phosphoinositides are quite well separated, but cephalin is distributed between the two fractions. Thus in present work when commercial crude phosphatides were extracted five times with a total of 6.5 ml. of isopropyl alcohol per gram, the combined extracts contained 61% acetone-soluble material, 23.2% lecithin, and 10.6% cephalin. Practically all the inositol-containing material and 77% of the sugar remained in the insoluble fraction.

It is obvious that the separation between oil and phosphatide components achieved by acetone extraction followed by isopropyl alcohol or ethyl alcohol cannot be obtained by the use of isopropyl alcohol alone; but the direct extraction with isopropyl alcohol gives a good separation of the inositol containing fraction from the other components and, in addition, uses a much smaller volume of solvents. If it is desirable, the oil may later be removed from the soluble portion by acetone in the usual way.

While five extractions and a total of 6.5 ml. of isopropyl alcohol per gram of phosphatides were used in this work, for some purposes a less complete extraction may be satisfactory. For example, by extracting only two times with a total of 2.5 ml. isopropyl alcohol per gram 40.8% of the crude phosphatides would be extracted as indicated by the data in Table I. This extract would contain 34% acetone-insoluble material and the remainder would be mainly oil. The fraction remaining after the isopropyl alcohol extraction would contain 91.8% acetone-insoluble material.

Separations of lecithin from cephalin in the soluble portion and of the phosphoinositides of the insoluble portion have been accomplished as yet only by methods suitable for small-scale laboratory purposes. However, current reports indicate that commercial fractionations of soybean phosphatides are following closely upon knowledge of composition and laboratory separations and that further developments depend upon the growth of more fundamental information.

Summary

Isopropyl alcohol has many advantages as a commercial solvent and gives useful separations of soybean phosphatides. When commercial crude phosphatides were extracted five times with a total of 6.5 ml. isopropyl alcohol per gram, 31.5% oil, 12% lecithin, and 5.5% cephalin were dissolved. Practically all the inositol-containing material and about 77% of the sugars remained insoluble. Further extractions with ethyl alcohol removed the remaining lecithin together with additional cephalin.

After removal of the oil from the crude phosphatides with acetone, the extraction of the isopropyl alcohol-soluble material required twice the amount of isopropyl alcohol used for the direct extraction described above.

Acknowledgment

The authors are indebted to H. H. Hall and Margaret C. Shekleton for the inositol bioassays and to C. H. Van Etten, T. A. McGuire, and Mary B. Wiele for Kjeldahl nitrogen and total phosphorous determinations.

REFERENCES

1. Atkins, L., Schultz, A. S., Williams, W. L., and Frey, C. N., Ind. Eng. Chem., Anal. Ed., *15*, 141 (1943).
2. Burmaster, C. F., J. Biol. Chem., *165*, 1 (1946).
3. Glick, D., J. Biol. Chem., *156*, 643 (1944).
4. Goss, W. H., "The German Oilseed Industry," p. 29, Hobart Publishing Company, Washington, 1947.
5. Markley, K. S., "Soybeans and Soybean Products," Vol. II, p. 604, New York, Interscience Publishers Inc., 1951.
6. Linteris, L., and Handschumaker, E., J. Am. Oil Chem. Soc., *27*, 260 (1950).
7. Scholfield, C. R., Dutton, H. J., Tanner, F. W., Jr., and Cowan, J. C., J. Am. Oil Chem. Soc., *25*, 368 (1948)
8. Scholfield, C. R., McGuire, T. A., and Dutton, H. J., J. Am. Oil Chem. Soc., *27*, 352 (1950).
9. Scholfield, C. R., Dutton, H. J., and Dimler, R. J., J. Am. Oil Chem. Soc., *29*, 293-298 (1952).
10. Somogyi, M., J. Biol. Chem., *160*, 61 (1945).
11. Thornton, M. H., Johnson, C. S., and Ewan, M. A., Oil and Soap, *21*, 85 (1944).
12. Truog, E., and Meyer, A. H., Ind. Eng. Chem., Anal. Ed., *1*, *136 (1929)*.
13. Van Slyke, D. D., J. Biol. Chem., *83*, 425 (1929).

