Ethanol-Extractable Nonprotein Material in Preparations of Peanut Protein

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IT has been shown recently (1) that the removal of water-soluble material from a peanut protein preparation changes the viscosity characteristics of dispersions made for industrial uses. Peanut protein, prepared by the usual commercial methods, may contain any or all of the nonprotein constituents present in peanut meal, including mineral salts, pigments, carbohydrates, phytin, and various lipid materials (2). Macheboeuf and Tayeau (3) have found that extraction with hot alcohol and ether removes some glucides and lipids from peanut protein preparations. They did not, however, attempt to further characterize the impurities. This report offers some information on the nature and amounts of the nonprotein constituents extracted by cold ethanol at the curd stage from proteins prepared from solvent-extracted peanut meals. In general, the proteins were prepared by methods and operations summarized recently by Burnett (4).

Preparation of Material

In a typical preparation, 100 pounds of peanuts with red testa were flaked to a thickness of approximately 0.008 inch and extracted with a commercial hexane in a large batch extractor. The residual solvent was removed from the meal without use of heat.

As shown diagramatically in Fig. 1, protein was extracted by suspending 6100 grams of the peanut meal in 60 liters of water and stirring vigorously while adjusting the suspension to pH 7.8 by the addition of sodium hydroxide solution. The stirring was continued for a half hour and then most of the residual meal was removed on a 30-mesh screen. A portion of this extracted material was dried under an infrared lamp in a stream of air and reserved for analysis. The screened extract was allowed to settle for 1.5 hours and the supernatant liquid siphoned off and filtered through a 2-inch layer of diatomaceous earth on a glass cloth filter in a large Büchner funnel. The protein was precipitated by bubbling sulfur dioxide into the filtrate while stirring vigorously until pH 5.0 was obtained. The precipitated protein was allowed to settle overnight and the supernatant liquid was siphoned off.

One weighed aliquot of the wet protein curd was dewatered by holding at 50° C. for the 3 hours required for it to coalesce (5). The coalesced curd was squeezed by hand to remove as much liquid as possible and divided into two portions. One portion was dried in a forced draft oven at 48° C. and then air equilibrated. The other was washed with four successive portions of ethanol, being allowed to stand in contact with the last portion of ethanol overnight. The ethanol washed coalesced protein was then dried in a vacuum oven at 50° C. and air equilibrated.

A second aliquot of the wet curd was redispersed in 20 liters of distilled water by adjustment to pH 7.8 with sodium hydroxide solution. It was reprecipitated at pH 5.0 with sulfur dioxide and allowed to settle overnight. The supernatant liquid was siphoned off and the curd dispersed and precipitated as before. The curd obtained after the third precipitation was divided into two weighed portions. Again one portion was dried in a forced draft oven at 48° C. and allowed to air equilibrate. The other portion was washed without previously dewatering with four successive portions of ethanol, being allowed to stand in contact with the last portion overnight. The ethanol-washed protein was dried in a vacuum oven at 50°C. and then air equilibrated.

The ethanol washings from the coalesced protein as well as those from the wet curd protein were filtered through paper to remove fines and were separately concentrated to a volume of 300 ml. by vacuum distillation in a water bath maintained at 50° C. Five hundred milliliters of water were then added to each and the mixtures again vacuum distilled to remove alcohol and concentrate them to about 100 ml. The concentrates were transferred to a separatory funnel and exhaustively extracted with a commercial pentane. The pentane extracts were washed with water and the solvent evaporated on the steam bath. The aqueous phases and washings were filtered to remove a trace of emulsion and evaporated to dryness in vacuum. Yields of aqueous and lipid extracts are shown in Fig. 1.

Similarly, additional lots of protein were prepared from red-skin peanut meal containing considerable residual lipids; from red-skin peanuts treated with alkali to remove the skin pigments (4); and from white-skin peanut meal. In these preparations the protein was precipitated only once and then coalesced before drying.

For comparison, protein was prepared from depigmented cottonseed meal in the same way as shown in Fig. 1, except that no attempt was made to dewater or coalesce the cottonseed protein.

Analysis and Properties of the Protein Preparations

The results of analysis of the original meal samples, the meal residues, the air-dried proteins, and the alcohol-washed proteins are shown in Table I. Moisture was determined by drying for 2 hours at 130° C. in a forced draft oven. Ash values were obtained by smoking off the sample at 250° C. and then igniting for 4 hours at 600° C. in an electrically heated, pyrometer controlled muffle. The Kjeldahl method with mercury as a catalyst was used for the nitrogen determinations. Phosphorus was determined by the reduced molybdate method after digestion with sulfuric acid and hydrogen peroxide. Lipids in the meal were determined by the A.O.C.S method (6) and in the pro-

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tein preparations by the A.O.A.C. acid hydrolysis method (7).

