Molecular Distillation of a Crude Soybean Oil

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I. Introduction

Molecular distillation has received considerable attention in this laboratory during the past several years and has proved to be a useful tool in studies of the composition of soybean oil (3, 7, 21).

The extensive literature on molecular distillation (5, 6) reveals that in the field of vegetable fats and oils most workers have used refined oil rather than crude oil as starting material. The desirability of preliminary removal of "break" material is frequently mentioned, while Embree (9) states that molecular distillation cannot be carried out on oils that contain more than traces of phospholipids and mucilaginous matter. For this reason, and to demonstrate the performance of the still used at this laboratory, it appears useful to present the results of a typical molecular distillation of a crude expeller soybean oil.

The oil was subjected to exhaustive fractional distillation; that is, the undistilled residue remaining after each cycle or pass over the distilling surface was recirculated and used as the starting material or distilland for the next cycle at the same or a higher temperature. The distillate from the various cycles was collected and analyzed to show the extent of fractionation of minor constituents such as free fatty acids, unsaponifiable matter, phosphatides, and pigments, as well as that of the component glycerides of the oil. Attention was also given to the oxidative stability of the various fractions.

II. Apparatus

The molecular still used in the present study was the same as that previously described (7) with two additional features. A solenoid-operated glass pump was installed in the system below the residue receiver to permit recirculation of undistilled residues. This pump was a modification of that described by Hickman (17), the hollow magnet-iron piston being enclosed within glass walls so that no metal could come into contact with oil in the system. A timing device permitted automatic intermittent operation. The capacity of the pump was about 5 g. of oil per stroke, or 50 to 60 g. per minute, and the hold-up in the chamber and riser was about 158 c.c.

A manostat placed in the line to the kerosene reflux system permitted precise control of the pressure therein, and hence of the temperature of the evaporating surface. Essentially the same as the design published by Hershberg and Huntress (16), the manostat made use of sulfuric acid with platinum contacts to actuate an electronic relay. Any increase over the established pressure served to operate an intermittent "flutter valve," or plug at the end of a capillary tube, which connected the system to a water aspirator or mechanical pump. With the manostat in operation it was possible to maintain a constant still temperature within 1°C.

III. Distillation Procedure

The original charge of crude soybean oil was 1865 g., representing about a third of the capacity of the still. This oil had been pressed from 1938 Illini beans in a half-commercial-size Anderson super-duo expeller. The maximum cage temperature during operation of the expeller was 261°F., considerably lower than temperatures usually obtaining in commercial expeller operations. As a consequence, and because it had been stored for 10 months prior to the distillation, the oil had a somewhat lower phosphatide content than commercially produced oil, which facilitated degassing prior to distillation. Degassing was accomplished by two or three hours of evacuation in the storage reservoir and passing the oil down the cold column prior to distillation.

Ordinary crude expeller oils are somewhat tedious to degas in the preliminary stages; however, with reasonably careful operating technique, proper adjustment of pumping speed and rate of flow, etc., no special difficulty is encountered in degassing or subsequent distillation, contrary to implications in the literature (9, 12, 33). No observable decomposition of phosphatides was encountered at temperatures up to 240°C., the maximum required for complete distillation of the triglycerides.

The operating data are presented in Table 1, in which each pass represents a single circulation of the distilland over the column except in the case of Pass 1, where the amount of distillate was so small that two cycles at the same temperature were combined; Pass 2, where three cycles were combined for the same reason; and Pass 31, where after the first cycle the residue was continuously recirculated until only the hold-up remained.

The temperatures recorded in the table represent maximum temperatures at the lower part of the evaporating surface, as measured by a thermocouple placed within the reflux column. Because kerosene was used as the heating medium, a temperature gradient existed along the column, usually of 11 or 12°C. The upper part of the column thus served partly as a preheater, most of the distillation taking place along the lower portion.

The temperature differential between evaporating and condensing surfaces was an almost linear function of evaporating temperature, being 73, 105, and 110°C. for evaporating temperatures of 156, 234, and 240°, respectively. Since such differentials are ample to ensure nearly complete condensation of distillate molecules, it was considered unnecessary to resort to artificial cooling of the condensing surface.

During the first pass traces of a distillate—presumably free fatty acids and tocopherols—were ob-

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served at a temperature of about 150°C. Solids also were collected during this pass; they included chiefly sterols, as well as a noticeable amount of material containing a red pigment. While this material doubtless contained carotenoids, it also contained a substance soluble in water but insoluble in common organic solvents, and having a distinct odor of apples.

Most of the remaining sterols were collected during the second pass, at about 185°. Triglycerides also began to distill at this temperature, but the amount of condensate was inconsiderable up to about 220°.

Above 220° the temperature in successive cycles was raised only as required to ensure a useful rate of distillation. In this manner all of the oil except the hold-up in the circulating pump was distilled at temperatures no greater than 240°, although other workers have indicated that temperatures as high as 260 (11), 267 (20), 270 (17), and even 295° (29), are necessary for the complete distillation of soybean and other oils containing triglycerides of C_{18} fatty acids.

Free air pressures within the system were measured with a McLeod gage, which was separated from the distilling column by a liquid air trap. An indication of the total pressure was obtained with a thermal conductivity gage placed near the bottom of the distilling column, where the pressure in the system might be expected to be a maximum. The latter instrument cannot be considered to give strictly accurate readings, since it was calibrated against air, and because it is influenced by variations in ambient temperature. It served, however, as a check on the distilland flow rate, to ensure that volatiles were not being evolved faster than the pumps could remove them.

It is observed in Table 1 that for Passes 5, 9, 13, 17, 22, and 29 the total pressures indicated by the thermal conductivity gage were relatively high, although the free air pressures were satisfactorily low. Prior to each of these passes the course of work had been interrupted so that the liquid air had evaporated from the cold trap, allowing volatiles to diffuse back into the system. As would be expected, this increase in pressure noticeably influenced the distillation rate.

Except in the foregoing cases and the early Passes 1 to 6, the total pressure appeared to have little relation to the amount of distillate.

TABLE 1

Operating Data

Pass No.	Temperature	Pressure		Distilland flow rate [1]	Distillate	Rate of distillation	Ratio,[1] distillate / distilland
		McLeod gage	Thermal conductivity gage				
	°C.	*Microns*	*Microns*	*g/hr.*	*g.*	*g/hr.*	*Percent*
1 [2]	156	0.051	5.6	615.7	2.3	0.4	0.07
2 [3]	185	.045	7.7	586.7	12.3	1.4	.2
3	217	.118	13.7	886.8	12.8	6.7	.8
4	218	.968	10.3	812.1	11.5	5.5	.7
5	215	.008	16.3	559.0	11.4	3.8	.7
6	223	.091	4.4	500.0	33.8	10.2	2.0
7	224	.079	2.9	632.0	28.2	10.9	1.7
8	229	.167	3.1	533.9	36.7	12.2	2.3
9	230	.006	13.4	507.9	31.9	10.4	2.0
10	231	.026	2.4	633.0	41.4	17.1	2.7
11	233	.013	2.0	389.0	65.7	17.2	4.4
12	234	.051	2.0	449.0	61.3	19.3	4.3
13	234	.059	9.3	429.5	45.8	14.4	3.4
14	235	.166	2.6	358.4	74.9	20.4	5.7
15	236	.080	1.7	372.3	79.8	24.0	6.4
16	237	.177	1.8	331.2	82.8	23.6	7.1
17	238	.480	6.2	189.7	120.5	21.2	11.2
18	238	.143	2.3	150.8	160.5	25.4	16.8
19	238	.065	1.7	264.5	80.1	26.7	10.1
20	238	.367	2.1	135.8	132.0	25.1	18.5
21	239	.006	1.9	193.4	85.8	28.6	14.8
22	239	.018	2.7	114.0	116.1	26.8	23.5
23	239	.005	2.0	110.2	86.0	25.1	22.8
24	238	.006	1.8	139.6	50.3	24.2	17.3
25	237	.008	1.7	87.0	62.9	22.9	26.3
26	239	.011	2.4	72.7	56.5	23.3	32.1
27	240	.037	2.3	101.5	37.1	31.7	31.2
28	239	.056	2.6	42.2	39.6	20.6	48.9
29	240	.005	5.8	70.3	13.1	22.6	32.1
30	240	.005	2.8	46.7	10.6	18.3	39.1
31 [4]	240	.005	3.1	85.2	14.1	36.2	96.2
31x [5]					1.1		

SUMMARY

	Temperature	Amount	
	°C.	*g.*	*Percent*
Original charge of crude soybean oil	.....	1865.0	100.0
Distillate:			
Fractions 1 to 5	156-220	50.3	2.7
Fractions 6 to 9	221-230	130.6	7.0
Fractions 10 to 31	231-240	1518.0	81.4
Hold-up in circulating pump	.....	146.5	7.9
Loss due to handling, etc	.....	19.6	1.0

[1] Corrected for losses due to handling, loss of volatiles, oil and solids left on column, etc.

[2] Because of the small amount of distillate, the distilland was circulated twice during this pass.

[3] Distilland was circulated three times during this pass.

[4] After the first cycle the distilland was recirculated until only the hold-up remained.

[5] Final drainings of distillate from condenser after Pass 31.

The data also indicate that in the latter stages where the temperature in successive passes was nearly the same and volatiles had been well removed from the oil, the distilland flow rate could be varied within rather wide limits (about 50 to 400 g. per hour) without greatly changing the rate of distillation (about 20 to 25 g. per hour). The percentage of distillate (distillate-distilland ratio) was markedly affected by changes in the distilland flow rate, since the latter determines the thickness of the oil film on the evaporating surface, and consequently the total number of molecules exposed during the course of a cycle to distillation on the surface of the film. The point is illustrated in Fig. 1.

IV. Analytical Results

Determinations of iodine value, unsaponifiable matter, free fatty acids, phosphorus, and other characteristics were made for various distillate fractions as well as for the original oil and the residue. The results are recorded in Table 2 and Figs. 2 to 5. In addition, determinations of component fatty acids were made as shown in Table 3 and Fig. 2.

