Comparison of Oxidative Stability of High- and Normal-Oleic Peanut Oils

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A peanut breeding line with high-oleic acid and an isogenic sister line with normal fatty acid composition were obtained. Oil was extracted with dichloromethane and processed in the laboratory by alkali neutralization and bleaching. Fatty acid compositions were determined by gas chromatography and application of theoretical response factors. Oils were extracted and processed in duplicate. The oxidative stability of the oils was measured by the Schall oven test (80°C), active oxygen method (AOM) (112°C) and by comparison of oxidation rates on thin-layer chromatography-flame ionization detector (TLC-FID) rods (100°C). Fatty acid analysis indicated that the high-oleic line had 75.6 and 4.7% oleic and linoleic acids, respectively, compared to 56.1 and 24.2% for the normal line. The induction times for the Schall test were 682 and 47 h for high- and normal-oleic oils ($P < 0.01$). The AOM induction times were 69 and 7.3 h for high and normal oils, respectively ($P < 0.01$). The times to reach 50% loss in triglyceride area on TLC-FID were 847 and 247 min for high-oleic compared to normal-oleic oils ($P < 0.01$). The results indicate that high-oleic peanut oil has much greater autoxidation stability as compared to normal oil.

KEY WORDS: Accelerated oxidation, AOM, high-oleic, peanut oil, Schall.

Modification of fatty acid composition of oilseeds to improve oxidative stability has been the focus of work on soybeans, sunflower, safflower and rapeseed (1-3). Similar work has not been successful in peanuts due to the lack of sufficient variability for low linoleic content (4-7). The known genetic range of fatty acid compositions of peanuts is 41-67% oleic acid and 14-42% linoleic (8). A recent survey of runner-type peanuts reported 49.6-56.3% oleic and 24.1-30.6% linoleic acid (9). A line of peanuts has been identified in the University of Florida breeding program that exhibits high-oleic characteristics with very low linoleic content (10,11). This line contains up to 80% oleic acid and 2-4% linoleic acid.

Peanut oil is one of the most stable vegetable oils to oxidation. This is partly due to the fatty acid composition, which is low in 18:3ω3 (12). The rates of oxidation of C18 fatty acids are approximately 1:10:100:200 for 18:0, 18:1, 18:2 and 18:3, respectively (13). The effect of fatty acid composition on oil oxidative stability in vegetable oils has been studied by a number of investigators. Liu and White (14) have studied the oxidative stability of several low-linolenic acid (1.5-6.5%) soybean varieties. They reported a significant positive correlation between linolenic content and peroxide value (PV) of oils stored at 60°C, and significant negative correlations between linolenic acid and flavor quality or intensity. The low-linolenic oils had greater oxidative stability than commercial oils. A decrease in linolenic acid in rapeseed oil from 7-10 to 3.1% resulted in improved odor scores during oxidation (1). Other research (15) has shown that the order of oxidative stabilities of sunflower, soybean and LEAR (low-erucic acid rapeseed; canola) oils are not constant and depend on experimental conditions. High-oleic sunflower oil was reported to have greater accelerated oxidation stability than other vegetable oils studied, and it performed as well as partially hydrogenated canola in deep-fat frying experiments (16). High-oleic sunflower oil and seeds are commercially available (Sigco Sun Products, Breckenridge, MN).

Fatty acid composition appears important in determining oxidative stabilities, but other factors are also involved. The purpose of this work was to compare the oxidation rates of high- and normal-oleic peanut oils by chemical accelerated oxidation techniques.

