**Particle Size Effects on Supercritical CO$_2$ Extraction of Oil-Containing Seeds**

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**ABSTRACT:** Rosehip seeds were milled, sieved, and extracted with 26.3 g/g substrate/h of supercritical carbon dioxide (CO$_2$) at 40°C and 300 bar. The extraction kinetics were characterized by an initial solubility-controlled period (8.78 g oil/kg CO$_2$ at 40°C and 300 bar), followed by a transition period to a final mass transfer-controlled process. The integral yield of oil approached an asymptotic value that was dependent on the particle size of the substrate: 57.1 g oil/kg dry oil-free substrate (large particles), 171.0 g/kg (medium-size particles), or 391.5 g/kg (small particles). Based on gravimetric determinations and microscopic analysis, our size-classification process segregated seed parts having different oil contents. Particles ≥0.85 mm were mainly composed of tough, lignified testa fragments devoid of oil, whereas particles ≤0.425 mm contained mostly brittle, oil-rich germ fragments. The segregation of seed in fractions with different oil contents may be a common occurrence in supercritical extraction experiments, especially for seeds with thick and/or hard testa and small germ, whose fractions can be separated by sieving.


**KEY WORDS:** Extraction, microstructure, oil, particle size, rosehip, supercritical CO$_2$.

Supercritical carbon dioxide (SC-CO$_2$) is a nontoxic alternative to organic solvents for oil extraction from plant material (1). Conventional solvent extraction produces low-quality oil requiring extensive refining operations (2), whereas deoiling by pressing is customary only for seeds containing ≥20% oil (3).

Rosehip (*Rosa aff. rubiginosa*) seed, an inexpensive natural source of unsaturated FA, is a potential candidate for SC-CO$_2$ extraction of oil. Rosehip oil finds use in cosmetics and other high-value applications. del Valle *et al.* (4) assessed the effects of process temperature, pressure, and time on the yield and quality of rosehip oil by using response surface methodology. Optimal conditions to extract high-quality oil were 40°C and 300 bar. Eggers *et al.* (5) reported that extraction rate and yield were the same for rosehip seeds milled with a blade grinder (Sautier mean or volume-surface mean diameter of 1.15 mm), or flaked in a roller mill (1 mm gap). The apparent solubility (g extracted oil/kg utilized CO$_2$, in the initial stages of extraction) was virtually unaffected by process conditions for extraction pressure ≥500 bar, at 40–80°C, and using 8.6–28.6 g CO$_2$/g substrate/h. However, the apparent solubility of rosehip oil decreased when the extraction pressure was decreased from 500 to 300 bar (5). Reverchon *et al.* (6) assessed and modeled the effects of process temperature and pressure, superficial solvent velocity, and substrate particle size on extraction kinetics of ground rosehip seeds. The apparent solubility at 40°C increased from 0.5 g/kg at 101 bar to 40.0 g/kg at 671 bar and was not affected by process temperature or solvent flow rate. Reverchon *et al.* (6) observed that the amount of oil that was available for immediate extraction increased as particle size was reduced, and attributed this to the associated increase in specific surface.

Besides the effect of the process temperature and pressure on the apparent solubility of oil in the extracting solvent, SC-CO$_2$ extraction of oilseeds depends strongly on substrate pretreatment (7). Prior to extraction, the oil-containing plant cells should be broken by flaking or some similar process. Compression and shear forces developed between smooth rollers that rotate at differential speeds during flaking flatten the seed cotyledon pieces, the end result being the extensive deformation and fracture of the cell contents and separation of cell wall from the cytoplasm (3). Fattori *et al.* (8) compared the effects of flaking, chopping, crushing, and other less effective pretreatments on the extraction rate and oil yield of canola seeds treated with SC-CO$_2$ at 55°C and 360 bar. The crushed seeds produced slightly lower oil yield than chopped or flaked seeds. In addition, there was no additional positive effect of 30 min cooking at 90°C on extraction of flaked seeds (8). Oil yield from flaked soybeans treated with SC-CO$_2$ at 50°C and 537 bar increased as flake thickness decreased, from 66.0% for 0.81-mm-thick flakes to 97.4% for 0.10-mm-thick flakes, which was attributed to the associated increase in cell distortion (9).

Particle size reduction by milling not only increases the specific area (surface area-to-volume ratio) of oilseed materials but also ruptures cell walls. In small particles with large specific areas, there is more oil on the surface than in inner, unbroken cells. Thus, since there is apparently no diffusion through undamaged cell walls (10), oil yield may be higher when extracting smaller rather than larger particles.

