Wax Constituents of the Winterizer Press Cake of Soybean Oil[1]

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IN the winterization of soybean oil there is removed only a small amount of substances which are solid at room temperatures. Although this trace of material apparently has no immediate commercial value, it has been examined as part of a comprehensive investigation of the composition of soybean oil.

A sample of the press cake from a filter used in the commercial winterization of soybean oil was extracted with petroleum ether in a one-bushel batch-type stainless-steel Soxhlet extractor.[4] The following operating data were reported:

Charge of press cake:	
Weight	75.9 lb.
Oil content	43.48 percent
Extracted residue of diatomaceous silica:	
Weight	50.6 lb.
Oil content	7.98 percent
Recovered oil	28.4 lb.
Number of extractions	6
Length of extractions	120 hr.
Average extraction temperature	31° C.
Total solvent through extractor	109 gal.
Extraction of oil	87.8 percent
Recovery of extracted oil	98.1 percent

It is of interest to note that spontaneous heating occurred in the extracted residue after its storage for several hours in sacks. This was caused by the rapidity of oxidation of the residual oil when adsorbed on the large surface of the diatomaceous silica.

The extracted oil had the following characteristics:[5]

Iodine number (Wijs—1/2 hr.)	124.8
Thiocyanogen number	78.5
Refractive index (25/D)	1.4739
Diene number	6.1
Acid number	0.88
Hydroxyl number	14.7
Specific gravity (25/25)	.9246
Saturated acids (Twitchell)	13.80 percent
Saponification number	198.8
Unsaponifiable	0.93 percent
Phosphorus	0.002 percent
Ash	0.0153 percent
Lovibond reading (133 mm. cell, 45° C.)	35 Y 7.1 R. 70 Y 6.9 R

With the exception of the hydroxyl and diene numbers, these values do not differ markedly from those of normal soybean oil.

Although this oil was clear when warmed, solids were suspended in it at room temperature. These were filtered from 7150 gm. of the oil, washed three times with acetone to remove adhering oil, and crystallized twice from benzene. The product (26 gm.) melted at 77° to 78° C. and had an iodine number (Wijs) of 6.3. After two treatments with activated carbon and a further crystallization from benzene, 21.5 gm. of material remained, having a melting point of 78° to 79° C. and an iodine number of 4.7.

Another filtration of the oil was carried out at 0° C., but only a small additional amount of solid material was obtained. After allowance for losses in washing and crystallization, the total yield of solid represented between 0.001 and 0.002 percent of the soybean oil originally winterized. This estimate is based on the assumption that 50 lb. of diatomaceous silica were used for the commercial winterization of 15,000 lb. of soybean oil. Although some wax undoubtedly remained in the press cake after the extraction, the quantity would probably not materially increase the foregoing estimate.

A Liebermann-Burchard test showed that the material contained no sterols. This result was considered of interest in view of a statement (1) that in the winterizing of soybean oil "small quantities of unknown substances (not stearines but quite possibly sterols) are removed."

The saponification equivalent of the material isolated was found to be 795, which at once suggested the presence of a wax. Upon examination of the saponification mixture, the presence of long-chain fatty acids and alcohols was established. A hydroxyl number determination was attempted on the wax, using the West-Hoagland-Curtis method (2). Duplicate samples did not check within reasonable limits because the viscosity of the reaction mixture prevented determination of the exact end point. However, the determination showed the absence of free acid in the sample and indicated the presence of about 10 percent of free alcohol, assuming an alcohol molecule containing 30 carbon atoms.

In order to separate the free alcohol as well as to effect, if possible, a fractionation of the wax, 13.5 gm. of the material was distilled in the molecular still illustrated in Figure 1.[6] Fourteen fractions were removed, at temperatures and pressures indicated in Table I. It should be noted therein that the McLeod gage readings represent only the pressures of noncondensable gases and that the total pressures indicated by the thermal

TABLE I
Data on Molecular Distillation of Wax. (13.5 gm.)

			Predominant pressures		
Fraction No.	Weight	Distillation rate	McLeod gage	Thermal conductivity gage	Predominant bath temperature
	gm.	gm./hr.	microns	microns	°C
1.	0.08	0.010	0.015	0.2	135[1]
2.	0.02	0.006	0.005	0.9	126
3.	0.03	0.020	0.02	0.7	142
4.	0.41	0.087	0.005	0.2	157
5.	0.14	0.046	0.02	0.3	154
6.	0.22	0.030	0.005	0.07	154
7.	0.05	0.022	0.01	0.08	154
8.	0.09	0.027	0.015	0.6	174
9.	0.18	0.033	0.005	0.5	185
10.	3.02	0.698	0.05	0.4	255
11.	1.01	0.303	0.07	0.7	248
12.	1.20	0.152	0.01	0.05	230
13.	1.38	0.190	0.04	0.1	250
14.	0.70	0.311	0.03	0.6	260
Residue	2.75				

[1] Traces of distillate were noted at 110° C.

