield was obtained with the smallest particle size examined in this study, 0.17 mm. Extraction yields were very similar for both Soxtec and SC-CO_2 extractions. The effect of particle size on SC-CO_2 extraction of lipids from other oilseeds such as soybeans and canola has been reported (13–15). Our experimental results support previous findings that the size-classification process segregates seed parts having different oil content (14).

Effects of pressure and temperature on the SC-CO_2 extraction yield of seed oil are shown in Figures 2 and 3. These experiments were carried out by using samples of 0.17 mm particle size since maximum extract yield was obtained by using this particle range in the previous experiments. Pressure had a

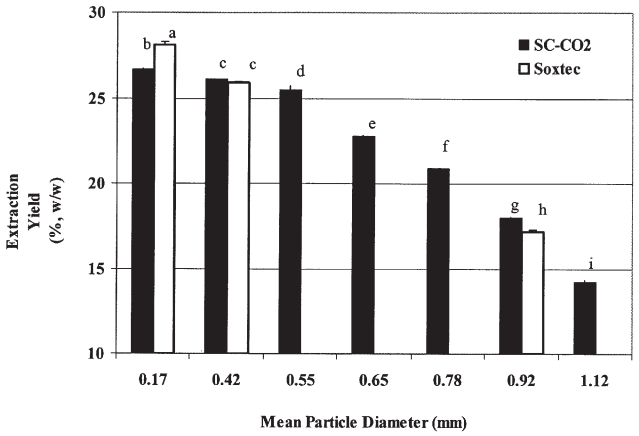

FIG. 1. Effect of particle size on Soxtec and supercritical CO_2 (SC-CO_2) extraction yields. Extraction conditions for SC-CO_2 experiments were as follows: 51 MPa, 100°C extractor and 80°C restrictor temperature. Bars with the same letter are not significantly different at $P = 0.05$ level.

significant effect on the extraction yield (Fig. 2). The amount of extract collected increased with increasing pressure. The maximal extract yield was 26.95% (w/w) at 68 MPa and 75°C, which were the maximum pressure and temperature examined in this study.

A typical oilseed SCF extraction curve (extract amount or yield vs. extraction time or solvent amount) is initially linear, is followed by a curved transition region, and finally approaches a fairly flat asymptotic phase (16). The linear region corresponds to the constant mass transfer rate from solid matrix to the SCF at the high solute concentration in the solid matrix. This region of the extraction curve is also referred to as the steady-state or solubility-controlled mass transfer region. Figures 2 and 3 displayed typical SC-CO_2 extraction curves for daphne seed as a function of pressure and temperature, respectively. The extract solubility in SC-CO_2 was calculated from the slope of the initial linear portion of the curves. Extract solubility increased exponentially with increasing pressure ($y = -5 \times 10^{-5} x^2 + 0.0069 x - 0.1036$, $R^2 = 0.9981$) and

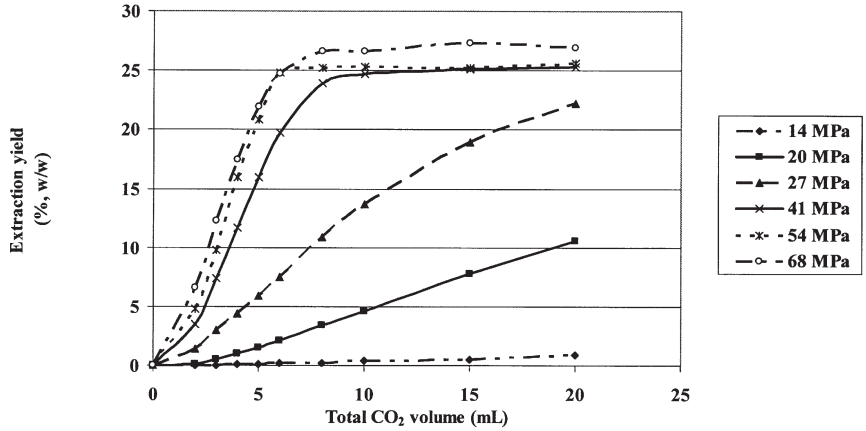

FIG. 2. Effect of pressure on SC-CO_2 extraction yield. All the experiments were carried out at 75°C. Particles with mean diameter of 0.17 mm were used for these experiments. For abbreviation see Figure 1.