The over-all accuracy of the principal analyses was checked by multiplying the weight of the original charge of oil by the analytical value concerned, and comparing this product with the sum of corresponding products for each of the distillate fractions plus the residue. Percentages of error were determined to be as follows (+ indicates that product for orig-

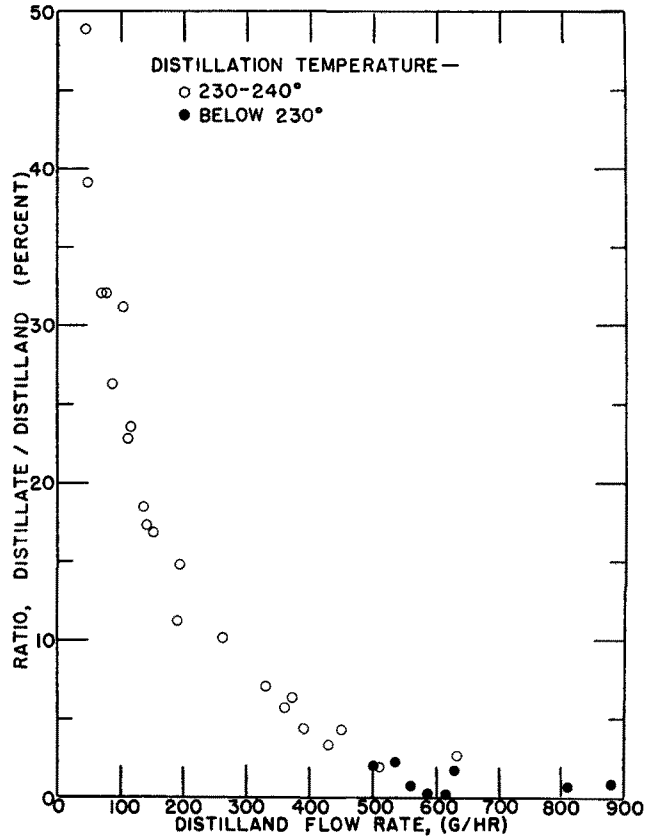

FIG. 1. Relation between distilland flow rate and percentage of distillate.

TABLE 2

Characteristics of Distillate Fractions

Fraction No.	Iodine number [1,2]	Unsaponifiable [1]	Free fatty acids [1,3] (as oleic)	Phosphorus [4]	Density, 25°C.[5]	Viscosity, 25°C.[6]	Refractive index,[7] n_D^{25}	Dispersion,[7,8] v^{25}
		Percent	*Percent*	*Percent*	*g./c.c.*	*Centipoises*		
Original oil [9]	133.1	0.62	0.29	0.0306	0.9182	50.1	1.4737	11.907
1	134.0	48.4	37.86	.....	.....	.....	.....	.....
2	132.0	34.2	10.04	.....	.....	.....	.....	.....
3	128.3	8.9	2.41	.....	.....	.....	1.4760	12.091
4	126.8	4.8	1.50	.0027	.....	.....	.....	.....
5	126.6	3.2	0.89	.....	.....	.....	1.4735	11.867
6	127.6	1.4	.45	.0024	.....	.....	1.4730	11.925
7	128.2	....	....	.....	.9172	52.1	1.4728	11.877
8	127.8	0.90	.14	.0029	.....	.....	1.4726	11.804
9	127.3	....	....	.....	.9166	.....	1.4727	11.735
10	128.4	.55	.11	.0023	.....	.....	1.4728	11.705
11	128.6	....	....	.....	.9162	51.7	1.4726	11.698
12	129.2	....	....	.....	.....	.....	1.4728	.....
13	129.1	....	....	.....	.....	.....	1.4728	.....
14	130.0	.33	.11	.0017	.....	.....	1.4727	.....
15	130.2	....	....	.....	.9162	50.1	1.4728	11.738
16	130.2	....	....	.....	.....	.....	1.4730	.....
17	131.4	....	....	.....	.....	.....	1.4730	.....
18	132.3	.19	.07	.0011	.....	.....	1.4730	.....
19	132.5	....	....	.....	.9163	49.1	1.4731	11.853
20	133.2	....	....	.....	.....	.....	1.4732	.....
21	134.2	....	....	.....	.....	.....	1.4735	.....
22	134.9	.21	.04	.00093	.....	.....	1.4735	.....
23	136.2	....	....	.....	.9162	48.2	1.4737	11.841
24	136.8	.17	.04	.0010	.....	.....	1.4737	.....
25	137.5	....	....	.....	.....	.....	1.4738	.....
26	138.3	.10	.05	.0009	.....	.....	1.4740	.....
27	138.4	....	....	.....	.9169	48.6	1.4739	11.807
28	138.4	....	....	.....	.9171	48.6	1.4738	.....
29	138.4	....	....	.....	.....	.....	1.4740	.....
30	138.4	.15	.05	.....	.....	.....	1.4740	.....
31	138.8	....	....	.0015	.....	.....	.....	.....
31x[10]	139.1	....	....	.....	.....	.....	1.4740	11.777
Residue	135.0	.33	.19	.276	.9271	62.8	1.4755	11.899

[1] A.O.C.S. official method.
[2] Wijs, 30 minutes.
[3] 5-g sample except Fractions 1 and 2.
[4] Truog and Meyer. Ind. Eng. Chem., Anal. Ed. *1*, 136 (1929).
[5] Göckel pycnometer, 10 ml.
[6] Fenske modification of Ostwald viscometer.
[7] Abbe refractometer.
[8] $v = (n_D - 1)/(n_F - n_C)$.
[9] Constants determined at time of distillation. The freshly-expelled oil had iodine number 133.0; free fatty acids 0.19 percent; phosphorus 0.0358 percent; $n_D^{25} = 1.4733$.
[10] Final distillate drainings from condenser after Pass 31.

TABLE 3

Analyses of Fatty Acids From Distillate Fractions

Fraction No.	Iodine value [1]	Thiocyanogen value [1]	Saturated fatty acids [2]	Unsaturated fatty acids [3]			
				Oleic	Linoleic	Linolenic	Total
Original oil	137.8	84.7	*Percent* 13.8	*Percent* 24.3	*Percent* 55.9	*Percent* 5.4	*Percent* 85.6
2 [4]	126.6	80.2	17.1	29.0	50.8	3.1	82.9
3 [4]	130.4	79.4	17.0	23.2	58.5	1.3	83.0
4 [4]	130.4	79.3	16.6	23.3	59.4	0.7	83.4
5 [4]	131.0	78.8	18.8	20.3	58.2	2.7	81.2
6	132.4	79.2	17.7	19.0	58.3	3.6	80.9
8	132.9	79.8	17.2	20.0	58.8	3.1	81.9
10	134.1	80.6	17.0	20.2	58.6	3.6	82.4
14	135.4	81.1	16.6	19.9	59.9	3.3	83.1
18	138.5	83.8	15.0	21.5	58.3	5.0	84.8
22	141.2	84.8	12.9	21.2	62.2	3.5	86.9
24	142.2	85.9	12.2	22.2	61.2	4.2	87.6
26	144.0	86.4	11.2	21.6	63.8	3.3	88.7
30	143.7	86.4	9.1	23.4	67.0	0.5	90.9
Residue	142.9	87.4	10.3	25.0	60.3	4.1	89.4

[1] A.O.C.S. official method.
[2] Crystallization method of Earle and Milner. Oil and Soap *17*, 106 (1940).
[3] Formulas based on values of 96.3 and 167.3 for thiocyanogen values of linoleic and linolenic acids, respectively. (Kass *et al.* Oil and Soap *17*, 118 [1940].)
[4] Unsaponifiable was removed before determining iodine and thiocyanogen values of mixed fatty acids. In other cases unsaponifiable was small and was not removed.

inal charge was higher than the sum of the individual products):

Iodine number	+1.3
Unsaponifiable matter	—10.7
Free fatty acids	+20.2
Phosphorus	+25.0
Saturated fatty acids	—1.7
Unsaturated fatty acids	+1.4

Data for the iodine numbers of the oil fractions (Fig. 2) indicate that a small but definite fractionation of triglycerides has taken place. The iodine number spread is 12.5 units, as compared with a range of 21 units obtained by Riemenschneider *et al.* (30) in the molecular distillation of refined cottonseed oil. The greater fractionation obtained by Riemenschneider is doubtless attributable to the fact that cottonseed oil has a larger content of palmitic acid than has soybean oil. That a spread of even 12.5 units was obtained, was due in part to the low temperatures employed, since in general the degree of separation of two substances will be better the lower the temperature (10).

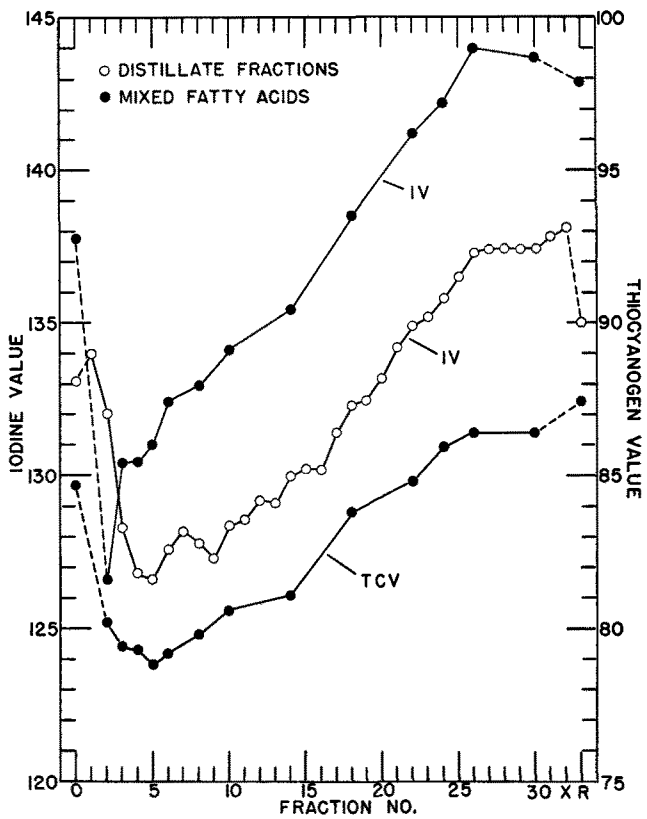

FIG. 2. Iodine and thiocyanogen values. (Note: In this and subsequent drawings, O refers to the original crude oil, R to the final residue, and X to Fraction 31x.)

Further indications of glyceride separation are given by the refractive index curve (Fig. 5).

The relation between iodine number and refractive index is given in Fig. 6. In the calculation of the curve of the function by least squares, values for samples below Fraction 7 were omitted because of their high content of unsaponifiable. It is of interest to note that the curve agrees nearly exactly with that obtained by Majors and Milner (25) for Butt-extracted oil from soybeans of the 1938 crop. For example, for iodine numbers of 130 and 140, the Majors-Milner curve shows refractive indices of 1.4729 and 1.4740, respectively. Corresponding values from the curve in Fig. 6 are 1.4729 and 1.4741.

The constituent fatty acids showed a slight but definite degree of fractionation. It is shown in Table 3 that the unsaturated fatty acids tended to concentrate in the later fractions, while the opposite was the case for the saturated acids.

Fig. 3 illustrates the concentration of unsaponifiable material—chiefly sterols—and of free fatty acids in the early fractions removed at temperatures below 220°. Such materials comprised 86 percent of Fraction 1 and 44 percent of Fraction 2. Of the total unsaponifiable matter present in the original charge of oil, 46 percent appeared in Fractions 1 and 2, and another 18 percent in Fractions 3, 4, and 5. Corresponding figures for free fatty acids are 39 and 11 percent.

