Quantitative Extraction of Pecan Oil from Small Samples with Supercritical Carbon Dioxide

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ABSTRACT: A procedure to determine total oil content of pecan was developed for samples weighing 500 and 10 mg by supercritical fluid extraction (SFE) with carbon dioxide as the extraction solvent, and chilled hexane as the trapping solvent. Fatty acid methyl esters (FAMEs) were prepared from the total lipid fraction by using either an aliquot (500 mg starting weight) or the entire extract (10 mg starting weight). Total oil content obtained for either sample size with SFE was similar to that obtained with an organic solvent extraction technique. The fatty acid composition for the total lipid fraction of oils extracted with SFE was the same as for oils extracted with organic solvents, and oil composition did not change during SFE. Both oil yield and fatty acid composition were similar to those reported previously for pecan. Samples could be extracted and placed into FAME-derivatizing reagents in one day, and fatty acid composition of the total lipid fraction could be determined by gas–liquid chromatography the next day. The procedure, as demonstrated for pecan, should be suitable for other oilseeds, especially those containing low amounts of water.

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KEY WORDS: Fatty acid methyl ester, oilseed, pecan, supercritical fluid extraction.

Oil is a major constituent of pecans, accounting for 55–75% of the total kernel weight (1). Pecan oil is particularly rich in 18:1 and 18:2 fatty acids, which make up about 32 and 17%, respectively, of the kernel weight, or almost 90% of the total fatty acids present in the oil (2). Short shelf life for shelled pecans has been attributed to the high unsaturated fatty acid content in pecan nutmeats and presents a major obstacle for market expansion. Understandably, oil content and composition have been extensively studied to elucidate possible means of extending their shelf life (3–5). The laborious and time-consuming methods presently used for pecan oil extraction and analysis present challenges for embarking on any studies involving large numbers of treatments. An alternative, rapid procedure for quantitative extraction of pecan oil and subsequent analysis of the total lipid fraction is needed.

Procedures for quantitative pecan oil extraction have traditionally relied on organic solvents, primarily based on chloroform, methanol, and aqueous salt solutions (1,2). With the use of carcinogenic and flammable solvents coming under increased scrutiny by governmental regulatory agencies, alternative oil extraction techniques are being sought for laboratory, as well as industrial, applications. One alternative currently being utilized successfully in both settings is supercritical fluid extraction (SFE) with carbon dioxide (CO_2) as extraction solvent. Supercritical CO_2 is a safe and efficient solvent for extracting oilseeds, especially those oilseeds that contain relatively low amounts of water (6). Although many reports have outlined use of supercritical CO_2 for large-scale extraction of oilseeds (7–9), only a few have addressed analytical-scale extractions (10,11), and none have addressed extraction of quantities suitable for direct fatty acid methyl ester (FAME) derivatization and gas-chromatographic analysis.

We now report an SFE procedure for quantitative extraction of pecan oil with CO_2, collection into chilled hexane, and subsequent fatty acid composition analysis. Sample sizes of 500 mg were utilized to determine extraction conditions needed for quantitative oil recovery, and were compared to results obtained by conventional organic extraction. The procedure was tested with sample sizes of 10 mg to address its utility for oil composition determination of samples small enough for FAME preparation and subsequent fatty acid analysis in the same vials used for oil collection.

EXPERIMENTAL PROCEDURES

Sample preparation. Food-grade native pecan halves were obtained from a local supplier and were stored sealed in a plastic liner at –80°C prior to use. One hundred-gram quantities of pecans were removed periodically (one-month intervals or less) for oil extraction. Samples were ground for one minute with a Waring blender (Waring, New Hartford, CT), sealed in glass jars, blanketed with nitrogen, and stored in a desiccator at –20°C to await extraction. Grinding samples for longer durations resulted in lower oil recovery from nutmeats, due to oil loss on grinder surfaces. The one-minute grinding duration was sufficient to reduce more than 90% of the sample to particle sizes of 0.3 mm or smaller, with the remainder present as larger (mostly >1 mm) pieces. The oil recovery (determined by organic solvent extraction) and fatty acid

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composition of oil obtained from the larger pieces were identical to those obtained from the smaller pieces, allowing us to remove and discard the large pieces prior to extraction. All samples were allowed to reach room temperature prior to opening, to prevent moisture condensation onto nutmeats. Nutmeat moisture content, determined periodically as previously described (1), ranged from 3 to 4% and was unchanged during the course of these experiments.

