A Solvent Extractor System for the Rapid Extraction of Lipids and Trace Bioactive Micronutrients in Oilseeds

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ABSTRACT: A low-cost laboratory extractor has been designed and constructed that selectively extracts polar and nonpolar components from oilseeds and other matrices. The extractor uses available high-performance liquid chromatography laboratory equipment for pumping the solvent into the extractor. Pressure, temperature, and valving arrangements are automatically controlled by commercially available components. Advantages of this system include low initial investment, reduced solvent consumption, shorter extraction times, quantitative lipid recovery, use of multiple extraction solvents, and reduction in cost per sample. The method has broader applications that include extraction of trace components from a variety of matrices, for example, the extraction of pesticides from foods and polychlorobiphenyls from soil. Class separation of components from different matrices can be achieved easily by selection of solvents with the appropriate polarity characteristics. Very small samples can be extracted simply by changing cell size or by adding an inert material to the cell to fill the void volume. Analyte collection can be accomplished by collecting in a test tube with an appropriate solvent, or on a solid-phase material. Optimization of extraction times, number of extractions, matrices, and solvent used is described. Neutral lipids were extracted from peanut meal in 70 min by the rapid extraction method compared to 1440 min required to extract the comparable amount of neutral lipids from a similar sample by the Soxhlet extraction method.

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KEY WORDS: Design, equipment, evaluation, matrix, oil, quantitation, rapid extraction, solvent.

Large amounts of organic solvents are normally required to extract certain analytes from solid matrices. Typical solvent volumes can vary from 100 to 800 mL, depending on the matrices, mass, analytes of interest, and extraction technique. Recently, there has been renewed interest in decreasing extraction times and reducing solvent consumption due to cost, environmental concerns, and regulations imposed by the Resource Conservation and Recovery Act. This act restricts the number of analyses that can be performed by laboratories using solvent-intensive methods such as Soxhlet extraction, separatory funnel, automated Soxhlet extraction, and sonic extractions, as described in the U.S. Environmental Protection Agency reports. Some of the accepted extraction techniques are discussed below.

Oilseeds consist of a complex mixture of triglycerides, diglycerides, monoglycerides, phospholipids, and other polar micronutrients. These components are important to the food industry because of the chemical properties and health benefits they impart to food products (1). The various lipid classes found in oil seeds vary in polarity, and quantitative lipid recovery requires polar solvents. To extract trace micronutrients from legumes and oilseeds, long extraction times and large volumes of polar solvents have been required. Organic solvents used for lipid extraction are hexane, methanol, ethanol, isopropyl alcohol, diethylether, methylene chloride, pentane, petroleum ether, and chloroform. Numerous combinations of these solvents have been used to extract lipids from many different matrices.

A combination of chloroform/methanol/water was found to be the best solvent combination to extract total lipids from fish meal using a sonication method of extraction (2). However, the use of chloroform-based solvents is now being discouraged because chloroform is believed to be a carcinogen (3,4). For total lipids, chloroform-based solvents have been found to be the best for most matrices.

Numerous extraction techniques have been used to extract analytes from solid samples. Sonication is an effective extraction technique but requires large amounts of solvent and is difficult to automate. Soxhlet extraction requires large amounts of solvent and lengthy extraction times that can be anywhere from 16 to 24 h. This time requirement makes it labor-intensive and limits the number of samples that can be processed.

Supercritical fluid extraction (SFE) replaces hazardous solvent consumption and is environmentally safe because it uses carbon dioxide as the extractant. However, there are several disadvantages to SFE. CO_2 is nonpolar, which limits it uses; the extracted oil is less stable and subject to oxidation, and initial cost and maintenance of equipment are high (5–7).

Microwave extraction reduces solvent consumption and generally affords complete extraction (8,9). However, this method becomes labor-intensive because samples must cool before further processing, and it is difficult to automate. The

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analyst must then filter, centrifuge, or decant the samples to remove the solvent from the solid material. Time saved due to the fast microwave extraction is lost in the cooling process, re-extraction, and preparation of the analyte for further analysis.

