TABLE I

The Oxidation of Soybean Oil Under Different Atmospheres

Time	Peroxide Value			
	100% O_2	21% O_2	0.5% O_2	0% O_2
hrs.				
0	0.7	0.7	0.7	0.7
0.5	6.8	4.7	2.1	....
1	14.8	9.1	2.1	....
2	28.2	16.3	2.8	....
4	63.6	33.3	4.0	0.8
8	132	76.6	6.4	1.7

234 mμ is due to oxidation products rather than the "reversion compounds" (1) of soybean oil.

Effect of Atmospheres of Different Oxygen Concentrations on the Flavor Changes Produced by Light Exposure

The data in Table I show the peroxide development in samples of soybean oil when exposed under varying oxygen concentration to the G-E reflector-drying lamp.

As expected, the oxidation rate decreased with decrease in oxygen concentration above the sample. Minute traces of oxygen were apparently present in the purified nitrogen as shown by the very slight but definite rise in peroxide value following very long exposures under the purified nitrogen.

Under each set of conditions it was found that reversion flavors were detectable after one-half hour of exposure. It may also be observed that under nitrogen, the reversion appeared at very low peroxide values. The various samples shown in Table I were examined organoleptically in three ways.

First, the effect of increasing exposure time on degree of reversion was noted. In each atmosphere studied, it was found that the reversion flavor became more pronounced as the exposure period increased.

Second, the samples exposed for equal lengths of time were compared with each other. Little or no difference could be detected between samples of oil maintained in oxygen and in air for the same period of time, despite the difference in their peroxide values. However, a marked difference was noted between the above samples and those exposed under nitrogen. The former acquired the grassy flavor generally associated with reversion, but in the latter, grassy flavors, though evident, were accompanied and superseded by a particularly disagreeable and persistent drying aftertaste not readily detectable in the soybean oil reverted in air or oxygen. Oil exposed under nitrogen for very long periods developed a flavor that resembled heat-reverted oil.

Finally, pairs of oil samples with approximately equal peroxide values were compared with each other. In general, it was found that the samples exposed for the greater length of time were more strongly reverted.

Summary

It is evident that the oxidation rate of soybean oil may be varied over a considerable range without influencing the organoleptic evaluation of the degree of reversion. Even when the rate of oxidation is greatly reduced by the use of inert atmospheres, there is no diminution in the tendency to revert. On the contrary, with low oxygen concentrations, a type of reversion is produced that is more persistent in taste than that resulting in air or oxygen.

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Extraction and Purification of Gossypol from Cottonseed Meats*

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THE literature indicates that some investigators (3) have encountered difficulties in precipitating gossypol extracted from cottonseed according to the modified method of Clark (5) and consequently have first extracted the oil from the crushed cottonseed with petroleum ether as described by Carruth (2, 3, 7). Murty, Murty, and Seshadri (7) developed another method, after finding their yields unsatisfactory with the latter, in which the gossypol was extracted from crushed cottonseed meats and precipitated with aniline. The dianilino-gossypol was converted to gossypol acetate by boiling for a few minutes with acetic anhydride. Boatner (2) reports that she was unable to remove all the red pigment from gossypol by two recrystallizations as the acetate and as gossypol, respectively.

Clark's methods of extraction (5) precipitation and purifications (6) have been satisfactory in this laboratory with certain modifications in technique to facilitate the procedure. A somewhat detailed description of the method is given because gossypol is precipitated with difficulty, if at all, unless precautions are taken to prevent its over-heating or scorching in the oil-ethyl ether extract; it is necessary to use peroxide-free ether at all times. (Ether in a wash bottle was repeatedly found to contain peroxides after standing in the laboratory a few days.)

Preparation of Cottonseed or Cottonseed Meats

Freshly crushed or rolled decorticated kernels or meats from prime quality seed collected before entering the cooker are screened as free from hulls as possible by means of a wire screen of 16 meshes per square centimeter and extracted with peroxide-free

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ether by percolation. If raw seeds covered with linters are used, the seeds may be crushed in a hammer feed mill, the Wiley mill without screens, or Bauer mill and the meats screened from the hulls and linters through a somewhat coarser screen. The meats are then ground two or three times by a meat chopper using the intermediate sized blades to prevent clogging and extracted as above. Damaged or immature seed should not be used as they are high in free fatty acids, and the gossypol acetate precipitates with difficulty.

Extraction of the Gossypol

A small quantity of seed or meats, 1.5 pounds, may be satisfactorily percolated in a larger dispensing burret or glass percolator. A wad of cotton is inserted above the stopcock and the latter adjusted to allow a flow of about 50 ml. of ether per minute. The meats should be kept covered with ether until percolation is practically completed as judged by the color of the extract, which changes from a cherry red to a pale yellow color. Remove the ether and make a precipitation test with acetic acid on 10 ml. of the oil in a test tube as outlined below. This test indicates the feasibility of proceeding on a larger scale.