[Received December 3, 1951]

Variations in the Chemical, Physical, and Organoleptic Properties of Soybean Oil Hydrogenated Under Widely Varying Conditions

REX J. SIMS, Swift and Company, Chicago, Illinois

IT is generally accepted that there is a relationship between the flavor stability of soybean oil and its oxidative stability. Consequently many workers in the field subscribe to the assumption that the flavor deterioration of fats is due to an autoxidative process of some type involving the polyunsaturated glycerides (1, 2).

In the work to be reported here soybean oil was hydrogenated to various degrees of hardness under a variety of conditions so as to produce fats of widely varying oxidative stability. No correlation was observed between flavor scores of fresh or aged products and their oxidative stabilities as measured by the active oxygen method (3, 4). Furthermore the content of polyunsaturated glycerides appeared to bear no relationship to flavor stability.

As reported by other workers (5), at moderate pressure chemical selectivity was found to vary directly with catalyst concentration and with temperature up to 200°C., and inversely with hydrogen pressure and rate of agitation. But at hydrogen pressures over 500 psi. the effect of changes in catalyst concentration and agitator speed was reversed. That is, selectivity then varied inversely with catalyst concentration and directly with agitator speed.

The percentage of high-melting trans isomers formed during hydrogenation (6) is also a function of pressure and varies inversely with it. Yet higher melting products were obtained, for a given drop in refractive index, when higher pressures were used. Apparently the amount of fully saturated glycerides

TABLE II

Sample No.	Hydrogenation Conditions				R. I.[a]	Melting Point		Softening Point (°C.)	Linoleic (%)[c]	Linolenic (%)[c]	Conj. Diene (%)[c]	A.O.M. Stability (hours)	Flavor Score	Trans Acids (%)
	Hydrogen (psi.)	Temp. (°C.)	Nickel Catalyst (%)	Time (Min.)		F.A.C. (°C.)	Wiley (°C.)							
Original Oil					51.3				55.6	8.0	0.44			
1	10	200	1	170	40.5	103	101	100	0.18	0.008	0.24		5	
2	225	200	1	30	40.5	104	102	101	0.02	0.00	0.21		6	
3	950	200-250	1	2	39.7	114	112	110	0.04	0.00	0.23		7	
4	10	200	1	240	41.8	96	96	93	1.28	0.00	0.50	215	6.5	54
5	225	195-205	0.05	38	41.6	118	104	98	5.37	0.13	0.32	115	8	44
6	700	200-225	0.05	3	42.1	122	123	109	12.30	0.64	0.46	70	7	30
7	600	200		135	41.7	125	119	114	7.37	0.29	0.44		6	
8	500	200-205	0.05	3	46.3	81	Too soft	Too soft	28.76	2.53	0.71		7.5	
9	10	200	0.25	165	46.3	Too soft	Too soft	Too soft	21.80	0.90	3.43		8	
10	400	198-205	1	5	45.9	98	87	Too soft	28.59	1.39	1.18		8.5	
11	400	125	1	30	45.8	98	87	Too soft	28.31	1.34	0.33		8.5	

[a] Refractive indices at 60°C. using a Butyro refractometer.
[c] Polyunsaturated by the A.O.C.S. Tentative Method Cd. 7-48.