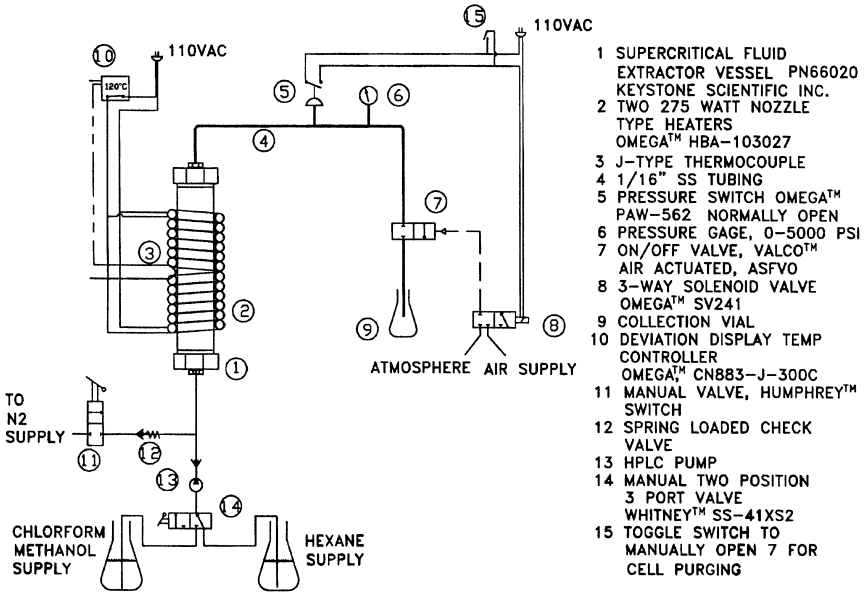
Another extraction technique that is gaining widespread attention is accelerated solvent extraction (ASE) (10). This technique uses pressure and temperature to extract solid samples. It greatly reduces extraction time and solvent consumption. Automation or semiautomation can be achieved to extract analytes from different matrices. Static or dynamic extractions can be performed separately or in combination. Even though commercial units are available, the cost of these instruments is prohibitive for most laboratories. Therefore, there is a need for an affordable, efficient laboratory extractor for lipids and other analytes from solid materials. An extractor that employs heat and pressure can significantly reduce solvent usage, cost per sample, and extraction time.

This paper describes the design and evaluation of a versatile, inexpensive laboratory extractor for the removal of lipids and polar components from solid materials. This extractor has been interfaced with existing laboratory equipment capable of using multiple solvents for selective extraction of compounds from solid materials. This unit has a temperature range from ambient to 300°C and a pressure range from atmospheric to 5,000 psi. Extraction times and solvent consumption have been significantly reduced for extracting lipids and trace polar compounds.

MATERIALS AND METHODS

Materials. Solvents used in the extraction and analysis of peanut lipids were high-performance liquid chromatography (HPLC)-grade and were obtained from Fisher Scientific (Fair Lawn, NJ). Reference phospholipid standards were obtained from Sigma Chemical Co. (St. Louis, MO) . The stainless steel extraction cell was obtained from Keystone Scientific, Inc. (Bellefonte, PA). The band heaters and temperature controller were obtained from Omega Corp. (Stamford, CT). Peanuts were obtained from the Peanut Research Station (Lewiston, NC). Soyflour and potato chips used for evaluation of the extractor were obtained from local markets.

