Residual Lipids of Hexane-extracted Rapeseed Meal[1]

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Abstract

The residual lipids of rapeseed meals produced by pilot-scale and commercial solvent-extraction processes were extracted with hexane (free lipids), then extracted with chloroform-methanol (bound lipids). Thin layer chromatography showed that the free lipids were similar to those previously reported for intact mature seed. The amount of residual free lipid varied with the method of meal production. Bound lipids were similar for all methods of production but differed in some respects from polar lipids of mature seed.

Introduction

A study of the free lipid (hexane-extractable) components of rapeseed has already been made (1). The present study of residual lipids of rapeseed meals (i.e. those not extracted by hexane in large-scale processes) was undertaken as part of a Food Research Institute study of rapeseed meals. Little has been reported on the lipid composition of rapeseed meal and lipids may well be an important factor in the acceptability and stability of the meals.

Experimental Procedures

Materials

Rapeseed meals were prepared at the Food Research Institute by the processes listed in Table 1 (2). These meals, prepared from *Brassica campestris* seed, were compared with two commercial meals, C-1 from *Brassica napus* and C-2 from *Brassica campestris* seed. The free lipid remaining after processing was extracted in a Virtis homogenizer with cold, deoxygenated hexane (20 vol followed twice by 10 vol). After this the bound lipids, i.e., those not released by hexane extraction, were similarly extracted with chloroform-methanol (2:1). The latter extract was purified by passage through Sephadex G25 coarse (3,4). The sample was applied to the column in chloroform-methanol-water (90:10:1) and the lipids eluted with 20 column volumes of the same solvent. Routine checks of the Sephadex method showed complete recovery of lipid and removal of nonlipid from known mixtures. Aliquots of the free lipid in hexane solution and of the bound lipid in chloroform-methanol were dried in vacuo and weighed to determine the amount of lipid obtained from the meals.

Chromatography

The free lipids in hexane solution were applied to plates coated with 0.25 mm layers of Silica Gel G (Res. Spec. Co.) and separated by means of the system hexane-diethyl ether-acetic acid (90:10:1) (5). The bound lipids were similarly separated by one-dimensional thin layer chromatography (TLC) with the two-stage system (6) of chloroform-methanol-30% ammonium hydroxide-water (140:50:7:3) followed in the same direction by chloroform-methanol-acetic acid-water (160:30:4:1.5). The following systems were used in two-dimensional TLC: A, chloroform-methanol-water (65:15:2) (7); B, chloroform-acetone-methanol-acetic acid-water (65:20:10:10:3) (7); C, chloroform-methanol-water (65:25:4) (8); D, butanol-acetic acid-water (60:20:20) (8); E, chloroform-methanol-7 N ammonium hydroxide (65:30:4) (9); F, chloroform-methanol-acetic acid-water (170:25:25:6) (9). General detection was by means of iodine vapor (10); molybdate (11) was used to detect phospholipids, Dragendorff (12) for choline, ninhydrin for amino groups and perchloric acid (7) to give characteristic colors. Three glycolipid sprays were also used, periodate-benzidine No. 18, diphenylamine No. 53 and trichloroacetic No. 143, according to Waldi (13). Thin layer separations were supplemented by the chloroform-acetone column separation of glycolipids (14). Deacylation was accomplished by means of a modified Dawson procedure (15). Paper (Whatman 1M) chromatograms were developed overnight in phenol saturated with water-acetic acid-ethanol (50:6:6) (16).

TABLE I
Proportion of Free to Total Residual Lipid

Meal[a]	Process	Free lipid (% of total)
RSM 2	Hexane only	48.5
RSM 4	Hexane followed by aqueous extraction	47.3
RSM 7	Aqueous extraction followed by hexane	53.0
RSM 8	Aqueous extraction followed by hexane	54.0
RSM C-1	Solvent (hexane) only	56.8
RSM C-2	Pre-press solvent method	57.6

[a] Meals 2, 7 were prepared from essentially dehulled seed, 4, 8 from whole seed, cracked and flaked for extraction.