EXPERIMENTAL PROCEDURES

Oil extraction. Peanuts (500 g) were blended for 4 min at high speed with 1.5 L dichloromethane in a Waring blender. The slurry was filtered through Whatman No. 1 filter paper (Maidstone, England), and the cake was re-extracted with 1 L dichloromethane. The combined organic phases were rotary-evaporated under vacuum at 40°C to remove solvent. The free fatty acid content of the oil was determined by the IUPAC official indicator method 2.201 (17). Hexane (500 mL) was added and the oil was dissolved. The oil-hexane micelle was alkali-treated (10% excess) with 0.8N NaOH and then washed with water until neutral. After separation of the final water wash, the micella was treated with silica adsorbent (10 g chromatographic grade silica gel, 100-200 mesh; Fisher Scientific, Fairlawn, NJ) for 2 h with stirring at room temperature. The silica was removed by filtration through Whatman No. 1 filter paper, and the hexane was removed by rotary evaporation. The oil was stored under nitrogen and protected from light at −10°C until used. Two separate batches of oil were prepared from high-oleic and normal-oleic isogenic peanuts. Nontriglyceride components were estimated by thin-layer chromatography-flame-ionization detector (TLC-FID) analysis of freshly extracted and processed oils (TLC-FID described below).

Fatty acid methyl ester (FAME) preparation. FAMEs were prepared by BF_3-catalyzed transesterification (18). Oil (50 mg) was accurately weighed into a 10-mL screw cap tube. Two mL benzene containing 2.5 mg/mL 17:0 free fatty acid internal standard (IS) (Sigma Chemical, St. Louis, MO) was added, along with 2 mL of 6% BF_3 in methanol (Supelco, Bellefonte, PA). After flushing the headspace with nitrogen, the tube was capped and placed on a boiling water bath for 60 min. After cooling, 2 mL water and 2 mL hexane were added. The tubes were shaken and then centrifuged for 3 min. The organic phase was placed into a new tube, evaporated to dryness with a slight stream of nitrogen gas and the FAMEs were dissolved in 1 mL hexane.

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Gas-liquid chromatography. A model GC-14A gas chromatograph equipped with a split-splitless injector and FID and a Chromatopac CR5A integrator were obtained from Shimadzu Scientific (Norcross, GA). The column was a DB-WAX (30 m × 0.25 mm i.d., 0.25 μm film) obtained from J&W (Folsom, CA). Helium carrier gas was used at a linear flow velocity of 25 cm/s. Split pressure was 0.5 kg/cc, producing a ratio of 80:1. The oven temperature was held at 155°C for 20 min, programmed to 220°C at 5°C/min, and held at final temperature for 20 min. Detector and injector temperatures were 275°C. Fatty acids were identified by comparison of retention times with authentic standards, cod liver oil and rapeseed oil (19), and by comparing calculated equivalent chainlengths (ECL) with the literature values (19). Fatty acid concentrations were calculated as weight percent by applying theoretical correction factors (20,21) and as mg/g by using the equation:

$$\text{mg/g fatty acid} = (\text{area FAME} \times \text{mg 17:0 FAME})/(\text{area 17:0} \times \text{mg oil}) \quad [1]$$

A correction factor was applied to the 17:0 to account for mass difference between free acid, added as IS, and FAME as measured by gas chromatography (GC) (the mg 17:0 was multiplied by mass 17:0 FAME/mass free acid).

Schall oven test. The oils were removed from the freezer, melted under running tepid water, mixed thoroughly, and 5 g was weighed accurately into replicate 250-mL Erlenmeyer flasks. The flasks were placed in an oven held at 80°C. Oils were protected from light during heating. Care was taken to ensure that glassware was clean and the flasks were distributed evenly in the oven. After various time periods, duplicate samples of high-oleic peanut oil (HOPO) and normal-oleic peanut oil (NOPO) were randomly removed. The PV was determined on the samples directly in the flasks by using the AOCS official method Cd 8-53 (22). Results were expressed as meq peroxide per kg oil.

Active oxygen method (AOM). Oil was added (40 mL) to 50-mL screw cap tubes that were then placed in an oil bath held at 112 ± 1°C. Two Pasteur pipettes were connected to a medical air tank *via* a plastic Y and clean tygon tubing. The pipettes were placed into the oil within 2 cm from the bottom. Air flow was started once the tubes were placed into the bath, and flow was measured. A flow rate of 234 mL/min was maintained for the two tubes. The two oils, HOPO and NOPO, were run in pairs by this procedure to ensure that all conditions (temperature, air flow, etc.) were exactly the same. After various time periods, 0.3 g was removed in duplicate, accurately weighed, and anisidine values were determined by the IUPAC official method 2.504 (17). The entire procedure was replicated. Induction times were determined by the X intercept of the PV time curve at the point of oxidation.

