Test Analyses

Standard oils were prepared, covering the range from 15–1200 p.p.m. soap calculated as sodium oleate by adding calculated quantities of sodium hydroxide solution (0.001, 0.01, or 0.1 M) to separate 100-g. samples of Wesson oil and blending in the Waring Blendor. Fairly stable emulsions were thus obtained. The 100-g. treated sample was immediately analyzed, avoiding such sampling problems as settling and inhomogeneity. An additional series of test analyses were made on Wesson oil which had been treated with sufficient oleic acid to bring the FFA to 1%. This assured complete conversion of sodium hydroxide to sodium oleate.

Excellent agreement was obtained with soap concentrations below 150 p.p.m. From 150 to 1200 p.p.m. the results tended to be somewhat low, probably because of the single extraction. Tests showed that further extraction did wash out more sodium. However it was felt that the saving in time warranted the simplified procedure. Refined oils will generally contain well under 150 p.p.m. soap, and the higher samples are so obviously contaminated that analysis is probably unnecessary. Certainly in this range the accuracy is adequate. Representative data, obtained on a Wesson oil containing 1% added oleic acid, are given in Table II.

TABLE II

p.p.m. Na taken (calc. as Na oleate)	p.p.m. Na oleate found
15	15
30	31
45	47
60	60
150	130
300	250
600	490
1,200	1,060

The applicability of the method to refined oils with soap contents below 15 p.p.m. was tested in the following way. Wesson Oil was extracted three times by our procedure outlined above, *i.e.*, concentrated HCl and petroleum ether, then water added and mixed in the Waring Blendor in the manner described. The water layers were discarded. The third water layer gave the same reading on the instrument as the reagent blank. Sodium hydroxide solution, the equivalent of 6 p.p.m. of soap, was added to the "soap-free" oil, and the resulting oil was analyzed for soap by our method. The soap found was 5 p.p.m. In the range of 5 p.p.m. of soap in refined oil the method gives values reliable to ± 1 to 2 p.p.m. Sensitivity can be increased by decreasing the volume of water for extraction or increasing the size of sample, but the latter would tend to make the procedure somewhat cumbersome.

We are making preliminary studies on direct aspiration of a petroleum ether solution of the oil sample into the flame photometer.

Summary

A method has been developed for the determination of sodium soap as sodium in refined vegetable oil. The oil is extracted with hydrochloric acid, and the sodium is determined in the aqueous extract by using the flame photometer.

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Selective Hydrolysis of Soybean Oil Phosphatides[1]

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PHOSPHATIDES obtained from degumming soybean oil are used, after suitable treatment, in a variety of food and industrial products where their emulsifying properties are necessary or important. Although the use of soybean phosphatides has increased year by year, a greater quantity than required for existing uses would be available if the entire production of soybean oil were degummed. Conversion of phosphatides to mono- and diglycerides by selective hydrolysis is therefore a potentially practical method to utilize excess phosphatides, which are now disposed of in soapstock.

Hydrolysis of phosphatides occurs at one or more points in the molecule according to the agents employed (9). Such hydrolytic reagents as aqueous acids or bases hydrolyze the glycerol-fatty acid bonds and the phosphoric acid-choline or ethanolamine bonds first, whereas the glycerol-phosphoric acid bond is the most resistant to attack. Enzymatic hydrolysis is generally more selective. For example, lecithin can be hydrolyzed to lysolecithin, glyceryl-phosphorylcholine, phosphorylcholine, or choline by the selection of appropriate phospholipases and conditions of treatment. Both a glycerophosphatase of takadiastase and a phosphatase derived from certain animal tissues have been reported to liberate phosphoric acid from egg-yolk lecithin. One product of the hydrolysis appears to be a diglyceride. MacFarlane and Knight showed that α-toxins of *Clostridium welchii* filtrates contain a lecithinase which hydrolyzes lecithin to a diglyceride and phosphorylcholine (4). This enzyme does not attack cephalins and certain other phospholipides. Enzymes are generally slow acting and they require buffering for maximum effectiveness. The present report describes a rapid method for converting soybean oil phosphatides to diglycerides by using ion-exchange resins.