Results

Comparison of Meals

An indication of the efficiency of the original extraction process is given by the proportion of free to total lipid in the residual lipid in the meals. As shown in Table I, the proportion of free lipid in the residual lipid varied with the process used in pre-

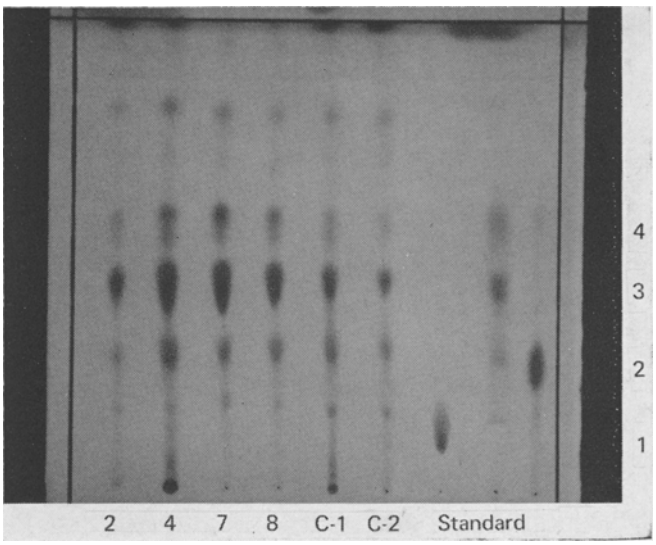

FIG. 1. Solvent systems; (6) chloroform-methanol-30% ammonium hydroxide-water (140:50:7:3) followed in the same direction by chloroform-methanol-acetic acid-water (160:30:4:1.5). Detection: iodine vapor. (10) Standards: 1. Lysophosphatidyl choline. 2. Phosphatidyl inositol. 3. Phosphatidyl choline. 4. Phosphatidyl ethanolamine.

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paring the meal.

In the free lipids of all meals, the lipid class distribution was similar to that reported for mature seed (1), almost entirely triglyceride with very small amounts of sterol, steryl ester, di- and monoglyceride, and polar lipid. The distribution of components of the bound lipids varied very little among the six meals (Fig. 1). Less triglyceride in RSM 7 and RSM 8 and a faint low R_f spot, which showed only with iodine, in RSM 4 and RSM C-1, were the only apparent differences. Likewise there was very little difference when the bound lipids of each meal were separated by two-dimensional TLC.

Bound Lipids

A more detailed study of the residual bound lipids was therefore carried out without regard to the processing method. The separation obtained by the Nichols procedure (9), system E in the X direction, followed by system F in the Y direction is shown in Figure 2. The largest single polar lipid component was phosphatidyl choline (Spot 4) identified by molybdate and Dragendorff. Steryl glycoside (Spot 10) was identified by its characteristic color with perchloric acid and its R_f in the Nichols and Lepage (7) (A followed by B) procedures, and in procedure IV (system C in the X direction followed by system B in the Y direction). These two prominent spots were useful in comparing the relative R_f values of fainter or less readily identified spots. Phosphatidyl ethanolamine, another main component, was identified by molybdate, ninhydrin, and R_f in the Nichols and Lepage procedures.

Most of the spots in Figure 2 were identified by specific sprays, and by R_f in three or all of the four procedures used. Phosphatidyl inositol (Applied Science), phosphatidyl serine (Supelco), diphosphatidyl glycerol (General Biochemicals) and cerebroside (Applied Science) were used in cochromatography to confirm identity. In system C, unidentified material was partially separated from the lyso phosphatidyl choline of Spot 1. Cochromatography with phosphatidyl serine, paper chromatography of deacylation products and partial separation in system C indicated that Spot 3 was phosphatidyl serine plus an unidentified phospholipid. The very faint spot, No. 5, corresponded to the sulfolipid obtained by Nichols; this spot was not detected in the other procedures.

The phospholipids in Spots 8, 11 and 12 corresponded to phosphatidyl glycerol, phosphatidic acid and diphosphatidyl glycerol, respectively. The presence of the glycolipids mono- and digalactosyl diglyceride and cerebroside in Spots 13, 6 and 9, respectively, was confirmed by column chromatography with chloroform-acetone (14). The low R_f Spots 1–3 were more clearly separated in the Rouser or IV procedures, whereas the Nichols or Lepage procedures separated Spots 5–14 more clearly.