TLC/FID. An Iatroscan TH-10 Analyzer (Mark 4) was obtained from RSS Inc. (Costa Mesa, CA). The system was used with T DATA SCAN software (version 2.41) running on an IBM compatible 80386 computer and a Strawberry Tree A/D card (RSS Inc.). Silica S-III Chromarods were spotted with 20 μg of either oil. The rods are used in sets of 10 and oils were alternately spotted. The order was switched for the replicate. In this manner, each oil was spotted on each of 10 rods. The rods were placed in an oven that was held at 120°C. After removal of the rods, they were cooled and developed in hexane/ethyl ether/formic acid (96:3:1, vol/vol/vol) for 20 min. The triglyceride (TG) peak was identified by comparison of ratio of fronts of standard tripalmitin. The area of the TG and of the polar material were determined by using the integration software. The spotting-heating procedure was repeated.

Statistics. Statistics (*t*-tests, least squares linear regression) were performed by using the analysis package of an Excel 4.0 spreadsheet (Microsoft, Redmond, WA) run on an IBM compatible personal computer.

RESULTS AND DISCUSSION

The fatty acid composition of the oils and a typical chromatogram of the unheated high-oleic oil are shown in Table 1 and Figure 1, respectively. The largest difference in the fatty acid composition was found to be the replacement of linoleic acid by oleic acid in the high-oleic peanut. There were highly significant ($P < 0.01$) differences in 16:0, 18:1ω9, 18:2ω6 and 20:0 and significant differences ($P < 0.05$) in 18:0, 18:1ω7, 20:1ω9 and 22:0. The total saturated fatty acids were slightly but significantly ($P < 0.05$) lower in high-oleic oil. This was due to lower palmitic levels, as the other saturates were slightly higher or similar in HOPO. Beside the fatty acids reported in Table 1, trace amounts (<0.05%) of 14:0, 19:0, 24:1ω9 and

TABLE 1

Fatty Acid Composition of Peanut Oils

Fatty acid	wt% fatty acid		$P <$
	High-oleic	Normal-oleic	
16:0	7.5	9.5	.01
16:1ω7	0.12	0.02	NS[a]
18:0	3.3	2.6	.05
18:1ω9	75.6	56.1	.01
18:1ω7	0.65	0.50	.05
18:2ω6	4.7	24.2	.01
18:3ω3	0.03	0.03	NS
20:0	1.6	1.3	.01
20:1ω9	1.6	1.3	.05
22:0	3.3	2.8	.05
22:1ω11	0.06	0.05	NS
24:0	1.5	1.5	NS

[a]Not significant.

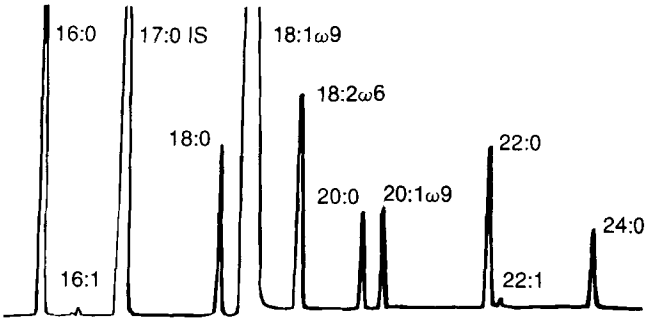

FIG. 1. Partial gas-liquid chromatography of high-oleic peanut oil. IS, internal standard.

26:0 were present. TLC-FID analysis of freshly extracted oils indicated that phospholipid and other nontriglyceride components were present only at trace levels (<0.05%).