TABLE III

Sample No.	Hydrogenation Conditions				R. I.[a]	Melting Point		Softening Point (°C.)	Linoleic (%)[c]	Linolenic (%)[c]	Conj. Diene (%)[c]	A.O.M. Stability (hours)	Flavor Score	Trans Acids (%)
	Hydrogen (psi.)	Temp. (°C.)	Nickel Catalyst (%)	Time		F.A.C. (°C.)	Wiley (°C.)							
Original Oil					51.3				53.65	7.49	0.31			
12	1800	225	None added	2 hr., 30 min.	46.6	108	Too soft	87	28.61	3.01	0.84	20	8	
13	1250	225-250	0.01	30 sec.	46.6	131	119	101	28.96	3.53	1.93	20	6	
14	30	225	0.1	10 min.	46.6	Too soft	Too soft	Too soft	29.46	2.58	1.29	27	7.5	
15	30	225	0.01	33 min.	46.7	Too soft	Too soft	Too soft	34.97	1.96	2.56	30	8	
Original Oil					51.5				51.89	7.69	0.48			
16	1100	200-230	0.05	30 sec.	45.7	135	130	115	28.40	3.49	1.85	35	8	18.0
17	550	200-230	0.05	45 sec.	45.6	130	123	111	27.81	3.16	1.26			19.3
18	225	200-215	0.05	80 sec.	45.7	123	118	106	25.30	2.84	1.79	35	7	25.1
19	30	200	0.05	7 min., 45 sec.	45.5	Too soft	Too soft	Too soft	23.48	1.24	1.35	40	7	31.8
20		200	0.05	57 min.	45.5	83	83	Too soft	21.26	0.98	0.91	50	7	38.8

[a] Refractive indices at 60°C. using a Butyro refractometer.
[c] Polyunsaturated by the A.O.C.S. Tentative Method Cd. 7-48.

TABLE IV

Sample No.	Hydrogenation Conditions					R. I.[a]	Iodine Value[b]	Melting Point		Softening Point (°C.)	Linoleic (%)[c]	Linolenic (%)[c]	Conj. Diene (%)[c]
	Hydrogen (psi.)	Temp. (°C.)	Nickel Catalyst (%)	Agitator rpm.	Time			F.A.C. (°C.)	Wiley (°C.)				
Original Oil						51.5					51.89	7.69	0.48
21	30	225	1	1140	8 min.	45.8	100.5	97	87	Too soft	21.64	1.21	1.04
22	30	150-165	1	1140	10 min.	46.1	102.3	90	Too soft	Too soft	29.86	2.05	0.52
23	550	200-230	0.05	1140	45 sec.	45.6		130	123	111	27.81	3.16	1.26
24	550	200-205	0.005	1140	66 min.	45.4		124	122	108	18.07	2.44	0.90
25	30	300	1	1140	28 min.	50.4	106.3				27.97	2.39	3.65
26	30	200-205	0.1	1140	7 min.	44.5		84	82	Too soft	17.99	0.86	0.94
27	30	200-205	0.1	520	70 min.	44.5		89	87	Too soft	15.87	0.44	1.7
28	750	200-215	0.05	1140	2 min.	43.9		131	128	121	15.0	2.2	0.6
29	750	200-207	0.05	520	9 min.	43.6		138	135	131	21.7	2.8	0.74

[a] Refractive indices at 60°C. using a Butyro refractometer.
[b] Iodine values using the Wijs method.
[c] Polyunsaturated by the A.O.C.S. Tentative Method Cd. 7-48.

rather than the amount of trans acids determined the final consistency of these fats.

Extent of Hydrogenation

Experiments on the relationship between flavor stability and extent of hydrogenation were done, using a carbon-steel, gas-fired converter of 5-l. capacity equipped with a mechanical agitator. A 3,500-g. charge of refined and bleached soybean oil was hardened at 200°C. and 30 psi. with 0.2% by weight of a commercial reduced nickel catalyst (containing about 25% Ni). At intervals, as indicated in Table I, 500-g. samples were withdrawn into an evacuated flask and allowed to cool to 80°C. before admitting air. Each was filtered with bleaching clay and filter aid until the filtrates gave no discoloration with boiling ammonium polysulfide solution.

The oils of each series were then deodorizeed concurrently for five hours at 200°C. and 1-mm. pressure in 1-l. glass laboratory deodorizers. Each was stabilized with 0.005% citric acid and then cooled. Then the freshly deodorized oils were graded by a flavor panel, using the scoring system developed at the Northern Regional Research Laboratory (7). Samples were aged in covered glass jars at 60°C. for three days and then graded again. This procedure was repeated four times (Series 1-4 of Table I), using a different lot of soybean oil each time.

