Quantitative Isolation of Lipids of Partially Defatted and Whole Peanuts by a Dry Column Method

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ABSTRACT

A dry column method of lipid extraction was found to be applicable to peanuts—partially defatted as well as whole. Total lipid was obtained from the peanut/sodium sulfate/Celite 545 columns by isocratic elution with dichloromethane/methanol 9:1. Moreover, neutral lipids were obtained by sequential elution that were completely free from polar lipids. First, dichloromethane eluted the neutral lipids, then the 9:1 solvent eluted the polar lipids—at times containing small quantities of neutral lipids. Total lipid values obtained by the column extraction method were slightly higher than those obtained by the standard Soxhlet extraction procedure. This was due in part to the more complete polar lipid isolation produced by the column method. In partially defatted peanuts produced by mechanical pressing, 99% of the polar lipids remained in the retained oil, and these were shown to be slightly less unsaturated than the neutral lipids.

INTRODUCTION

While studying the oxidative stability and related phenomena in partially defatted peanuts (1), the fate of the polar lipids during mechanical oil separation was investigated. To isolate the peanut lipids and concomitantly separate the polar from the neutral lipids, a dry column method recently developed for meat tissues was used (2,3). The efficacy of this method for lipid quantitation and separation in whole and partially defatted peanuts was evaluated, and the results were compared with those obtained by traditional lipid isolation techniques.

EXPERIMENTAL PROCEDURES

Partially defatted peanuts (PDP) were prepared by pressing 100 g unroasted, seed quality Comet type Spanish variety peanuts in a 2.5-in. id test cylinder outfit in a Carver hydraulic press at 3,000 psi for 30 min. Oil contents were determined either by AOCS official method Ab 3-49 (4), the method of Folch et al. (5), or by the dry column method, as indicated below.

The column had the dimensions shown in Fig. 1. Glass wool was inserted into the column's tip. The column was then charged with 10 g of a 9:1 well-blended mixture of Celite 545 and $CaHPO_4 \cdot 2H_2O$; this was tightly compressed. Either whole peanuts (WP) or PDP were ground in a CRC micro-mill (Chemical Rubber Company, Cleveland, OH) for 3-15 sec for uniformity in sampling. Ground PDP (3.5 g) or ground WP (2 g) were mixed with 5 times their weight of anhydrous Na_2SO_4 in a 750-ml mortar, and ground thoroughly. Celite 545 (15 g) (non-acid washed, Johns Manville Co., Maryland Heights, MO) was added and grinding was continued until the mixture was thoroughly blended. The mixture was transferred with the aid of a metal spoon to the column, on top of the Celite 545 and $CaHPO_4 \cdot 2H_2O$ mixture previously placed there. The column packing was tamped down to a height of 17-18 cm. The mortar, pestle and spoon then were washed with the solvent used in the extraction, which subsequently was transferred to the column using a disposable pipette. When room temperature was above 27 C, the column was cooled with running tap

water.

For total lipid extraction, the column was charged with 75 ml of 9:1 CH_2Cl_2/CH_3OH (CH_2Cl_2 reagent ACS grade), a volume just sufficient to fill the void space of the packing. The lipids were extracted with an additional 150-ml charge of the same solvent mixture to the column bed.

When neutral and polar lipids were to be eluted sequentially, the column was charged initially with 75 ml of CH_2Cl_2 and the neutral lipids were extracted with an additional 150 ml of CH_2Cl_2. When extraction of the neutral lipids was completed, 225 ml of 9:1 CH_2Cl_2/CH_3OH was added to extract the polar lipids.

Blanks were run in a manner identical to the above, but without sample. The quantities of solvents required were determined in a preliminary experiment using 10- to 25-ml increments of solvents. The solvents in all fractions were evaporated under nitrogen; the residues were dried to constant weight in a vacuum oven at 50 C and weighed. For further characterization, the residue was usually dissolved in $CHCl_3$ to yield 50 mg/ml of dry extract, and 2 μl was spotted on 5 × 20 cm, 250-μm-thick commercial Silica Gel G plates (Analtech, Inc., Newark, DE). The developing solvents used were: hexane/ethyl ether/acetic acid 70:30:2 and chloroform/acetone/methanol/acetic acid/water 3:4:-1:1:0.5. Identification of polar compounds was aided by ninhydrin, Phospray and Dragendorff spray reagents. Lipid phosphorus was determined by the Vaskovsky et al. method (6), i.e., spectrophotometric measurement (830 nm) of the resulting heteropolyphosphomolybdate blue color, using a Bausch and Lomb Spectronic 21 DV spectrophotometer.

Fatty acids were methylated by the method of Christopherson and Glass (7) and analyzed by GLC in a 5710 A Model Hewlett-Packard gas chromatograph using a flame ionization detector. The stainless steel column was 6 ft by 1/8 in. id packed with 10% DEGS on 80/100 Chromosorb W/AW. Carrier gas (N_2) flow rate was 20 ml/min. Column, injector and detector temperatures were 195 C, 250 C and 300 C, respectively.

RESULTS AND DISCUSSION

Results in Table I show total and sequential lipid analyses of WP and PDP. It is immediately apparent that the concentration of polar lipids was lower in WP than in PDP—an observation that was confirmed by a determination of lipid phophorus in the pressed peanut oil. Pressing essentially removed only neutral lipids; the phosphorus content of the pressed oil was found to be only 0.016%. The column method yielded reproducible extraction of total lipids and of sequentially separated polar and neutral lipids. Column extraction of both samples resulted in a higher total lipid content than that obtained by the Soxhlet procedure (4) and, as expected because of a higher polar lipid content, the difference was greater for the PDP than for the WP. For both types of peanuts, the difference is believed to be caused by a more complete extraction of polar lipids by the column method. For both WP and PDP, the gravimetrically determined sum of polar and neutral lipids by the sequential dry column method is in reasonably good agreement with nonsequentially obtained total lipid values: 47.45% vs 47.51% in WP and 25.25% vs 25.49% in PDP.