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[2] Now associated with Northern Regional Research Laboratory, Peoria, Illinois.

[3] A cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

[4] The authors are indebted to Mr. V. F. Pfeifer of this laboratory for carrying out the extractions.

[5] The preliminary examination of this material was made by Mr. F. G. Dollear, now associated with Southern Regional Research Laboratory, New Orleans, Louisiana.

[6] The still was designed by Dr. K. S. Markley, formerly of this laboratory, and the junior author. Of several pot-type stills employed in this laboratory, this one has been found most advantageous for the distillation of small amounts of materials where the use of a column molecular still was impracticable, and especially for substances of relatively high melting point.

conductivity gage are not strictly accurate since the instrument was calibrated against air. The bath temperatures listed are probably a few degrees higher than the temperature of the material under distillation, but are considered more accurate than readings of the thermometer in the well of the still.

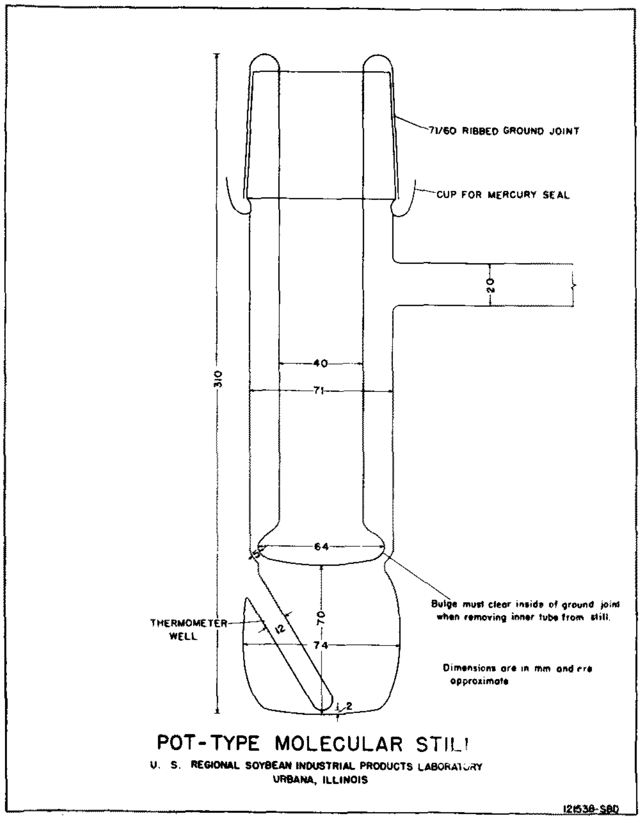

Figure 1.—Pot-type molecular still.

The still is connected to the high-vacuum system through a 29/42 gound joint and a stopcock of 15 mm. bore. A second stopcock connecting to the atmosphere permits removal of fractions without interrupting the pumps. Joints and stopcocks are especially ground for high-vacuum work. Heat is supplied by a woods-metal bath mounted on a hot plate. The side wall between the bath and the constriction is wrapped with resistance wire to prevent formation of condensate thereon. The condensing dome is cooled with air, water, or dry ice, according to the temperature differential required.

Fractions 2 and 3 were so small that their examination was restricted to melting point determinations. The distillation behavior and melting points of fractions 6, 7, and 8 indicated that the fractions were similar and might be combined.

Melting points and saponification equivalents were determined for 11 fractions, including the combination of fractions 6, 7, and 8. The saponification reaction mixtures were evaporated to dryness and extracted repeatedly with hot acetone to remove the alcohols. The acetone solutions were concentrated to about 20 ml., cooled, and filtered to recover the alcohols. Acetates were prepared from the alcohols by heating for 2 hours with acetic anhydride and sodium acetate. After recrystallization of the acetates from petroleum ether, their melting points and saponification equivalents were determined. Thereafter the alcohols were recovered as before.

