TABLE II

Matrix of Simple Correlation Coefficients for Fatty Acids and Oil Content of Castorbean Seed Varieties

	Stearic	Oleic	Linoleic	Linolenic	Ricinoleic	Oil
Palmitic	0.79*	0.13	0.61*	-0.06	-0.80*	-0.11
Stearic	–	0.19	0.43*	-0.13	-0.75*	-0.07
Oleic	–	–	-0.06	-0.04	-0.37*	0.18
Linoleic	–	–	–	-0.26	-0.59*	-0.17
Linolenic	–	–	–	–	-0.07	0.21
Ricinoleic	–	–	–	–	–	0.04

*:$P < 0.05$.

The castorbean varieties showed a low variation for fatty acid composition, but more surveying may result in greater variability. However, the variability in seed oil content indicates that further selection or breeding could be carried out.

ACKNOWLEDGMENT

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Oxidative Stability of Soybean Oil at Different Stages of Refining[1]

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ABSTRACT

Oxidative stabilities of soybean oil samples at 7 different stages of commercial refinement were measured by weight increases. Also, a method was developed for isolating essentially pure soybean triglyceride, and its oxidative stability was measured. Crude oil was most stable and the highly purified soybean triglyceride was least stable, with other samples being intermediate in stability. Adding phospholipids and tocopherols to the highly purified soybean triglyceride gave its oxidative stability, and in combination they were synergistic in delaying oxidation. The weight increase method demonstrated that surface exposure is an important variable in rates of autoxidation.

INTRODUCTION

Understanding of the causes of oxidative stability in soybean oil would help produce better quality soybean oil and possibly improve processing technology.

The effect of processing technology on oxidative stability has been investigated by Going (1), who established that alkali refined and bleached soybean oil oxidized faster than crude soybean oil, and that hydrogenation after bleaching and alkali refining increased oxidative stability. Going's results were based on changes in peroxide values.

Cowan (2) observed that bleached soybean oil is less susceptible to off-flavor development than non-bleached oil. This result was based on peroxide values and sensory evaluation of 2 types of fully refined soybean oil: one that had been bleached and one that had not been bleached.

Park et al. (3) found that oxidative stability of soybean oil was greatly decreased by purification, although it was not clear what kind of soybean oil was used as starting material for the purification.

We have studied changes in the oxidative stability of soybean oil due to refining. The results are presented herein.

EXPERIMENTAL METHODS

Materials

Soybean Oils. Seven samples of soybean oil were received from Riceland Foods (Stuttgart, Arkansas) and were used without further treatment. They were: crude oil (SBO-1), degummed oil (SBO-2), alkali-refined oil (SBO-3), bleached oil (SBO-4), deodorized oil (SBO-5), partially hydrogenated

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oil (SBO-6) and partially hydrogenated oil with antioxidants added (SBO-7).

Phospholipids and Tocopherols. A crude phospholipid mixture was obtained from Riceland Foods (Stuttgart, Arkansas) and solubilized in 1% isopropanol in hexane. Amounts of phospholipids were determined according to Bartlett (5) and expressed as phospholipid phosphorus (PL-P).

Mixed tocopherols (67%-D-α-tocopherol TOC) in soybean oil were obtained from Sigma Chemical Co. (St. Louis, Missouri) and were solubilized in hexane.

Procedures

Purification of Soybean Oil Triglycerides. To compare oxidative stability of soybean oils in various stages of refining, we wanted to prepare soybean triglyceride that was free of contaminants. A procedure by Park et al. (3) was tried, but we found that it resulted in an overloading of soybean triglyceride on the Florasil column. Working from that procedure (3), we devised a method that effectively separated soybean triglycerides from hydroperoxides and gave satisfactorily low $E^{1\%}_{1cm}$ values at 233 nm.

