The Fatty Acid Composition of Purified Fractions of Cold-Pressed Peanut Oil

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Abstract

Cold-pressed peanut oil was separated into chromatographically homogeneous fractions by column chromatography. The fatty acid composition of the major fractions was determined by gas chromatography. The phosphatides, and especially the cephalins, had a higher palmitate content than did the triglycerides. Palmitate was the dominant fatty acid in the phosphatidylserines.

Introduction

Triglycerides are the major components of both plant and animal lipids and have received major emphasis in studies of the composition of natural fats including peanut oil, with relatively little consideration given to the phosphatides. The development in recent years of gas chromatographic methods for analytical separation of methyl esters of the component fatty acids has stimulated re-examination of the composition of many natural fats. These gas chromatographic methods are simple, relatively rapid, and capable of accuracy and precision superior to older methods. Since the introduction of highly sensitive ionization detectors it has become possible to detect and to estimate the concentration of components present at a small fraction of 1% of the total.

The relative ease and small sample requirements of gas chromatographic methods has stimulated such studies as those of Worthington and Holley (1). Their investigation of the controversy relating to the occurrence or absence of linolenic acid in peanut oil has yielded evidence that this acid does exist in trace amounts in all of the seven varieties examined. Of equal interest are the values which they report for the variations in proportions of 12 fatty acids in the oils from these varieties. It was their conclusion that variations in the tendency of the oils of different varieties of peanuts to develop oxidative rancidity cannot be explained on the basis of variations in linolenic acid content.

Fore et al. (2) found that, in general, the stability of peanut oils was inversely related to the linoleic acid content, but postulated that an antioxidant other than the known tocopherols may exist in peanut oils.

The role of phosphatides in oxidation of lipids is not clear. Under certain conditions milk phospholipids are oxidized in preference to the triglycerides, whereas under other conditions the reverse is true (3). Hornstein et al. (4) characterize the phospholipids as among the most unstable components of meat. These latter authors present data to show that their partially purified phosphatide fractions were higher in linoleic and linolenic acids than were the neutral fats, and state that the phosphatide fractions rancidified more rapidly than did either the whole lipid or the neutral fat. On the other hand, Woodroof et al. (5) recommended the addition of 0.1% of lecithin to peanut oil to retard rancidity.

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The confusion which exists in the relationship of phosphatides to the development of rancid odors and flavors in commercial fats suggested that the fatty acid composition of purified phosphatide fractions of peanut oil should be determined.

Materials and Methods

Peanut Oil

U.S. number 1 grade in-shell Virginia-type peanuts were obtained on the local market, hand shelled, and imperfect and immature kernels removed by hand picking. The raw peanuts were pressed in a Carver laboratory press at 20,000 psi on the ram until no further oil was expressed. The usually faintly turbid oil was vacuum filtered on fritted glass of fine porosity. Samples not fractionated immediately were held under nitrogen in a deep freeze.

Fractionation of Crude Oil

The batch adsorption method for separating neutral lipids from phospholipids described by Choudhury and Arnold (6) was used to obtain the crude triglyceride and phosphatide fractions. The triglyceride fraction contained no detectable phosphorus, but did contain pigments and other impurities. The phosphatide fraction contained substantial amounts of triglycerides, as well as pigments and other impurities. Both fractions were purified further by column chromatography.

Chromatography

Silicic acid (Mallinckrodt, 100 mesh, analytical reagent) was suspended in water and fines decanted, filtered on glass, washed with acetone and chloroform, dried in air, then activated overnight at 115 C. Columns were packed from a slurry in chloroform. Separation of lipid samples into fractions was by the method of Rouser et al. (7), except that triglycerides were eluted with chloroform and addition of NH_4OH was omitted. Fractionation was followed by placing a spot of eluate on a Silica Gel G thin layer plate, allowing the solvent to evaporate, and using an appropriate reagent to detect the column fractions which contained the desired component. Triglycerides were detected with iodine vapors, cephalins with ninhydrin, and lecithins with Dragendorf's reagent. Appropriately pooled fractions were rechromatographed until thin layer chromatography on Silica Gel G showed only a single spot. Thin layer plates of neutral lipids were developed with 1,2-dichloroethane and of phospholipids with chloroform-methanol-water (65:25:4). Pooled fractions were concentrated in a rotating evaporator under water pump vacuum, taken up in chloroform, and stored in a freezer under nitrogen. All solvents were reagent grade, freshly redistilled through a fractionating column packed with glass helices (8).

