Pressurized Liquid Extraction of Polar and Nonpolar Lipids in Corn and Oats with Hexane, Methylene Chloride, Isopropanol, and Ethanol

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ABSTRACT: Samples of freshly ground corn kernels and freshly ground rolled oats were extracted *via* pressurized liquid extraction (accelerated solvent extraction) using four different organic solvents [hexane, methylene chloride (also known as dichloromethane), isopropanol, and ethanol] at two temperatures (40 and 100°C). Lipid yields varied from 2.9 to 5.9 wt% for ground corn and from 5.5 to 6.7 wt% for ground oats. With ground corn, more lipid was extracted as solvent polarity was increased, and for each individual solvent, more lipid was extracted at 100°C than at 40°C. With ground oats, the same temperature effect was observed, but the solvent polarity effect was more complex. For both corn and oats, methylene chloride extracted the highest levels of each of the nonpolar lipid classes. In general, for both corn and oats, increasing solvent polarity resulted in increasing yields of polar lipids, and for each solvent, more of each lipid class was extracted at 100°C, than at 40°C. Among the lipids in corn extracts, the phytosterols may be the most valuable, and total phytosterols ranged from about 0.6 wt% in the hot ethanol extracts to about 2.1 wt% in the hot hexane and methylene chloride extracts. Total phytosterols in all oat extracts were about 0.1 wt%. Digalactosyldiacylglycerol was the most abundant polar lipid in the oat extracts; its levels ranged from 1.6 wt% in the cold hexane extracts to 4.3 wt% in the hot ethanol extracts.

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KEY WORDS: Accelerated solvent extraction, ASE, corn, lipids, oatmeal, oats, pressurized solvent extraction, PSE.

Various methods are available for the solvent extraction of lipids from biological samples. A recent report (1) compared the extractions of several common oilseeds (soybean, rapeseed, and sunflower) using supercritical CO_2 extraction, accelerated solvent extraction, microwave-assisted extraction, solid fluid vortex extraction, Soxhlet, and Soxtherm extraction (petroleum benzene was used as the solvent in the latter five methods). The comparative data for the six extraction methods included oil content, tocopherol content (total and individual tocopherols), FFA, and DG (1). Recently we reported high levels of two polyamine conjugates (diferuloylputrescine and *p*-coumaroyl-feruloylputrescine) in corn kernels and exceptionally high levels of them in corn bran that were extracted with hot polar solvents [methylene chloride and isopropanol (IPA)] using pressurized solvent extraction (2). Reports on other uses of pressurized solvent extraction (accelerated solvent extraction) have included measurement of herbicides in soybeans (3), determination of polyphenolics in cider apples (4), extraction of taxols (5), extraction of five common medicinal plants (6), extraction of phenols, sterols, and carboxylic acids in environmental and biomass samples (7), and determination of oxysterols in egg-containing foods (8). Based on our recent finding of the significant increase in extractability of polyamine conjugates with polar solvents at elevated temperatures (2), the current study was undertaken to compare the various types of nonpolar and polar lipids that are extracted with four common organic solvents by pressurized solvent extraction of corn and oats.

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MATERIALS AND METHODS

Plant material. Corn kernels (yellow dent hybrid Pioneer 3394) grown during the 2000 growing season at the Agricultural Engineering Farm, University of Illinois at Urbana–Champaign, were field dried to 14–16% moisture content, harvested, and stored at 4°C. Rolled oats were purchased at a local market. Both were ground to 20 mesh in a Wiley mill (Thomas Scientific) immediately before extraction.

Extraction. All extractions were performed on a Dionex ASE 200 Accelerated Solvent Extractor (Sunnyvale, CA) using 11-cc extraction vessels. Samples (2 g) were mixed with an equal volume of sea sand and placed in the extraction vessel. The remaining dead volume was filled with sea sand. The extraction cell was fitted with a cellulose filter at the inlet and stainless steel frit at the outlet. The conditions for extraction were: pressure 1000 psi, temperature of 40 or 100°C, heating period of 5 min, extraction (static) time of 10 min, with three static cycles per sample, and a total recovered solvent volume of 22 mL. For lipid yield data, the entire solvent extract was dried under a gentle stream of nitrogen at 40°C, and the mass was measured on an analytical balance.