TABLE I

Series 1

Time (min.)	R.I.[a]	A.O.M. (hours)	Flavor Panel Score	
			Fresh	Aged (3 days, 60°C.)
0	51.1	25	9	6
3	47.5	40	9	6
6	43.9	60	9	7
9	42.2	110	9	6
24	40.2	375	9	7.5
49	38.4	Over 400	9	7

Series 2

Time (min.)	R.I.[a]	Iodine Value[b]	%[c] Linoleic	%[c] Linolenic	%[c] Conj. Diene	Flavor Panel Score	
						Fresh	Aged (3 days, 60°C.)
0	50.8	132.1	53.4	8.40	0.30	9	6
4	47.0	107.3	25.2	2.60	1.24	9	5
7	43.6	89.7	9.9	0.50	0.63	9	7
11	41.1	73.4	1.8	0.03	0.28	9	7.5
17	39.6	63.9	0.26	0.00	0.14	9.5	7.5
31	37.5	49.6	0.01	0.00	0.12	9	7
Series 3							
0	51.2	132.9	48.7	8.12	0.31	9	6
3	46.7	105.1	27.0	2.70	0.81	9	5
5	43.8	87.4	11.9	0.65	0.37	9	5
8	41.5	76.4	2.7	0.04	0.24	9	6
13	40.2	66.5	0.2	0.03	0.12	9	7
28	38.0	52.6	0.00	0.00	0.09	9	7
Series 4							
0	51.2	131.7	55.6	8.0	0.44	9	7
3	46.5	103.9	27.6	2.6	0.75	9	7.5
5	43.1	84.4	10.4	0.45	0.33	9	7.5
8	41.1	73.7	2.9	0.05	0.20	9	4
12	39.7	63.2	0.3	0.0	0.12	9	6
24	37.5	48.0	0.0	0.0	0.10	9	8

[a] Refractive indices at 60°C. using a Butyro refractometer.
[b] Iodine values using the Wijs method.
[c] Polyunsaturates by the A.O.C.S. Tentative Method Cd. 7-48.

It would quite naturally be assumed that as the refractive index and the content of polyunsaturated glycerides decreased, the flavor stability would gradually increase. Instead, in at least three cases, Series 2-4, the stability actually fell off as hydrogenation proceeded, passing through a minimum, and then rose again. Possibly soft oil reversion is not as objectionable qualitatively to panel members as that of a slightly hydrogenated oil. In any case no correlation was found between flavor stabilities and oxidative stabilities or contents of polyunsaturates.

Variation in Conditions of Hydrogenation

Experiments involving changes in hydrogenation conditions were done, using a 2-l. electrically-heated, high pressure stainless steel autoclave (Table II). The agitator was set to rotate at 520 rpm. so that the surface of the charge was almost motionless. Temperature control at high pressures and high catalyst concentration was almost impossible even at this slow rate of agitation, e.g.

> To a 1500-g. sample of refined and bleached soybean oil was added 15 g. of nickel catalyst flakes. This was heated to 200°C., the heaters were shut off, and 1800 psi. of hydrogen was admitted. In 10 seconds the temperature had risen to 330°C. Then the agitator was stopped, and the converter was evacuated. The product had the following constants: R.I. (60°C.), 32.2; Titer, 64.3°C.; I. value, 6.5; FAC M.P., 154°F.

The oils prepared as described in Table II were filtered from catalyst, deodorized, and stabilized with 0.005% citric acid. Each received a flavor panel score of 9 or better when fresh. The values reported in Table II were obtained after three days' aging at 60°C.

Of samples 1, 2, and 3 from Table II, sample 3 has a melting and softening point considerably higher than would be expected on the basis of its slightly lower R.I. (0.8 unit). Apparently, relatively large quantities of high-melting glycerides form during high pressure hydrogenation. Note the improved flavor stability of 3 over 1.

Samples 4, 5, and 6 again emphasize the high melting and softening points of oil hardened at the higher pressures. The percentages of trans acids in the three samples were determined by the spectrophotometric method of Swern (6). Since the oils hardened at high pressure actually contain less of the high-melting trans acids than those hardened at low pressure, the higher melting points of the former must be due to increased amounts of fully saturated fatty acid radicals. Note also the spread between melting and softening points in the case of sample 6. Evidently products with long plastic ranges are formed during high pressure hydrogenation.

