TABLE I

Effects of Analysis on Iodine Value and Refractive Index

Fatty Acids	Iodine Value				Refractive Index at 25°C.			
	ASTM		Federal		ASTM		Federal	
	Initial	After analysis	Initial	After analysis	Initial	After analysis	Initial	After analysis
Linseed	177	176	177	173	1.4675	1.4688	1.4675	1.4693
Soya	139	139	141	138	1.4633	1.4640	1.4633	1.4636
Tall oil	132	129	131	131	1.4663	1.4649	1.4663	1.4665

TABLE II

Effects of Alkyd Resin Processing on Iodine Value and Refractive Index

Alkyd resin	Hours of processing	Iodine Value		Refractive Index at 25°C.	
		Resin processed at 400°F.	Resin processed at 450°F.	Resin processed at 400°F.	Resin processed at 450°F.
Linseed	2	170	174	1.4696	1.4700
	5	172	171	1.4693	1.4696
	9	172	168	1.4685	1.4695
Soya	2	141	137	1.4638	1.4645
	5	141	138	1.4637	1.4638
	9	135	137	1.4643	1.4639
Tall oil	2	126	124	1.4651	1.4665
	5	127	125	1.4650	1.4648
	9	126	125	1.4651	1.4649

iodine values or refractive indexes resulted from any analysis. Generally the analyses resulted in a slight decrease in iodine values and a slight increase in refractive indexes.

Effects of Alkyd Resin Processing. Iodine values and refractive indexes of the fatty acids separated from the alkyd resins are listed in Table II. Only minor changes in the iodine values and refractive indexes of the fatty acids resulted from the alkyd resin processing. Slightly more changes occurred in the linseed fatty acids and in the fatty acids from resins processed at 450°F. Decreases in iodine values were accompanied usually by increases in refractive indexes except for the tall oil acids. Decreases in iodine values of tall oil acids were accompanied generally by decreases in refractive indexes.

Acknowledgment

The authors wish to express their gratitude to the Resin Research Department for the preparation of the alkyd resins used in this investigation. They also acknowledge the helpful suggestions of C. A. Lucchesi and G. G. Schurr.

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Effect of Autoxidation Prior to Deodorization on Oxidative and Flavor Stability of Soybean Oil

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DEODORIZATION of vegetable oils is effective in removing volatile odor components and in destroying fat hydroperoxides (1,2,3,24). The effect of the hydroperoxide decomposition products on the subsequent oxidative stability of the oil is less well understood.

One effect of heating oxidized fats under deodorizer conditions is the formation of a reducing material that interferes with the Emmerie-Engel tocopherol determination (9). Fat hydroperoxides are known to undergo rapid destruction when the temperature is raised above 100°C. (7), and for this reason most workers (4,8,11,18,22,23) have used lower temperatures in both the oxidation and isolation procedures used to study products derived from fat autoxidation. However temperatures as high as 120°C. have been employed (13,21). O'Neill (17) and Williamson (25) studied the thermal decomposition of fat peroxides and purified methyl linoleate hydroperoxide at 100°C. Even after heating for 23 hrs., followed by molecular distillation at 110°C., Williamson found that a large amount of active peroxidic material remained in the distillate.

Chang *et al.* (5,6) concluded that oxidative polymers introduced into soybean oil during processing could serve as the precursors of flavor reversion compounds. Johnson *et al.* (15) studied the autoxidation of ethyl linoleate oxidative polymers and indicated that this reaction was a possible source of volatile flavor-reversion compounds in soybean oil. Holm *et al.* (14) have stated that high-molecular-weight carbonyl compounds, formed through the breakdown of oxidation products, were the substances responsible for poor oil stability and flavor reversion. They believed that the concentration of carbonyl compounds in a fat or oil was indicative of both the amount of oxidation that an oil has undergone and the future stability of a finished margarine. Some of these secondary

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oxidation products must be stable to processing conditions because such substances are found in raw, refined, and finished oils.

This paper reports the effects of mild oxidation prior to deodorization on the quality of edible soybean oil. Changes in stability were related to the formation of decomposition products derived from fat hydroperoxides.