Larger amounts of cottonseed meats are percolated in a galvanized sheet-iron cylinder 4 feet long by 8 inches in diameter to the bottom of which is soldered a cone of the same material fitted with a stopcock for controlling the rate of percolation to about 125 ml. per minute. A wad of cotton is placed above the stopcock.

Place fifty pounds of screened meats in the cylinder and percolate with peroxide-free ethyl ether.[1] Maintain the ether above the level of the seed until the extraction is practically complete as judged by the color of the extract which is collected in 4-liter bottles. Then drain the ether from the extractor and express the ether-gossypol mixture from the residue with a fruit press. The last one or two gallons of the extract as well as that expressed from the residue are used over again on a new batch of meats.

Separation of Ether from the Oil-Gossypol-Ether Extract

Four liters of the oil-ether extract mixture is poured into a 12-liter round bottom flask containing an ebullition tube and placed in a water bath. The water in the water bath should be approximately level with surface of the oil-gossypol-ether extract, and its temperature should not exceed 75° C. This prevents over-heating of the very sensitive gossypol in the mixture. Distillation of the ether is discontinued when the extract in the distilling flask reaches a temperature of approximately 55° C. At this temperature the extract begins to foam although the ether continues to distill over quite freely. If this residual oil-gossypol mixture is over-heated, the gossypol acetate is precipitated with extreme difficulty, if at all. The ether remaining in the oil-gossypol mixture is further removed by filtering the hot extract with suction through a Büchner funnel.

This filtration is greatly facilitated by the use of a prepared diatomaceous earth, Hyflo Super-Cel from Johns Manville Corporation. A mat is formed with a ¼-inch layer of Hyflo Super-Cel on a moistened filter paper placed in a deep Büchner funnel. The extract, in which a small quantity of Hyflo Super-Cel has also been suspended, readily filters through this mat without clogging.

[1] *Test for Peroxides in Ether.* The Vanadic Acid Test (1) is used. One-tenth gram of Vanadic oxide heated on the steam bath with 2 ml. of H_2SO_4 for 10 to 15 minutes is cooled and diluted to 50 ml. with water. Shake 1 to 2 ml. of this solution with 5 to 10 ml. of ether; a pink color indicates peroxides.

Precipitation of Gossypol Acetate

To each volume of the filtered extract add equal volumes of glacial acetic acid and of petroleum ether (Skellysolve F). Stir thoroughly and let stand until precipitation is complete. With prime quality seed this occurs within a few hours or is generally complete after standing overnight. Occasionally a longer time is required. Overheating retards or prevents precipitation.

When the precipitate has settled, filter through a Büchner funnel using C. S. & S. filter paper No. 597 or its equivalent. Wash the precipitate with either Skellysolve F or petroleum ether. On standing small amounts of gossypol acetate may separate from the filtrate.

Separation of the Gossypol from the Acetate

The acetate may be removed from the gossypol by suspending 50 gms. of the gossypol acetate in 400 ml. of ethyl ether over the surface of 1600 to 2000 ml. of distilled water in a three-liter beaker containing a small amount of Sodium Hydrosulphite (3, 4) and the ether stirred until the gossypol is dissolved. The gossypol remains in the ether layer while the acetic acid goes into the water layer. Transfer the mixture to a 3- or 4-liter enamelled mixing bowl and remove the ether by placing the mixing bowl in a water bath heated to about 75° C. The mixture is stirred continually during the evaporation of the ether. The gossypol at first separates into a sticky mass which breaks up into particles as the ether is driven off. The larger surface exposed in the mixing bowl gives a product which breaks into particles of about 2 to 5 mm. in diameter more readily than when a beaker is used.

When the water in the mixing bowl reaches about 60° C. decant through a Büchner; replace with cold distilled water to cool the gossypol; after standing a minute or two, decant again through the Büchner. The gossypol precipitate is crushed with a spatula, transferred to the Büchner, and washed several times with distilled water.

Recrystallization as the Acetate

Transfer the gossypol to a beaker and dissolve in the smallest possible amount of ether, then decant from the small amount of water onto a small Büchner funnel containing a mat of hydrochloric acid washed Hyflo Super-Cel (8). The small amount of ether in contact with the remaining water is separated from it with a separatory funnel and filtered. Place the stoppered filter flask containing the filtrate in warm water and concentrate to about 350 ml. under partial vacuum. Then transfer to a liter beaker and add an equal volume of glacial acetic acid. Mix thoroughly by stirring. Precipitation takes place immediately and is complete in 10 to 20 minutes. Filter on a Büchner funnel and wash slightly with a mixture of acetic acid and ether (1:1), then remove the filtrate and wash thoroughly with Skellysolve F. The precipitate should be recrystallized as gossypol acetate two or three times, or until the red color is removed.