Organic solvent extraction. Extractions were performed as described in method 2 of Hubbard *et al.* (12) with some modifications. Pecan samples (1.00 g) were ground in 20 mL chloroform/methanol (2:1, vol/vol) for two 2.5-min durations in an Omnimixer equipped with a 50-mL grinding vessel (Omni International, Waterbury, CT). The extract was then filtered through a medium scinterred-glass funnel, rinsed with approximately 10 mL chloroform/methanol and placed into a 50-mL separatory funnel. A volume of aqueous 0.8% KCl (equal to about 25% of the total extract volume) (2) was added, vigorously shaken, and the chloroform layer was recovered. An equal volume of extraction medium was added to the aqueous layer and recovered two more times. The combined organic layers were then filtered though phase-separation paper (Type 1PS; Whatman International, Ltd., Maidstone, England) to remove all water, and rinsed with a minimum volume of the chloroform/methanol extraction solvent. The filtrate was then dried in a tared, round-bottom flask *in vacuo* to constant weight by means of a rotary evaporator at 40°C. In most cases, 75 min of rotary evaporation was adequate for solvent removal. Oil content was determined gravimetrically to the nearest mg.

SFE. The apparatus used for SFE consisted of a Dionex 703 extractor (Dionex Corp., Sunneyvale, CA) with 2.5-mL extraction vessels (7.9 mm × 50 mm) rated to 69 MPa (Keystone Scientific, Inc., Bellefonte, PA). Coleman-grade CO_2 with a 14 MPa He headspace and dip tube was obtained from Air Products (Air Products and Chemicals, Inc., Allentown, PA). Clean empty vessels were installed prior to extraction, and a blank extraction was conducted to purge the SFE system components of oil remaining from prior extractions. Samples of 500 or 10 mg were loosely packed into the extraction vessels between glass wool plugs, inserted at the inlet and outlet sides to retain the sample inside the cell. Extractions were carried out simultaneously in four extraction vessels at 69 MPa (final pressure) and 75°C with 250 mL/min restrictors for specified durations. At the beginning of each run, a two-stage ramp in pressure from 0 to 25 MPa and from 25 to 50 MPa, with durations of 2 min each, was necessary to prevent restrictor clogging by the extract. Restrictors were maintained at 150°C. The effluent from each extraction vessel was channeled into vials that contained 15 mL of chilled hexane (2°C). Expanded CO_2 flow rate and total flow were determined from on-board flow meters for each vessel. Flow rates ranged from 510 to 680 mL/min at 69 MPa.

Upon completion of extraction, the extracts were quantitatively transferred into tared two-dram vials with hexane and dried *in vacuo* with a Speed Vac sample concentrator equipped with an ultralow sample condenser and an organic vapor trap (Savant Inc., Farmingdale, NY). The Speed Vac system, as opposed to a rotary evaporator, was preferred because all extracted samples could be simultaneously dried in vials suitable for FAME preparation.

FAME preparation and analysis. Oil (1–2 mg) from the chemical extraction or from the 500-mg SFE was transferred into one-dram vials that contained 600 nmoles heptadecanoic acid as internal standard. The entire oil yield from 10-mg SFE samples (6–7 mg) was utilized for FAME preparation, and the amount of heptadecanoic acid was adjusted to the weight of oil. For each mg of oil, 200 μL of methanolic HCl (3% HCl in methanol, prepared by adding 2.8 mL acetic anhydride to 56 mL methanol) and 50 μL methyl acetate (as a water scavenger) were added. Vials were sealed with Teflon-lined caps and incubated for 2 h at 90°C. In preliminary experiments, we determined that this time period was necessary for complete fatty acid methylation as judged from response factors, obtained from standard methanolized fatty acids vs. their nonmethanolized derivatized counterparts, and from yields of fatty acids from extracted oils. It was necessary to mix the vial contents by vortexing during the first 15 min of the incubation period to assure equilibration of the oil samples into a single phase for methanolysis. After incubation, ten drops of tertiary butanol per mg of oil were added to co-evaporate the HCl, and samples were dried with N_2 gas. FAMEs were brought up into 300 μL hexane per mg oil, and a 1-μL aliquot was utilized for gas chromatography. Samples were methanolized, evaporated, and diluted for injection in the same vial.