HPLC of nonpolar lipids. Nonpolar lipid HPLC analyses were performed using a DIOL column employing the method of Moreau *et al.* (9). With this method, the retention times of the nonpolar lipids were as follows: phytosterol fatty acyl

esters (1.8 min), TAG (4 min), free linoleic acid (11 min), γ-tocopherol (20 min), free phytosterols (22 min), and ferulate phytosterol esters (29 min).

HPLC of polar lipids. Polar lipid HPLC analyses were performed on a Hewlett-Packard Model 1100 HPLC, with autosampler, and detection by both an HP Model 1100 diode-array UV-visible detector (Agilent Technologies, Avondale, PA) and a Sedex Model 55 Evaporative Light Scattering Detector (Richard Scientific, Novato, CA), operated at 40°C and a nitrogen gas pressure of 2 bar. The column was a LiChrosorb, 3 mm diameter and 100 mm length, 7 µ DIOL (Chrompack, Raritan, NJ). The ternary gradient had a constant flow rate of 0.5 mL/min, with Solvent A = hexane/acetic acid, 1000:1; Solvent B = IPA; and Solvent C = water. Gradient timetable: at 0 min, 90:10:0 (%A/%B/%C); at 30 min, 58:40:2; at 40 min, 45:50:5; at 50 min 45:50:5; at 51 min, 50:50:0; at 52 min, 90:10:0; and at 60 min 90:10:0. With this method the retention times of the polar lipids were these: acylated sterol glycosides (5 min), sterol glycosides (SG; 11 min), PE (21 min), digalactosyldiacylglycerol (DGDG: 24 min), and PC (30 min). All experiments were performed at least two times with triplicate extractions for each data point. The data presented in the figures are means, and the error bars indicate SD.

RESULTS AND DISCUSSION

Lipid yields for ground corn varied from 2.9 to 5.9 wt%, and for ground oats from 5.5 to 6.7 wt% (Fig. 1). With ground corn, more lipid was extracted as solvent polarity was increased, and for each individual solvent, more lipid was extracted at 100°C than at 40°C, especially with IPA and EtOH. With ground oats the same temperature effect was observed, but the solvent polarity effects were minor.

Quantitative analysis of the six major nonpolar lipids in corn extracts (Fig. 2) revealed that the highest levels of TAG were extracted with methylene chloride at 100°C, with the next highest levels extracted with five conditions: methylene chloride at 40°C, hexane at both temperatures, and IPA at both temperatures. Lower levels of TAG were extracted with ethanol. Comparison of the levels of extraction of the three phytosterol lipid classes (acyl esters, free, and ferulate esters) revealed that the highest levels were extracted with methylene chloride at 100°C, followed by the same solvent at 40°C. Slightly lower levels of total phytosterols were extracted with hexane, followed by IPA, and finally ethanol. Among the lipids in the corn extracts, the phytosterols may be the most valuable, and total phytosterols ranged from a low of about 0.6 wt% (calculation based on 100 g kernels yielding 35 mg total phytosterols and 5900 mg oil) in the hot ethanol extracts to a high of about 2.1 wt% in the hot hexane and methylene chloride extracts.