The data in Table I show that ethanol washing lowered the ash content slightly but was without significant effect on the phosphorus content of peanut and cottonseed protein preparations. The most striking effect of the ethanol washing was the reaction in the lipid content. This effect was more marked when the protein is washed with ethanol in the wet curd state without coalescing. In the protein from the red-skin peanut meal that had not been thoroughly extracted with commercial hexane, considerable lipids were retained even after washing the coalesced protein with ethanol. As expected from the lowered lipid and ash contents, the total nitrogen content of the proteins showed an increase as a result of washing with ethanol. This increase was larger for the preparations that were washed without previously dewatering.

TABLE I.

Analysis of Meal, Meal Residue, and Protein. (Moisture-free basis)

Material Analyzed	Moisture	Ash	Nitrogen	Phosphorus	Lipids
	%	%	%	%	%
Solvent-Extracted Red-Skin Peanuts "A"					
Meal	6.44	5.33	9.10	.....	0.72
Meal residue	11.70	5.70	5.85	0.50	0.52
Dewatered protein air dried	9.62	1.82	16.30	0.53	1.06
Dewatered protein ethanol washed	9.80	1.71	16.65	0.53	0.46
Reprecipitated protein air dried	9.55	3.30	16.34	0.51	1.09
Reprecipitated protein ethanol washed	9.79	1.54	17.11	0.50	0.12
Solvent-Extracted Red-Skin Peanuts "B"					
Meal	10.23	4.36	8.26	0.59	18.50
Meal residue	11.87	5.21	7.24	0.51	3.49
Dewatered protein air dried	8.11	1.31	15.71	0.48	3.21
Dewatered protein ethanol washed	9.50	1.27	16.18	0.49	1.25
Solvent Extracted Alkali-Decolorized Red-Skin Peanuts					
Meal	15.59	5.19	10.72	0.73	0.63
Meal residue	11.74	6.03	8.39	0.65	0.52
Dewatered protein air dried	8.95	1.51	17.20	0.45	0.38
Dewatered protein ethanol washed	10.45	1.42	17.31	0.45	0.17
Solvent-Extracted White-Skin Peanuts					
Meal	7.65	4.83	10.85	0.70	2.91
Meal residue	9.96	4.66	8.44	0.54	0.82
Dewatered protein air dried	9.04	1.58	16.98	0.46	0.54
Dewatered protein ethanol washed	9.21	1.52	17.18	0.46	0.24
Solvent-Extracted Depigmented Cottonseed Meal					
Meal	7.60	7.79	8.94	1.51	0.15
Meal residue	11.70	9.20	10.70	1.91	0.09
Reprecipitated protein air dried	10.81	10.27	12.80	1.49	0.44
Reprecipitated protein ethanol washed	8.98	6.15	14.46	1.70	0.09

Viscosity measurements (1) showed that ethanol washing increased the maximum viscosity considerably and shifted the maximum to a slightly lower pH value.

The solubility of some of the protein preparations was determined in 0.5 molar sodium chloride solution and in dilute sodium hydroxide and dilute hydrochloric acid at several pH values (8). In each case 2.5 grams of finely ground protein was weighed into a centrifuge bottle and 100 ml. of the solvent introduced with a pipette. The suspension was shaken thoroughly to wet the protein and the shaking repeated at frequent intervals during a 2.5-hour period. The suspensions were then centrifuged and the supernatant liquid decanted through dry filter paper. The pH of the filtrate was obtained with a glass electrode and nitrogen determined on an aliquot. The data in Table II show that the solubility of the protein was somewhat less after ethanol washing. The pH values for the neutral and alkaline solutions of the air-dried preparations were lower than those of the corresponding ethanol-washed protein, indicating the possibility that the ethanol washing removed a small amount of an acidic constituent.

No attempt was made at an exact evaluation of the color of the protein preparations, but the ethanol-washed peanut and cottonseed preparations appeared lighter in color. The ethanol-washed preparations were easily dried friable materials while the other protein preparations were dried with difficulty. Both of these differences were more marked when the protein was washed with ethanol without previously coalescing the curds by dewatering and were apparent in both the peanut and cottonseed protein preparations.

Lipid Fraction

Analytical values for the lipid fraction obtained by extracting the concentrated ethanol extracts with commercial pentane are shown in Table III.

Wijs iodine number, thiocyanogen number, and unsaponifiable material were determined by the methods of the A.O.C.S. (6). Neutralization equivalent and saponification equivalent were determined by a semi-micro modification of Gorbach's method (9). Phosphorus and nitrogen were determined colorimetrically on aliquots of a sulfuric-acid-hydrogen peroxide digest of the lipid material.