The soaps remaining after the acetone extraction were dissolved in hot water and filtered. The fatty acids were liberated, recovered from the solution, and recrystallized from benzene. The first nine fractions gave such small amounts of acids that they were not considered. Melting points and neutralization equivalents were determined on the acids from the last five fractions. The melting points ranged from 72° to 74° C. and the neutralization equivalents were found to vary between 352 and 395 without showing any regular trend. The expected neutralization equivalents, calculated from the saponification equivalents of the wax fractions and of the acetates, varied from 321 to 350 and showed a progressive increase. The difference is probably caused by loss of the shortest-chain alcohols in the preparation of the acetates, loss of the shortest-chain acids in the preparation of the acids, and contamination of the acids by inert materials, probably alcohols.

The analytical results are shown in Figures 2 and 3. The melting points plotted are the temperatures at which the last portion of solid disappeared when heated in the usual capillary melting point tubes. As Chibnall and coworkers have pointed out in their series of publications on the vegetable waxes, data of the type presented here can seldom be used to establish the identity of the specific alcohols and acids in natural waxes, but they do permit a number of generalizations.

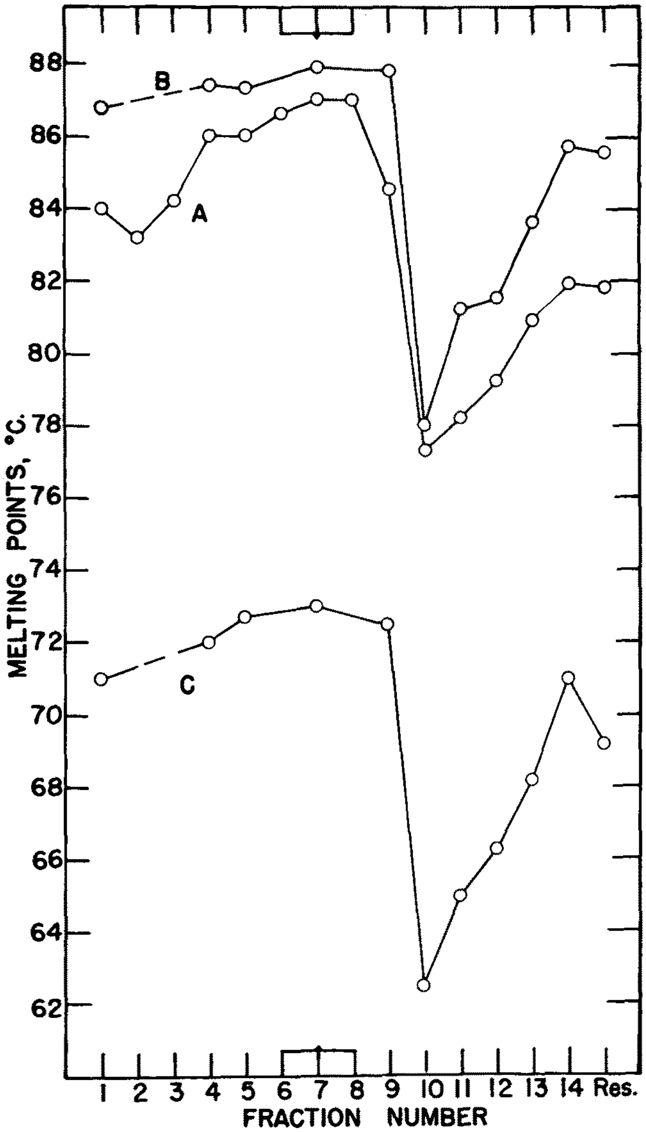

Figure 2.—Melting points.

A. Wax fractions.
B. Alcohols obtained by saponification of wax fractions.
C. Acetates prepared from alcohols.

The saponification equivalents of the wax fractions indicate that the first eight fractions contain chiefly unsaponifiable material (alcohols) with perhaps 5 to 15 percent of true wax. Fraction 9 is apparently composed of about equal amounts of alcohol and wax while fraction 10 is composed almost entirely of wax. The last four distillate fractions apparently do not contain significant amounts of free alcohols.

The saponification equivalents of the acetates prepared from the alcohols of the first nine fractions show that the average molecular weight of the alcohols varies from 450 to 467, corresponding to a molecule containing 30 to 32 carbon atoms. The assumption that the alcohols are of the usual straight chain type is supported by the melting points of the alcohols and acetates. The alcohols of the combined fractions 6, 7, and 8 have an average molecular weight of 467, corresponding to an alcohol of 32 carbon atoms, and their melting point, 87.6° to 87.9° C., approaches the temperature of 89.2° C. reported as the melting point of dotriacontanol (3). The melting point of the acetates of these combined fractions is 72.2° to 73.0°, which is essentially the same as the value 72.6 to 72.8° reported for dotriacontanyl acetate (4). Although the analysis indicates that the alcohol in these three fractions is the C_{32} straight-chain alcohol, the material must be considered a mixture containing some longer and some shorter homologs.