Ion-exchange resins have been shown to catalyze a variety of organic reactions. Sussman reported in 1946 that cation resins may be substituted for acid catalysts in esterification, alcoholysis, hydrolysis, and other reactions (6). A continuous flow reactor employing a cation resin catalyst for converting acetone to mesityl oxide was described by Klein and Banchero

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[1] Presented at the 49th Annual Meeting, American Oil Chemists' Society, Memphis, Tenn., April 21–23, 1958.

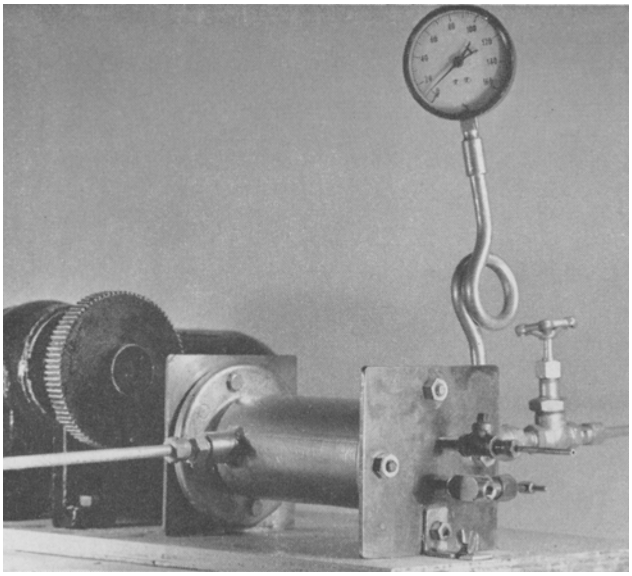

FIG. 1. Stainless steel reaction vessel.

(2). Hamilton and Metzner hydrated ethylene oxide to ethylene glycol over a cation resin in a vapor-phase reaction at temperatures up to 180°C. (1). Sutton and Moore demonstrated the use of a cation resin to promote the hydrolysis of tallow with water at 100°C. (7). They found it necessary to add an emulsifier to the reaction mixture to promote contact between the liquid phases and the resin.

Since phosphatides emulsify with water, it was considered possible to catalyze the hydrolysis of phosphatides in nondegummed soybean oil, using a cation resin without adding emulsifier. It was found that the phosphatides both in the oil or after their separation by degumming are largely hydrolyzed without attack on the oil itself. If the acidity of the water phase is controlled by the addition of a strong anion resin to the reaction mixture, the phosphatides may be largely hydrolyzed free of phosphorus without an appreciable increase in free fatty acid content, indicating that the reaction products are diglycerides and phosphoric acid or its esters with choline, ethanolamine, or inositol.

Experimental

The hydrolysis experiments were conducted either in a 1-liter round-bottom flask or in a small stainless-steel apparatus equipped with a steam jacket and agitator (Figure 1). In either case the reactor was charged with nondegummed soybean oil or soybean gums, water, and freshly activated resin. Often a hydrocarbon solvent, such as heptane or a kerosene distillate, was also added.

When the stainless-steel reactor was used, the reactor headspace was evacuated and placed under nitrogen; the charge was heated to the desired temperature, which was measured by a thermocouple and potentiometric temperature recorder; thorough agitation was provided. Samples were removed through a sampling valve or, after cooling, the whole charge was removed. After the sample or entire reaction mixture was removed, a small amount of salt water was added and the reaction mixture was centrifuged in 250-ml. bottles in an International centrifuge.[2] The oil was then separated from the water by syphoning as close to the interface as possible without disturbing the water layer. The water layer was washed five times with 50-ml. quantities of petroleum ether by shaking with the solvent, centrifuging, and syphoning off the solvent layer. The solvent was removed by evaporation, and the extracted oil was added to the original oil phase for analysis.

In several instances the water layer and resin were evaporated to dryness, then refluxed with alcoholic potassium hydroxide for 1 hr., followed by evaporating the alcohol, dissolving the residue with hot water, acidifying, cooling, and extracting several times with petroleum ether to recover fatty acids. The amount of free and combined fatty acids recovered from the water layer was generally less than 0.3% of the original nondegummed oil charged to the hydrolyzer. The oil phase including the desolventized petroleum ether extracts was thoroughly mixed and analyzed for free fatty acid and phosphorus. Phosphorus was determined by the method of Truog and Meyers after ashing a sample with alcoholic magnesium nitrate (8).