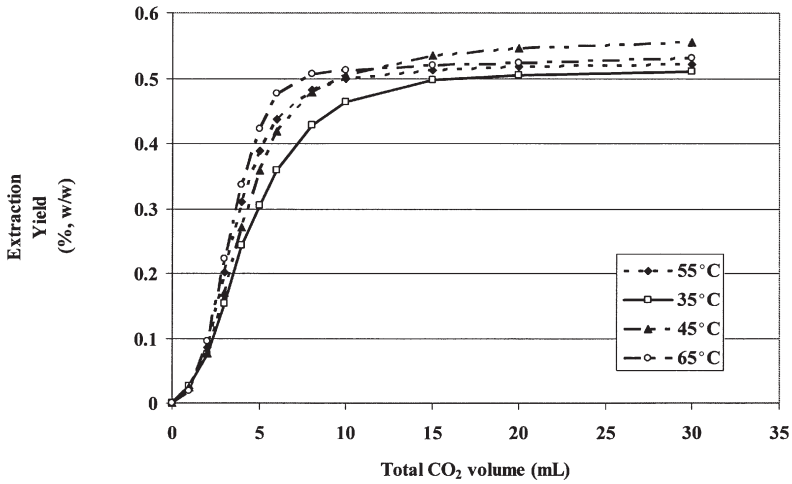

FIG. 3. Effect of temperature on SC-CO_2 extraction yield. All the experiments were carried out at 68 MPa. Particles with mean diameter of 0.17 mm were used for these experiments. For abbreviation see Figure 1.

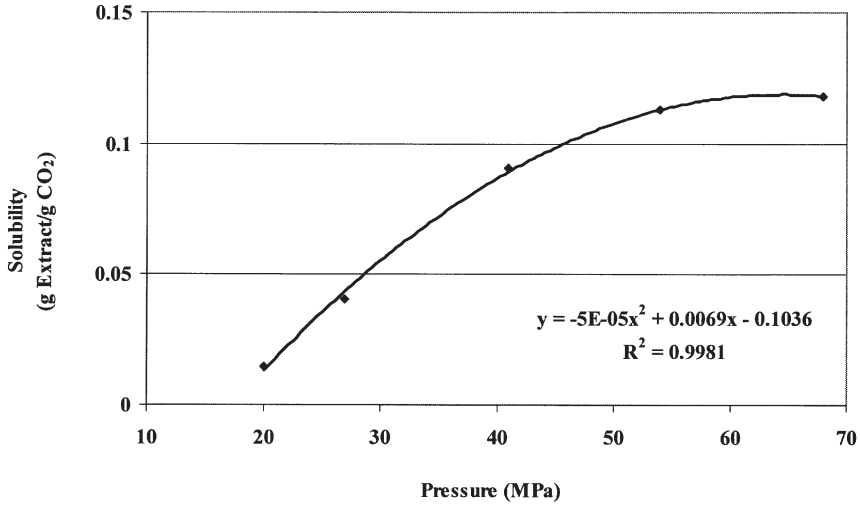

FIG. 4. Extract solubility as a function of pressure. All the experiments were carried out at 75°C. Particles with mean diameter of 0.17 mm were used for these experiments.