TLC analysis confirmed that the pressed oil was virtually identical in composition to the neutral fraction from the dry column extraction of the tissue. Polar lipid contaminants were negligible in the pressed oil and undetectable in the neutral fraction, as revealed by TLC. In some experiments, a small holdup of neutral lipid in the polar fraction was detected by TLC, but this did not interfere with separation of the individual polar components. Such a holdup of neutral lipid was reported earlier for the analysis of meat by sequential dry column extraction (3).

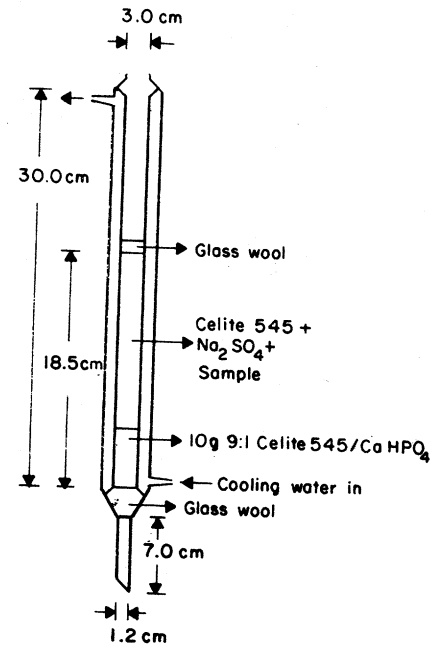

FIG. 1. Diagram of Celite column.

Although no effort was made to identify all the lipid components thoroughly, the presence of some constituents was tentatively established. The neutral fraction from both WP and PDP definitely contained triglycerides and diglycerides, free fatty acids and possibly sterols. When held-up neutral lipids from PDP were found in the polar fraction, only triglycerides were present.

Phosphorus analysis of the PDP extract (using a conversion factor of P × 25 = phospholipid) indicated that 2.7% of the total lipid was phospholipid, whereas the weight of the polar fraction represented 3.96% of the total lipid. The difference in these percentages was due in part to the presence of glycolipids and neutral lipids in the polar fraction. The absence of nonlipid material was suggested by the solubility of the polar fraction in hexane and the absence by TLC analysis of ninhydrin-sensitive nonlipid components, and of mineral deposits at the origin. The phospholipid content of the total lipid was equivalent to 0.49% of the weight of the original tissue (as WP). This is comparable to 0.62% reported in the literature (8).

In contrast to the results obtained by the dry column method, the Folch extraction (5) of retained oil from partially defatted peanuts yielded an oil that by phosphorus

TABLE I

Lipid Contents of Whole and Partially Defatted Peanuts[a]

Extraction method	Whole peanuts	Partially defatted peanuts
Dry column		
Isocratic elution (total lipid)	47.51 ± 0.07	25.49 ± 0.03
Sequential elution, neutral fraction	46.53 ± 0.12	24.24 ± 0.06
Sequential elution, polar fraction	0.92 ± 0.02	1.01 ± 0.02
Soxhlet (4)	46.82 ± 0.29	24.51 ± 0.15

[a]Expressed as % of tissue, ± standard deviation. Each value is the mean of three determinations.

TABLE II

Fatty Acid Composition of Oil Fractions from Spanish Peanuts

Fatty acid[a]	Pressed oil (%)	Retained oil[b] Neutral fraction (%)	Retained oil[b] Polar fraction (%)
16:0	11.4	11.5	21.4
18:0	2.2	2.4	1.9
18:1	42.0	41.5	43.2
18:2	37.6	37.6	33.0
20:0	1.7	1.6	0.3
20:1	1.2	1.2	–
22:0	2.6	2.0	–
24:0	1.0	1.1	–

[a]Data are averages of duplicate analyses of GLC determinations (normalized peak area) on fatty acid methyl esters.

[b]By sequential dry column extraction of partially defatted peanuts.

analysis contained only 0.58% phospholipid, whereas the dry column method gave 2.7% phospholipid for the same sample. Since the Folch method was originally described for brain tissue, which has a higher moisture content than PDP (about 8% for PDP), this method does not appear to be suitable for the isolation of total lipid from peanuts. This observation was further supported by gas liquid chromatography (GLC) analysis of the Folch extract; the fatty acid profile was similar to that observed for pressed oil (Table II).

Additional information regarding the composition of pressed and retained oils from peanuts may be obtained by using the dry column extraction method. The pressed oil from Spanish peanuts was transesterified to methyl esters (7) and the composition of the oil was determined by GLC. The remaining partially defatted peanuts were then extracted sequentially by the dry column method to obtain neutral and polar lipid fractions. These fractions were transesterified, analyzed by GLC, and the results were compared with those obtained from the pressed oil sample (Table II). Apparently, the composition of the neutral fraction from the partially defatted peanuts closely parallels that of pressed oil, which is further evidence that pressed oil consists essentially of neutral lipids. The polar lipid fraction shows marked differences in composition from that of the neutral fraction, such as twice the amount of 16:0, slightly less in overall unsaturation and the absence of C-20-24 components.

The dry column method makes separation of neutral and polar lipids a routine procedure, which in this study yielded useful information on the fate and composition of lipid fractions of peanuts following mechanical pressing. The fact that the polar lipids remained in the retained oil, along with the slightly lower unsaturation of the polar lipid fraction, was useful in subsequent work to explain the oxidative stability of various forms of peanuts (1).

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