Effects of Variety and Maturity on Lipid Class Composition of Peanut Oil

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ABSTRACT

Oils extracted from three varieties of mature peanuts with chloroform/methanol (2:1, v/v) or petroleum ether and oils extracted with petroleum ether from one variety at eight distinct physiological maturity stages were fractionated by TLC. The three varieties of mature peanuts were quantitatively similar in relative percentages of lipid classes regardless of extraction solvent; however, oil composition of the one variety changed with maturity. Generally, triacylglycerol percentage increased and percentage of all other fractions decreased with maturity. The calculated weight per seed of most fractions increased from the lowest maturity stage tested up through stage 10 or 11, which corresponds closely with apparent physiological maturity of the seed.

INTRODUCTION

Current trends in the consumption of vegetable oils necessitate an understanding of the factors that affect oil stability, nutrition, and quality. The importance of individual lipid components in peanut oil has not been extensively studied, although some researchers have suggested that they play a significant role in oil stability and quality (1-4).

Lipid classes of many seed oils have been examined, and significant changes in their relative amounts with changes in maturity have been reported (5-10). Several studies of peanut oil fatty acid composition and its changes with broad maturity levels have been reported (4, 11-15). In those studies, the oils were either pressed or extracted with a variety of organic solvents from peanuts. Few solvent systems extract oilseed lipids with equal efficiency. This investigation was initiated to closely monitor the effect of peanut maturity on oil composition, compare lipid class composition of three peanut varieties, and compare the effects of two different extractants on lipid class composition. The purpose of these investigations was to provide a basis for identifying factors important to the stability and quality of peanut oil.

MATERIALS AND METHODS

Florunner, Florigiant, and Starr varieties of peanuts were grown, harvested, and cured in Headland, Alabama, as part of the 1976 National Peanut Performance Trials. Peanuts were mechanically shelled, and seed riding a shaker screen with 0.635 x 1.905 cm openings were sealed in plastic bags and stored at 4 C. Florunner peanuts from the 1977 crop at Tifton, GA, were hand harvested, separated according to physiological maturity stages (16), and the seed immediately frozen in plastic bags. Physiological maturity stages 5-12 were examined. Stage 5 seed were somewhat flattened and white, or just turning pink at the embryonic-axis end of the kernel, and the inner pericarp tissue was soft and uncracked. Stage 9 inner pericarp was white but beginning to contain light brown splotches while dark brown splotches were evident in stage 12 (16). Stage 12 in this study also included stage 13, as described by Pattee et al. (16). Seed were removed from -16 C storage and freeze-dried to constant weight before lipid extraction.

Random 10 g samples of sound, mature, intact peanuts of each variety were extracted with either 100 ml petroleum ether (PE) (BP 35-60 C) or 150 ml chloroform/methanol (CM) (2:1, v/v) with butylated hydroxytoluene added to inhibit autoxidation. All solvents were redistilled before use. Peanuts were blended with petroleum ether for 3 min, left to stand 5 min, and then vacuum filtered through glass fiber filters (Reeve Angel 934 AH). The residue was washed on the filter several times with petroleum ether. The filtrate volume was reduced by rotary evaporation and then adjusted to 100 ml. Seed extracted with $CHCl_3$/MeOH were ground to fine consistency in the solvent in a mortar and pestle. The mixture was quantitatively transferred to a blender jar, allowed to stand for 10 min, and then blended for 4 min. The mixture was filtered as above and the solvent removed by rotary evaporation. The oil residue was dissolved in chloroform (125 ml) and washed with saturated sodium chloride (50 ml). After the

TABLE I

Weight Percentages of Lipid Classes in Oil Extracted from Florunner, Florigiant and Starr Peanuts with Petroleum Ether and Chloroform/Methanol (2:1)[a]

	Florunner		Florigiant		Starr	
	Weight %					
Lipid class	PE	C:M	PE	C:M	PE	C:M
Hydrocarbon, sterol ester	0.26	0.32	0.90	0.27	0.18	0.15
Triacylglycerol	97.27	95.07	96.57	95.65	97.02	96.44
Free fatty acid	0.44	0.60	0.40	0.42	0.25	0.20
Sterol	0.59	0.94	0.67	1.22	0.98	0.75
Diacylglycerol	1.32	2.04	1.22	1.48	1.48	1.53
Monoacylglycerol	0.09	0.09	0.08	0.07	0.03	0.05
Polar Lipid	0.11	0.94	0.16	1.04	0.12	0.89

[a]Each value is the mean of 2 replications.

chloroform layer was removed, the aqueous salt phase was washed twice with chloroform (25 ml). The chloroform extracts were combined, dried over anhydrous sodium sulfate, and filtered through Whatman No. 4 filter paper. The volume of solvent was reduced and then adjusted to 100 ml. Weighed, freeze-dried seed without seed coats from each maturity stage were ground in petroleum ether with mortar and pestle and then blended as other petroleum ether extractions. Percent oil extracted was estimated from duplicate aliquots of each extract. The aliquots were dried with N_2 then placed in a vacuum oven at 40 C until constant weight was obtained. Percent oil of the three varieties was also determined by AOCS Official Method Ab 3-49 (17). Oil was calculated as percent of dry weight, as determined from the weight of mature seed dried 6 hr at 130 C, or of the freeze-dried seed of each maturity stage.