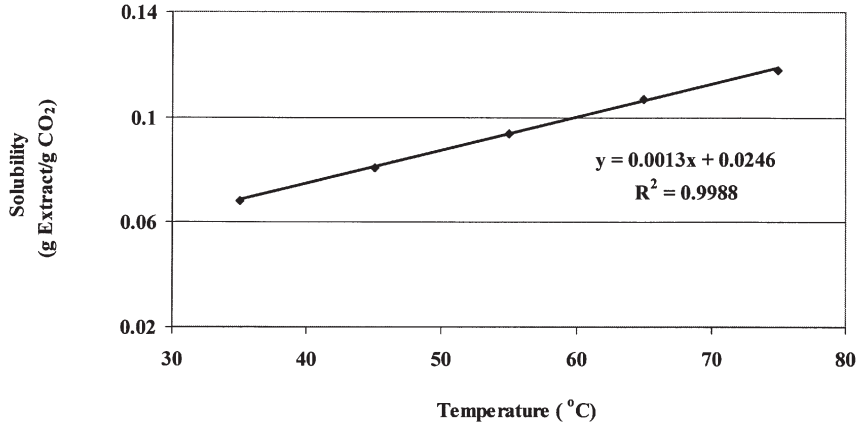

FIG. 5. Extract solubility as a function of temperature. All the experiments were carried out at 68 MPa. Particles with 0.17 mm were used for these experiments.

reached a maximum at 68 MPa (Fig. 4). Increasing pressure at a given temperature increases CO_2 density and consequently increases SC-CO_2 solvent power. The solubility of the extract in SC-CO_2 increased from 1.5 to 11.8% (w/w) when pressure was raised from 20 to 68 MPa at 75°C.

The effects of temperature on SC-CO_2 extraction of daphne seed oil yield are shown in Figure 3. Higher extraction yields were obtained at higher temperatures, indicating that extraction was dependent on solute (extract) vapor pressure, which increases with an increase in temperature. Maximal extraction yield was obtained at the highest temperature (75°C) examined in this study. Extract solubility in SC-CO_2 increased linearly with increasing temperature ($y = 0.0013x + 0.0246$, $R^2 = 0.9988$) (Fig. 5). Solubilities of daphne seed extracts in SC-CO_2 were higher than the vegetable oil solubility data reported in the literature (17–19). This might be due to the extraction of water and other volatile daphne seed components with SC-CO_2 along with seed oil. In fact, moisture/volatile analysis of daphne seed samples before (7.5%, w/w) and after (4.4%, w/w) SC-CO_2 extraction indicates significant moisture/volatiles loss from the samples. Co-extraction of water with SC-CO_2 along with seed oils has been reported (20,21). Furthermore, daphne seed contains oil-soluble aromatic compounds (1) that might contribute higher SC-CO_2 extraction yields.

Both SC-CO_2 and petroleum ether extracts of daphne seeds were rich in lauric acid (about 45%) (Table 1). Oleic, linoleic, and palmitic acids were also present in significant amounts in the extracts. These results are within the range of FA composition reported in the literature for daphne seed oil (2). HPLC analysis of SC-CO_2 and petroleum ether extracts showed extremely high

TABLE 1
FA Composition (GC area %) of Supercritical CO_2 (SC-CO_2) and Hexane-Extracted *Laurus nobilis* L. Seed Oil

FA name	SC-CO_2 extract	Petroleum ether extract
Lauric (C12:0)	43.1 ± 0.9	44.8 ± 0.8
Myristic (C14:0)	0.1 ± 0.01	0.1 ± 0.02
Palmitic (C16:0)	4.9 ± 0.2	4.5 ± 0.2
Oleic (C18:1)	37.2 ± 0.6	37.3 ± 0.3
Linoleic (C18:2)	14.7 ± 1.4	13.3 ± 0.5

TABLE 2
Lipid Composition (HPLC area %) of Daphne Seed Oil Extracts

Compound	SC-CO_2 extract	Petroleum ether eextract
Phytosterol FA Esters	2.8 ± 0.07	2.1 ± 0.04
TAG	31.3 ± 0.6	32.7 ± 0.9
FFA	56.8 ± 0.5	55.7 ± 0.4
Free phytosterols	0.08 ± 0.05	0.12 ± 0.01
Compounds not identified	9.0	9.4

FFA content in the oil, over 50% (Table 2). This might be due to poor handling of daphne fruit after harvest since these samples were collected from a local market in Turkey. Unfortunately, there are no data on the FFA content of daphne seed oil in the literature with which to compare our results. The majority of the phytosterols present in seed oil were in the form of esters (FA esters). Further research is needed to characterize daphne seed oil composition.

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