The method consisted of dissolving SBO-2 (degummed soybean oil) in 3x its volume of hexane and stirring overnight with activated charcoal (MCB Manufacturing Chemists, Inc., Cincinnati, Ohio) equal in weight to the soybean oil. The mixture was filtered, hexane evaporated, and 2g of the oil was chromatographed on a deactivated Florasil column (2 x 25cm, 7% water, 100-200 mesh). The eluting solvent was 0.55-0.6% isopropanol in HPLC grade hexane. Flow rate was 4 ml/min, and 20 ml fractions were collected for analysis by HPLC (4). The fractions with only soybean oil triglyceride (SBO-TG) were pooled, solvent evaporated and stored (-18 C).

Hydroperoxides produced during solvent evaporation and storage were conveniently removed by dissolving 0.5g SBO-TG samples in hexane and passing them through Sep-Pak silica cartridges (Waters Associates, Inc., Milford, Massachusetts).

Determination of Oxidative Stability. The Olcott and Einset method (6) was used to measure oxidation in the soybean oil samples. Amounts ranging from 10mg to 1g were weighed into 10ml Pyrex beakers, and weight change was measured with time. The samples were incubated in a convection oven at 60 C or 45 C and were allowed to come to room temperature before weighing.

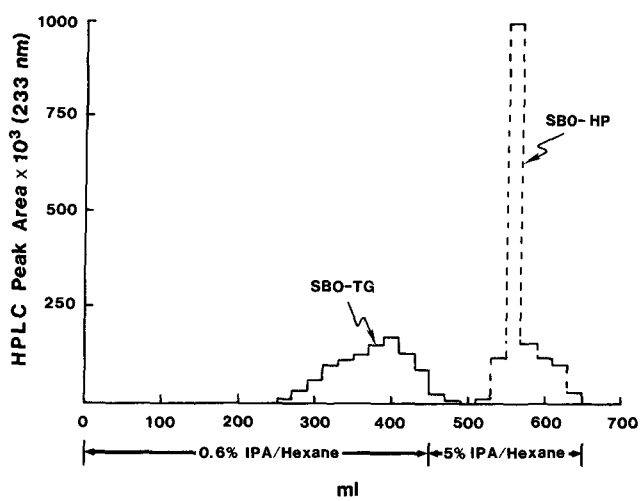

FIG. 1. Elution pattern of SBO-TG and soybean oil hydroperoxides (SBO-HP) from a Florasil column.

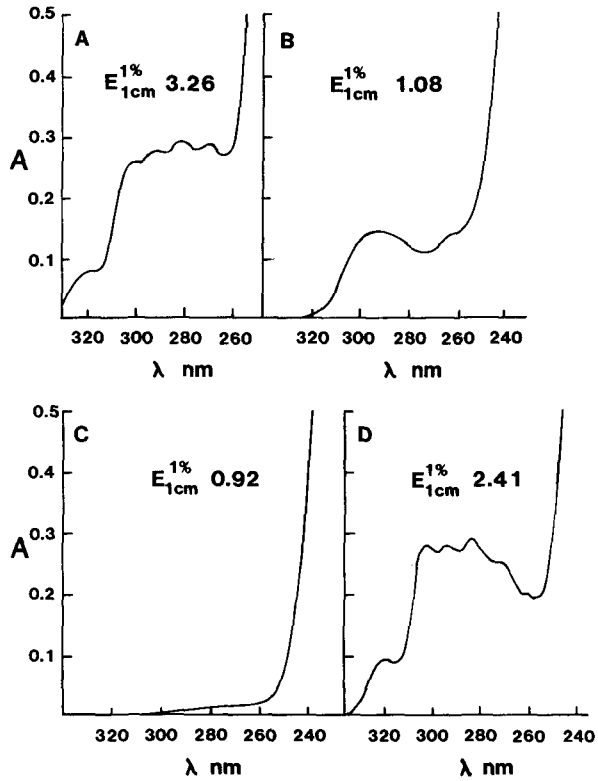

FIG. 2. UV spectra of various fractions from Florasil chromatography. A = SBO-2 (The starting material); B = SBO-TG (260-280 ml); C = SBO-TG (360-380 ml); D = SBO-TG (446-460 ml). All $E^{1\%}_{1\ cm}$ values at 233 nm.