Description of extraction apparatus. A diagram of the extraction apparatus, including associated plumbing and electrical circuitry, is shown in Scheme 1. The 32-mL stainless steel extractor vessel (#1) has a pressure rating of 10,000 psi. Two band heaters (#2) were placed over the stainless steel extractor vessel leaving enough room to allow the removal of both ends of the vessel, to remove spent material and refill the vessel with new material to be extracted. A thermocouple (#3) was placed into a short piece of 1/8-in. copper tubing that was placed around the extraction vessel between the two band heaters and connected to a deviation display temperature controller (#10). Both band heaters were connected to the same controller. These heaters have a temperature range of ambient to 300°C. The exit end of the extraction vessel was plumbed into a pressure switch (#5) and then into a pressure gauge (#6) and finally through a static valve (#7) to the receiving flask (#9). Solvent was supplied to the extraction vessel by a single-piston Waters (Milford, MA) HPLC pump (#13). The pump input line was fitted with a two-position three-way valve (#14) fed from the solvent supply flask. A nitrogen supply was attached to the extraction vessel between the pump and vessel *via* a spring-loaded check valve (#12). This nitrogen supply was used to blow out the residual solvent at the end of the extraction process. A manual valve (#11) was used to turn the nitrogen supply on and off. The pressure limit of the vessel was controlled by a pressure switch (#5) set at 3,000 psi. Solvent was pumped into the cell with the exit cir-



SCHEME 1

cuit (static valve #7) manually open until all the air was flushed out. The static valve (#7) was then manually closed and solvent was pumped until the pressure reached 2,700 psi, at which time the pump was stopped. The band heaters were then used to heat the cell to 120°C. During heating, thermal expansion of the solvent causes the pressure to increase. When the pressure exceeded the 3,000 psi set pressure limit, the on/off static valve (#7) was opened and closed (pilot operated) by a three-way solenoid pneumatic valve (#8). Extracted analyte was released into the collection vessel (#9). At the end of the extraction time, a manual toggle switch (#15) was used to release the extractant and then purge the extraction vessel with fresh solvent. This switch was connected in parallel with the pressure switch (#5) and allowed manual operation of the static valve (#7). The components of the extraction system were connected with 1/16-in. stainless steel tubing (#4).

Determination of cell heat-up time. In the initial design stages, temperature vs. time was predicted analytically using a 2-D axisymmetric finite element model of the extractor cell. The model included the stainless steel cell with hexane filling the volume. This was done with Ansys® software (Cannonsburg, PA) by using a plane 75-element type and two sets of material properties, one for the stainless steel cell and one for the hexane volume. All model nodes were set to an initial temperature of 23°C. The temperature of the outer surface nodes (where the band heaters were in contact) was varied using multiple load steps to simulate the rise of the band heaters during the heat cycle. The stainless steel portion of the model was assumed to have a density of 0.28 lbm/in.^{3} (pounds mass per cubic inch), a specific heat of 0.2 BTU/(lbm-°C), and a thermal conductivity of 0.25 BTU/(min-in.-°C). Hexane was assigned a density of 0.0238 lbm/in.^{3} and a specific heat of 0.957 BTU/ (lbm-°C).

Because of the importance of allowing for adequate heat-up time an experimental measurement of temperature vs. time at the cell centroid also was done as explained below.

The outlet tubing in the top of the cell was temporarily removed. The compression fitting in the end of the cell was modified by drilling a 1/8-in. diameter through-hole. A 1/8-in. diameter stainless steel tube was welded in this fitting so that its length extended down to the centroid of the cell volume. This tube was open at the top but welded shut at the lower end. The tube and compression fitting thus sealed in the high-pressure solvent and formed a well into which a thermocouple could be inserted. This arrangement positioned the thermocouple on the centerline of the cell near the volume centroid. The thermocouple was inserted along with thermal (heat sink) grease to ensure good thermal contact. The thermocouple was connected to a data acquisition system driven by LabVIEW™ (National Instruments Corp., Austin, TX) software on a laptop PC. Hexane was pumped into the cell, the temperature controller for the band heaters was turned on, and the temperature setpoint was adjusted to 120°C. Temperature readings were taken once per second and written to a file for plotting later *via* a spreadsheet. The cell was then cooled and reloaded with hexane and peanut meal. The temperature measurements were taken again as described above. Peanut meal, soyflour, and potato chips were used as materials to evaluate the extraction efficiency of the accelerated extractor.