Most of the phosphorus present was concentrated in the residue. Because of the low concentrations of phosphorus encountered, the determinations cannot be considered accurate. However, the regular decrease of phosphorus in successive distillate fractions indicates the precision of the determinations, and indicates further that the oil may have contained at least

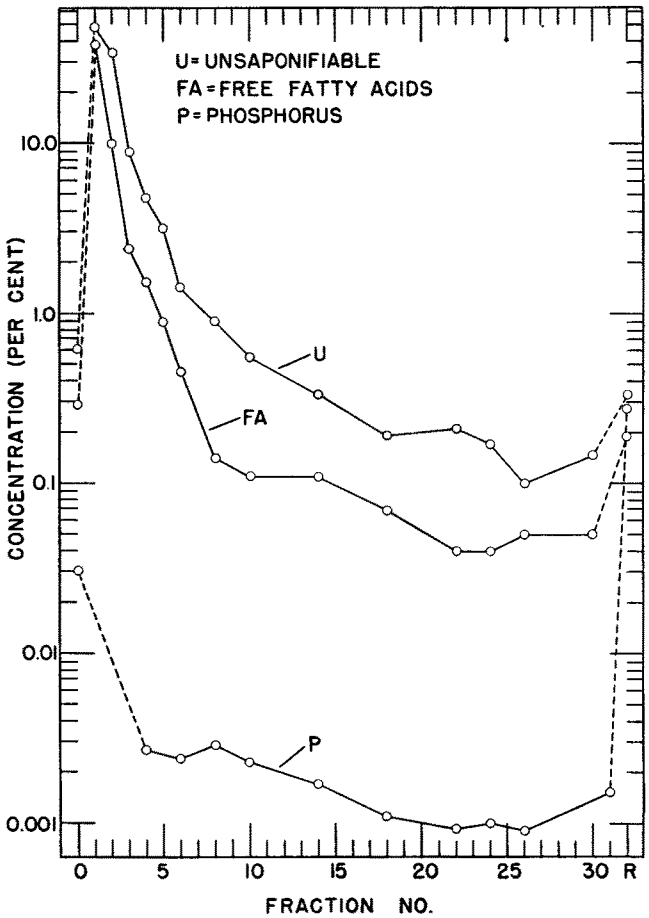

FIG. 3. Unsaponifiable matter, free fatty acids, and phosphorus.

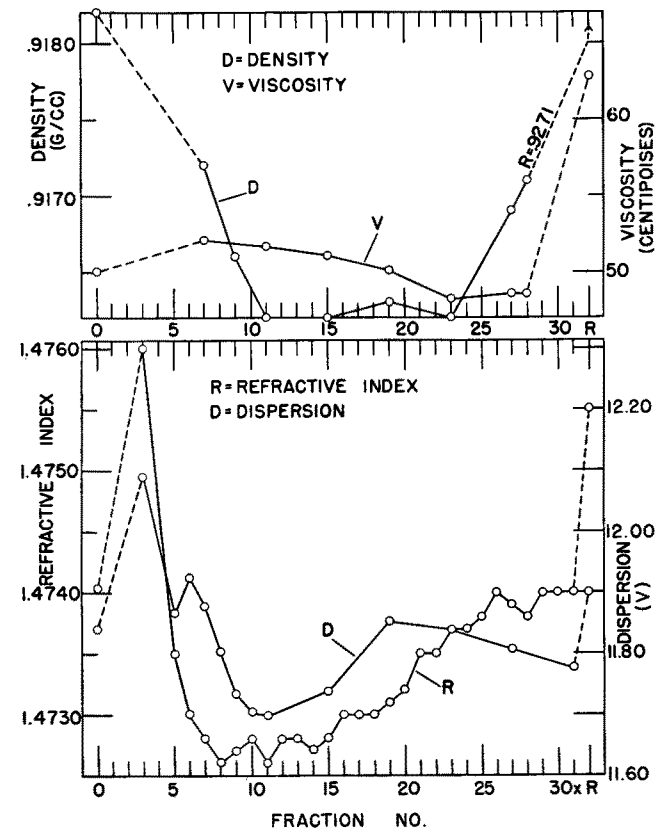

FIG. 4 (above). Density and viscosity. FIG. 5 (below). Refractive index and dispersion.

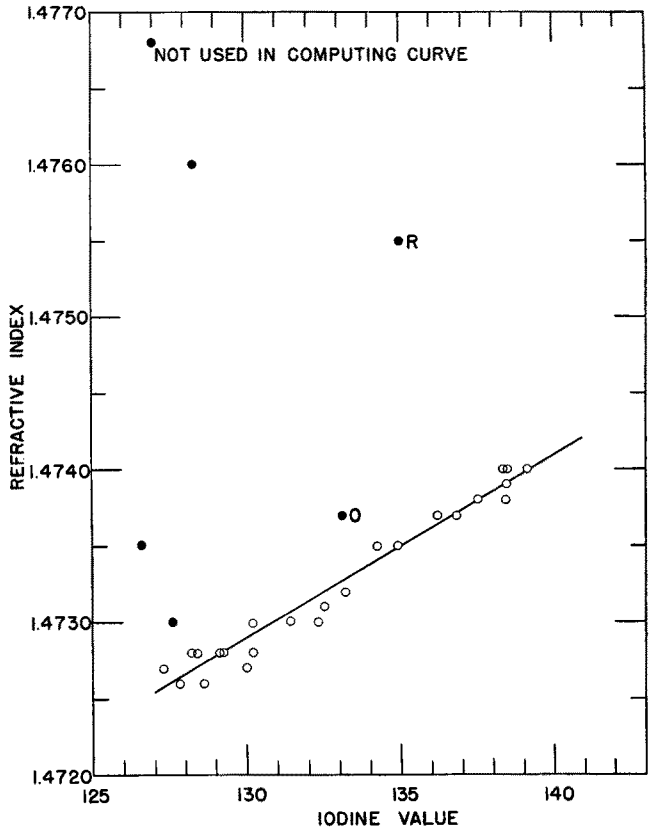

FIG. 6. Relation between iodine value and refractive index.

two phosphorus compounds. One, present in very small quantities, was readily distillable and was concentrated in the early fractions, while the other and much more abundant compound was undistillable.

The curves for density, viscosity, and dispersion (Figs. 4 and 5) give further evidence of fractionation in the successive distillate fractions. The increase in density in the later fractions may be attributed to increasing unsaturation, since Wheeler *et al.* (35) have shown that at 40° the density of triolein is 0.8988 and the density of trilinolein is 0.9184.

In general, the density, viscosity, and refractive index values of the fractions would indicate that polymerization was negligible during the distillation and after the distillates had been collected. The increased viscosity of the residue is only slight, compared to oils which have been appreciably bodied, and may possibly be attributed to the concentration of phosphatide material.

Smoke, flash, and fire points were determined for the original oil, the residue, and two distillate fractions, by methods previously described (8).

	Smoke point		Flash point		Fire point	
	°F.	°C.	°F.	°C.	°F.	°C.
Original oil	385	196	602	317	677	358
Fraction 16	475	246	630	332	685	363
Fraction 20	495	257	635	335	685	363
Residue	475	246	545	285	545	285

The values for the original oil were considerably higher than those previously reported (8) for crude expeller soybean oil—a reflection of the lower-than-usual content of free fatty acids and "break" material. Values for the two distillate fractions were

higher than average results for commercially refined oils.

"Break" material in the original oil, as determined by a modified Gardner heat break method, was found to be 0.16 percent. This relatively small amount was retained in the residue, which had a "break" content of about 3.6 percent, as estimated on the sample used in the fire point test. When certain distillate fractions were heated above the "break" temperature, 245-50°, they not only showed no traces of deposit but also were bleached water-white.

The separation of pigments and antioxidants will be discussed in subsequent sections.

V. Spectral Characteristics

Visual observations. Most of the carotenoid pigments were concentrated in the first six distillate fractions, which had a decided red color. Fraction 7 was colored light orange and Fraction 8 golden yellow. Succeeding fractions progressively lightened to a pale straw color intermediate between a commercial alkali-refined oil and a bleached oil. Beginning with Fraction 18 the oils were observed to have a slight greenish cast. Fractions 27 to 31 had a slightly more intense yellow color than the fractions just preceding.

The distilland began to darken during the first pass, and after the fifth pass was a very dark brown.

Lovibond values. Lovibond readings of the original oil and Fractions 4 to 31, inclusive, were made in a Stevenson type colorimeter (31) using standardized N″ red glasses (15) and unstandardized yellow glasses. Because of the small quantity of many of the fractions, measurements were made in a 1-inch oil thickness instead of the standard thickness of 133 mm. The results, shown in Table 4, confirm the visual observation of a slight darkening of color in the last few fractions.

Spectral transmittance. Spectral transmittance measurements of the original oil and Fractions 6 to 28, inclusive, were made with a General Electric recording spectrophotometer, using an absorption cell 2.516 cm. long and dry carbon tetrachloride as a reference standard. The curves are presented in Figs. 7a to 7d.

In the curve for the original oil (Fig. 7a) the principal absorption band in the red region, characteristic of crude vegetable oils, has its point of minimum transmittance at 668 mmu. This absorption may be due at least in part to pheophytin *a* rather than to chlorophyll *a*, since some or all of the latter may have been thermally degraded during the expeller treatment of the soybeans. For the major red absorption Zscheile and Comar (4, 36, 37) give wavelength values of 666.0 and 660.0 mmu. for pheophytin *a* and chlorophyll *a*, respectively. Although their measurements were made in dilute ether solution, spectrophotometric curves of the crude soybean oil mixed with various amounts of ether have shown that, with increasing dilution, the 668 mmu. absorption band does not shift along the wavelength scale to an extent that would account for the discrepancy noted.

In the early distillate fractions, there is little absorption at 668 mmu. Beginning with Fraction 15, however, there is a steady increase in absorption, indicating that most of the pigment was distilled in the later passes. This point is illustrated in Fig. 8, which shows extinction values at 668 mmu. converted to a 1 cm. thickness of oil.

The minor absorption band at 609 mmu. is possibly due to pheophytin *a* (Zscheile gives values of 608.0 mmu. for pheophytin *a* and 614.0 mmu. for chlorophyll *a* in ether solution).

TABLE 4

Spectral Characteristics

Fraction No.	Lovibond value [1]		Trichromatic coefficients [2]		Brightness	Dominant wave length	Purity
			Red	Green			
	1-inch cell						
	Y	R	*Percent*	*Percent*	*Percent*	*mmu.*	*Percent*
Original oil	70	4.89 [3]	58.23	40.79	52.57	584	98
4	70	17.84	.....	.....	.....	598 [4]	....
5	70	13.70	.....	.....	.....	594 [4]	....
6	70	7.95	62.22	37.11	42.65	588	98 [5]
7	70	6.72	60.82	38.42	47.50	587 [5]	98
8	70	5.92	59.47	39.67	55.19	586	98
9	70	5.42	59.03	40.06	55.02	585	98
10	70	3.99	57.15	41.73	64.05	583	98
11	70	3.22	56.09	42.65	68.45	582	97 [5]
12	70	2.76	55.67	42.98	70.79	582	97
13	70	2.72	55.07	43.44	72.19	581	97
14	70	2.22	54.49	43.87	74.01	580	97
15	70	1.72	53.64	44.55	78.20	578 [5]	96 [5]
16	70	1.20	52.89	44.98	80.16	578	96
17	35	1.10	51.59	44.96	80.94	577	93
18	15+ 20—	0.50	48.67	43.95	83.88	576	84
19	10+ 15—	0.61	47.98	43.50	84.81	576	81
20	5+	0.50	44.58	41.55	86.78	576	67
21	3+	0.28	43.16	40.65	88.12	575	61
22	3—	0.28	42.08	39.91	88.07	575	55
23	3—	0.28	41.56	39.55	88.51	575	53
24	3—	0.41	41.70	39.61	86.94	575	53
25	3—	0.41	41.55	39.42	85.88	575 [5]	52 [5]
26	3—	0.28	41.55	39.41	84.79	575 [5]	52 [5]
27	3+	0.61	42.58	40.14	83.48	576	57 [5]
28	3+ 5—	0.92	43.95	40.56	76.59	577	63
29	5+	1.22	.....	.....	.....	.....	....
30	3+ 5—	0.71	.....	.....	.....	.....	....
31	5—	0.50	.....	.....	.....	.....	....