Aliquots of the oil (75-150 mg, depending on maturity stage) were streaked onto 20 x 20 cm glass plates coated with 0.5 mm Silica Gel G (Brinkmann). Chromatograms were developed in a N_2 atmosphere in petroleum ether/diethyl ether/acetic acid (80:20:1, v/v/v), and the resulting bands were made visible with iodine vapor. Typical Rfs of the lipid classes were: hydrocarbons-sterol esters, 0.98-0.96; triacylglycerols, 0.9; free fatty acids, 0.43; *sn*-1,3-diacylglycerols, 0.29; sterols, 0.22; *sn*-1,2(2,3-diacylglycerols, 0.17; monoacylglycerols, 0.08; polar lipids, 0.0. Lipid classes were identified by comparison of their Rfs with those of standards (TLC Mix-1, TLC Mix-8, cholesterol; Applied Science Laboratories, Inc.) under identical conditions. Sterol and sterol ester bands were also identified by spraying the plates with sulfuric acid/acetic acid (1:1, v/v) and heating at 90 C for 15 min (18). The bands made visible with iodine were marked, iodine was evaporated, and the bands were scraped from plates. Lipids were eluted three times with high purity chloroform/methanol (2:1, v/v), and methyl esters of fatty acids of each class except sterols were prepared (19). Methyl heptadecanoate was added as an internal standard and the samples were analyzed by GLC. The gas chromatograph was equipped with a FID and a 3.17 mm x 1.83 m stainless steel column that was packed with 5% DEGS-PS on 100/120 Supelcoport (Supelco, Inc.). Appropriate correction factors (20) were applied to convert the calculated total fatty acid weight to weight of lipid in each class. Sterols were determined gravimetrically. Some error is assumed in the calculations, since, lacking true compositional data, the hydrocarbon-sterol ester fraction was calculated totally as esters of β-sitosterol, the most abundant sterol in peanuts (21), and polar lipids were calculated arbitrarily as phosphatidylcholine. Data reported for the varieties are the mean of two replications, and seed from three separate harvests (three replications) were used for the maturity study.

RESULTS AND DISCUSSION

Oils, as a percent of dry weight, extracted from Starr, Florunner, and Florigiant varieties, respectively, by three different methods were: CM extraction, 49.4, 51.5, 50.0; PE extraction, 50.1, 49.5, 51.6; and AOCS Official Method Ab 3-49, 50.2, 52.2, and 50.5. The relative weight percentages of lipid classes in the oils extracted with CM and PE are presented in Table I. Differences in oil among the varieties extracted with the same solvent were relatively small. Triacylglycerols accounted for over 95% of the oil extracted from each variety, and diacylglycerols were the second largest fraction regardless of solvent used. Diacylglycerols as reported included both *sn*-1,3- and *sn*-1,2(2,3)-diacylglycerols which were separated by the TLC procedures employed. In each oil, *sn*-1,2(2,3)-diacylglycerol

TABLE II

Influence of Maturity on Quantity and Composition of Petroleum Ether-Extracted Oil from Florunner Peanuts

Maturity stage	Dry weight per seed, g	Oil, % dry weight	Hydrocarbon sterol ester[a]	Triacylglycerol[a]	Free fatty acid[a]	Diacylglycerol[a]	Sterol[a]	Monoacylglycerol[a]	Polar lipid[a]
5	0.03	25.3	0.6	85.3	4.5	4.7	2.4	0.5	2.0
6	0.08	30.8	0.4	89.3	3.1	3.5	1.8	0.6	1.4
7	0.15	34.4	0.5	88.3	2.5	3.6	1.3	0.8	1.9
8	0.32	42.8	0.6	90.8	1.8	3.0	1.4	0.8	1.6
9	0.45	45.6	0.6	92.6	1.3	2.2	1.0	0.9	1.3
10	0.57	46.7	0.3	94.3	0.9	2.0	0.8	0.6	1.0
11	0.59	48.4	0.4	94.8	0.7	1.9	0.9	0.5	0.7
12	0.59	48.2	0.4	95.8	0.7	1.7	0.5	0.3	0.6

[a]Relative weight percent. Each value is the mean of 3 replications.

percentages were slightly higher than *sn*-1,3-diacylglycerols (data not presented). The range of relative weight percentages of free fatty acids (FFA), 0.2-0.6, agree generally with the reported range of 0.02-0.6% as determined by titration with a standardized NaOH solution (22). FFA determinations of petroleum ether extracted Florunner, Florigiant, and Starr peanut oils by a standard titration procedure, AOCS Official Method Aa 6-38 (17) resulted in 0.22, 0.23 and 0.24% FFA as oleic acid, respectively. Monoacylglycerol made up the smallest weight percentage of the oils. Pohle et al. (23) reported that peanut oil contained 0.4% monoacylglycerol as determined by oxidation with periodic acid. The average polar lipid percentages for the three varieties were substantially lower when the oils were extracted with petroleum ether than with chloroform/methanol (0.13 vs. 0.95). No further separation of this fraction was attempted. Brown et al. (4) found that oven stability of peanut oil was related to the solvent used for extraction, and Hokes (24) found that removal of phosphatides from the oil by precipitation reduced oven stability. Thus, differences in the relative amounts of polar lipids extracted by different solvents might explain the findings of Brown et al. (4).