Sample preparation. Peanut seeds were ground in a Waring blender and/or a coffee grinder to as small a particle size as possible without producing peanut butter and placed in a sealed plastic bag and stored in the freezer. Soyflour, which was already milled very fine, was used from the package. Potato chips were ground into particle size by using a mortar and pestle prior to being placed in the extraction cell. Approximately 10 to 11 g of each material was used for each extraction.

Rapid extraction of different matrices. Peanut meal was loaded into the cell and hexane was pumped into the extraction cell with an HPLC pump using a set flow rate of 6 mL/min. The temperature was raised to 120°C. Heating of the extraction cell causes expansion of the solvent, and the system automatically opens static valve #7 at 3,000 psi. The pressure equilibrates and controls at approximately 2,700 psi. Pressure inside the extraction cell was monitored with a pressure gauge. As the static valve automatically released pressure, the excess solvent plus extracted oil were collected in a flask. The cell heat-up time allowed was 10 min, and the static extraction time was 15 min. In this study four static extractions were used. At the end of each static extraction the pressure was released by a manually switched solenoid valve which opened the static valve, and the extracted oil was collected in a flask. Four static extractions were made on each sample. A 100% flush volume of hexane was used after each static extraction and a nitrogen blowout time of 2 min was used after the last static extraction. Potato chips and milled soyflour were extracted as described above. The hexane lipid solutions from each sample were placed in tared beakers and the solvent was removed under a fume hood. The extracted lipid was weighed, and the percentage oil extracted was calculated. Five replications were made on each of the materials extracted. To determine the amount of oil extracted for each static extraction, the collection flask was changed after each static extraction. The amount of oil extracted as a function of time was determined as explained above.

Soxhlet extraction. Oil was extracted from peanut meal, soyflour, and potato chips by Soxhlet extraction (standard extraction technique) and compared to the rapid extraction method. The sample size for each product was the same as used for accelerated extraction. The prepared samples were placed in an extraction thimble and the loaded thimble placed into the Soxhlet extractor. Six hundred milliliters of hexane was placed in a 1,000-mL flask. Heat was applied to the flask and the extraction continued for 24 h. Solvent was removed by rotary evaporation and the lipid sample placed in a tared beaker and weighed to determine the amount of oil extracted. Five replications were made on each material extracted.

Extraction and HPLC separation of peanut phospholipids. The nonpolar lipid fraction was first removed from the peanut meal by accelerated extraction using hexane. The solvent was

switched to chloroform/methanol (2:1) and the polar fraction extracted. A portion of this fraction was injected onto a HPLC silica column (250 × 2.1 mm). Phospholipids were separated on a silica column using two binary solvent mixtures [solvent A: 2-propanol/hexane (4:3, vol/vol); solvent B: 2-propanol/hexane/water (8:6:1.5, vol/vol/vol)] and were detected using an ultraviolet (UV) detector at 210 nm. A gradient program was used for the separation starting at 100% solvent A and ramping to 100% solvent B in 20 min at a flow rate of 1 mL/min.

Extraction of other peanut bioactive compounds. Bioactive compounds were extracted from peanut meal and peanut hearts using different combinations of polar solvents. After the removal of lipid material using hexane in the extractor, a valve was switched and a polar solvent was pumped into the extraction cell. Methanol, chloroform/methanol (2:1), and 99% methanol were used. Extracts were reduced in volume and analyzed by HPLC/fast atom bombardment (FAB) mass spectrometry and/or by direct-probe FAB mass spectrometry. The solvent system used for HPLC/FAB mass spectrometry was the same as used for the separation of molecular species of peanut phospholipids (13).

RESULTS AND DISCUSSION

Temperature profiles. Figure 1 contains the analytical and experimental temperature profiles for the extraction cell heat-up times. Curve A shows the predicted temperature vs. time for the finite element model at a node near the centroid of the hexane volume. The 6-min heat-up time is longer than the actual experimental times shown in Curve B and Curve C. The finite element model did not account for any convective flow currents within the solvent during heating; this lengthened the predicted time to temperature. Also, it was difficult to estimate the actual heater band surface temperature vs. time used as the boundary condition in the model. Any underestimate of this temperature rate of rise would result in a conservative temperature rise time prediction.