In this work we assessed an alternative hypothesis for the effect of sample particle size on extraction rate and yield of SC-CO$_2$ extraction processes, namely, that seed parts with different oil contents may be segregated during sample preparation. Rosehip seeds were used as a model system, and microscopic evidence was gathered. Microscopy may help in
assessing the effect of sample pretreatment on the kinetics and yield of SC-CO₂-based extraction processes. Examples that include the use of microscopy for assessing extraction effectiveness for oil-containing seeds exist (6,9,11,12).

MATERIALS AND METHODS

Extraction substrates. Rosehip (Rosa aff. rubiginosa) seed samples were processed by Novbelttec S.A. (Santiago, Chile) in a roller mill with a 0.5 mm gap. Milled samples were size classified in a Ro-Tap test sieve shaker (W.S. Tyler, Mentor, OH). Three fractions were separated: −8/+20 mesh Tyler (0.85 mm < particle diameter [Dp] < 2.36 mm); −20/+35 mesh Tyler (0.425 mm < Dp < 0.85 mm); and −35/+100 mesh Tyler (0.150 mm < Dp < 0.425 mm). Samples were kept in sealed plastic bags in a refrigerator up to the time of analysis.

Supercritical extraction. Experiments were carried out using a Thar Designs (Pittsburgh, PA) SFE-IL process development unit, equipped with an automatic control system for controlling the extraction temperature and pressure. Liquid CO₂ (≥99.8% pure) from AGA S.A. (Santiago, Chile) was used as the solvent. Extraction vessels (20 mm diameter; 50 cm³ volume) were loaded with ca. 26 g milled substrate and placed in a convection oven set at 40°C. After a 2-min static extraction period, when extraction pressure (300 bar) had been reached, a P-200A-220V pump (Thar Designs) was set to the desired flow rate (11.4 g CO₂/min). The extraction pressure was subsequently maintained by a BPR-A-200B1 back-pressure regulator (Thar Designs). The outlet line of the BPR was connected to the inlet port of a Swagelock (Solon, OH) SS-43YF2 six-port, two-way valve that allowed periodic switching of oil collection between 15-cm³ capacity glass vials with polytetrafluoroethylene silicone septa (Supelco, Bellefonte, PA). Twelve unequally spaced samples were taken in all cases, and the total extraction time was 90 min. These glass vials were kept in a thermostated bath set at 50°C. The outlet port of the six-port, two-way valve was connected to an Omega (Stamford, CT) FMA5700 flowmeter equipped with an Omega DPF65 totalizer. Extraction experiments were performed in duplicate.

The oil yield was expressed in units of dry oil per unit mass (dried and oil-free) of substrate. In order to remove water from extract samples, vials were dried in an oven (Binder WTC, Tutlingen, Germany) set at 70°C prior to weighing. Recovered oil was assessed gravimetrically by difference with cleaned and dried vials. Percentage recovery of extract was estimated by determining the mass and moisture content was determined gravimetrically by drying in the oven (105°C) to a constant final weight (ca. 24 h). Oil content was determined gravimetrically by extracting to exhaustion with technical-grade hexane (TCL, Santiago, Chile) in a Soxhlet apparatus. Hexane was mostly recovered in a Fisatom (São Paulo, Brazil) rotary evaporator that was operated with a Vacuubrand (Wertheim, Germany) vacuum pump, and residual solvent traces were removed in the oven (ca. 2 h at 100°C).

Microscopy. Sample preparation for light microscopy was done according to standard procedures (14). Untreated seeds were moistened to assist in sample preparation. Moistened seeds and milled seed samples were fixed for 48 h using a 1:1:1.8 mixture of formalin, acetic acid, and 70% aqueous ethanol and then dehydrated by a 30-min immersion in a series of aqueous solutions with increasing ethanol concentration (50, 70, 95, and 100%), followed by 15 min immersion in pure tert-butanol. Dehydrated samples were then embedded with a liquefied mixture of tert-butanol and paraffin prior to cutting thin slices (18 µm thick) using a manual microtome (Jung, Heidelberg, Germany). Thin slices were fixed to slides with the aid of an albumin preparation, and paraffin was removed by treating samples with xylol, a series of aqueous solutions with a decreasing ethanol concentration, and distilled water. Staining was done with safranine (to redden cell chromosomes, nuclei, and lignified walls) and fast green (to mark other cellular structures with green color), followed by washing out excess stain with eugenol. A Nikkon (Kawasaki, Japan) Optiphot 142915 light microscope equipped with a Nikkon FX-35A photographic camera was utilized to view and record representative images.

RESULTS AND DISCUSSION

Figure 1 shows integral oil extraction yields of rosehip seed as a function of solvent usage and sample particle size. Trend