Fraction 10, the first fraction which was essentially all wax, yielded a mixture of alcohols having an average molecular weight of 405, corresponding to an average chain length of 27.7 carbon atoms. The melting point, 77.0° to 78.0° C., is somewhat less than the value 79.2° C. reported for the C_{26} alcohol, but the melting point of the mixed acetates is 60.5° to 62.5° C., slightly higher than the value 60.0° to 60.1° reported for the acetates of the C_{26} alcohol (4). The alcohol portion of fraction 10 must therefore contain an alcohol at least as short as C_{26} mixed with the longer alcohols such as occur in the earlier fractions.

Fractions 11 to 14 show progressive fractionation of the wax, the alcohols of the last fraction being similar to those of the first eight fractions.

The analyses of the fractions showed such differences in their alcohols that X-ray patterns were obtained by Dr. J. N. Mrgudich, of the University of Illinois Chemistry Department, in an effort to identify definitely the alcohols present. The patterns, however, were ill-defined, indicating that the alcohols were complex mixtures, and no further conclusions could be drawn. Patterns of the acids from fractions 10 and 14 and of the ethyl esters of these acids were somewhat more definite and indicated the presence of the C_{26} or C_{28} acid.

The iodine number of the residue was 8.9. This accounts for only half of the unsaturation in the original wax. A portion of the remainder was certainly lost in the crystallization of the residue, and another portion was perhaps lost through polymerization in the still. It is thought probable that the unsaturation, as well as the lowered saponification equivalent of the residue, can be attributed to partially polymerized soybean oil which was not removed by the washing and crystallization procedures.

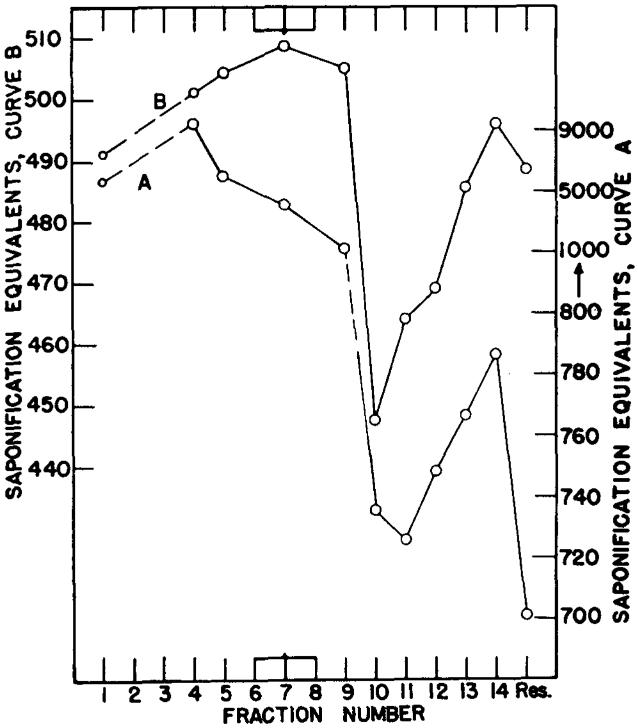

Figure 3.—Saponification equivalents.

A. Wax fractions.
B. Acetates prepared from alcohols of wax fractions.

Any hydrocarbons in the wax should have appeared with the alcohols. The agreement between the saponification equivalents of the acetates and the melting points of the alcohols and acetates may be taken as evidence that hydrocarbons are not present in significant amounts.

Summary

Wax from the winterizing press cake of soybean oil contains approximately 10 percent free alcohol in addition to true wax. No free acids and apparently no hydrocarbons are present. The principal alcohols range from C_{32} to below C_{28}, while the acids have an average chain length of approximately 22 carbon atoms. The wax amounts to not more than 0.002 percent of the original oil.

REFERENCES

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3. Francis, F., Collins, F. J. E., and Piper, S. H., Proc. Roy Soc. A, *158*, 691 (1937).
4. Piper, S. H., Chibnall, A. C., and Williams, E. F., Biochem. J. *28*, 2179 (1934).

CORRECTION

We regret that the formula at the top of the right hand column, page 106, May 1941 issue, in the article Copra Quality Under New Rules by P. W. Tompkins was printed incorrectly. It should read:

Corrected F.F.A. to compare with 7% oil in cake =

$$\frac{0.615 \times 6.45\% + (0.0539-0.02) \times 1.05\%}{(0.6689-0.02)} = 6.17\% \quad \text{(Reported as 6.2\%)}$$