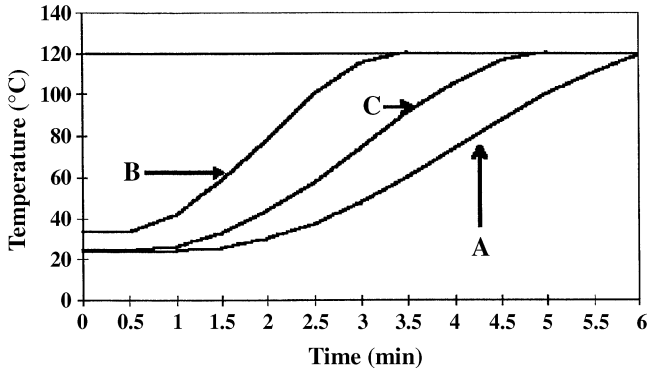
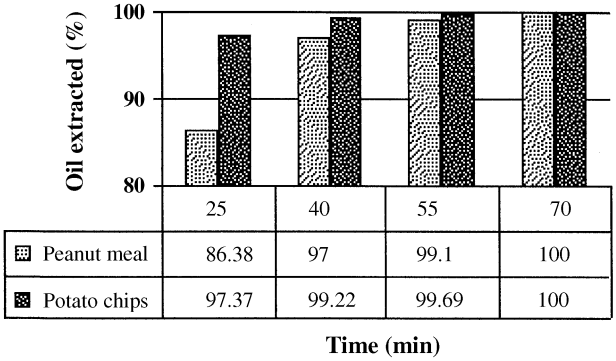


FIG. 1. Temperature curves for extraction heat-up times: (A) Finite element predicted temperature for a node at the cell centroid with hexane; (B) temperature measured at centroid with hexane; (C) temperature measured at centroid with hexane and peanut meal.

The experimental heat-up time was evaluated with only hexane (Curve B) in the extraction cell and then with hexane and peanut meal (Curve C) loaded in the cell. During these temperature measurements the cell temperature setpoint was maintained at 120°C and the pressure at 3,000 psi. A temperature reading of 120°C was reached in 3.1 min with only hexane in the extraction cell. When the extraction cell was loaded with peanut meal and hexane, a temperature of 120°C was reached in 4.7 min. There was a slight overshoot of temperature; however, equilibration occurred quickly. The temperature heat-up times for the extraction cell were very fast. It is desirable for the matrix to reach the proper temperature rapidly since this reduces the extraction time and increases the extraction efficiency.

Accumulative amounts of oil removed using static extraction. The accumulative amount of oil removed during the successive static extractions for the rapid extraction of peanut meal and unbound oil in potato chips is shown in Figure 2. The major portion of the lipid fraction found in potato chips is due to unbound oil. In the first 25 min, 86.38% of the total amount of oil in the sample was extracted from peanut meal. This time frame includes the allowed cell heat-up time (10 min) plus the 15-min static extraction time. Since peanut meal has a relatively high concentration of oil, it is an excellent candidate for evaluating the extractor. Three subsequent static extractions (15 min each) were used to remove the remaining oil from the meal.

Figure 2 also shows the percentage of unbound oil extracted from potato chips for increasing extraction times. Ninety-seven percent of the oil was extracted in the first extraction time of 25 min. Potato chips, which contain primarily unbound oil, were included to demonstrate the usefulness of the extractor for extracting unbound oil in a food matrix of an entirely different texture. Actually, a shorter extraction time could have been used for this extraction; however, for comparative purposes, the same extraction times were used for all products. Subsequently, other tests have revealed that solvent extractions can be reduced to 45 min by allowing a cell heat-up time of 5 min and a static time of 10 min. The amount of lipid extracted from peanut meal using a 5-min heat-up time and a 10-min static extraction time equals the



	25	40	55	70
Peanut meal	86.38	97	99.1	100
Potato chips	97.37	99.22	99.69	100

FIG. 2. Percentage of peanut oil and unbound oil from potato chips extracted per static extraction time (accumulative percentage) using the rapid extraction method.

amount of lipid extracted from a similar peanut meal sample with the longer heat-up cycle and static extraction time.