Results and Discussion

Treatment of nondegummed soybean oil with water and a cation resin in the hydrogen form under several conditions is summarized in Table I. The resin was separated and regenerated for subsequent tests. In the first example 90% of the phosphorus was split off from the phosphatides to a water-soluble, hydrocarbon solvent-insoluble form. At the same time there was a substantial increase in free fatty-acid content of the oil. As a check on the procedure for recovering the oil phase following hydrolysis, a test was made without resin, and the phosphorus and free fatty-acid content of the recovered oil were the same as for the original oil. This check test showed that the results obtained with the resin were not caused by degumming.

TABLE I
Hydrolysis of Phosphatides in Nondegummed Soybean Oil Using a Cation Exchange Resin

	Examples					
	1	2	3	4	5	6
Reactants						
Soybean oil, nondegummed, g.	100[a]	100	100	100	100	100
Distilled water, g.	25	25	25	4	25	10
Cation resin, ml.[b]	20	20	10	10	5	10
Hydrocarbon solvent, g.		100	100	100		
Emulsifier, g.					0.25	
Salt, g.						0.7
Hydrolyzed oil[c]						
Free fatty acid, %	2.40	1.84	1.44	2.64	17.2	3.2
Reduction in phosphorus, %	90	94	84	96	87	91

[a] Reactions were conducted 4 hr. at 100°C., except example five was conducted 1 hr. at 121°C.

[b] Ten ml. of wet resin weighed 2.9 g. when dried 2 hr. at 100°C. Dowex 50 × 4, 50–100 mesh, H^+ form was used.

[c] The free fatty-acid content of the original nondegummed soybean oil was 0.73%, and the oil contained 0.095% of phosphorus.

Other examples in Table I indicate the effects of adding a hydrocarbon solvent, an emulsifier, or sodium chloride to the reaction mixture, and the result of reducing the amount of water used. None of these factors result in appreciably different phosphorus removal although the solvent reduces free fatty-acid formation; also the amount of water has an effect on fatty acid hydrolyysis. It is estimated that complete hydrolysis of the fatty acids from the phospha-

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[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

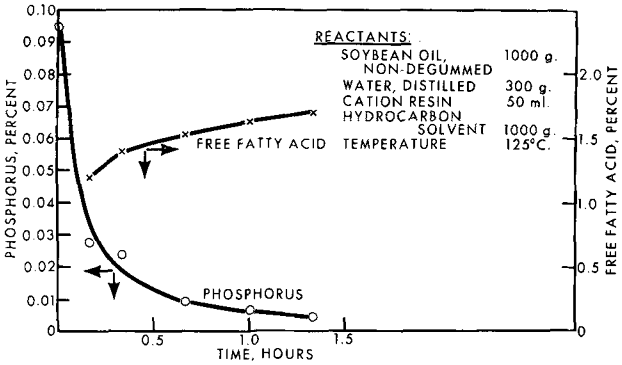

FIG. 2. Hydrolysis of crude soybean oil with a cation resin.

tides present in the crude oil would produce about 2.2% free fatty acids in addition to those originally present in the oil. Therefore, in examples 1, 4, 5, and 6, nearly complete hydrolysis of the phosphatide fatty acids must have occurred although complete hydrolysis is improbable since no phosphorus would then be in a petroleum ether-soluble form.

Figure 2 summarizes results obtained when the hydrolysis was conducted at 125°C. on a larger scale in a stainless-steel autoclave. Samples were removed during the reaction for treatment and analysis. Most of the phosphorus is split off from the phosphatides in the first 10 min. of reaction. Fatty acid hydrolysis shows a steady rate of increase. In this reaction a 200-400 mesh cation resin was used, which may have contributed to a faster reaction. The color of the oil was not visibly altered by treatment at this temperature.