[1] Stevenson colorimeter.
[2] Since r + g + v = 100 percent, only r and g values are listed.
[3] In 133 mm. cell = 70 Y + 14.94 R.
[4] Estimated from Lovibond values.
[5] The superscript 5 means about ½.

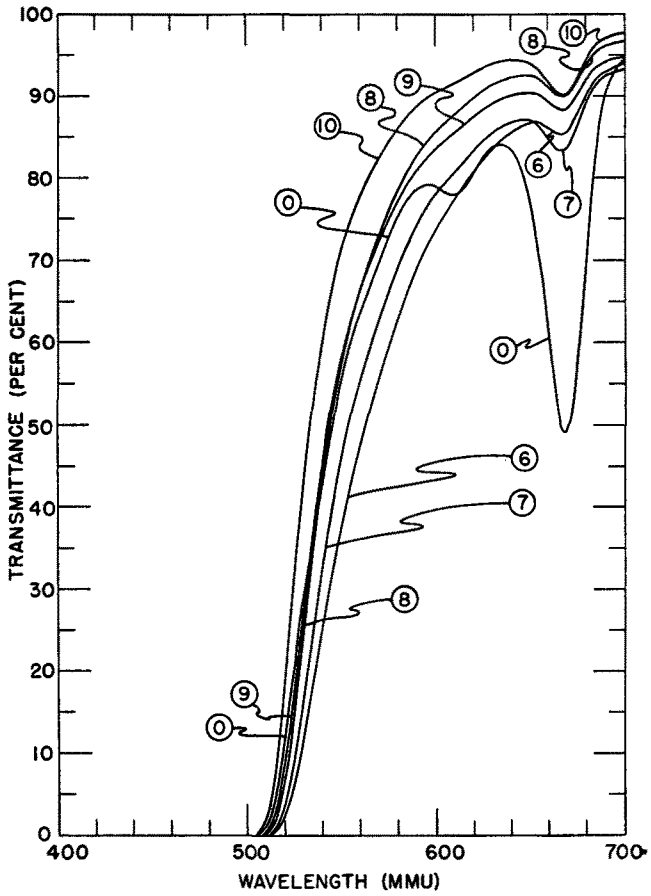

FIG. 7a. Spectral transmittance of original oil and Fractions 6-10.

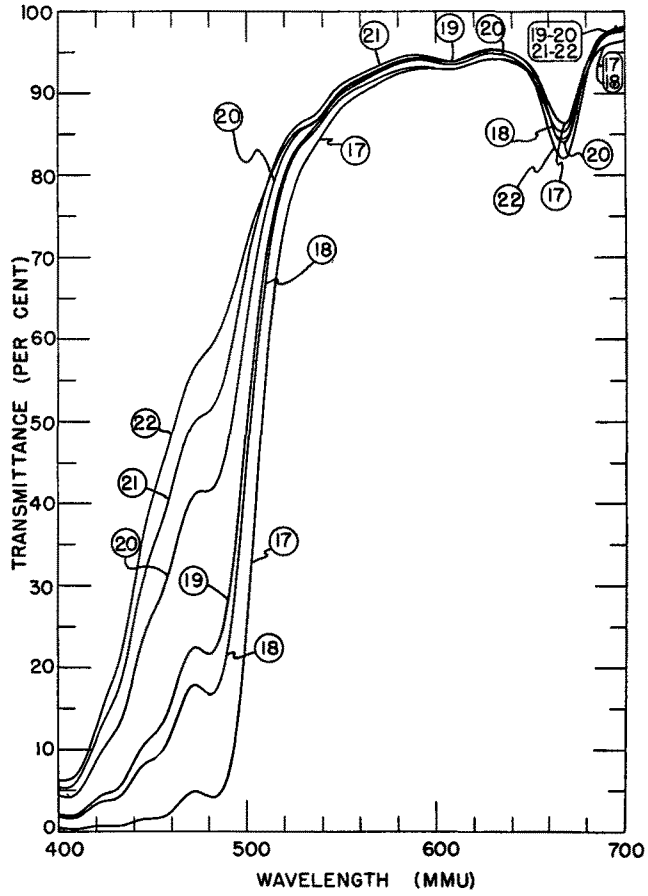

FIG. 7c. Spectral transmittance of Fractions 17-22.

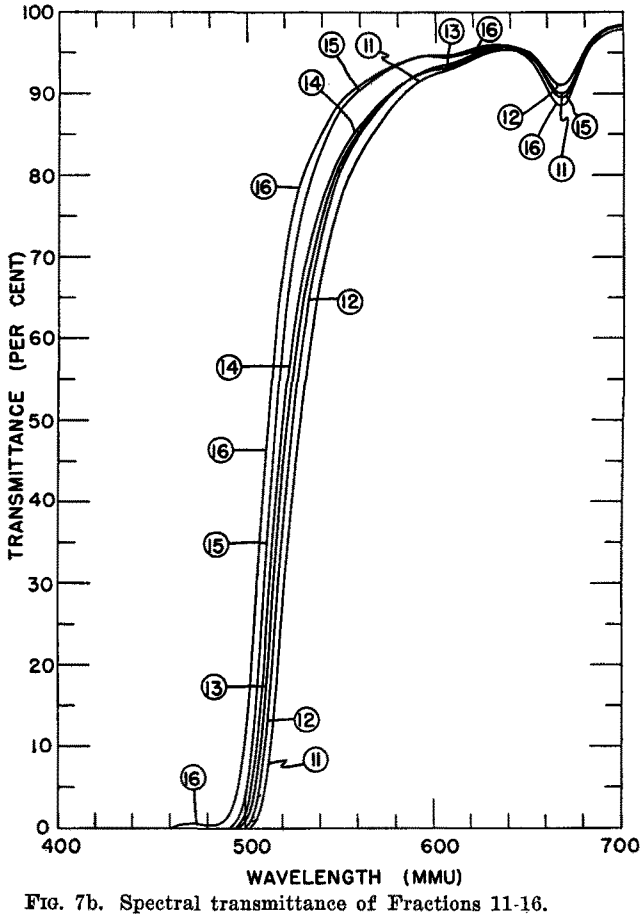

FIG. 7b. Spectral transmittance of Fractions 11-16.

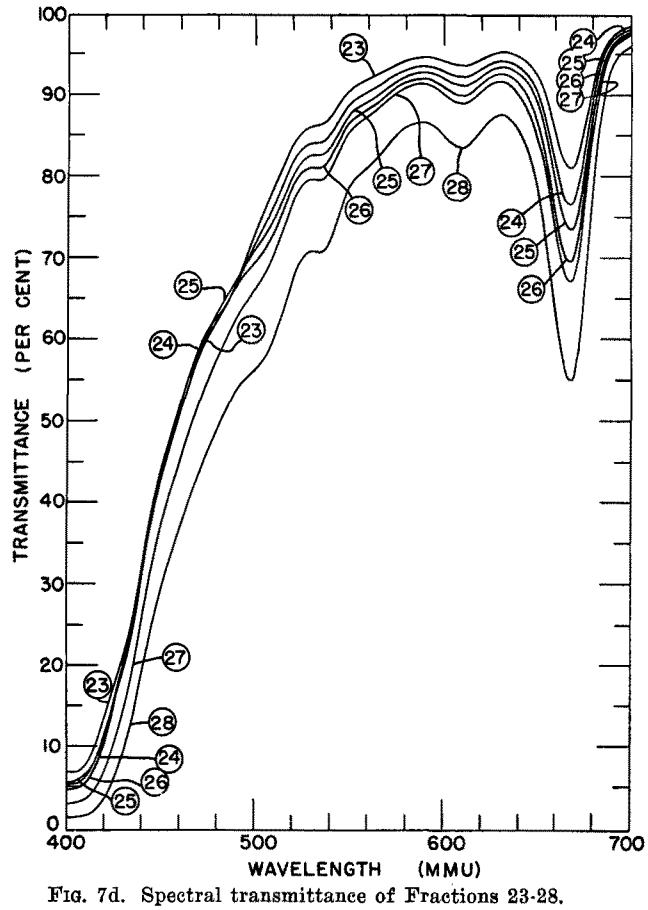

FIG. 7d. Spectral transmittance of Fractions 23-28.

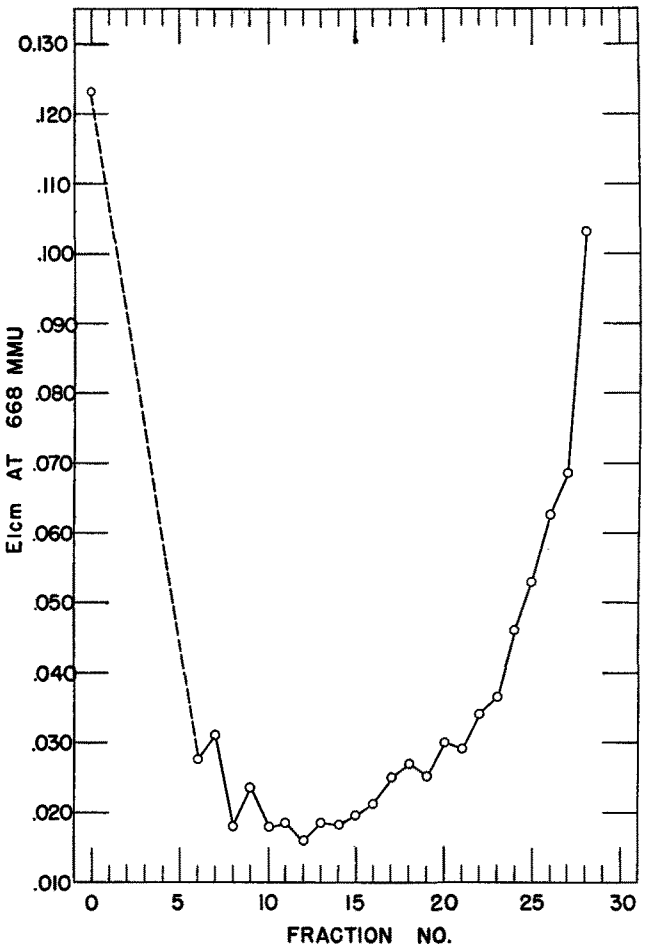

FIG. 8. Distribution of pheophytin.