Comparison of rapid extraction to Soxhlet extraction. Figure 3 compares rapid extraction of peanut meal with Soxhlet extraction. Seventy minutes were required to extract 100% of the oil from peanut meal by rapid extraction, whereas 1,440 min was required to extract the oil from peanut meal by Soxhlet extraction. For comparison purposes, Soxhlet extraction was done for four time periods on the same sample. The amount of solvent required for rapid lipid extraction was approximately 150 mL, which included a 100% solvent flush of the cell. In using Soxhlet extraction, 600 mL of solvent was used for each extraction. Rapid extraction significantly reduces the amount of solvent used, as well as the amount of time and labor required to remove the solvent from the sample.

Comparison of total lipid extracted by rapid extraction and Soxhlet extraction. Three different food matrices were used for both extraction methods for comparative purposes and evaluation of the rapid extractor. Peanut meal, soyflour, and potato chips were selected because of the differences in texture, fineness of grind, and the amount of lipid contained. The results for the amount of lipid extracted from each matrix and the precision are given in Table 1. Rapid extraction of lipid material from peanut meal was comparable to the amount of lipid extracted from a similar peanut meal sample by Soxhlet extraction. Standard deviations for both methods were very small. Soyflour was used to evaluate the extractor because the soyflour was milled to a much finer particle size than full-fat peanut seed can be ground. Comparable amounts of lipids were extracted by both rapid and Soxhlet extraction methods. Standard deviations were comparable for both methods. Potato chips were used to demonstrate the extraction of unbound oil from food matrices, and this product had an entirely different texture and porosity from peanut meal and soyflour. The amount of lipid extracted from potato chips (Table 1) by both methods is comparable. The standard deviation for the rapid extraction method was slightly lower than for Soxhlet extraction. The time required for rapid extraction was 70 min vs. 24 h for Soxhlet extraction. A significant reduction in time, cost per sample, and solvent usage can be realized by a rapid extraction method.

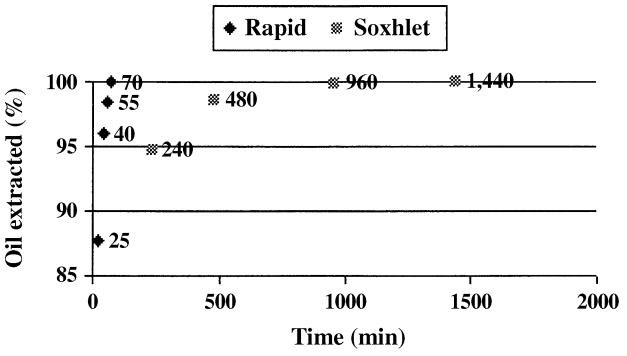


FIG. 3. Comparison of rapid extraction of oil from peanut meal with soxhlet extraction.

TABLE 1
Comparison of Rapid Extraction to Soxhlet Extraction[a]

	Rapid extraction		Soxhlet extraction	
Matrix	Lipid (avg. %)	SD	Lipid (avg. %)	SD
Peanut meal	48.03	0.56	45.2	0.69
Soy flour	19.58	0.5	19.9	0.25
Potato chips	20.3	0.8	21.4	1.43

[a]Average of five repetitions.

Rapid extraction of peanut phospholipids. Figure 4 shows a typical chromatogram of peanut phospholipids resulting from the rapid extraction of the polar fraction from peanut meal after the removal of the nonpolar lipid fraction. This chromatogram shows the separation of the peanut phospholipid fraction into the individual components, namely, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine. Individual phospholipids were identified by retention time using authentic phospholipid standards. Peanut phospholipids had been previously separated and identified by thin-layer chromatography (11) and electron impact mass spectrometry (12). Only 10 g of material was required and 15 mL of solvent was used for the static extraction of phospholipids after the removal of the nonpolar lipid fraction. This fraction was separated without further purification or concentration. Phospholipid content in peanut seeds is low, therefore it is usually necessary to concentrate this fraction prior to HPLC analysis to obtain a useful chromatogram (14,15). This method eliminates preconcentration steps such as solid-phase extraction, open-column technology, and other previously used methodology for the extraction and analysis of phospholipids. Both extraction time and solvent consumption were significantly reduced using the rapid extraction method.