A three-fold difference in A.O.M. stabilities was found between samples 4 and 6 with almost a ten-fold difference in content of linoleic acid. Yet the flavor panel actually preferred sample 6.

Sample 7 was prepared without using any added catalyst although actually traces were present in the agitator stuffing box from previous runs. The reaction chamber itself was washed free of oil and catalyst between runs. An analysis of product 7, subsequent to hydrogenation and prior to filtration, showed less than 6 parts per million of nickel to be present (a). Evidently very little active catalyst surface is necessary for hydrogenation at high pressure.

Surprisingly, these was little difference in the selectivity of hydrogenation over a wide range of temperature at 400 psi. (runs 10 and 11). Also flavor stabilities of the two oils were comparable.

Table III contains data on samples of soybean oil which were hardened in the high pressure autoclave with nickel catalyst flakes as described above. However for this series the agitator speed was increased to 1,140 rpm. This gave violent turbulence at the surface of the charge.

Samples 12-15 again illustrate the lack of correlation between flavor stability and oxidative stability. As before, the oils were graded after three days' aging at 60°C. Sample 12 was obtained by high pressure hydrogenation at a very low catalyst level. The final oil, before filtration, contained less than 6 parts per million of nickel.[1]

Runs 16-20 demonstrate the effect of extreme changes in pressure on the rate and selectivity of hydrogenation. Sample 20 was obtained by bubbling hydrogen through the oil while agitating it under 20 inches of vacuum. Note that this oil is evidently higher melting than sample 19 in spite of the fact that it was hydrogenated more selectively than 19. This is probably the result of its high content of trans acids (6).

Effect of Catalyst Concentration

Samples 14 and 15 of Table III show the direct relationship which holds between catalyst concentration and selectivity at normal pressures. That is, the content of polyunsaturated glycerides at a given refractive index or iodine value varies inversely with the amount of catalyst used in the hydrogenation. Samples 23 and 24 of Table IV illustrate how this effect is reversed at high pressure.

Effect of Temperature

The change in selectivity with temperature at moderate pressure is shown by comparing samples 21 and 22 of Table IV. At 300°C. (sample 25) considerable polymerization takes place, which accounts for the small drop in refractive index. Selectivity is low at this extreme temperature. Samples 10 and 11 of Table II seem to indicate that selectivity is almost independent of temperature at 400 psi.

Effect of Agitation

Samples 26 and 27 of Table IV illustrate the effect of changes in agitator speed at low pressure on chemical selectivity. At high pressure, samples 28 and 29, the effect is reversed.

Summary

No correlation was found between the flavor stability of partially hydrogenated soybean oil and its oxidative stability or its content of polyunsaturated glycerides. Fats having unusually high melting and softening points with considerable spread between melting and softening points were obtained by high pressure hydrogenation. At moderate pressures chemical selectivity was found to vary directly with catalyst concentration and inversely with agitator speed, as reported by previous workers. However at high pressures the effect of these variables was reversed. That is, selectivity then varied directly with the amount of agitation and inversely with the catalyst concentration.

REFERENCES

1. Bailey, A. E., "Industrial Oil and Fat Products," 2nd edition, Interscience Publishers, New York, 1951.
2. Black, H. C., and Mattil, K. F., Ch. XIX, in "Soybeans and Soybean Products," Markley, K. S., ed., Interscience Publishers, New York, 1950.
3. King, A. E., Roschen, H. L., and Irwin, W. H., Oil and Soap, *10*, 105 (1933).
4. Mehlenbacher, V. C., Oil and Soap, *19*, 137-139 (1942).
5. Bailey, A. E., Feuge, R. O., and Smith, B. A., Oil and Soap, *19*, 169-176 (1942).
6. Swern, D., Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chem. Soc., *27*, 17 (1950).
7. Moser, H. A., Jaeger, C. M., Cowan, J. C., and Dutton, H. J., J. Am. Oil Chem. Soc., *24*, 291-296 (1947).