Gas chromatography was conducted with a Tracor model 540 gas chromatograph (Tracor Instruments, Austin, TX), equipped with a split-injection port (split ratio of 50:1) and flame-ionization detector. Separations were performed on a DB 23 fused-silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness; J&W Scientific Inc., Rancho Cardova, CA) with helium carrier gas at a linear flow rate of 20 cm/s. The injector temperature was 275°C, and the detector temperature was 300°C. The initial column temperature was 50°C for 2 min. FAMEs were then separated with a linear temperature program from 50 to 180°C at 10°C/min, a hold at 180°C for 5 min, and a second linear temperature program from 180 to 240°C at 5°C/min and a hold at 240°C for a final 5-min period. Individual FAME peaks were identified according to coelution with an authentic standard (FAME preparation 2; Sigma Chemical Co., St. Louis, MO). Peak areas were obtained with a Spectra Physics 4990 integrator (Spectra Physics Inc., San Jose, CA) and quantitated relative to heptadecanoic acid as internal standard.

RESULTS AND DISCUSSION

Initial SFE experiments were conducted with 500-mg samples to determine the effect of extraction duration on oil yield (Fig. 1) and on oil extraction rate (Fig. 2) from ground pecans. Oil was recovered after four consecutive 5-min extractions (including an additional 4-min pressurization step, as indi-

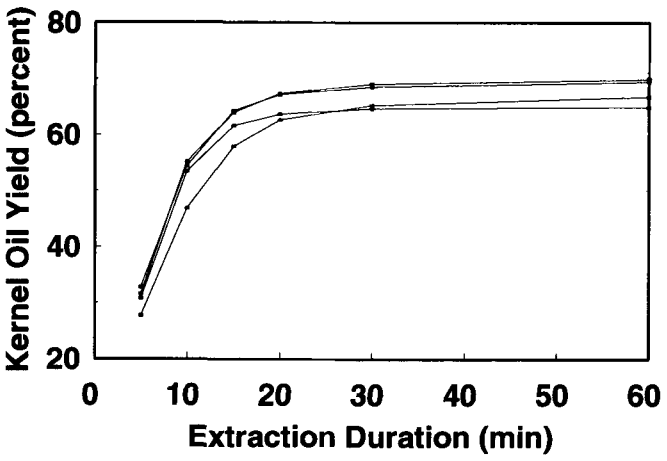

FIG. 1. Effect of extraction duration on kernel oil yield for ground pecans. Results indicate average oil yield for four simultaneous extractions of 500-mg samples. Average oil yield 65.7% (w/w).

cated in the Experimental Procedures section) during the first 20 min, followed by a 10-min extraction and a final 30-min extraction. Oil yield was exponential with respect to time, and all extraction vessels with at least 500 mL/min expanded CO_2 flow rates exhibited similar responses (Fig. 1). A flow rate below 500 mL/min indicated extraction cell frit, restrictor and/or exit port clogging, resulting in reduced oil extraction rate. Oil extraction was incomplete under severely limited CO_2 flow (200 mL/min or less). Essentially all (98–99%) of the oil could be recovered within the first 30 min of extraction. Extraction rates declined during the course of an extraction from 30 mg/min during the initial 5-min extraction period to less than 1 mg/min after a 30-min extraction period (Fig. 2). The high initial oil extraction rate caused considerable restrictor clogging (four out of eight restrictors became clogged within three 1-h extractions of 500-mg samples). The 4-min stepped pressure gradient was necessary and sufficient to prevent restrictor clogging.

Oil solubility [(wt oil/wt CO_2) × 100] also exhibited a decline during extraction from 2.8% during the first 5 min to 0.06% after a 30-min extraction period (Fig. 3). Our initial pecan oil solubility is about one-half of that reported for peanuts (5.5%) under similar temperature and pressure conditions (5), and is about three-quarters of the value reported for vegetable oil extracted at 69 MPa and 50°C (13). The lower oil solubility for our system may be due to low CO_2 flow and large sample particle size. Extraction pressure, temperature, sample moisture content, sample particle size, and supercritical CO_2 flow rate are all known to affect oil solubility (13). We utilized a pressure and temperature combination that had been used previously for quantitative oilseed extractions (10). Our sample moisture content of 3–4% did not vary during the course of these experiments, and is well within a range previously reported to have no effect on oil solubility in supercritical CO_2 (6). Our sample particle size of 0.3 mm and smaller was slightly larger than that recommended for the best extraction rates [0.25 mm and below (10)], but allowed for quantitative oil recovery when SFE results were compared with organic solvent extraction. We recognized that extraction efficiency could be enhanced by reducing sample particle size and tried various grinding durations, ranging from 0.5 to 5 min. Oil yields increased in samples ground for times up to one minute, but decreased when ground longer. We attribute the lower oil yields obtained with longer grinding durations to oil loss inside the grinding vessel. Supercritical CO_2 flow rate may have contributed to the decreased oil solubility. In preliminary experiments, we used 500 mL/min flow restrictors, which exhibited between 1.7 to 2 times more CO_2 flow than the 250 mL/min restrictors. Although we observed more rapid oil extraction of samples at these flow rates (40–50 mg/min; apparent extraction efficiency of 2.5%), quantitative oil recovery from the hexane solvent trapping system proved to be impossible due to oil loss through the CO_2 venting system. Lower recovery of oils and transfer of oils into the flow detection tubing led to plugging of the vent