Discussion

A larger number of polar lipid components were

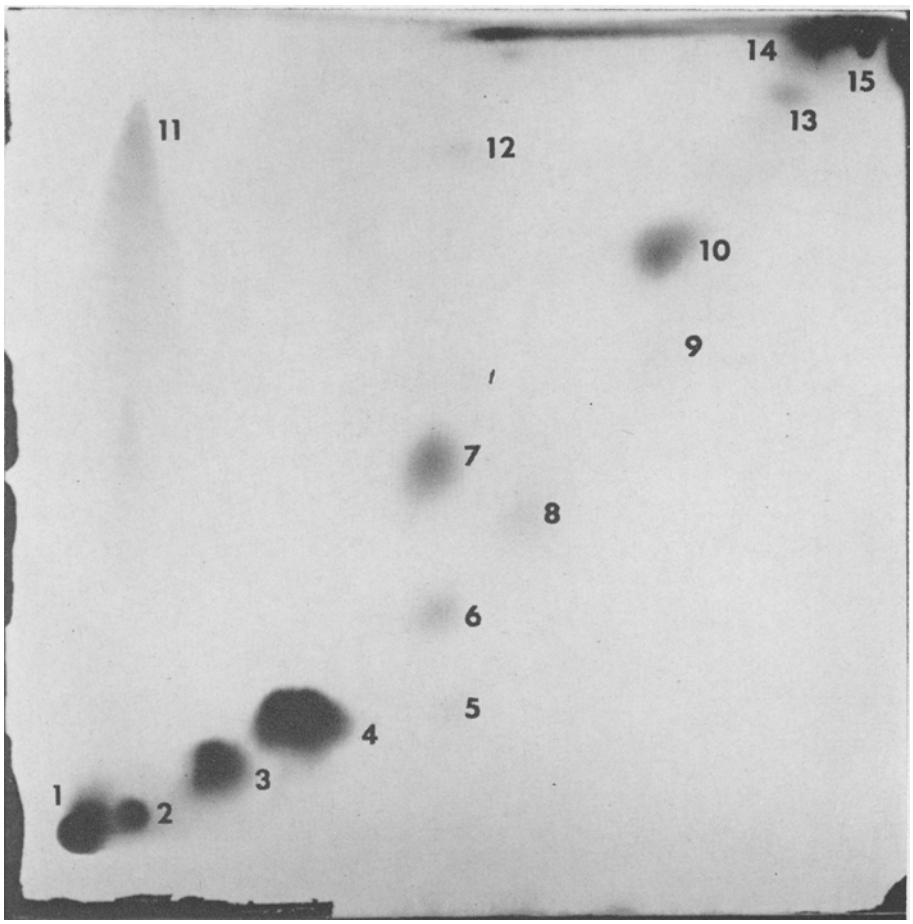

FIG. 2. Solvent system; X direction, chloroform-methanol-7 N ammonium hydroxide, Y direction, chloroform-methanol-acetic acid-water (9). Detection: iodine vapor. 1, Lysophosphatidyl choline + unidentified. 2, Phosphatidyl inositol. 3, Phosphatidyl serine + unidentified. 4, Phosphatidyl choline. 5, Sulfolipid. 6, Digalactosyl diglyceride. 7, Phosphatidyl ethanolamine. 8, Phosphatidyl glycerol. 9, Cerebroside. 10, Steryl glycoside. 11, Phosphatidic acid. 12, Diphosphatidyl glycerol. 13, Monogalactosyl diglyceride. 14, Esterified steryl glycoside. 15, Neutral lipids, mainly triglyceride.

found in the bound residual lipids than were previously found in the polar lipid of the hexane extract of mature rapeseed. Some difference is apparent in the proportions of glycolipids and steryl glycosides, more steryl glycoside and esterified steryl glycoside were found in the bound residual lipid while more mono- and digalactosyl diglyceride were found in the polar lipid of mature seed. The bound residual lipids of the meals and the polar lipids of the hexane extract of mature seed had similar phospholipid fractions. In both, the main phospholipid component was phosphatidyl choline, and phosphatidyl ethanolamine was also present in appreciable quantity.

The polar lipid of the hexane extract of mature rapeseed was separated chromatographically in small quantity from large amounts of neutral lipid, whereas the bound residual lipid was removed from the meal after the free lipid was extracted. Despite these differences in methods, the polar lipids of the bound residual lipid may reasonably be compared with the polar lipids of the hexane extract.

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