RESULTS AND DISCUSSION

We devised a method based on the procedure of Park et al. (3) that is described in the Experimental Methods to obtain soybean oil triglyceride (SBO-TG). This method gave results shown in Figure 1. The purity of the SBO-TG was monitored by the pattern on HPLC and by $E^{1\%}_{1cm}$ values at 233 nm. Our main objective in the column treatment of SBO-TG was to separate contaminating hydroperoxides, and Figure 1 shows that a separation was achieved with a solvent system of 0.6% isopropyl alcohol in hexane.

The $E^{1\%}_{1cm}$ values for the various SBO-TG fractions differed. The smallest $E^{1\%}_{1cm}$ values were in the middle of the peak of Figure 1. Ultraviolet spectra along with $E^{1\%}_{1cm}$ values are shown for 4 SBO-TG fractions in Figure 2. The higher $E^{1\%}_{1cm}$ values correspond with spectra that show evidence of conjugated trienes and tetraenes.

Relatively low $E^{1\%}_{1cm}$ values were achieved only when SBO-TG were isolated from degummed oil. When bleached oil was the starting material, the $E^{1\%}_{1cm}$ values were higher and spectra showed larger quantities of trienes and tetraenes.

Pooled fractions of SBO-TG were incubated at 60 C along with soybean oil samples taken at various stages of refining. The weight increases were measured, and results are shown in Figure 3.

It is known that refining soybean oil decreases its oxidative stability (1), and the extent is shown in Figure 3. Degumming (SBO-2) decreased oxidative stability, but oils that were degummed and alkali refined (SBO-3), or degummed, alkali refined, bleached and deodorized (SBO-5) were even less stable.

The result that bleached oil (SBO-4) was not more stable than alkali refined oil (SBO-3) seems to be at variance with the result of Cowan (2) who found that bleaching signif-

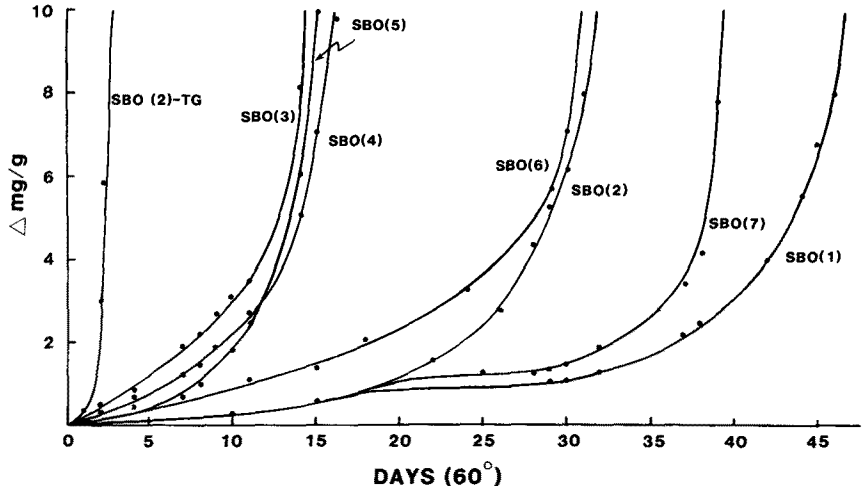

FIG. 3. Autoxidation of various soybean oil samples (g) (see text for description of symbols) as measured by weight increase.