Gas Liquid Chromatography

Fatty acid methyl esters were prepared by Metcalfe's boron trifluoride method (9) as modified by van Wijngaarden (10), except that only smaller

TABLE I
Fatty Acid Composition of Triglycerides and Phosphatides of Cold-Pressed Peanut Oil

Fatty acid	Fatty acid methyl esters, per cent of total			
	G3[a]	Eth[a]	Ser[a]	Chol[a]
16:0	8.1	24.7	33.9	12.9
16:1	Trace	.1	.2	Trace
17:0	Trace	.2		Trace
18:0	1.5	2.6	4.7	2.8
18:1	49.9	39.5	30.9	47.0
18:2	35.4	28.2	27.5	35.6
18:3	Trace	.1	Trace	Trace
20:0	1.1	.1	.2	.3
20:1	.9	1.0	.2	.4
22:0	2.1	.5	.5	.3
22:1				.1
24:0	1.0	3.0	2.0	.5
24:1				.1

[a] Abreviations: G3, triglycerides; Eth, phosphatidylethanolamines: Ser, phosphatidylserines; Chol, phosphatidylcholines.

samples of the phosphatide fractions were available. Completeness of methyl esterification was checked by heavily spotting a thin layer plate with the concentrated organic layer, developing with the chloroform-methanol-water mixture and visualizing with both iodine vapors and phosphomolybdic acid. No spots were visible below the solvent front, and it was assumed that esterification was essentially complete. The hexane extract of the methyl esters was concentrated at room temperature in a stream of dry nitrogen to obtain a concentration of the order of 10% for injection. Samples of 0.2 μliter were injected with a 1 μliter syringe. Columns used were 8 ft of ¼ in. (o.d.) tubing containing 15% stabilized diethylene glycol succinate (DEGS) on 80/100 mesh Gas Chrom P. Carrier gas was nitrogen at 75 ml/min. Column temperature was 180 C with injection port and hydrogen flame detector at 250 C. Some samples were analyzed on 8 ft by ⅛ in. (OD) columns of 15% stabilized DEGS on 70/80 mesh Anakrom ABS, oven temperature 180 C, nitrogen flow rate 25 ml/min. No trace of carbonization or polymerization was detected in the stainless steel injection port liners under these conditions.

Quantitation was by peak height times width at half height and internal normalization, except that small peaks were measured by planimetry. The contribution of the minor components was estimated by injection of three separate aliquots of the same sample and recording the detector response at three different attenuations. The relative proportions of the areas of those peaks remaining on scale were determined for each tracing and the contributions of the several peaks to the total fatty acid ester content of the sample determined by computation without reference to the degree of attenuation. One aliquot was separated at 160 C to improve resolution of arachidic acid.

Peak identification was by comparison of retention times with commercial mixtures of fatty acid methyl esters and by carbon number determinations, repeated each day that determinations were made.

Results and Discussion

Cold pressed peanut oil differed somewhat in appearance from sample to sample. In some cases the recovered oil was quite clear, but in other cases it was faintly turbid. Estimations of phosphorus content indicated that the turbid samples were much higher in phosphate than the clear samples. All samples were filtered on fine porosity fritted glass with water pump vacuum, and were clear after filtration. The nature of the insoluble material was not investigated. The limited results obtained when estimations of phosphorus were attempted indicate that a detailed study of the microscale estimation of phosphate in lipids containing very low concentrations of phospholipid is necessary before satisfactory phosphate distribution results can be reported. Results reported in this paper are of fatty acid contents only of the fractions as isolated.

The triglyceride fraction was obtained in water-white condition by repeated chromatography on silicic acid or by chromatography of partially purified fractions on acid-washed florisil (11). Phosphatide fractions could not be obtained in a colorless condition. Phosphatidylcholine and phosphatidylserine fractions could be obtained essentially free of odor with careful handling, but the phosphatidylethanolamine fractions always had a slightly rancid odor. All phosphatide fractions darkened slowly on storage in glass vials, but the triglyceride fractions remained colorless for months. Recoveries from the columns were not quantitative, since silicic acid recovered from exhausted columns became slightly gray when heated below red heat. No indication of the nature of the strongly adsorbed material was obtained, since no effective method of elution was found. Losses appeared to be quite small.

The fatty acid composition of the several purified fractions is presented in Table I. Each value represents an average of three determinations. The most striking difference is in the relatively high concentration of palmitate found in the phosphatides, particularly in the phosphatidylethanolamines and phosphatidylserines, with a lesser concentration of oleate and linoleate. The ratio of linoleate to oleate was approximately 0.7 for all fractions except the phosphatidylserines, in which case the ratio is nearly 0.9. In the phosphatidylserines, palmitate is the dominant fatty acid. These results differ appreciably from those reported by Sreenivasan (12) for a crude peanut lecithin fraction, most notably in the ratios of linoleate to oleate. In view of the wide variation reported by Worthington and Holley (1) in this ratio among the oils from several varieties of peanuts, it appears reasonable to ascribe the differences in phosphatide results principally to variety.

Deviations in fatty acid composition found from those claimed for fatty acid methyl ester standard mixture RM-3 (Supelco, Inc.) did not exceed 3% for the major or 10% for the minor components.

The fatty acid composition obtained for the triglycerides does not agree very closely with any of the known varieties of peanuts reported by Worthington and Holley (1). The history and variety of the peanuts used in the present investigation is unknown. The variations reported by the previous authors within a given type, notably the two Runner varieties, together with the variations reported in this paper in fatty acid composition of the several lipid types, suggest that it may be desirable to study the effects of variety, cultural, curing and storage conditions on the composition and stability of the oil. The data available to date indicate that a high content of polyunsaturated fatty acids in either the triglycerides or the phosphatides is not the only important factor in the development of oxidative rancidity in peanut oils.

It will be of interest, also, to correlate changes in fatty acid distribution of the several lipid fractions with flavor deterioration during staling of processed peanut products.

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