Because most of the carotenoid pigments were removed in the early distillates, the later fractions were sufficiently transparent in the blue region to expose several additional adsorption bands, at about 560, 535, 481, 453, 430, and 407 mmu. There is some indication that the 560, 535, and 407 mmu. bands are due to pheophytin *a* and the 481 and 453 mmu. bands to carotene. While the 430 mmu. band is uncertain, it might be caused by the presence of pheophytin *b* in minor amounts.

Monochromatic and trichromatic color specifications. To obtain a more quantitative picture of color relationships between the various distillate fractions, trichromatic coefficients (r, g, v) were calculated from transmittance values of the spectrophotometric curves by means of O.S.A. primary excitations (14, 34). The results are tabulated in Table 4 and are illustrated on the r-g chromaticity diagram in Fig. 9. Also included in the figure are a part of the locus for saturated spectrum colors and McNicholas' r-g curve for the locus of N″R with 35Y Lovibond glasses (24).

In Table 4 and Fig. 10, the brilliance, hue, and saturation of the various fractions are compared in terms of relative brightness, dominant wavelength, and purity, respectively.

Both Figs. 9 and 10 illustrate again the progressive change in hue in successive fractions, beginning in the red end of the spectrum and proceeding through deep yellow to a light yellow. Beginning with Fraction 17 a rapid decrease in saturation takes place. Fractions 23 to 26 inclusive are the most nearly water-white of the samples. Fractions 27 and 28 are seen to have increased hue and saturation. Presumably their darker color was due to increased concentrations of pheophytin *a*.

In Fig. 9 the r-g value for Fraction 17 falls nearly on the curve for Lovibond glasses, at 35Y—0.9R. This calculated value compares reasonably well with the

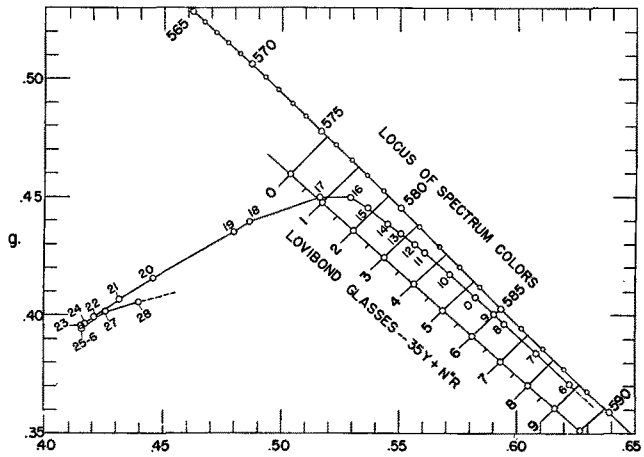

FIG. 9. Chromaticity diagram.

reading of 35Y—1.1R obtained visually with the Lovibond colorimeter, considering that the 35Y glass used at this laboratory has not been standardized against the Bureau of Standards glass used by McNicholas in calculating the r-g locus for 35Y+N″R.

VI. Stability

Organoleptic observations. The early distillate fractions, freshly removed from the still, possessed a very concentrated but not unpleasant odor of crude soybean oil. This characteristic odor progressively

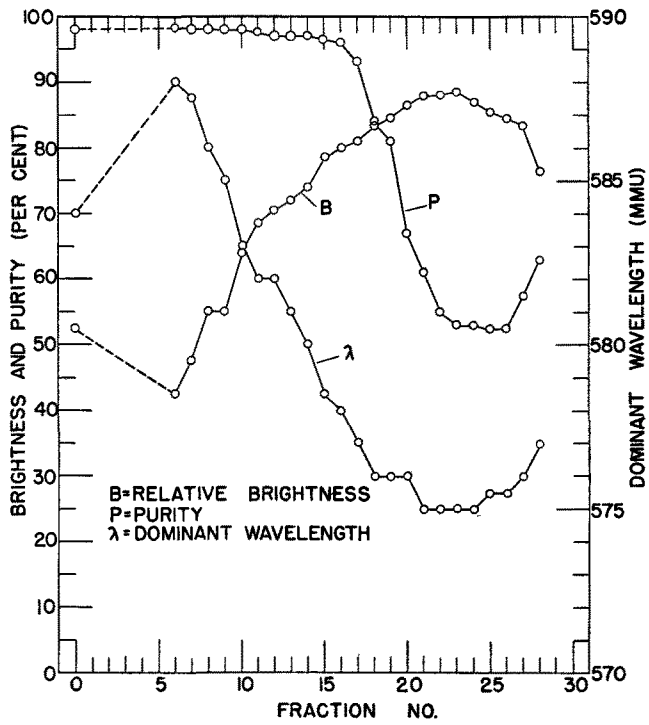

FIG. 10. Monochromatic color specification.

decreased in the later distillates, but was faintly evident even in the last fraction. The later fractions also had a slight underlying burnt odor.

The residue as removed from the still had a faint, fishy odor and an oily taste. The volatile products collected in the liquid air trap had the characteristic odor of crude soybean oil, but so concentrated that it was decidedly unpleasant. Underlying this odor there was detected also a concentrated hay-like odor.

The distillate samples were stored in a vacuum desiccator (pressure about 1 mm.) at refrigerator temperature (5°C.), and were only infrequently exposed to light. After storage for about six months they were evaluated by a tasting panel for evidence of organoleptic changes.

The first four or five fractions still possessed the concentrated odor of crude oil, apparently having undergone no change during storage. In succeeding fractions, Nos. 6 to 15, this odor steadily decreased; there were slight suggestions of other odors, but the oils were not definitely unpleasant.

Definite but erratically distributed odors were apparent in Fractions 16 to 31. Except that they were frequently unpleasant, there was little agreement among the members of the panel as to the nature of these odors; they were variously described as resembling stale milk, or grassy, sharp, tallowy, fishy, painty, sour, or branny. In a few cases the typical odor of a rancid, edible oil was said to be present.

The residue had the very rank odor characteristic of decomposed nitrogen compounds.

Methylene blue reduction tests. The antioxygenic activity of the unsaponifiable material concentrated in the low-temperature molecular distillates of vegetable oils has been observed by a number of workers (11, 13, 18, 22, 30). Such activity resides in the nonsterol portion of the unsaponifiable fraction (27) and, more specifically, in the tocopherols. Olcott (26) states: "It appears justified to conclude at this time that some if not most of the antioxidant activity present in the unsaponifiable portion of vegetable lipids can be attributed to the tocopherols." And Quackenbush *et al.* (28) have reported the isolation from crude soybean oil of a mixture of tocopherols. It was to be expected, therefore, that the oils in the early distillate fractions of the present study would display considerable stability toward oxidative rancidification.

To obtain an estimate of the concentration of antioxidant materials and the consequent stability of the distillates, the so-called methylene blue reduction test was employed. Although for the most part this method has been applied to refined edible oils, Bickford (1) has made preliminary mention that antioxidant-rich molecular distillates obtained in this laboratory from crude soybean oil, definitely enhanced the stability of an edible soybean oil to which they were added, as judged by the methylene blue method.

Limited attention was given to other minor constituents of the crude oil whose concentration or absence in the various distillate fractions might be expected to influence their stability. The antioxygenic properties of phosphatides appear to be well established (23, 27). Less well established is the effect of carotenoid pigments and chlorophyll in accelerating or retarding the onset of rancidification.[3]

[3] Columbic has recently reported (*J. Am. Chem. Soc. 64*, 2337 [1942]) the isolation from partially oxidized soybean oil of a red quinoid substance possessing antioxygenic properties.

Apparatus and method. In carrying out the methylene blue tests, the photometer assembly previously described by Bickford (2) was used. However, Bickford's original technique was modified in certain respects, the procedure being as follows:

Forty-nine ml. of an oil was placed in the absorption cell, which was connected to the constant- temperature bath and let stand for 20 minutes in the dark to bring the oil to the standard temperature, 50°C. Thereafter 2 ml. of a methylene blue solution [4] was added to the oil, a black cloth was placed over the cell, and it was shaken vigorously for 1.5 minutes. Thereafter the cell was quickly returned to its housing. At the end of the second minute the light shutter was opened (zero time) and microammeter values were read as a function of time.

Since only comparative relationships were desired, the original meter readings were used in plotting the results, without correction for the nonlinearity of current output as a function of light intensity at the photocell. It was determined, however, that for meter readings of 60 to 130 mua. a linear relation existed according to the equation:

$$\text{Corrected value (mua.)} = 4.0 + 0.6 \times \text{meter reading (mua.)}.$$

For meter readings below 60 mua. the relation was approximately linear according to the equation:

$$\text{Corrected value (mua.)} = 0.15 + 0.66 \times \text{meter reading (mua.)}.$$

The light source was adjusted to give a meter reading of 160 microamperes when the absorption cell was removed from the system and the photocell was fatigued.

Photocell fatigue. In general, the photocell was well fatigued at the start of each test. However, it was not exposed to light during the 20-minute period during which the oil sample was brought to temperature, or in the subsequent 2-minute interval between addition of the methylene blue and zero time. This may have resulted in slight recovery of the cell.

Because the initial amperage readings for the various samples were not uniform (range 9.6 to 30.8 microamperes) and because during many tests the values fell considerably below the initial readings, the matter of photocell fatigue was given further consideration. The unfatigued photocell was irradiated under the following conditions:

1. Absorption cell was removed from system.
2. Absorption cell contained 0.6 ml. standard methylene blue solution in water (0.3 mg. methylene blue).
3. Absorption cell contained 2.0 ml. standard methylene blue solution in water (1 mg. methylene blue).

Bickford has previously shown that methylene blue in water is stable upon prolonged irradiation.

Time-amperage curves were obtained with the results given in Table 5.

[4] The methylene blue was J. T. Baker Company's U.S.P. medicinal grade, the same as that used by Bickford. In preparing the stock solution 50 mg. of methylene blue was dissolved in 100 ml. absolute ethanol. Two ml. of this solution consequently contained about 1 mg. of methylene blue.

TABLE 5

Photocell Fatigue

Condition No.	Meter readings during irradiation (mua.)								
	Time (h:m)								
	0:00	0:01	0:05	0:10	0:30	1:00	3:00	3:30	4:55
1	199.0	188.6	181.2	177.2	169.0	164.0	160.4	160.0	160.0
2	41.0	39.4	38.4	38.0	37.5	37.0	35.4	.....	.....
3	8.0	8.0	8.0	8.0	8.0	8.0	.....	.....	.....

It will be seen from these data that at high light intensities the fresh photocell is rapidly fatigued, the time-amperage curve decreasing exponentially. At very low initial intensities (8.0 mua.) the cell is not affected at all, while at somewhat higher initial intensities (41.0 mua.) only minor fatigue occurs. Since in the various methylene blue-oil tests there were no initial intensities higher than 31 mua., cell fatigue appears to have played little if any part in causing variations in initial readings, which instead apparently were due to dark-reduction of the methylene blue prior to irradiation. Further, any decreases below initial values during the tests may have been due to reoxidation of the methylene blue.