Because peanut fruiting is indeterminant, several maturity stages are present in any lot of harvested peanuts. The quality of each lot depends on the overall or average maturity level of the peanuts. Changes in starch, sugar, and lipid content with increasing maturity have been reported (16), and data in Table II indicate that oil composition also changes significantly.

Dry weight of the seed increased rapidly during the early maturity stages, and these increases corresponded directly to an increase in petroleum ether-extractable oil content per seed (Table II). The most rapid increase in dry weight and percent oil occurred between stages 7 and 8. Pattee et al. (16) indicated that below stage 7, seeds accumulate starch more rapidly than lipids, but that at stage 7 and beyond, lipid synthesis becomes the dominant reserve accumulation mechanism. They reported that at stage 7, variety NC-2 contained ca. 27% lipid. Varietal differences, as well as environmental effects and/or slight variation in separation of the various maturity stages, may account for the higher value of 34% reported here.

Fractionation of the total oils from seed at eight maturity stages revealed that all lipid classes changed somewhat with maturity. Triacylglycerols increased from 85 to 96% of the lipid present, and the increases were highest during the more immature stages examined. This pattern of increase in triacylglycerols is common in seed oils (5-8) and is the single largest reason for the rapid increase in oil content of the seed.

The relative weight percentage of FFA decreased from ca. 4.5% to 0.7% as the peanut seed matured. This type change has also been reported in wheat (5), rape, and crambe (6). The osmotic shock caused by freezing often disrupts cell membranes to the extent that lipids become exposed to enzymes from which they are normally protected. Lipolytic enzymes may hydrolyze the lipids on prolonged standing, even at -20 C, and presence of large amounts of unesterified acids may be an indication of tissue damage and subsequent deterioration of lipids (25). The 0.7% FFA reported here for stage 12 may indicate very slight lipid deterioration during the freezing, storage, freeze-drying, and extraction processes employed, undoubtedly; however, the percentage of FFA did decrease with peanut maturity.

The diacylglycerol fraction was the second largest lipid component at all stages of maturity. In the early maturity stages, *sn*-1,3-diacylglycerols were more abundant than *sn*-1,2(2,3)-diacylglycerols, but by stages 9-12 the percentages, though not markedly different, had reversed.

Percentages of extracted polar lipids in the oil decreased with maturity. Polar lipids of rape (6), crambe (6), flax (7), safflower (7), and corn (8) extracted with a variety of solvents were found to decrease with maturity. The differences noted in polar lipid percentages for the mature cured (Table I) vs. the most mature frozen (Table II) seed may have been due in part to changes during the normal curing process which render this fraction less extractable and/or the different grinding techniques used in the extractions.

Although the percentages of minor components decreased with maturity, the calculated weight per seed indicated an opposite trend until about stage 10 (Table III). Maximum seed dry weight (Table II) was attained at stage 11, and the stage 10-11 range might be considered that state of maturation when the rates of many physiological and biochemical mechanisms begin to decrease or cease completely. The relative percentage of the hydrocarbon-sterol ester fraction was maximum at stage 9 and then fluctuated over the next two stages, while triacylglycerols did not reach maximum until about stage 11. The rate of increase in triacylglycerols decreased considerably after stage 10. Free fatty acids, monoacylglycerols, and polar lipids were all maximum at stage 9, then decreased. Diacylglycerols and sterols increased through stage 11 then decreased in stage 12.

This study indicates that the percentages of various oil fractions, some thought to influence oxidative stability, vary considerably with peanut maturity. It further suggests that

TABLE III

Lipid Composition of Petroleum Ether-Extracted Oil from Maturing Florunner Peanuts

Maturity stage	Hydrocarbon, sterol ester	Triacylglycerol	Free fatty acid	Diacylglycerol	Sterol	Monoacylglycerol	Polar lipid
			g/100 seed[a]				
5	0.005	0.65	0.03	0.04	0.02	0.004	0.02
6	0.01	2.20	0.08	0.09	0.04	0.01	0.03
7	0.03	4.56	0.13	0.19	0.07	0.04	0.10
8	0.08	12.44	0.25	0.41	0.19	0.11	0.22
9	0.12	19.00	0.27	0.45	0.21	0.18	0.27
10	0.08	25.10	0.24	0.53	0.21	0.16	0.27
11	0.11	27.07	0.20	0.54	0.26	0.14	0.20
12	0.11	27.24	0.20	0.48	0.14	0.08	0.17

[a]Based on data in Table II.

processes in which immature peanuts are utilized should be closely monitored for high oil quality.

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