A small amount of the gossypol acetate may be further recovered from the filtrate by adding an equal volume of water in a separatory funnel. A greater part of the acetic acid passes into the water layer and is drained off. On concentrating the ether layer more gossypol acetate separates out. This recovered gossypol is kept separately until enough has accumulated to work up a batch by removing the two molecules of acetic acid of crystallization and recrystallizing as the acetate as given above until this is sufficiently pure to combine with the purer grade of gossypol above for further recrystallization. Any shade of cherry red color (5) observed in the ether solution of the gossypol should be removed by recrystallization as the acetate.

The free gossypol is prepared by removing the acetic acid from the recrystallized gossypol acetate as described above. It is dissolved in ether and filtered through a small Büchner funnel containing the acid washed Hyflo Super-Cel mat. To the filtrate add two volumes of Skellysolve F and concentrate under reduced pressure with the flask standing in warm water.

When crystals begin to form readily, transfer the solution to a beaker and add one-half volume of Skellysolve F. Crystallization takes place rapidly. Filter when precipitation is practically complete (15 to 20 minutes. Then wash slightly with Skellysolve F. After removing the filtrate, wash thoroughly.

The first crop of crystals of gossypol are recrystallized two or three times until the ether solution has a bright canary yellow color. It is essential that peroxide-free ether be used throughout the purification process.

Preparation of Solvent-Free Gossypol

Prepare the solvent-free gossypol (6) by dissolving 50 gms. of the purified gossypol above in 400 ml. of ether, filter, place the filtrate over 2 liters of distilled water containing a trace of sodium hydrosulfite in the mixing bowl, evaporate the ether while constantly stirring, by placing the mixing bowl in a water bath maintained at 75° C., continuing the heating until the water in the mixing bowl reaches approximately 60° C. Decant through a Büchner funnel, pour cold distilled water over the gossypol, decant this water through the Büchner. Repeat this process, first crushing the lumps of gossypol with a spatula. Wash the crushed gossypol onto the Büchner with distilled water and drain thoroughly, partially dry at room temperature and finally in a vacuum oven at 60° C. Then grind in mortar and store in a dark cold place.

Discussion

In the precipitation and purification of gossypol Skellysolve F, a petroleum ether with a boiling range of 30 to 60° C. (Skelly Oil Co., Kansas City, Missouri) is used. Petroleum ether of a higher boiling range may be substituted, however.

Crushed cottonseed kernels containing 0.7 to 1.0 per cent gossypol yields 0.4 to 0.6 per cent crude gossypol when extracted with ethyl ether without previously extracting the oil with petroleum ether. This is a yield of 60 to 65 per cent of that in the cottonseed meats. Approximately 0.3 gm. gossypol acetate per liter is dissolved in a mixture of equal parts Wesson oil, petroleum ether, and acetic acid.

In purifying the crude gossypol the following yields were obtained: 80 to 85 per cent for each recrystallization as the acetate, about 70 per cent for the first crop of crystals as the gossypol, and about 30 per cent for the final purified product. The crystals coming down after the first crop are collected and reprocessed. The precipitates should be filtered off immediately after the rapid precipitation has subsided, and washed.

Over-heating the extract of gossypol while removing the ether from the oil causes difficulties in precipitation. When 200 gms. of crushed cottonseed were percolated with 1000 ml. of ether and the ether removed under reduced pressure with maximum temperatures of 48° C. in the water bath and 37° C. for the extract, the gossypol acetate began to precipitate immediately with the addition of equal parts of Skellysolve F and glacial acetic acid. The yield was 0.52 per cent gossypol after precipitating for 24 hours, which was increased to 0.57 per cent in 48 hours. A yield of 0.49 and 0.55 per cent was obtained for 24 and 48 hours, respectively, with maximum temperatures of 64° C. in the water bath and 50° C. in the extract. A third batch having maximum temperatures of 80° in the water and 75° C. in the extract began to precipitate more slowly and gave only 0.34 per cent after 24 hours. This increased to 0.56 per cent in 48 hours. With larger batches, the extracts are subjected to the heat for longer periods of time.

Murty, Murty, and Sheshadri (7) added water to the crushed cottonseed before extracting with ethyl ether. This is unnecessary for crushed cottonseeds; however, it does increase the amount of gossypol extracted from cottonseed meal or cake; neither is it necessary to add water and alcohol to the U. S. P. ethyl ether before extracting cottonseed.

The purity of the gossypol is determined by an electometric titration with standard sodium hydroxide and hydrochloric acid using glass-calomel electrodes. Precautions must be taken to avoid the effects of oxygen and carbon dioxide. Pure gossypol solutions should be free from the red color associated with crude gossypol. Gossypol prepared by the above method had a molecular weight of (a) 518.3, (b) 518.4 by titration.

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