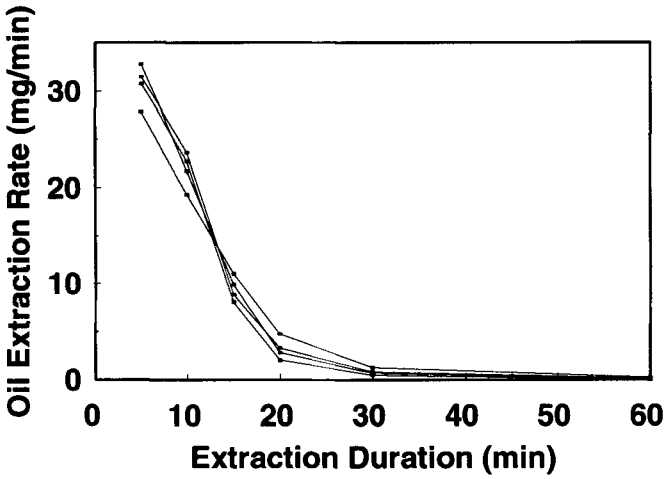

FIG. 2. Effect of extraction duration on pecan oil extraction rate. Results indicate average extraction rate for four simultaneous extractions of 500-mg samples.

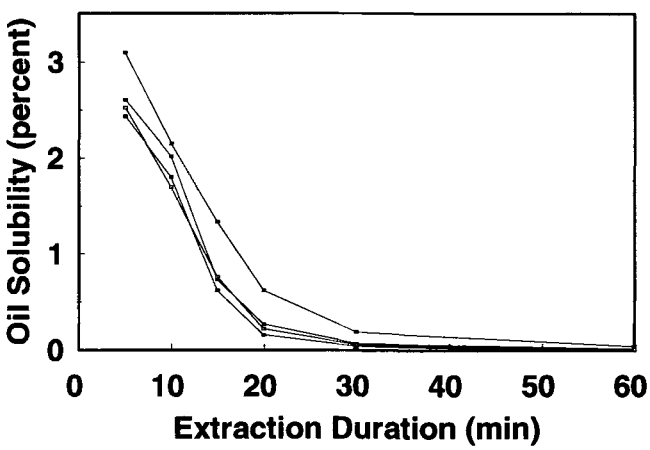

FIG. 3. Effect of extraction duration on pecan oil solubility in supercritical carbon dioxide (CO_2). Results indicate average percent oil solubility (oil wt/CO_2 wt × 100) for four simultaneous extractions of 500-mg samples.

TABLE 1
Percent Oil Recovery from Ground Pecans Extracted with Supercritical Fluid Extraction (SFE) and with Organic Solvent[a]

Extraction number	SFE		Organic solvent	
	Pecan oil content	Percent recovery of added oil	Pecan oil content	Percent recovery of added oil
1	67.1		62.6	
2	65.8		65.2	
3	64.7	101	63.8	98
4	66.7	101	63.5	100
5	63.7	98	64.7	100
6	66.5	103	64.5	101
Mean	65.7		64.3	
SD	2.5		1.8	

[a]All results represent the mean for four extractions, and oil contents are expressed in weight percent.

needles. Replacement of the hexane solvent trap with glass wool packing in the oil collection vials resulted in 10–15% oil loss, which also caused excessive plugging of vent needles. We observed little to no oil loss into the CO_2 venting system when using the 250 mL/min flow restrictors in combination with the chilled hexane solvent trap, making quantitative oil recovery possible.