The data show that all of the lipid fractions were high in free fatty acids. This is in line with the high free fatty acid content of the lipids in the meal from which the proteins were prepared. Glyceride content was therefore low, although qualitative tests for glycerine were positive. Spectrophotometric (10) analysis

TABLE II.

Solubility of Peanut Protein Preparations.

Solvent	White-Skin Peanut Protein				Red-Skin Peanut Protein "B"				Alkali-Decolorized Red-Skin Peanut Protein			
	Ethanol Washed		Air Dried		Ethanol Washed		Air Dried		Ethanol Washed		Air Dried	
	% Soluble N	pH	% Soluble N	pH	% Soluble N	pH	% Soluble N	pH	% Soluble N	pH	% Soluble N	pH
0.5 N NaCl	7.3	5.05	20.9	4.98	3.8	4.83	12.0	4.72	5.7	5.00	18.3	4.90
0.4 N HCl	9.8	0.55	17.6	0.55	8.0	0.70	12.4	0.72	11.0	0.50	14.7	0.50
0.02 N HCl	86.4	3.00	89.3	3.08	74.9	2.69	75.5	2.70	84.7	2.92	83.6	2.97
0.0015 N HCl	3.6	4.70	10.5	4.71	2.3	4.20	5.7	4.33	2.0	4.60	7.0	4.60
0.003 N NaOH	12.7	6.20	20.3	5.90	11.3	6.35	10.3	6.22	13.9	6.20	15.4	6.17
0.011 N NaOH	76.5	8.16	91.9	7.40	52.5	8.94	51.5	8.20	83.2	8.25	76.7	7.88
0.017 N NaOH	94.8	10.07	96.4	9.12	72.7	10.22	75.0	9.50	94.2	10.08	92.8	9.44