[Received November 29, 1951]

---

[1] Spectrophotometric method, involving the development of a colored complex with dimethylglyoxime.

ABSTRACTS . . . . Don Whyte, Editor

• Oils and Fats

R. A. Reiners, Abstractor

Reagents for idometric determination of peroxides in fats. L. Hartman and Margaret D. L. White (Dept. Sci. Ind. Res., Wellington, N. Z.). *Anal. Chem.* 24, 527-29(1952). It is suggested that a 10% solution of citric acid in a mixture of tert. butyl alcohol and carbon tetrachloride be used as solvent in the Lea method for peroxide determination in fats in order to reduce the blank.

Estimation of 2- and 3- tert-butyl-4-hydroxyanisole isomers. J. H. Mahon and R. A. Chapman (Dept. Nat. Health Welfare, Ottawa). *Anal. Chem.* 24, 534-536(1952). The method is based on the fact that the 3-tert. butyl-isomer reacts to form a more highly colored complex with 2,6-dichloroquinone chloroimide-borax reagent than the 2-tert. butyl-isomer and that the reverse is true on reaction with ferric chloride-1,1-bipyridine reagent. The 95% confidence limits for the determination were ± 3.0% units.

The component fatty acids and glycerides of jute-seed oils. M. L. Meara and N. K. Sen (Univ., Liverpool). *J. Sci. Food Agr.* 3, 237-240(1952). The component acids from the seed fats of *Corchorus capsularis* and *C. olitorius*, two varieties of jute, have been shown to consist of palmitic, 12.2, 16.9; stearic 4.6, 3.7; arachidic 2.2, ...; behenic ...., 1.8; lignoceric 0.9, 1.1; cerotic 1.2, ...; oleic 28.7, 9.1; linoleic 41.3, 62.5; linolenic 4.7, 0.9; and C_{20} monoethenoid 4.1, 4.0% (wt.) respectively. The component glycerides of the *C. olitorius* seed oil, which resembles sunflower seed oil, consist of 11% disaturated-monounsaturated glycerides, 64% monosaturated-diunsaturated glycerides mainly saturated-dilinoleins, and 25% triunsaturated glycerides consisting mainly of oleodilinoleins and trilinolein.

Semimicromethod for the determination of plant sterols. D. Waghorne and C. E. Ball (Ontario Agr. College, Guelph, Ontario). *Anal. Chem.* 24, 560-564(1952). The method uses dichromate oxidation of the digitonides, followed by titration of the excess dichromate with ferrous solution, and yields results with a precision better than ± 3%. Samples containing as little as 0.1 mg. total sterol are adequate for a single determination. The sensitivity of the method approaches that of the colorimetric methods and is unaffected by slight changes in the structure of the sterol molecule.

The constitution of some wool wax esters. J. Tiedt and E. V. Truter (Univ. Leeds). *Chem. Ind.* **1952**, 403. By fractional crystallization from methyl ethyl ketone there has been isolated from wool grease cholesteryl 24-methylhexacosanoate, cholesteryl 26-methyloctacosanoate, cholesteryl 28-methyltriacontanoate and an as yet unidentified diester of a hydroxy acid.

Lipids of the central nervous system. G. H. S. Stanley (Maudsley Hosp., London). *Biochem. J.* 50, xxiv(1952). Tracer experiments using deuterium showed that there is a significantly rapid turnover of the lipids in the central nervous system.

The composition of the depot fats of a pig fed on a diet rich in whale oil. G. A. Gorton, T. P. Hilditch and M. L. Meara (Univ., Liverpool). *Biochem. J.* 50, 517-23(1952). The depot fats from a pig whose diet had included 50% whale oil consisted of a mixture of whale oil with the typical fat synthesized by the pig from non-fatty food. Crystallization of the perinephric fat from acetone at —40° gave 26% of a soluble fraction which approximated the composition of whale oil and 74% of insoluble material which closely resembled normal pig fat.