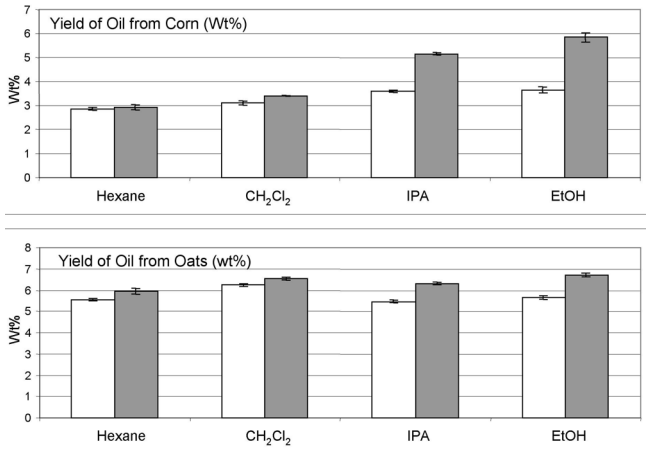

FIG. 1. Yield of oil from corn and oats. The white bars represent the 40°C extraction and the gray bars represent the 100°C extraction, for each solvent. Abbreviations: CH_2Cl_2, methylene chloride; IPA, isopropyl alcohol; EtOH, ethyl alcohol. Error bars represent SD.

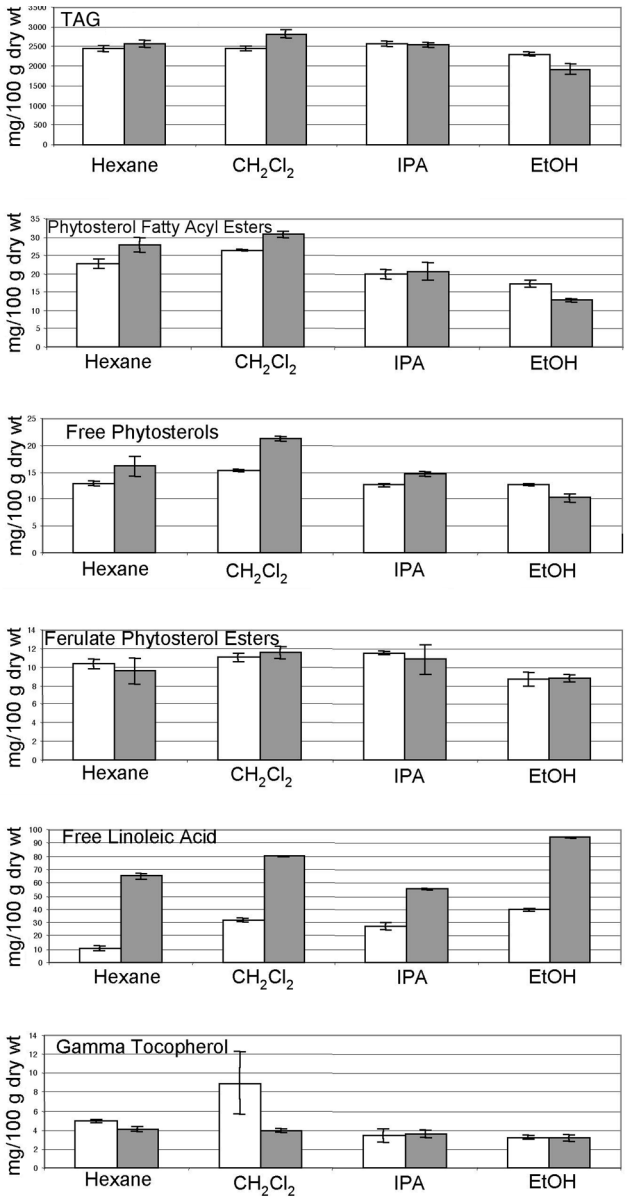

FIG. 2. Nonpolar lipids in corn extracts. For temperature designations and abbreviations, see the legend to Figure 1.

Increasing the solvent temperature from 40 to 100°C resulted in severalfold higher levels of free linoleic acid with each of the four solvents; and among the four solvents, the ethanol extraction had the highest yields of linoleic acid. Free oleic and palmitic acids were also present in the extracts at lower levels, and identical solvent and temperature effects were observed for them.