During the hydrolysis reaction increased acidity of the water phase is noted and undoubtedly results from the formation of free phosphoric acid. As previously stated, acidity in the aqueous phase is conducive to fatty acid hydrolysis; therefore a means of controlling acidity is desirable. Methods of controlling the pH of the aqueous phase by using buffer salts or by neutralization with alkali are believed to be ineffective in the presence of a hydrogen form of cation resin because the resin would exchange hydrogen ions for the salt cations. Use of a mixture of hydrogen-form cation resin and sodium-form cation resin, the first to catalyze the reaction, the second to exchange sodium ions with phosphoric acid formed in the reaction, was found to be only slightly effective for preventing free fatty formation in two tests. However when a mixture of hydrogen-form cation resin and a strong anion resin in the hydroxyl form was used in the reaction, it was found that a substantial amount of the phosphorus may be split off from the glycerol radical before the free fatty-acid content increases appreciably.

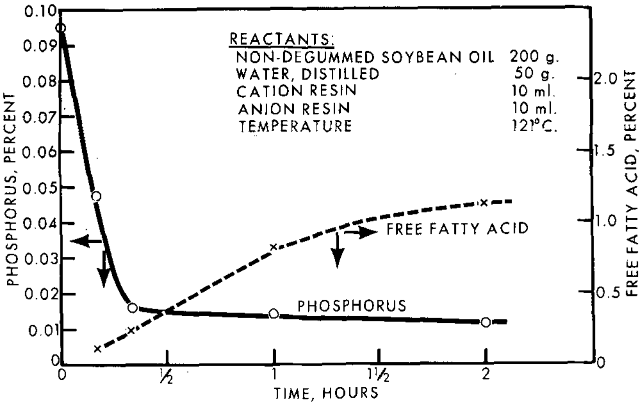

FIG. 3. Hydrolysis of crude soybean oil with mixed cation and anion resins.

Results of a test using mixed resins are shown in Figure 3. After 20 min. at 121°C., 83% of the phosphorus was split off while the free fatty-acid content was only 0.23%. Since the free fatty content of the original crude oil according to titration was 0.73%, it is indicated that most of this titration was caused by phosphatides. Although very little further reduction in phosphorus content of the oil occurred at longer reaction times, the free fatty-acid content had increased to 0.82% after 1 hr. and to 1.13% after 2 hr. The optimum reaction time at 121°C. for maximum phosphorus reduction with minimum free fatty-acid formation was therefore about 20 min.

TABLE II
Hydrolysis of Soybean Oil Gums

	Examples						
	1	2	3	4			
Reactants							
Soybean oil gums, g.	50	[a]	50	5	—	—	—
Distilled water, g.	50		50	50	—	—	—
Cation resin, ml.[b]	25		25	5	—	—	—
Anion resin, ml.[c]				10	—	—	—
Hydrocarbon solvent, g.	150		150	50	—	—	—
Conditions of treatment							
Temperature, °C.	121	121	107	121	—	—	—
Time at temperature, hr.	2	2	2	½	1	2	4
Hydrolyzed gums[d]							
Free fatty acid, %	54.4	54.4	52.9	13.9	18.1	19.0	25.5
Reduction in phosphorus, %	98	98	89	45	90	—	95

[a] The oil phase recovered from example one was re-hydrolyzed under the same conditions with the same amounts of fresh water and solvent.
[b] Dowex 50 ×, 200–400 mesh, H^+ form.
[c] Dowex 1 × 4, 50–100 mesh, OH^- form.
[d] The original gums analyzed 5.2% as free fatty acid and 2.02% phosphorus.

Results of several tests conducted on the hydrolysis of soybean oil gums obtained by degumming a crude oil with 2% of water are given in Table II. In the first example where a cation resin catalyst is used, about 95% of the phosphorus was split off after a 2-hr. treatment at 121°C. However the high free fatty-acid content of the hexane-soluble phase indicates that most of the fatty acids have also been hydrolyzed from the glycerol radical. Column 2 lists conditions of treatment and product analyses when the hydrolyzed gums from example 1 were treated with fresh water, resin, and solvent. No increase in free fatty acid but a further reduction in phosphorus is noted. Lowering the reaction temperature resulted in less hydrolysis of phosphorus in example 2, column 3. In the third example where a mixture of cation and anion resins was used and samples were taken at ½, 1, 2, and 4 hr. the phosphorus was reduced about the same amount as for example 1, but the free fatty-acid content of the hexane-soluble phase was substantially less. If a larger proportion of anion resin and a reaction time less than 30 min. had been used, it is probable that the free fatty-acid content would have been still less although this was not determined.