Methods

Several lots of commercially refined edible soybean oil and laboratory prepared methyl esters of soybean and safflower fatty acids were used. The esters were prepared by alkaline methanolysis of the oil, followed by the usual purification and distillation procedures. Oxidations were conducted at room temperature and at 60°C. in a thermostatically controlled bath by bubbling oxygen through the fat or ester. High peroxide levels were obtained in the methyl esters by ultraviolet light catalysis.

Peroxides were destroyed during the usual laboratory conditions of deodorization used in preparing samples for taste panel evaluation (16), which consist of heating the oil for 3 hrs. at 210°C. at a pressure of 1 mm. or less. The methyl ester hydroperoxides were decomposed by heating small quantities of the oxidized esters under nitrogen for 15–20 min. at 210°C. Unoxidized esters and monomeric materials were separated from the high-molecular-weight hydroperoxide breakdown products by molecular weight distillation in a falling film still at 120°C. and under 10 microns of pressure. The high-molecular-weight residue was distilled at 200°C. and under 5–10 microns to yield about 70% of a dimeric product. Both the undistilled residue and the distilled dimers were utilized in oxidative and flavor-stability studies that will be described later.

The concentration of breakdown material in an oil or fat derived from thermal decomposition of the peroxides can be determined by the reducing power, as described previously (9), or by the increased carbonyl content of the fat system (14). The initial peroxide value is also a partial measure of residual material since only about 10% of the weight of the hydroperoxide is lost through volatilization during deodorization and 90% forms high-molecular-weight, fat-soluble materials.

Unsaturated carbonyls were determined by the method of Henick *et al.* (12), using crotonaldehyde as a standard and measuring the absorbance at 460 mμ. The method described by Sidwell *et al.* (20) was used to determine the thiobarbituric acid (TBA) values in the oils submitted for taste-panel evaluation.

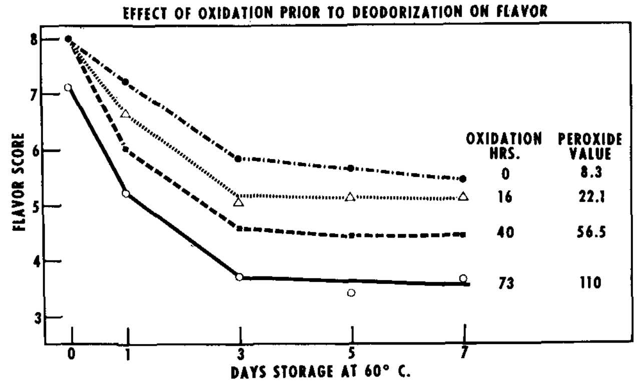

FIG. 1. Effect of autoxidation prior to deodorization on the flavor stability of soybean oil.

Experimental Results

Figure 1 shows the results of an organoleptic evaluation of a sample of refined bleached soybean oil that was submitted to three degrees of oxidation prior to its deodorization. Several different lots of soybean oil gave identical experimental results, and these duplications are not reported. The oil in this particular experiment (Figure 1) was oxidized with oxygen at 60°C. for 16, 40, and 73 hrs. and the resulting peroxide levels, as measured by the ferric thiocyanide method, increased to 22, 57, and 110 me./kg., respectively, above the initial value of 8.3. Tocopherol content of the oil dropped 4.8, 7.2, and 13.7%, respectively, from an initial value of 1,420 γ/g. Oils were evaluated by the taste panel after deodorization and after aging for 1, 3, 5, and 7 days in a 60°C. oven. An organoleptic evaluation by a 15-member taste panel detected no significant impairment in the initial quality of the deodorized oils at the two lower levels of oxidation. At the highest level of oxidation (P.V. of 110) a significant lowering of the initial flavor score was observed.

After 1 day's aging the panel distinguished between the flavor of the respective samples. The decrease in flavor score of oils was in direct relationship to the amount of oxidation they had undergone prior to deodorization. All oils behaved in a typical manner in showing the greatest loss in quality during the first 3 days of storage. At all levels of oxidation the flavor scores of the most highly oxidized samples were always significantly lower than those of the control.