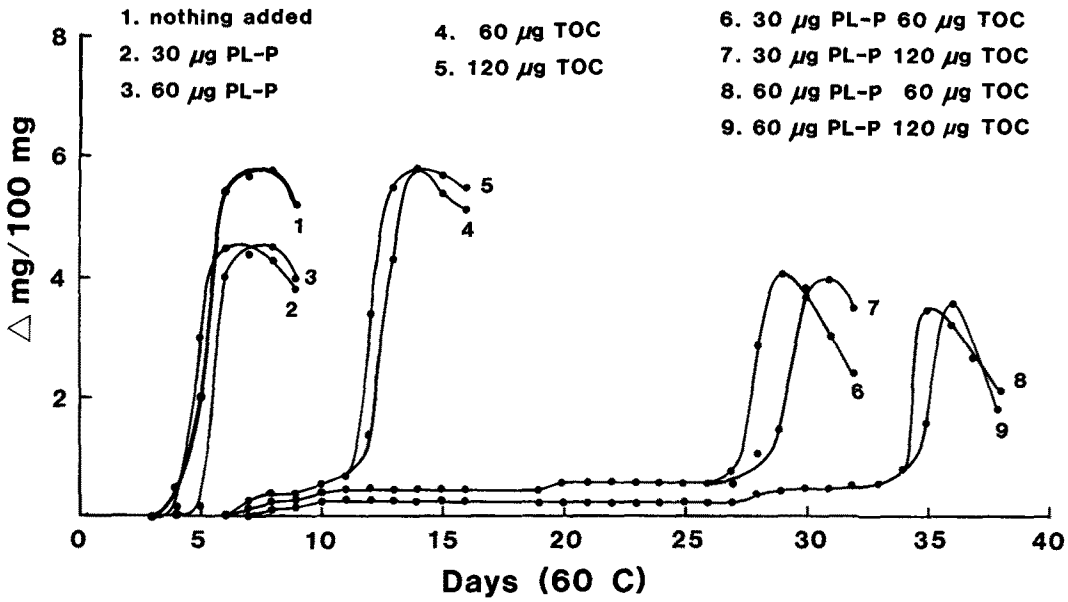

FIG. 4. Autoxidation of SBO-TG (100 mg) before and after addition of various combinations of phospholipids and tocopherols.

icantly increases the flavor stability of the fully refined oil with and without the bleaching step. When the bleaching step was omitted, the final oil had less flavor stability. This is a different experimental procedure than ours, in which oxidative stability was measured by change in weight immediately after the bleaching step. The difference probably is due to removal by deodorization in Cowan's experiment of prooxidant conjugated compounds produced by the bleaching step.

Oxidative stability was increased, as would be expected, by hydrogenation (SBO-6), and it was increased further by adding antioxidants to the hydrogenated oil (SBO-7). The hydrogenated oil with antioxidants had stability approximately equivalent to the original crude soy oil (SBO-1).

Oxidative stability of the SBO-TG was only about 1/15 that of the crude oil based on time to reach a weight increase of 1%. The most likely reason for the decreased oxidative stability of the highly purified soybean triglyceride was the loss of inherent components such as phospholipids and tocopherols. But there also is the possibility that prooxidants such as iron and copper could have contaminated the oil during purification. Such prooxidants could account for the decreased oxidative stability. We tested the oxidative stability of the purified triglyceride fraction in the presence of citric acid and found no difference due to citric acid. Consequently, we concluded that removal of phospholipids and tocopherols was the most likely reason for decreased oxidative stability.

There has been considerable emphasis placed on breeding soybeans for low linolenic acid (7) with the goal of increasing oxidative stability. We believe the results shown in Figure 3 emphasize another aspect of soybean oil stability that has not been recognized sufficiently. That is that naturally occurring components in soybean oil can have a large effect (15-fold) on its oxidative stability without changing the fatty acid composition. If flavor deterioration in soybean oil is tied to specific compounds resulting from linolenic acid oxidation, then the goal of a low linolenic acid soybean is valid. However, oxidation of linoleic acid can also lead to off-flavor compounds, thus the potential benefit from optimizing the antioxidative properties inherent in crude soybean oil may be more readily