Rapid extraction of bioactive compounds. Saponins and other flavonoid glycosides are amphipathic compounds like phospholipids; however they are more polar because of the number of sugar moieties present on the molecule. Figure 5 shows the negative FAB mass spectra of Soyasaponin I extracted from the peanut meal with the extraction device as de-

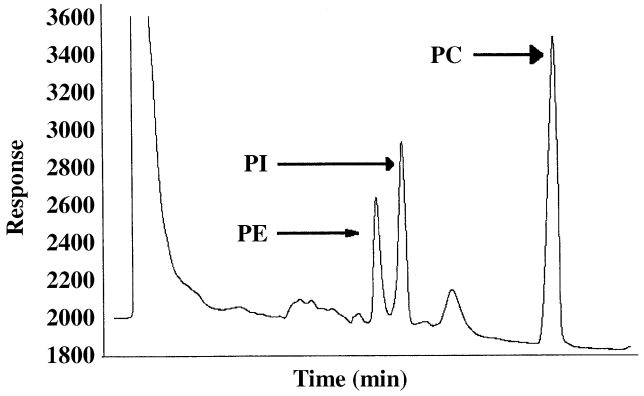


FIG. 4. High-performance liquid chromatogram of peanut phospholipids isolated by the rapid extraction technique; phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylcholine (PC).

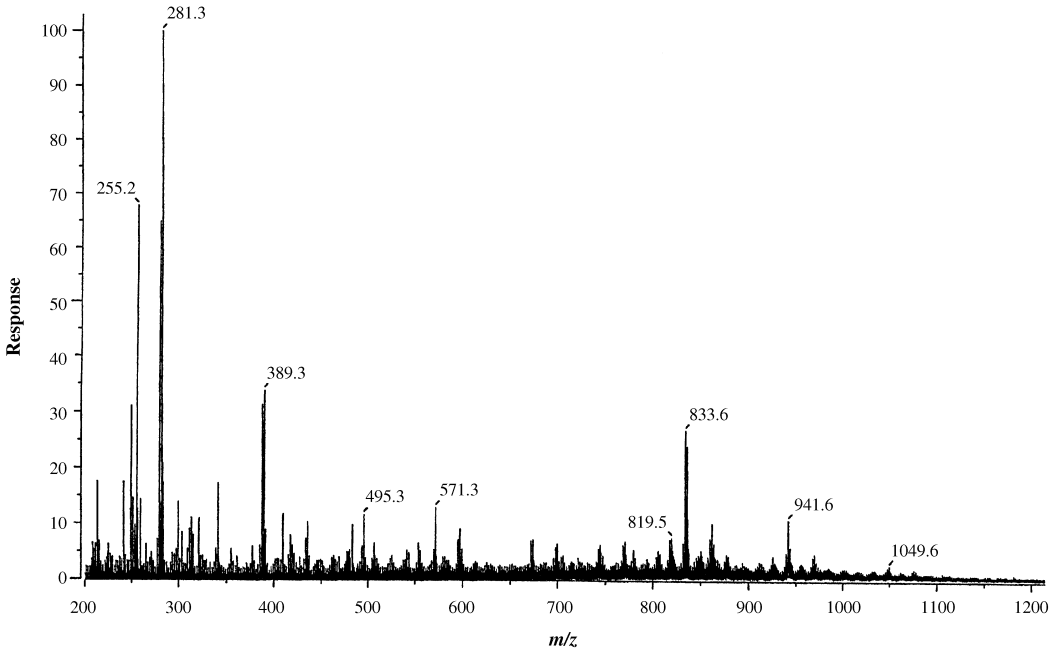


FIG. 5. Mass spectrum of Soyasaponin I extracted from peanut meal.