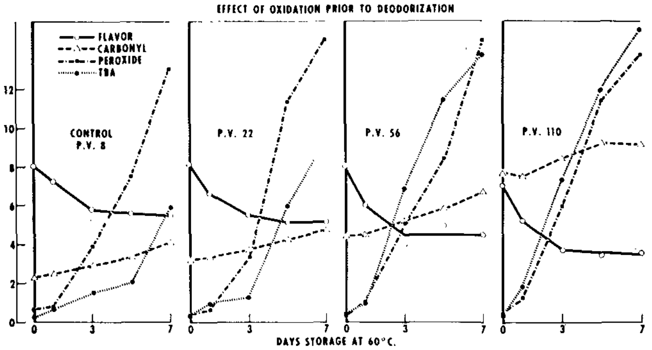

FIG. 2. Effect of autoxidation prior to deodorization on the oxidative and flavor stability of soybean oil. Units: flavor 0–10; carbonyl mg./kg.; TBA absorbance at 530 mμ × 10^{-1}.

Chemical data obtained in the evaluation of these aged samples and the comparison with organoleptic data are presented in Figure 2. Peroxide development curves are similar for each of the four samples, showing an induction period of approximately 1 day and a rather uniform and rapid rise in peroxide thereafter. TBA values follow the peroxides rather closely and also show an induction period, which indirectly corresponds to the amount of oxidation received prior to deodorization by each of the respective samples. This induction-period response for the TBA test has also been observed in other studies.

Carbonyl values show a constant rate of increase regardless of the level or rate of prior exidation. The initial value of each of the three oxidized samples increased over the unoxidized control in direct proportion to the degree of prior oxidation. This increase in carbonyls during storage for 1, 3, 5, and 7 days did not show a linear relationship to increase in peroxides.

The association of the peroxide value before deodorization to the carbonyl content after deodorization is shown in Figure 3. A good correlation is evident in the conversion of the hydroperoxide to carbonyls at high temperatures. This linear relationship holds at all peroxide levels investigated. The rate of carbonyl formation may change with the nature of the fat peroxides and the conditions of oxidation, such as temperature, presence of metals and metal inactivators, exposure to light, and other factors influencing fat oxidation. Under conditions of oxidation where peroxides are readily decomposed (*i.e.,* high temperatures and high metal contents) high carbonyl values are produced per unit of peroxide. The lowest carbonyl values were determined in freshly deodorized and unoxidized oils. The initial values in Figure 3 are about average for soybean oil, but some soybean oils gave values ranging from 400 to 600 mg./kg. Oil from freshly extracted beans has shown carbonyl values somewhat less than 100; however the precision of measurement at this level is not good (± 20%). Even when freshly distilled and prepared ordinarily, soybean fatty acid esters have shown about this level of carbonyl values. Low carbonyl values were obtained only with highly purified esters. On the basis of current data it cannot be indicated whether the carbonyl value obtained upon extrapolation to a 0 peroxide level is a base value or whether it is indicative of an equivalent amount of prior oxidation. The resolution of this problem will offer an interesting study in autoxidation.

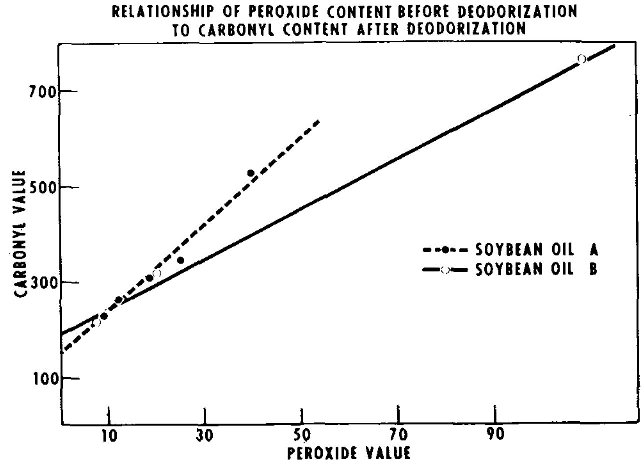

FIG. 3. Relationship of the peroxide content of soybean oil before deodorization to the carbonyl content after dedorization.