In corn, the γ isomer is the most abundant tocopherol, and although the highest mean values were observed with the 40°C methylene chloride extraction, the SD for this treatment was quite high. A conservative interpretation is that very similar levels of γ-tocopherol were extracted with all eight extractions. Previously, we reported that heat pretreatment of corn fiber, prior to extraction, caused a 10-fold increase in the levels of γ-tocopherol in the hexane extracts (10). Increasing solvent temperature did not cause a significant increase in γ-tocopherol in the extracts of any of the solvents.

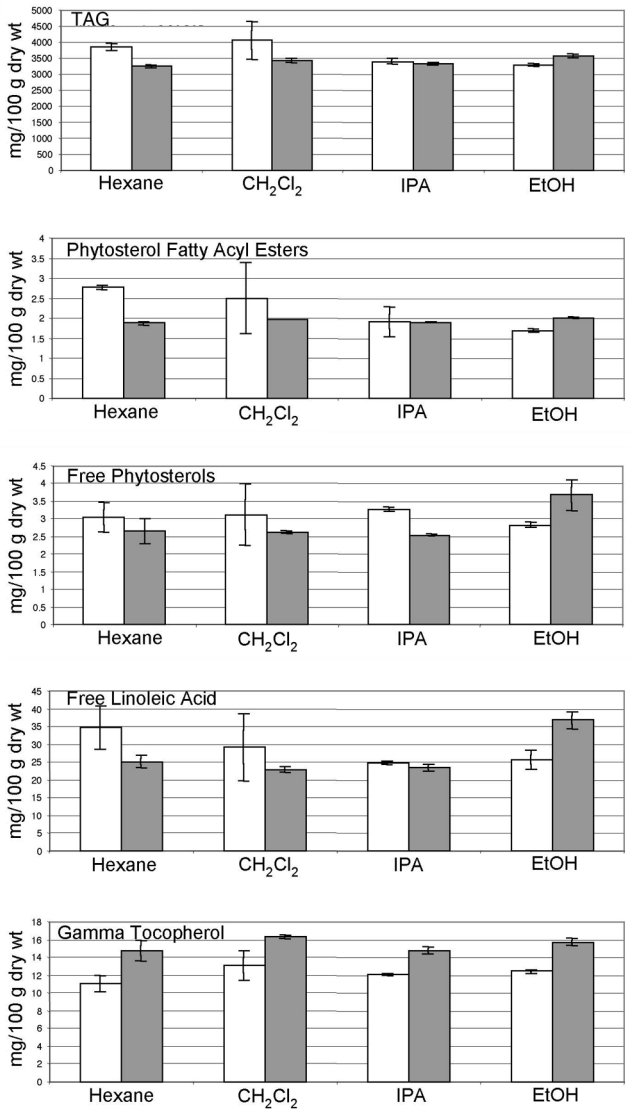

FIG. 3. Nonpolar lipids in oat extracts. For temperature designations and abbreviations, see the legend to Figure 1.

Quantitative analysis of the five major nonpolar lipids in oat extracts (Fig. 3) revealed that the highest levels of TAG were extracted at 40°C with both hexane and methylene chloride, with nearly identical TAG levels extracted with the other six treatments. Oats have nearly 10-fold lower levels of free and fatty acyl phytosterols (only about 0.08 wt% total phytosterols, a value that was nearly constant for all extraction conditions) than corn and have no ferulate esters. Most of the solvent–temperature effects were minor, but there did appear to be higher levels of several of the phytosterols at 40°C than at 100°C with hexane, methylene chloride, and IPA. Examination of free linoleic acid levels revealed a temperature enhancement with ethanol, but no clear trends with the other six treatments. The levels of γ-tocopherol were about two times higher in oat than in corn extracts, and there was a significant temperature enhancement with each solvent, but no significant difference among the four solvents at the same temperature.

Examination of the three major polar lipids in corn extracts (Fig. 4) revealed that the 40°C hexane extract contained no detectable SG, and very low levels of PE and PC. The general trend was that increasing solvent polarity increased the levels of the three polar lipids in the extracts. Also, with each solvent, more of each polar lipid was extracted at 100°C than at 40°C. Previously, Hojilla-Evangelista *et al.* (11) developed a "sequential extraction process" (SEP) to extract proteins and other components from corn kernels, and the first step in the sequence involved extraction of the flaked kernels with hot ethanol. Although these authors did not report the composition of their SEP "kernel oil," its composition was probably similar to that of the ethanol extracts in this current report.