The results for the Schall test are shown in Figure 2. Initial experiments resulted in increased PV for NOPO but little change in HOPO. Once the induction time for HOPO was estimated, experiments were conducted using the longer times necessary for HOPO. The induction time for Schall and AOM tests were estimated by the intersection of the linear portions of the PV time curve (after induction) with the X axis. This procedure allowed estimates of error to be determined by linear regression and allowed simple statistical comparisons to be made. Others (23) have used the time for PV = 100 as the induction point. The induction times for the high- and normal-oleic oils were 682 and 47 h, respectively ($P < 0.01$). Even 550 h after the induction time of NOPO, the HOPO sample still had PV values less than 20. Both oils had rather sharp induction times and rapidly increased in PV after the induction occurred. This was most noticeable in HOPO.

Figure 3 illustrates the AOM curves for both oils. Again, the HOPO had greater stability than did NOPO. The induction times were 69 and 7.3 h, respectively ($P < 0.01$). It was necessary to pair the samples in the apparatus constructed in the laboratory to ensure that exactly the same air flow conditions were observed for both oils. In preliminary experiments, difficulties in maintaining exact flow rates made comparisons impossible.

The oxidation of oils can be followed by increases in polar material (24). A typical TLC-FID chromatogram is shown in Figure 4, and the change in polar material measured by TLC-FID with heating time is shown in Figure 5. The time for 50% loss in triglyceride area was determined from a plot of TG area *vs.* time. The plots were linear, with no apparent induction point. This indicates that TG loss on TLC-FID rods followed zero-order kinetics. Some lipid oxidation reactions may follow zero-order kinetics (25). The times for 50% TG loss ($TG_{50\%}$) were 847 and 247 h, respectively, for HOPO and NOPO. The difference in $TG_{50\%}$ times was not as striking as the differences in induction period for Schall and AOM tests. The stability values of HOPO were 3.4, 9.5 and 14.5 times greater for TLC-FID, AOM and Schall tests, respectively.

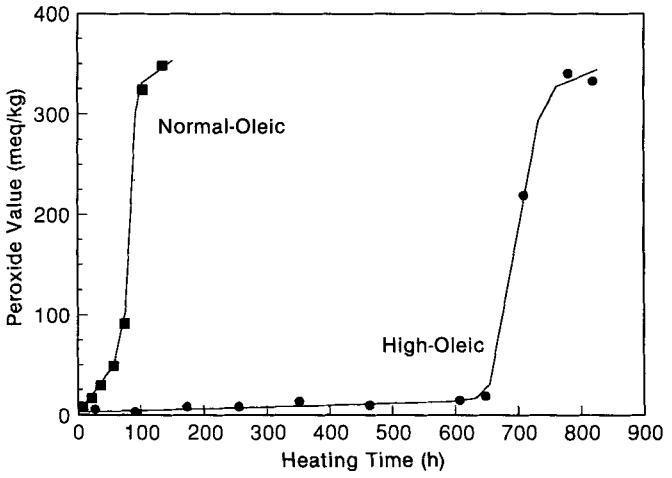

FIG. 2. Schall oven stability of normal- and high-oleic peanut oils.

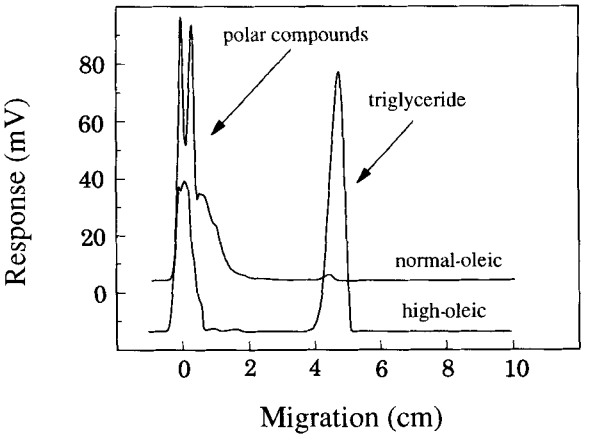

FIG. 4. Thin-layer chromatography-flame-ionization detection chromatogram of peanut oils on silica chromarods.