The recovery and repeated re-use of a cation resin from a hydrolysis reaction presents no difficulties and is a familiar technique. However regeneration of mixed cation and anion resins presents problems. In one method of regenerating mixed resins used for demineralizing water the exhausted, intimately mixed cation and anion resins are separated into layers by hydraulic classification and regenerated by appropriate treatment of each layer with solutions of acid or base, followed by rinsing and remixing (3).

In a catalytic process where considerable agitation is employed, attrition of the resins occurs, which probably prevents their successful re-classification for regeneration since their separation is governed by Stokes' law of settling. According to this law, the rate of settling of a spherical particle in a quiescent fluid medium is a function of the square of its diameter and of its density. The same principle applies to hydraulic classification. Therefore anion and cation resin particles, if equal or nearly equal in density and if the same size, should not be amenable to hydraulic classification. Even though cation and anion resins of a different size range were used initially, they would probably be inseparable or only partially separable after several re-uses on account of attrition.

In one experiment a mixture of anion and cation resins, recovered from the reaction mixture by centrifugation following a hydrolysis reaction, was washed on a Buchner funnel with acetone, followed by eight washings with distilled water. When these resins were used to catalyze a hydrolysis reaction without further treatment, 84% phosphorus removal from a nondegummed soybean oil was effected in 30 min. During this treatment the free fatty-acid content increased to 0.86%.

In another test the mixed resins were regenerated by washing with acetone followed by one wash of 50 ml. of one normal hydrochloric acid, three washes with distilled water, one wash with 25 ml. of one normal sodium hydroxide, and five washes with distilled water. The regenerated mixed resins catalyzed the hydrolysis of 71% of the phosphorus from nondegummed soybean oil in 30 min. at 121°C. The final free fatty-acid content of the oil was 0.19%.

A sample of the nondegummed soybean oil used in the tests and a sample of soybean oil recovered from a hydrolysis reaction, using mixed resins, were alkali-refined with 0.2% excess of 10% aqueous sodium hydroxide. The refined oils were analyzed for mono-, di-, and triglycerides by the adsorption method of Quinlan and Weiser (5). The refined oil contained 3.1% monoglycerides, 13.1% diglycerides, and 80.8% triglycerides while the hydrolyzed, refined oil contained 4.0% monoglycerides, 14.7% diglycerides, and 78.5% triglycerides. These data indicate that mono- and diglycerides are formed by the hydrolysis treatment although the unexpectedly high amounts of mono- and diglycerides in both samples may have been caused by oxidized glycerides which were adsorbed and eluted from the silica gel column together with the mono- and diglycerides.

Summary

A rapid method is described for treating nondegummed soybean oil with water and a mixture of cation and anion exchange resins at elevated temperatures. In less than 30 min. 85% of phosphatide phosphorus is converted to a water-soluble, petroleum ether-insoluble form. Very little free or combined fatty acids are associated with the phosphorus thus split off from the phosphatide, and only a moderate increase in free fatty acid in the oil phase occurs. It is proposed that the phosphatides are largely converted to diglycerides by the selective hydrolytic treatment.

Acknowledgment

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Storage of Cottonseed

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LOSSES on account of cottonseed deterioration during storage have been estimated at around $1 per ton. This figure may be considerably higher or lower in a given locality or during any specific season. Storage losses are caused primarily by two factors. a) Enzymes or microorganisms (1, 2) break down glycerides with the formation of free fatty acids and glycerol. This hydrolysis is accelerated in the presence of high moisture, high acid, or damaged seed. It is also accelerated by increased temperature up to the point where the biologically active agents are destroyed. b) Heat developed (from the biological activity) affects the seed pigments so that they are not satisfactorily removed during subsequent refining and bleaching.

Despite more than 50 years of commercial operation, in many respects cottonseed storage is still an "art," without sufficient scientific background. Some of the most interesting and intensive work done to date has yielded negative results in that no chemical inhibition of hydrolysis caused by enzymes or microorganisms has proven practical on a commercial scale. The inhibitors are either toxic or not sufficiently active (1).

Cottonseed is usually stored in seedhouses or silos. The seed houses may be of wood construction, but the modern types are made of steel with metal sides and roof. The roof is sloped at 45° to equal the angle of repose of seed. Cooling facilities are normally provided by large fans connected to ventilating ducts