Reference standard. As a reference standard for comparing the stability of the distillate fractions there was chosen a commercial edible soybean oil from a partly empty can which had been opened two weeks previously. This oil [designated on graphs as "reference standard (lot 1)"] was described by qualified tasters as being of good quality. The oil having been consumed during the tests, another can of the same batch of edible oil was obtained [designated on graphs as "reference standard (lot 2)"]. This new can had not previously been open since leaving the processor.

Five tests of each reference oil were made, and the microammeter readings for each set were averaged to give the two curves shown in Fig. 11. Here it is indicated that standing for two weeks in a partially empty can has had a noticeable effect on the stability of the first oil.

Some idea of the precision to be expected from the methylene blue method is given in Table 6, in which are recorded, for the individual tests of the reference standard (lot 2), values for initial reading; induction time (time to a definite and steady rise in the curve); inflection time (time to the point where the sigmoid curve changes from concave upward to concave downward); and maximum rate of rise (at the inflection point). The last four runs were made successively on the same day, four days later than Run 1.

TABLE 6

Individual Tests of Reference Standard (Lot 2)

Run No.	Initial reading	Induction time	Inflection time	Max. rate of rise
	mua.	*min.*	*min.*	*mua./min.*
1	29.4	26	36	6.8
2	23.2	24	34	12.6
3	23.4	23	33	12.2
4	23.4	27	37	8.4
5	23.4	27	35	7.5
Av.	24.6	25	35	

Distillate fractions. Methylene blue reduction tests were made with distillate Fractions 11, 17, 21, and 25, in each case using 49 ml. of the oil. Similar tests could not be made with earlier fractions because of the limited quantities of distillate available.

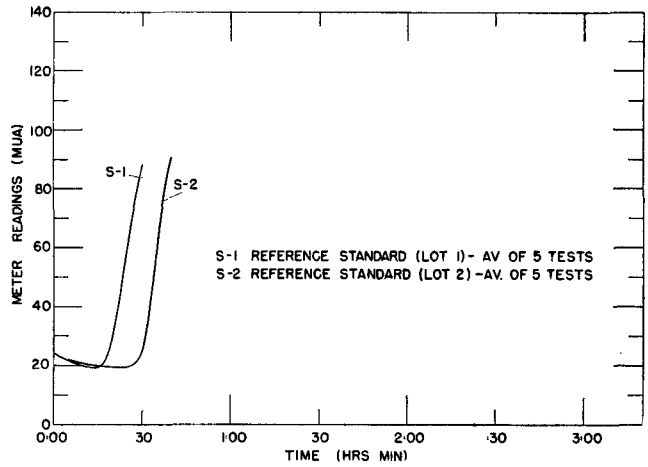

FIG. 11. Methylene blue reduction curves for reference standards.

The resultant curves are shown in Fig. 12, in comparison with curves for the original crude oil and the average for the reference standard. In the case of the crude oil the initial value was rather low, doubtless because of the relatively dark color of the oil. Thereafter, without decreasing, the curve rose very gradually. Between 3 and somewhat more than 5 hours the rate of rise was nearly constant. A final reading at 17 hours showed that the curve had already reached its maximum, and was either maintaining a constant value or was decreasing. The induction period was too indefinite to be determined.

Examination of the curve for Fraction 11 indicates that in comparison with the reference standard the oil possessed considerable stability, since the induction period was 1:15 compared with 0:15 for the edible oil. This result is surprising, since it would be expected that the volatile tocopherols had long since been removed from the distilland; moreover, the fraction contained only small amounts of pigments and almost no phosphorus compounds.

Another point of interest is the initial rapid rise in the same curve and the subsequent slow decrease to a minimum, before the onset of the final rise marking the end of the induction period. The cause of the initial rise, which was observed neither in the crude oil nor in the edible oil, is at present obscure.

Possibly the initial rise is the so-called "dark reaction" mentioned by Bickford (2) in connection with the reduction of methylene blue in oil, prior to its exposure to light. In Bickford's experiments, irradiation was begun 22 minutes after addition of the methylene blue. In the present case irradiation was begun and meter readings were taken only 2 minutes after addition of the methylene blue, and apparently before the "dark reaction" had ceased.

In the case of the edible oils (Fig. 11) the "dark reaction," if it occurred, was slight and was completed during the first two minutes after the addition of the methylene blue. An exception was one test of the reference standard (lot 1), in which a slight reduction and subsequent reoxidation was noted within the first 3 minutes after irradiation had begun.

Presumably the decrease in the curve for Fraction 11 (Fig. 12) after the initial rise was due to dissolved oxygen, although the distillate had had little exposure to air during its storage and preparation for the test.

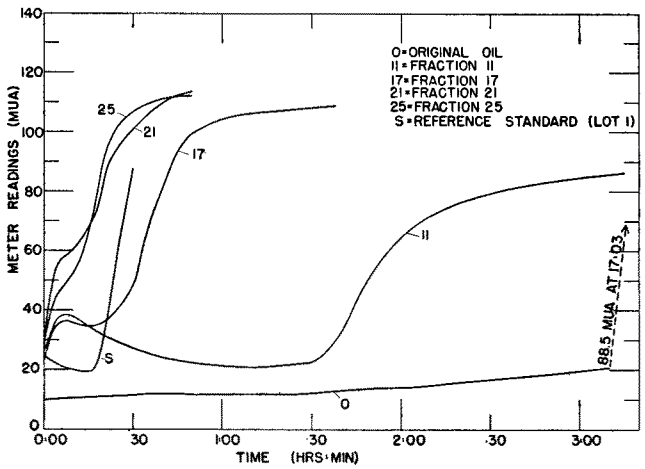

FIG. 12. Methylene blue reduction curves for original oil and Fractions 11, 17, 21, and 25.

The curves for Fractions 17, 21, and 25 (Fig. 12) likewise show the initial rapid reduction of methylene blue, followed by a slower reoxidation, before the onset of the final rise marking the end of the induction period. Apparently even Fraction 17 was more stable than the edible oil. Fractions 21 and 25 appear to have been less stable than the edible oil, although the induction periods are difficult to determine.

Fractions added back to reference standard. In order to compare the behavior of a more representative series of distillate fractions, additional tests were carried out in which 1.3 ml. of a given fraction was added back to 47.7 ml. of the reference standard. In the case of Fraction 2, the resultant mixture had an unsaponifiable content only slightly greater than that of the original crude oil. Such tests were carried out for mixtures of the reference standard with Fractions 2, 3, 5, 11, 17, 21, 25, and 29, as well as with the original crude oil and the final residue. The results are shown in Table 7 and Figs. 13a and 13b.

The curve for Fraction 2 (Fig. 13a) indicates that the oil had a longer induction period and a slower rate of methylene blue reduction than did the original crude oil. The curves for Fractions 3, 5, and 11, in the same figure, show that the oils had progressively shorter induction periods and faster rates of methylene blue reduction, indicative of decreasing stability. At the same time, even Fraction 11 shows greater stability than does the reference standard.

The curve for Fraction 17 (Fig. 13b) indicates this oil to be the least stable of the samples tested. Thereafter, the oils (Fractions 21, 25, and 29) show progressively longer induction periods. Possibly because of its high content of phosphatides, the residue shows marked stability, although the induction time is not well defined by the curve. It should be noted again that this residue at the time of testing had the very rank odor characteristic of decomposed nitrogen compounds; at the same time, there could be detected no evidence of the ordinary odor of a rancid oil.

TABLE 7

Distillate Fractions Added Back to Reference Standard (Lot 1)

Fraction No.	Induction time	Inflection time	Maximum rate of rise
	h:m	*h:m*	*mua./min.*
Original oil	1:18	1:47	1.7
2	1:32	1:58	1.5
3	:54 [1]	1:57	3.3
5	:39	1:04	4.6
11	:26	:38	9.6
17	:21	:29	11.2
21	:23	:30	14.5
25	:26	:33	15.0
29	:30	:40	12.8
Residue	1:00	1:41	2.0
Standard (Av. of 5)	:15	:21	

[1] Rise was very gradual from this point up to 1:31.

For the oils composed of distillate fractions added back to the reference standard, none of the curves exhibits the initial rapid rise shown in the curves of Fig. 12 for the concentrated distillates. Instead, like the reference oil, they steadily fall below the threshold value until the end of the induction period.

When the induction times were plotted against the distillate fraction numbers they were found to give a regular curve, as shown in Fig. 14. The induction time is not a function of the fraction number, of course, except insofar as various components of the original oil having an influence upon stability were more or less regularly segregated among the different fractions during the course of the distillations. Curves of nearly the same regularity were obtained by plotting the fraction number against the inflection time and against the maximum rate of rise.

For Fractions 2, 3, 5, 11, and 17, a regular curve is also obtained (Fig. 15) when the induction time is plotted against the logarithm of the percentage of

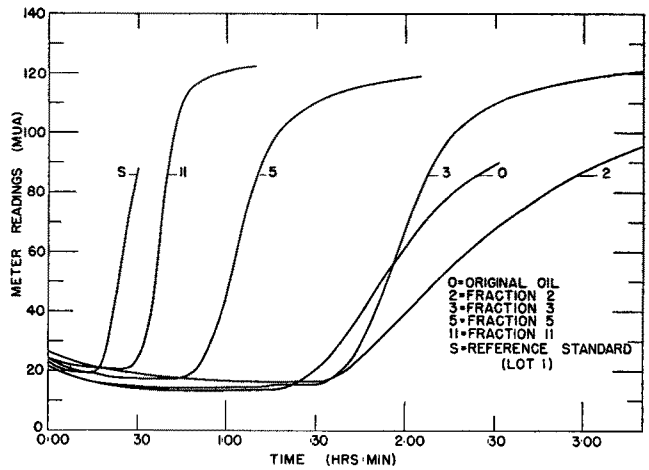

FIG. 13a. Methylene blue reduction curves for original oil and Fractions 2, 3, 5, and 11 diluted with reference standard.

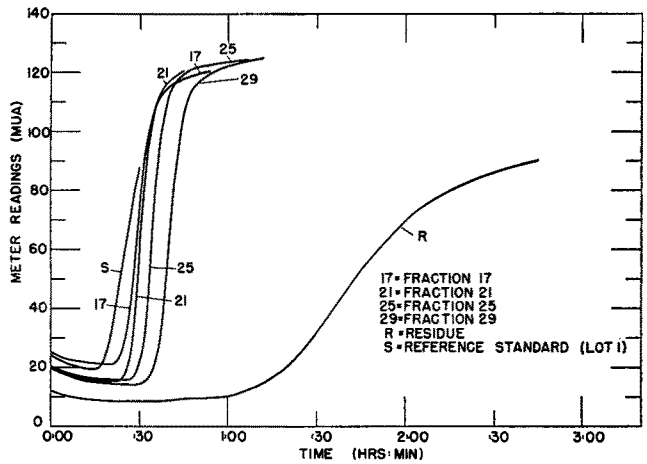

FIG. 13b. Methylene blue reduction curves for residue and Fractions 17, 21, 25, and 29 diluted with reference standard.

unsaponifiable matter. Apparently even in Fraction 17 the stability was largely influenced by the small amounts of unsaponifiable present. In the same figure, the induction times for Fractions 21, 25, and 29 are not a function of unsaponifiable matter, and it is evident that other factors are responsible for the increasing stability of the fractions toward the end of the distillations.