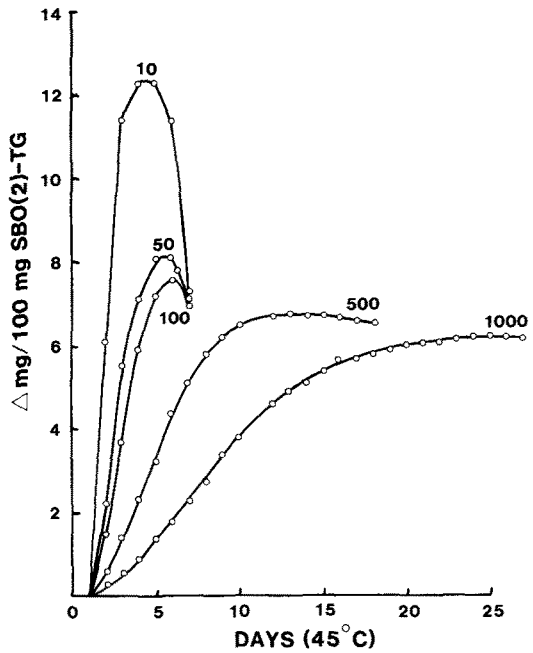

FIG. 5. Effect of sample size or increase in weight as calculated on 100 mg sample basis. The numbers indicate the mg of SBO(2)-TG present in a 10 ml glass beaker.

achieved than the benefit from a low linolenic soybean oil.

As a first step toward improving oxidative stability by use of inherent compounds in soybean oil, we have experimented with adding back phospholipids and tocopherols to highly purified SBO-TG (Fig. 4). Adding either 30g or 60g of PL-P (equivalent to about 1 to 2% phospholipid) to SBO-TG (100 mg) did not appreciably change the susceptibility to oxidation, although with 60g of added PL-P the onset of oxidation was delayed 1 day.

When tocopherols alone were added in 60g and 120g quantities (giving approximately the concentrations of tocopherols found in refined and crude soy oil, respectively), the onset of oxidation was delayed.

By combining the phospholipid and tocopherol additions, a definite synergism was noted. Interestingly, the doubling of tocopherol concentration seemed to have relatively little effect on the delay of onset of oxidation, but changing the phospholipid concentration did have a noticeable effect in the presence of tocopherols.

For practical purposes, adding 1 to 2% phospholipid to refined soybean oil would lead to the same problems that phospholipids cause in crude soybean oil. Those are a gummy precipitate forming with time and browning reactions during deodorization. However, a smaller amount of phospholipid may still contribute to a significant increase in oxidative stability.

During the course of these experiments we noted that the rate of weight increase depended on the amount of sample being tested. Figure 5 shows the rate of weight increase/100 mg of oil for 6 sample sizes. We interpret these results to mean that the rate of weight increase/unit weight and consequently the rate of oxidation of the oil depend on the surface area of oil exposed.

Olcott and Einset (6) noted a decrease in induction period with an increase in surface area of oil using the weight increase method. Going (1) noted an increase in peroxide value with an incrase in surface area/volume for 3 oil samples stored identically with respect to time and temperature. Our results confirm these (1,6) and indicate the need for control of surface area in studies of rates of oil oxidation.

Peroxide values are calculated on the basis of a kg of oil without reference to amount of surface exposed. Based on the results noted above, we recommend that reports on the rate of change of oxidative stability of oils include information on the size of the container and the size of the sample, so that surface effects can be reproduced.

The oxidative stability of soybean oil is an important quality attribute, and these results: the greatly decreased oxidative stability of highly purified soybean oil, the change in oxidative stability due to refining steps, the interaction of tocopherols and phospholipids in restoring oxidative stability, and the influence of surface area exposed in calculating oxidation rates, should be useful in subsequent research on and the understanding of soybean oil oxidative stability.

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