The quantity of oil recovered from ground pecans, expressed on a weight-percent basis by SFE is in good agreement with results from the chloroform/methanol organic solvent procedure (Table 1), and is similar to published pecan oil content (1). The SFE procedure required less time than the organic solvent extraction procedure (1 h vs. 4 h), and solvent evaporation was simpler and more rapid (all samples could be processed at once with the Speed Vac system, whereas samples had to be dried individually with the rotary evaporator). The low boiling point of hexane made solvent evaporation relatively rapid, but also made it necessary to apply vacuum to samples slowly to prevent excessive boiling and subsequent sample loss during the initial phase of drying. We tested the chloroform/methanol extraction solvent as an alternate trapping solvent for pecan oil recovery. This solvent provided a suitable collection solvent, but removal of chloroform with the Speed Vac was slow, requiring 24 h or more for complete removal. Hexane could be removed with the same system within 6–7 h and had the additional advantage of decreasing from 15 mL initial volume to about 3 mL ending volume after a 1-h SFE extraction period. Including sample preparation, extraction, and solvent evaporation, the SFE procedure required 7–8 h, whereas the organic solvent extraction procedure required 2–3 d, to process the same number of samples.

To assure complete oil extraction with either technique, we also added oil to ground pecan samples (equal to one quarter of the expected sample oil yield) and determined oil recovery (Table 1). Oil recoveries ranged from 98 to 103%, indicating that both techniques were quantitative.

The fatty acid composition of samples was determined by gas–liquid chromatography analysis of methyl esters prepared from the total lipids (Table 2). We investigated conditions needed for completion of fatty acid methyl esterification and found that a minimum of 1 h at 90°C was necessary to obtain maximum yields from authentic fatty acid standards, and that a minimum of 2 h was necessary for maximum yields from pecan oil samples. Continuation for up to 24 h resulted in no degradation in response. Oleic (65–66%) and linoleic (25–26%) acids were the major fatty acids present, making up about 90% of the total fatty acids on a weight-percent basis. The fatty acid compositions of oils extracted by organic solvent or SFE were essentially identical. Our fatty acid compositions were similar to those previously reported for pecan (2). The fatty acid composition for the total lipid fraction obtained by SFE did not change during the course of the 1-h extraction period. SFE sample size appeared to have little to no effect on fatty acid composition.

One of the objectives of this work was to develop a rapid procedure for quantitative oil extraction of pecans in quantities suitable for direct FAME derivatization of the extract. To this end, we attempted extraction of sample sizes small enough for derivatization of the entire sample extract, yet large enough for reliable gravimetric determination of oil content. We settled on 10-mg samples because they were within the analytical range of many common analytical balances, and the expected oil yield (6–7 mg) was suitable for FAME preparation in the same vials used for gravimetric oil yield determination. The oil recoveries for such samples were essentially identical to those illustrated in Table 1 for 500-mg samples. In preliminary extractions, we obtained results that indicated higher oil yields than previously found for 500-mg extractions and attributed the increased oil weight to residual

TABLE 2
Fatty Acid Composition of the Total Lipids from Pecan Samples Extracted by SFE and Organic Solvent Extraction

Extraction method	Extraction weight (g)	Fatty acid percentages (%, w/w)[a,b]				
		16:0	18:0	18:1	18:2	18:3
SFE	0.50	5.1	2.2	66.0	25.6	1.1
SFE	0.01	6.1	2.8	65.4	24.6	1.0
Organic solvent	1.00	5.2	2.4	65.3	25.9	1.2

[a]Fatty acids: 16:0 (palmitic), 18:0 (stearic), 18:1 (oleic), 18:2 (linoleic), 18:3 (linolenic). Abbreviation as in Table 1.
[b]Results represent the mean of duplicate determinations for oils recovered from eight extractions.

oil from prior extractions. We adopted the practice of precleaning the system with a blank SFE run to remove any residual oil from the previous run. Fatty acid compositions for oils obtained from 10-mg extractions were similar to oils obtained by using either SFE of 500-mg samples or chemical oil extracts.

Oil content for oilseed has been determined by SFE for sample sizes as small as 3.0 g (10). We measured oil content with ground pecan sample sizes of 500 mg and 10 mg and present a simplified procedure for FAME preparation by using either a subsample (500-mg extractions) or the entire (10-mg extraction) extract. Quantitative recovery of oil, as determined by comparison to a standard organic solvent extraction procedure and by recovery of added oils from extracts, was possible with chilled hexane as collection solvent, followed by solvent evaporation in a Speed Vac sample concentrator. Extractions could be completed in as little as 30 min with recovery of 98–99% of the total oil. For a flow restrictor-based SFE system, we found it essential to include a stepped-pressure gradient at the beginning of each extraction to prevent restrictor clogging. The FAME preparation procedure was demonstrated for determination of fatty acid compositions in the total lipid fractions. The method should also be applicable for analysis of fatty acid composition of the different acyl lipid classes.

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