Effect of dilution. Another series of tests was made to determine the usefulness of the induction time, inflection time, and maximum rate of rise for the quantitative evaluation of methylene blue reduction curves. Fraction 11 was mixed with the reference standard in various concentrations, and curves were made with the results shown in Table 8 and Figs. 16 and 17.

TABLE 8

Fraction 11 Added to Reference Standard (Lot 1) in Varying Concentrations

Sample No.	Amount of Fraction 11	Amount of standard	Concentration of Fraction 11 [1]	Induction time	Inflection time	Max. rate of rise
	ml.	*ml.*	*Percent*	*h:m*	*h:m*	*mua./min.*
1	49	0	96.1	1:15	1:44	2.2
2	7.0	42	13.7	:49	1:06	4.6
3	5.0	44	9.8	:43	1:04	5.0
4	1.3	47.7	2.5	:26	:38	9.6
5	0	49	0.0	:15	:21	

[1] Total volume was 51 ml., including 2 ml. methylene blue solution.

Fig. 16 shows the progressive decrease in stability with decreasing concentration of Fraction 11. The rapid initial apparent reduction of methylene blue, shown in the curve for the 96 percent concentration of Fraction 11, does not appear in the other curves. Unfortunately, not enough material was on hand to carry out the test at intermediate concentrations.

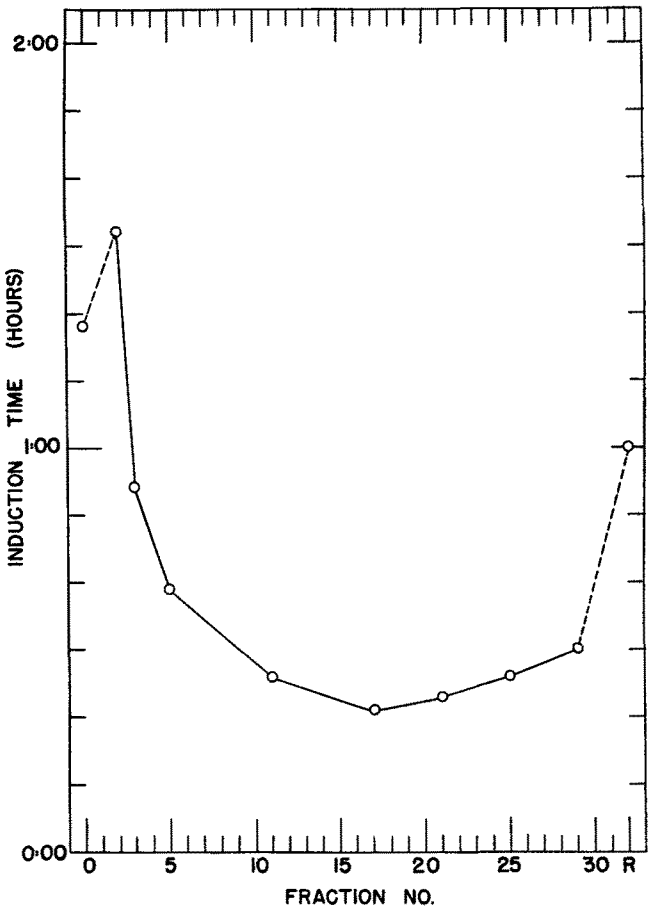

FIG. 14. Relation between induction time and fraction number.

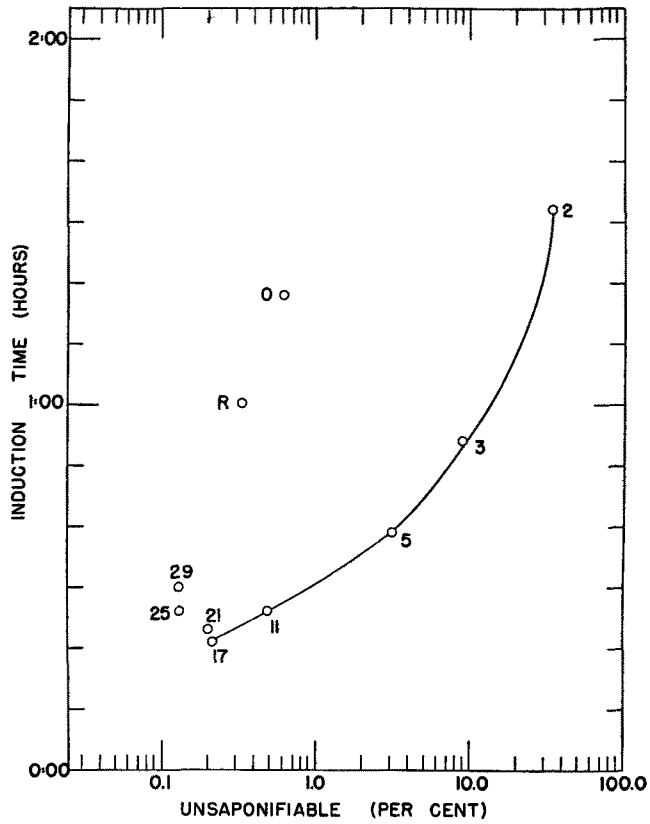

FIG. 15. Relation between induction time and content of unsaponifiable matter.

Fig. 17 indicates that induction time and inflection time are logarithmic functions of concentration, within a reasonable tolerance. The curve for the maximum rate of rise is more complex but nevertheless fairly regular.

Phosphatides. Crude phosphatides were prepared by washing the crude soybean oil with water, centrifuging, drying the water suspension under vacuum, and washing well with acetone. The material so prepared was dissolved in the reference standard in concentrations of 37 and 1400 mg./l. Fig. 18 shows the markedly increased stability of these oils as tested by the methylene blue method. However, since unsaponifiable matter may not have been completely absent, it is questionable whether the effect was due entirely to phosphatides or whether some synergistic action may not have been involved.

Chlorophyll. Chlorophyll *a* was dissolved in the reference standard in concentrations of 1.08 and 4.17 mg./l. Methylene blue reduction curves for the resultant solutions are shown in Fig. 19. It will be observed that for the lower concentration there was no increase in stability, while at the higher concentration a very definite increase in stability occurred. The concentration of chlorophyll *a* (or pheophytin) in the original crude oil and in the later distillate fractions was estimated to be in the neighborhood of 2 mg./l. Hence it is possible that the increased stability of Fractions 21, 25, and 29 (Figs. 13b, 14) was caused by chlorophyll (or pheophytin). More

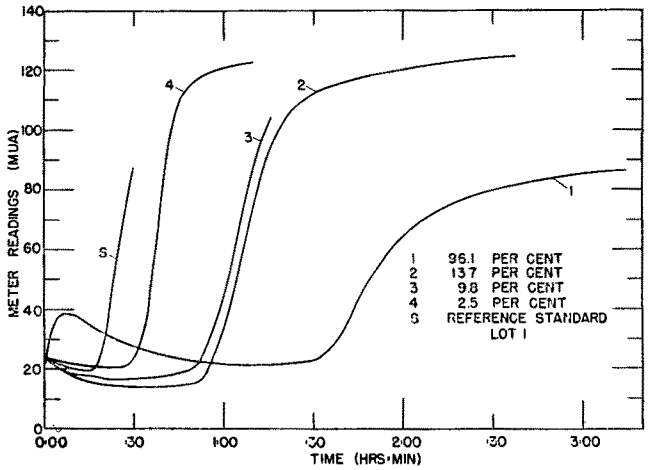

FIG. 16. Methylene blue reduction curves for Fraction 11 diluted with reference standard in various concentrations.

detailed studies along these lines appear to be warranted, in view of statements by other workers that chlorophyll acts as a prooxidant (27).

The chlorophyll used in the tests was originally pure and had been stored in an evacuated capsule; however, it was several years old, and may have undergone some deterioration.

Carotene. β-Carotene also was dissolved in the reference oil, in concentrations of 0.7, 2.9, and 14.3 mg./l. The induction periods for the three methylene blue curves were nearly the same, and only 6 to 10 minutes longer than the induction period of the reference oil. Hence, the results are considered inconclusive, and the curves are not shown.

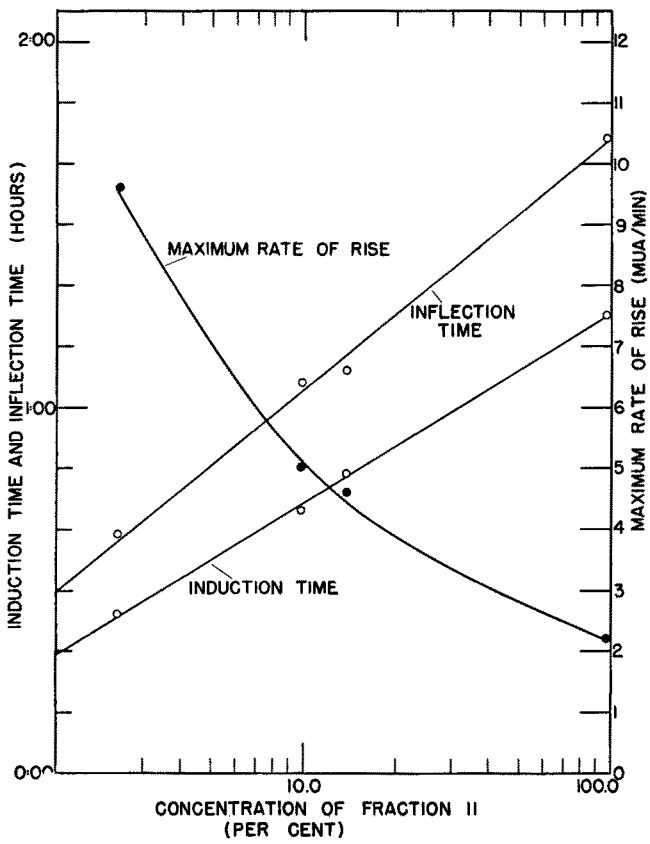

FIG. 17. Relation of induction time, inflection time, and maximum rate of rise to concentration of Fraction 11 in reference standard.

Oxygen absorption. A limited amount of work was done on the oxygen absorption characteristics of distillate Fraction 2 compared to the original crude oil and the edible reference oil. The absorption was measured at 60° in a Warburg apparatus in a manner similar to that described by Johnston and Frey (19), except that mercury was used as manometric fluid, with reaction flasks of about 15 ml. volume. The samples weighed approximately 1 g.