Examination of the five major polar lipids in oat extracts (Fig. 5) revealed unique effects for DGDG: significant levels in the 40°C extract (1.6 wt%); higher levels of extraction with solvent polarity increase; and a temperature enhancement for each solvent (with the highest levels in extracts being about 4.3 wt%, with the 100°C ethanol extraction). Among the other four polar lipid classes in oat extracts, increasing solvent polarity increased yields of each lipid class, and temperature enhancement was observed for each lipid class with each solvent.

The stability of lipids at high temperatures is a concern. Palma *et al.* (12) studied the effect of extraction with superheated solvents on the stability of several phenolic compounds, and they reported low rates of degradation of common phenolics at 100°C and moderate levels of degradation at 150°C. In this study we did not observe any clear evidence of lipid instability during 100°C extractions.

Comparison of the overall solvent and temperature effects for corn revealed that, if the goal is to extract the three nonpolar phytosterols, hexane and methylene chloride are the preferred extraction solvents. Higher levels of the three most abundant polar lipids in corn were extracted with polar solvents, but these do not appear to have much commercial value at this time. However, for oats, the high levels of DGDG (and

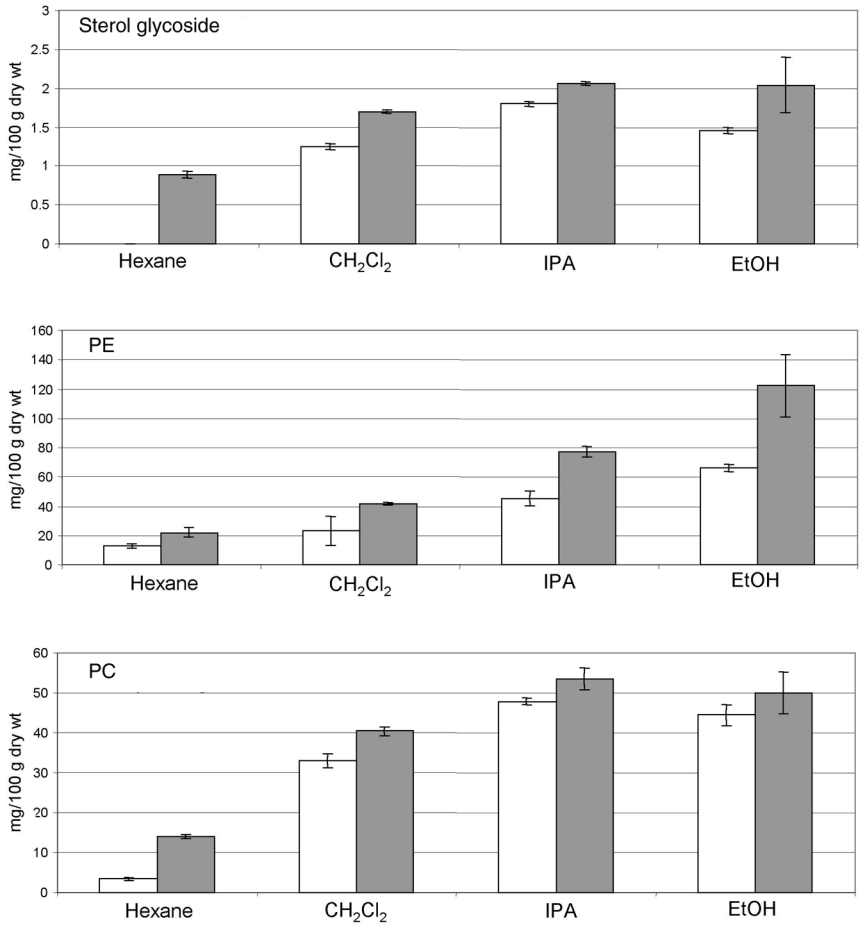

FIG. 4. Polar lipids in corn extracts. For temperature designations and abbreviations, see the legend to Figure 1.