Effects of Added Thermal Products. The deleterious effect on flavor resulting from the addition of the distilled dimeric fraction to soybean oil is presented in Figure 4. The loss in flavor quality is seen to be in direct relation to the concentration of distilled dimeric material that was added to the oil. Although the addition of 0.5% of the material did not lower the flavor score significantly, each increment caused a lowering of quality, which was detected by the taste panel. The higher the percentage of added material, the lower was the initial flavor score and the poorer the quality of the oil upon aging.

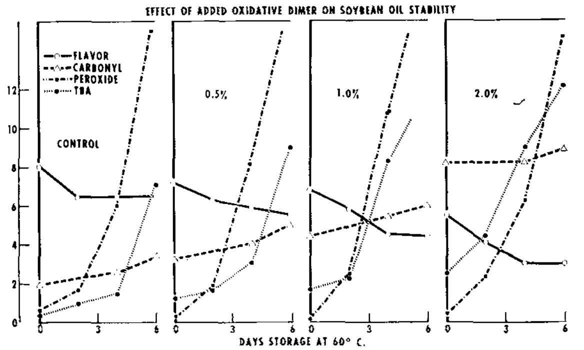

FIG. 4. Effect of added distilled oxidative dimer on the organoleptic and chemical stability of soybean oil. Units are the same as in Figure 2.

Figure 4 depicts chemical data similar to that discussed in Figure 2 but obtained from a different lot of soybean oil containing 0.5, 1.0, and 2.0% of added distilled dimeric material. TBA values indicate a similar type of induction period as observed previously, and the rate of development corresponds to the increase in peroxide values in Figure 2. Since the added dimeric material contains carbonyl groups, each sample of oil shows the corresponding increase in the initial carbonyl level. The rate of development of new carbonyl materials during the aging of the oil is not influenced by the amount of dimeric carbonyl added. The rate appears to remain constant and approximately equal to that in the earlier experiments.

In previous work it was shown (9) that these heat decomposition products of peroxides could be removed by chromatography on silicic acid. Figure 5 presents the results of chemical and flavor evaluations of soybean oil treated with silicic acid to remove carbonyls. Although the carbonyls are reduced to 20% of the original, the rate of carbonyl development during storage of the oil is the same in treated or untreated samples. However the rapid drop in flavor scores of the aged silicic acid-treated samples and their poor oxidative stability could be attributed to the chromatographic operation. The next pair of graphs show the same oils, one untreated and the other treated with silicic acid. These two oils were deodorized in the presence of 0.01% citric acid. Under these conditions flavor scores and the oxidative stability values of the two are about the same. The effect citric acid had in stabilizing the silicic acid-treated oil indicates that metal contamination occurred during the chromatographic procedure. It also appears that citric acid is

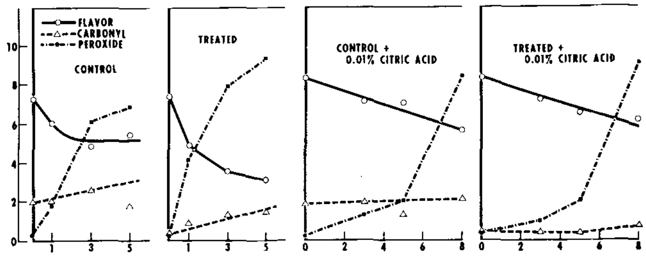

FIG. 5. Effect on the stability of soybean oil through removal of aldehydes by silicic acid. Units are the same as in Figure 2.

effective in preventing the development of carbonyls upon the aging of oils. The metal-inactivating action of citric acid appears to prevent or reduce the rate of peroxide decomposition which results in a decreased carbonyl formation.

Figure 6 shows the rate of peroxide development in air at 60°C. for samples of edible soybean oil submitted to oxidation prior to deodorization. Small samples (2-g.) were shaken in a Warburg apparatus, from which 20-mg. samples were removed periodically for the peroxide determination. Figure 7 shows the oxidation curves for soybean oil containing added distilled oxidative dimer. Oxidation rates in both sets of experiments indicates a lowered stability corresponding to the amount of dimer added or to the extent of prior oxidation. These experiments confirm results obtained on the taste-panel samples.