Fig. 20 shows the rate of oxygen absorption, in ml. per gram of oil, for the original crude oil, the edible reference oil, and Fraction 2. The tests were carried out in diffuse light. Fig. 21 shows the results of similar tests in which Fraction 2 was dissolved in the reference oil in a 2-percent concentration. The

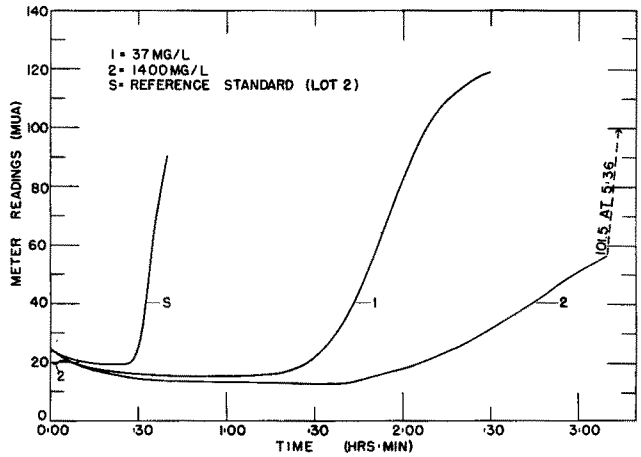

FIG. 18. Methylene blue reduction curves for crude phosphatides added to reference standard.

unsaponifiable content of this mixture (0.69 percent) was about the same as that of the original crude oil. These tests were conducted in the dark.

It is of interest to note in Fig. 21 that for the first 110 hours the crude oil absorbed oxygen at a faster rate than did the edible oil. Ultimately, in both series of tests, the crude oil absorbed at a slower rate, but the difference is less than might be expected. This may be attributable to the relatively large size of the samples in comparison with their surface area.

The curves for Fraction 2, both concentrated and diluted with the reference oil, furnish additional evidence of the presence of antioxidants. In each case, for the first 60 hours, the samples absorbed at a faster rate than did either the edible oil or the crude oil. The effect is similar to that reported by Riemenschneider, Swift, *et al.* (30, 32) using the peroxide value to study the oxidation characteristics of fractions of molecularly distilled alkali refined cottonseed oil.

The curve for the diluted Fraction 2 (Fig. 21) was erratic between 170 and 240 hours, and duplicate samples did not check well.

VII. Summary

A crude expeller soybean oil was subjected to exhaustive distillation in a column type molecular still. Numerous analyses and physical measurements

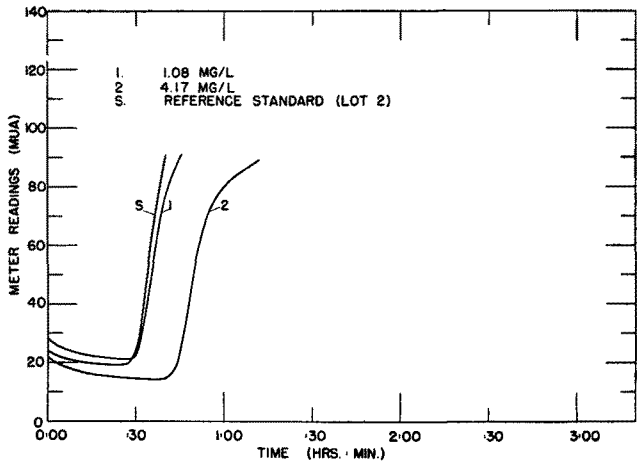

FIG. 19. Methylene blue reduction curves for chlorophyll *a* added to reference standard.

of the several distillates were made to determine the extent of fractionation of such components as triglycerides, free fatty acids, unsaponifiable matter, phosphatides, pigments, and antioxidants. The stability of certain fractions was measured by means of the methylene blue reduction test and the Warburg oxygen absorption test.

VIII. Acknowledgments

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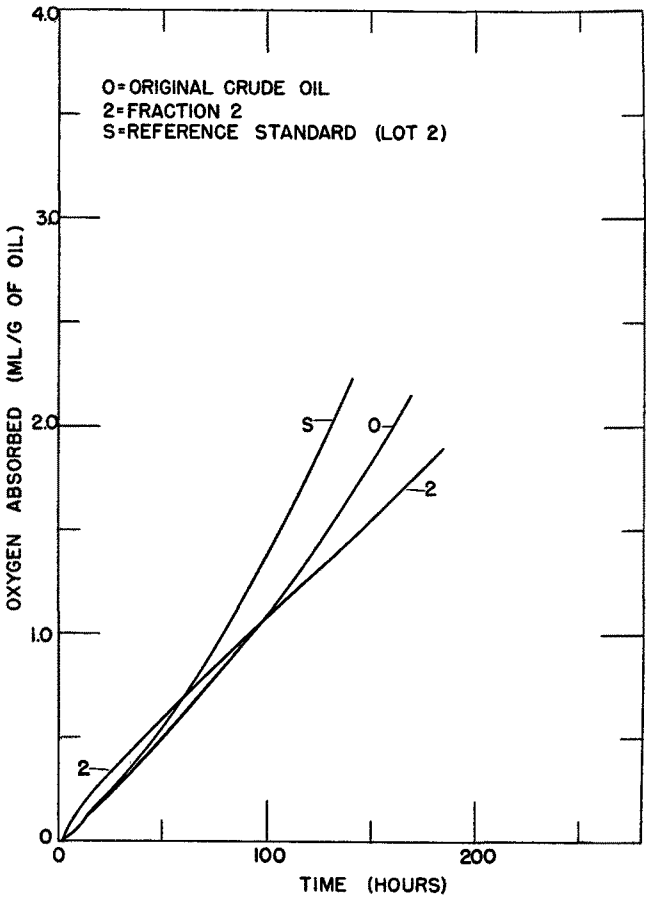

FIG. 20. Oxygen absorption curves for original oil and Fraction 2.

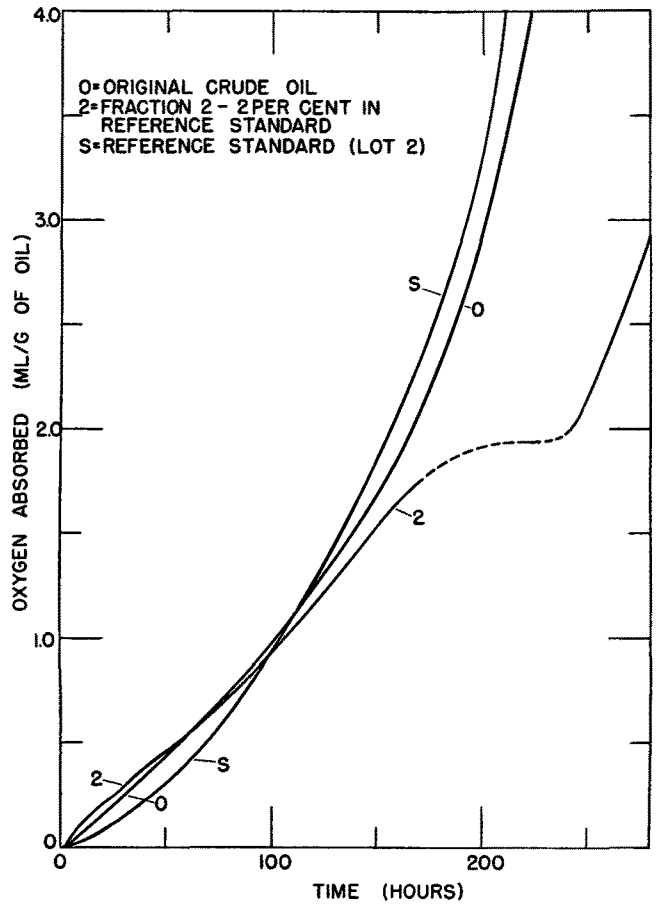

FIG. 21. Oxygen absorption curves for original oil and Fraction 2 diluted with reference standard.

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Apparatus for the Storage and Use of Carbonate Free Alkalies

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It is frequently desirable to have available solutions of carbonate free alkali without the necessity of taking up valuable bench space in the laboratory by the usual elaborate systems which involve the use of side arm burets, connecting tubes and storage reservoirs.

The apparatus shown in Figure 1 can be used to store small quantities of carbonate free alkali without the necessity for an elaborate set up. When not in use a pinchcock is placed on the rubber tube through which the buret tip is inserted and the buret is removed. The solution can then be stored like any other reagent in a cabinet or other safe place.

The operation of the apparatus is simple. Carbonate free alkali can be prepared by one of the usual methods (1). Assuming a flask of carbonate free alkali to be at hand the stock bottle is freed of carbon dioxide by attaching the tube in which the buret is subsequently to be mounted to a vacuum line. Air is drawn in through the release valve (situated just above the rubber bulb), thence through the ascarite trap and finally through the bottle itself sweeping it clear of all carbon dioxide. The prepared alkali is then siphoned into the stock bottle through the tube used for insertion of the buret and the rubber connection closed with a pinchcock.

For use, select a suitable buret provided with a guard tube, attach the tip to a vacuum line and draw air through the ascarite filled guard tube for several minutes. Close the buret pinchcock, slip the tip of the buret into the rubber connection, forcing the pinchcock to open a small way and proceed to fill the buret with the alkali in the usual manner employed in utilizing automatic burets.

Using the apparatus in this manner, carbonate free barium hydroxide solution, for example, can be preserved for long periods of time without change in normality.

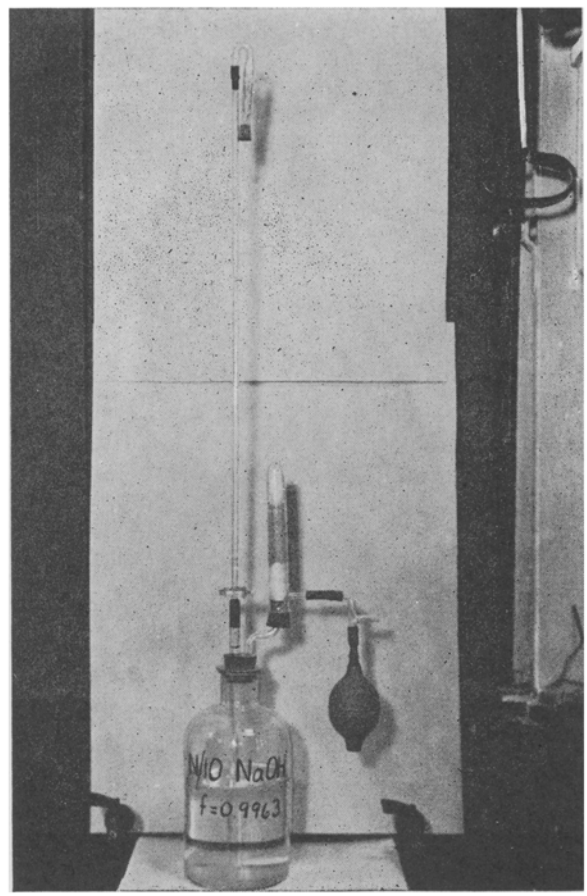

FIG. 1.

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