scribed in the Materials and Methods section of this manuscript. This compound is identical to Soyasaponin I, which has been isolated and identified from soybean meal. Other relatively high-molecular-weight (1,000 to 1,400 mass units) ions are present in some of the spectra, as well as smaller molecular weight ions (400 to 600 mass units) that are indicative of minor saponins and other glycosides that have not yet been identified. This rapid extraction technique will permit the screening of plant material for bioactive compounds with a significant reduction in initial investment, extraction time, solvent consumption, labor, and cost per sample.

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REFERENCES

1. Herslof, B.G., Analysis of Industrial Phospholipids Materials, in *Phospholipids: Characterization, Metabolism, and Novel Applications*, edited by G. Cevc and F. Paltauf, AOCS Press, Champaign, 1995, pp. 1–14.
2. Gunnlaugsdottir, H., and R.G. Ackman, Three Extraction Methods for Determination of Lipids in Fish Meal: Evaluation of a Hexane/Isopropanol Method as a Alternative to Chloroform-Based Methods, *J. Sci. Food Agric. 61*:235–240 (1993).
3. Radin, N.S., Extraction of Tissue Lipids with a Solvent of Low Toxicity, in *Methods in Enzymology*, edited by J. Lowenstein, Academic Press, New York, 1981, pp. 5–7.
4. Nelson, C.G., Isolation and Purification of Lipids from Biological Matrices, in *Analyses of Fats, Oils, and Lipoproteins*, edited by E.G. Perkins, American Oil Chemists' Society, Champaign, 1991, pp. 20–59.
5. List, G.R., and J.P. Friedrich, Oxidative Stability of Seed Oils Extracted with Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc. 66*:98–100 (1989).
6. Calvo, L., M.J. Cocero, and J.M. Diez, Oxidative Stability of Sunflower Oil Extracted with Supercritical Carbon Dioxide, *Ibid. 71*:1251–1254 (1994).
7. Ganzler, K., A. Salgo, and K. Valko, Microwave Extraction: A Novel Sample Preparation Method for Chromatography, *J. Chromatogr. 371*:299–306 (1986).
8. Chen, S.-S., and M. Spiro, Kinetics of Microwave Extraction of Rosemary Leaves in Hexane, Ethanol and a Hexane + Ethanol Mixture, *J. Flavor Frag. 10*:101–112 (1995).
9. Later, D.W., B.E. Richter, and J.L. Ezzell, An Accelerated Solvent Extraction System for the Rapid Preparation of Environmental Organic Compounds in Soil, *Amer. Lab. 27*:24–28 (1995).
10. Ezzell, J.L., B.E. Richter, W.D. Felix, S.R. Black, and J.E. Meikle, A Comparison of Accelerated Solvent Extraction with Conventional Solvent Extraction for Organophosphorus Pesticides and Herbicides, *LC-GC 13*:390–398 (1995).
11. Singleton, J.A., and H.E. Pattee, Computation of Conversion Factors to Determine the Phospholipid Content in Peanut Oil, *J. Am. Oil Chem. Soc. 58*:873–875 (1981).
12. Singleton, J.A., and L.F. Stikeleather, High-Performance Liquid Chromatography Analysis of Peanut Phospholipids. I. Injection System for Simultaneous Concentration and Separation of Phospholipids, *Ibid. 72*:481–483 (1995).
13. Singleton, J.A., and L.F. Stikeleather, High-Performance Liquid Chromatography Analysis of Peanut Phospholipids. II. Effect of Post Harvest Stress on Phospholipid Composition, *Ibid. 72*:485–488 (1995).
14. Singleton, J.A., and L.F. Stikeleather, U.S. Patent Number 5,462,660 (1995).
15. Mounts, T.L., and A.M. Nash, HPLC Analysis of Phospholipids in Crude Oil for Evaluation Soybean Deterioration, *J. Am. Oil Chem. Soc. 67*:757–760 (1990).

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