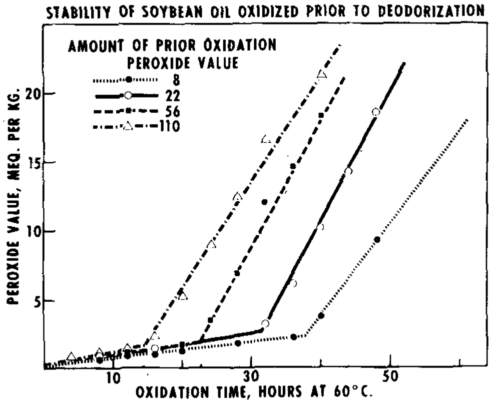

FIG. 6. Oxidative stability of soybean oil oxidized to different levels prior to deodorization.

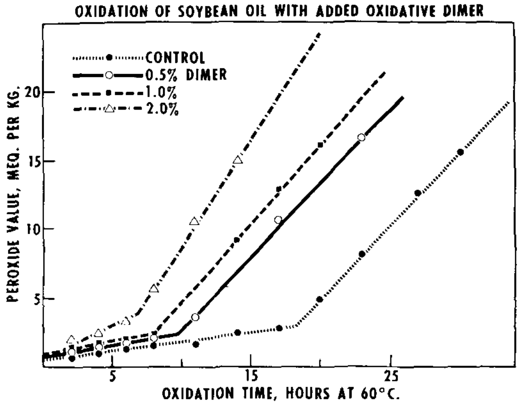

FIG. 7. Oxidative stability of soybean oil containing added distilled oxidative dimer.

Discussion

No differences could be established in the flavor or oxidative stability of soybean oil whether the products of thermal decomposition of the hydroperoxides were developed *in situ* or added to the deodorized oil. Organoleptic evaluations indicate that at low levels of oxidation unusual flavors or odors did not result from these thermal-derived products. Flavor and odor descriptions are those normally encountered in describing aged soybean oils. The pure distilled dimeric material isolated from the methyl ester hydroperoxide has a mild, tangy, bitter, and persistent taste that clings to the tongue. When this material is added at high levels (2%) to an oil, bitterness is the common flavor description. Although the lowering in flavor scores parallels oxidative treatment, statistically significant differences in scores were usually obtained only between the control sample and the most highly oxidized sample. Thus, in the flavor examination of soybean oil, where the oxidations were carried only to a peroxide level of 10, no differences were obtained between the control and the oxidized samples in either the initial flavor score or in the aged flavor scores. Our taste-panel data would indicate that, prior to deodorization, peroxide levels of 20 or 25 are necessary before a detectable difference in the flavor of aged oils can be established. Higher initial peroxide levels would be required to show significant differences in the flavor of freshly deodorized oils.

The chemical tests used to evaluate oxidative stability of the various oils gave results that were in agreement with the oxidative treatment of the samples, even at the lowest oxidation levels. These tests, although high in sensitivity like the taste test, are however nonspecific as to the cause of the lowered stability. In a similar way Holm *et al.* (14) used a benzidine test which measures the concentration of oxidation products thought to be unsaturated nonvolatile aldehydes. They interpreted the test as a measure of the future stability of the fat and propose this test as a measure of the oxidative history of crude and refined oils. Roschen *et al.* (19) obtained results on lard similar to that presented here. They showed that the stability of fresh lard was impaired by dilution with lard oxidized prior to deodorization. These workers found that the catalytic effect of the deodorized lards was not caused by peroxides and believed rather that the effect was caused by nonvolatile aldehydes.