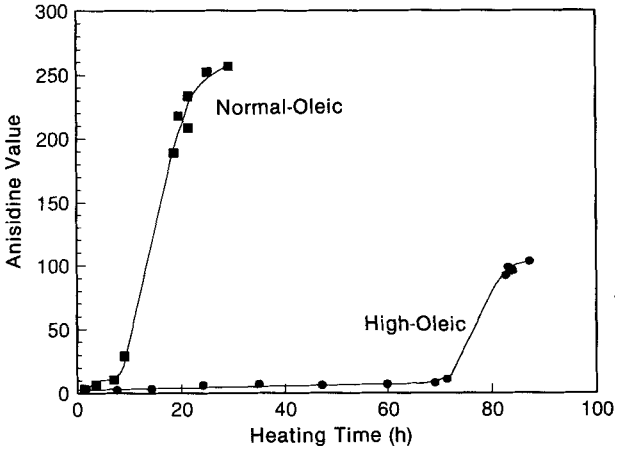

FIG. 3. Active oxygen method stability of high- and normal-oleic peanut oils.

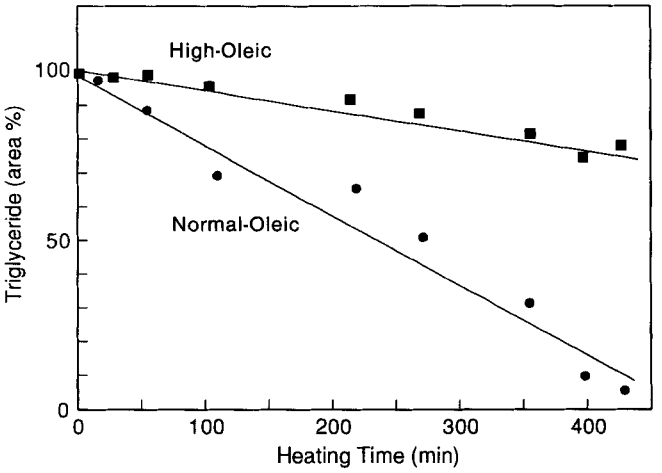

FIG. 5. Thin-layer chromatography-flame-ionization detection stability of high- and normal-oleic peanut oils.

Chemical measures of oxidative stability are sometimes poor indicators of stability in actual food products. However, in most instances, the correlation between sensory and chemical determination of oxidation are quite good. Peroxide values were significantly correlated with flavor intensity of soybean oils having modified fatty acid composition and approached significance for flavor intensity (14). PVs correlated well with induction time during oxidation of eight different vegetable oils (26). The total volatiles and hexanal determined by direct injection techniques correlated with sensory evaluation of soybean oils (27).

Factors other than fatty acid composition could be responsible for some of the difference in oxidative stability observed. There is some evidence that altering the distribution of fatty acids on the glycerol molecule by interesterification could affect stability; however, chemical but not enzymatic randomization has been reported to decrease stability in some vegetable oils (28). The reason for this was unclear, but could have been due to interactions between fatty acid composition and possible protective factors that are removed in chemical but not in enzymatic randomization. Frankel and co-workers (29) have recently reported that volatiles derived from linolenic acid (Ln; L = linoleic) oxidation were higher for LnLnL compared to LnLLn, illustrating a possible importance of positional effects in highly unsaturated triglycerides. The effect of fatty acid position on the stabilities of HOPO and NOPO is unknown, but it is likely to be small. No differences were found in oxidation for LLnL or LLLn (29), suggesting that the positional effect is related to fatty acid unsaturation level.

The data indicate that the oxidative stability of HOPO is much better than that of NOPO. Further experiments are needed to determine if the extent of the differences measured with chemical techniques are also found with sensory methods. Sensory analysis was not carried out on the oil samples after heating due to the small amount of peanut oil available.

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