perhaps other glycolipids) in all oat extracts appear to produce a unique product, especially since there is recent evidence that the polar lipids (of which DGDG is the most abundant) in oat oil may impart to it beneficial baking properties (13). The current study revealed that if the goal is to obtain oat oil with the highest levels of DGDG, then the 100°C ethanol extraction is clearly advisable. Comparison of the extraction effects with seeds of two different species of grains indicated that the extractability is greatly dependent on the polarity and temperature of the solvent, but the complex matrix of each plant material also contributes to the extractability of some lipids.

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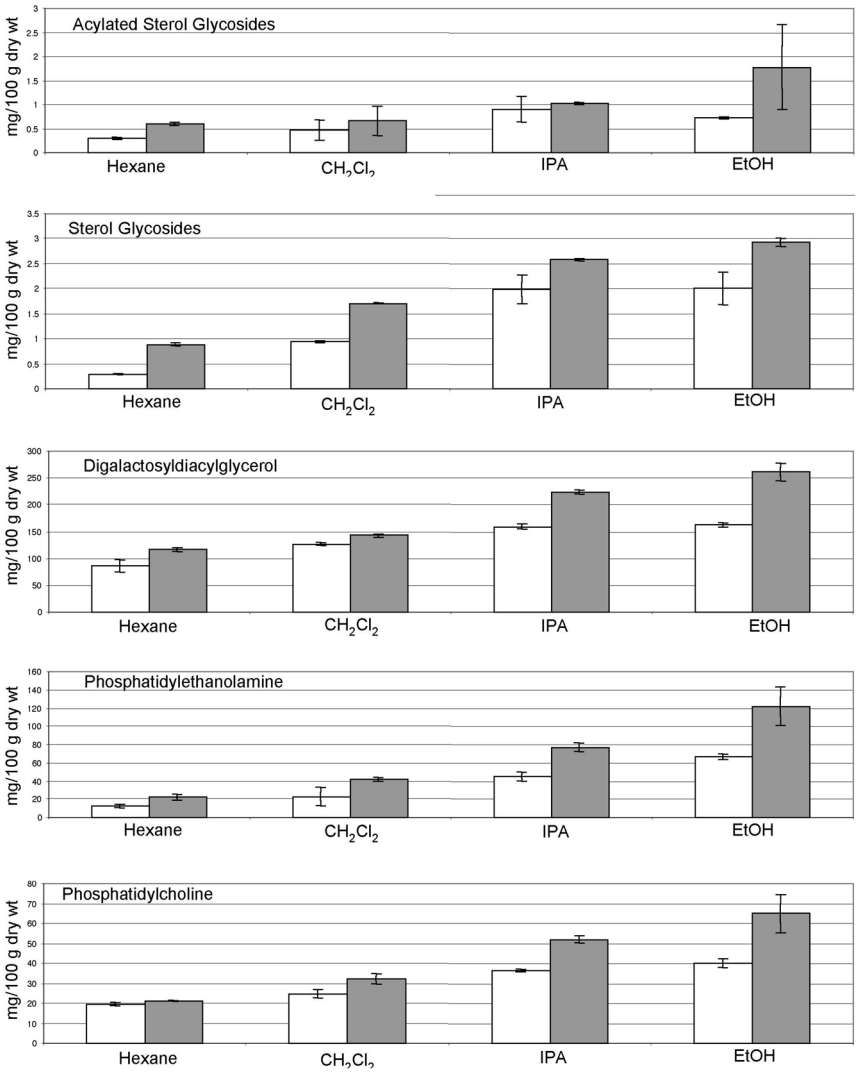

FIG. 5. Polar lipids in oat extracts. For temperature designations and abbreviations, see the legend to Figure 1.

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