When the carbonyl test used in our experiments is employed on freshly deodorized oils, the increase in carbonyl content over the unoxidized control is in agreement with the past oxidative treatment of the samples. Initial carbonyl values for soybean oil varied from 200–400 γ/g. Whether these materials reacting as carbonyl compounds result only from the breakdown of hydroperoxides cannot be stated at this time. Until the nature and structure of these materials are known and the mechanism of their formation is understood, it would not be justifiable to extrapolate such data. All types of hydroperoxides are decomposed under deodorization conditions, and any volatile, unstable aldehydes or ketones would not be measured.

Lowered flavor quality and poor stability of soybean oil cannot be attributed to the loss of antioxidants at these relatively low levels of oxidation. This is evident because 87% of the tocopherol remained in the oil at the highest levels of oxidations studied (P.V. of 110 FeCNS method), and, in other experiments where thermal decomposition products were added, there was no reduction in the tocopherol content. Earlier we reported that, by removing one-half to two-thirds of the tocopherol, the flavor stability of soybean oil is occasionally improved and the oxidative stability is usually improved (10).

The experimental evidencee presented here indicates, that, during the active oxidation of soybean oil, both peroxides and carbonyl material showed an increase but that no simple direct relationship existed between the two. However Henick *et al.* (12) showed a linear relationship during the oxidation of both soybean oil and corn oil. Sidwell *et al.* (20), in applying this method, found no increase in carbonyls during the oxidation of butter but did find an increase during the oxidation of lard and vegetable oils. The labile nature of aldehydes, especially the α,β-unsaturated and the dialdehydes formed in fat oxidation products, makes any determination of these compounds difficult to reproduce. Better and more specific methods, along with improved techniques, are definitely needed to evaluate and interpret chemical data in flavor work.

Summary

Oxidation prior to deodorization was shown to be detrimental to the flavor and oxidative stability of soybean oil. The increase in the nonvolatile carbonyl content of freshly deodorized oils was proportional to the peroxide value of the oils before deodorization. Rate of loss of flavor and oxidative stability of the oil were related to the extent of carbonyl development. All oils, whether or not they had been submitted to any known oxidation, contained some nonvolatile carbonyls. The loss in stability was not due to a loss of the antioxidant tocopherol.

Oxidized soybean oil methyl esters were shown to develop nonvolatile carbonyl compounds upon heating at deodorization temperatures. The addition of isolated methyl ester peroxide decomposition products to deodorized soybean oil reduced its flavor and oxidative stability in proportion to the amount added. The results obtained were parallel and similar to those obtained by oxidizing soybean oil prior to deodorization.

Flavor deterioration and undesirable flavors were typical of aging soybean oil whether or not the oils were oxidized before deodorization or whether an equivalent amount of nonvolatile thermal decomposition products was added to the oil. These oxidatively derived, nonvolatile carbonyl materials are believed to enter into the sequence of reactions that contribute to flavor instability and quality deterioration of soybean oil. The structure of these materials is not known.

This work indicates the importance of minimizing autoxidation in soybean oil particularly before deodorization to insure good oxidative and flavor stability.

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Quantitative Gas Chromatography of Fatty Derivatives. Relative Detector Responses to C_6-C_{14} Saturated Methyl Esters

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The relative response of a thermal conductivity cell, in terms of peak area produced per mole of compound, has been determined for the fatty methyl esters caproate through myristate. The values were found to be a linear function of molecular weight.

IN GAS CHROMATOGRAPHY of fatty derivatives, using thermal conductivity detectors, claims have been made that the peak areas are proportional to the weights of the corresponding components of the sample, and this assumption has been applied in the quantitative analysis of such materials (2,5). Increasingly sophisticated approaches to quantitative gas chromatography have revealed that the situation is not as simple as previously thought, and two different effects have been postulated to account for the fact that, in homologous series, the peak areas become progressively smaller than would have been predicted. One mechanism is that advanced by Orr and Callen (8): transesterification occurring between the sample components and polyester column liquids results in a decrease in the amounts of these components actually reaching the detector. The extent of the reaction, and corresponding decrease in the amounts eluted, would be progressively greater for those substances with longer retention-times; the effect was emphasized at higher temperatures. Other workers have attributed this trend to "minor errors associated with the technique of calculating areas" (6).

The second mechanism has been elegantly documented by Rosie *et al.* (7,9) for an impressive variety