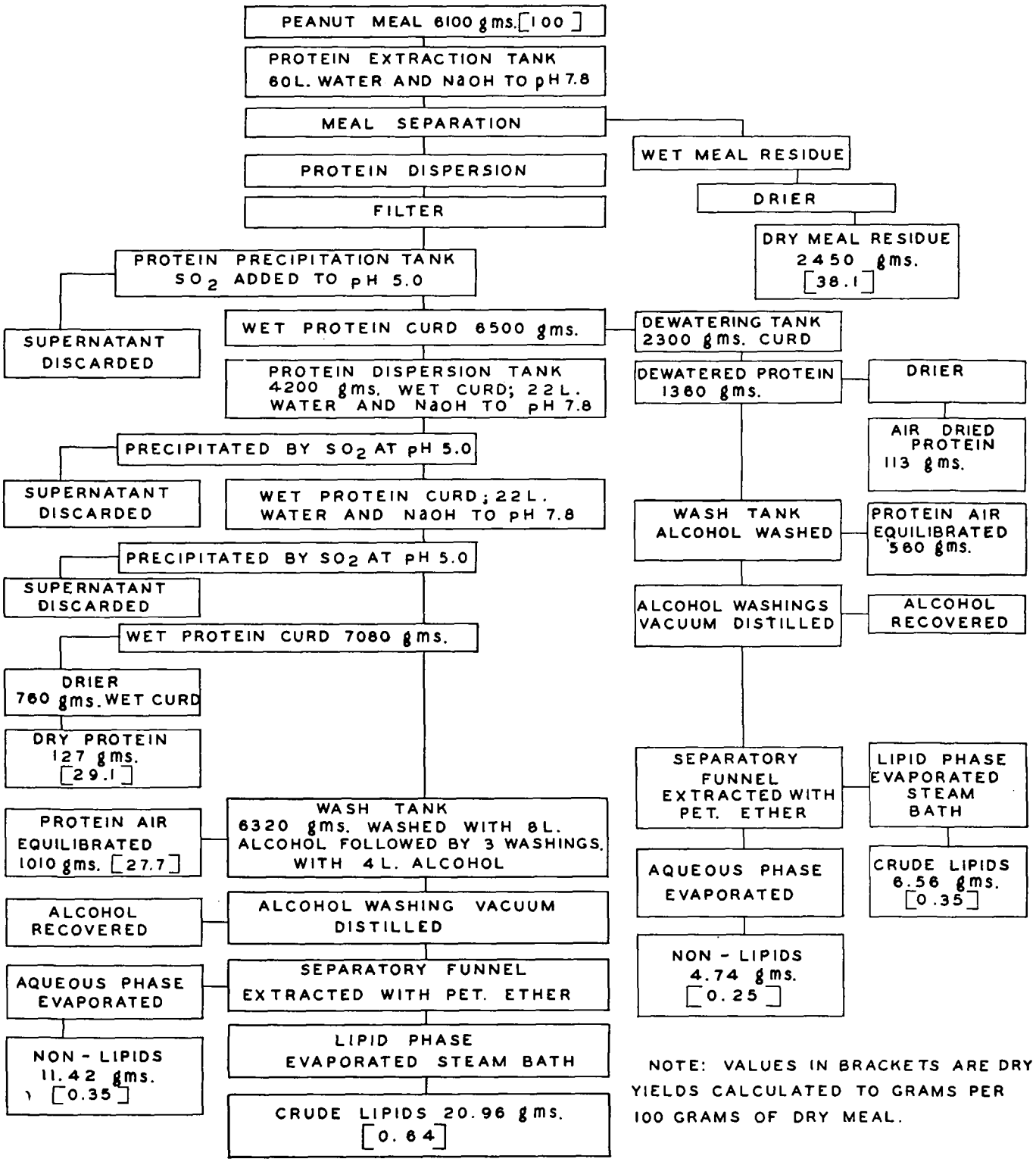

FIG. 1. Preparation of protein from peanut meal.

TABLE III.

Analytical Values Obtained on Lipid Fraction Removed by Washing Protein with Ethanol.

Determination	Red-Skin Peanut "A" Dewatered Protein	Red-Skin Peanut "A" Reprecipitated Protein	Red-Skin Peanut "B" Protein	Alkali-Treated Red-Skin Peanut Protein	White Skin Peanut Protein	Cottonseed Protein
Saturated acids %	.....	.....	37.4	29.2	30.8	.....
Iodine number (Wijs)	69.6	59.5	82.2	83.7	74.6	84.3
Free fatty acid (as oleic) %	32.6	15.8	60.1	59.8	61.8	27.6
Saponification equivalent	319	500	286	279	295	441
Thiocyanogen number	.....	.....	66.9	70.5	73.6	.....
Unsaponifiable matter %	.....	.....	1.24	3.84	3.49	.....
Phosphorus %	0.78	1.01	0.25	0.53	0.28	1.14
Nitrogen %	0.62	0.34	.....	.....	.....	1.52

of the lipids indicated an unsaturated fatty acid composition similar to that reported for peanut oil.

Portions of the lipid fractions were saponified, the unsaponifiable fraction removed, and the fatty acids isolated. The saturated and unsaturated fractions were separated by low temperature crystallization from acetone (11). The unsaturated acids were hydrogenated using platinum as the catalyst and acetic acid as the solvent. After hydrogenation the fatty acids were thrown down with water, recrystallized from acetone, and from alcohol-water mixtures. X-ray diffraction patterns of the hydrogenated acids and of the saturated acids were characteristic of a mixture of a number of long chain fatty acids, with an effective chain length of approximately 18 carbon atoms. Neutralization equivalents indicate some longer chain lengths.

The phosphorus content of the lipid fractions obtained from the ethanol washings of the dewatered protein was lower than that of the lipid fractions obtained from protein which had not been dewatered. The presence of appreciable quantities of both nitrogen and phosphorus was indicative of the presence of some phospholipids, particularly in the lipids from the cottonseed protein. There seemed to be a correlation between high phosphorus content and high saponification equivalent.

Water-Soluble Fraction

The material obtained by evaporation of the aqueous phase after extraction of the lipid fraction was analyzed for ash, total sugars, reducing sugars (7), total nitrogen, and phosphorus. The nitrogen distribution was determined by the Van Slyke method (12) and found to be very similar to that reported for peanut protein (2). However, the humin nitrogen fraction and nonamino nitrogen of the filtrate were somewhat high. As shown in Table IV this fraction contained considerable sugars, most of which were reducing sugars. The high nitrogen content showed that a large proportion of the material consisted of nitrogen compounds. It probably contained a mixture of amino acids as it was soluble in dilute trichloracetic acid. The increase in amino nitrogen on hydrolysis indicated some peptide linkages.

TABLE IV.

Retention of Fatty Acids by Protein on Reprecipitation.

Source of Ethanol-Washed Protein to Which 2.0% of Fatty Acid Was Added Prior to Redispersion	Fatty Acid Added	Lipid Found in Reprecipitated Protein	
		Washed With Ethanol	Not Washed With Ethanol
		%	%
White-skin peanuts	None	0.06	0.27
	Stearic acid	0.10	0.30
	Oleic acid	0.19	0.68
Red-skin peanuts	None	0.10	0.31
	Stearic acid	0.15	0.36
	Oleic acid	0.15	0.54
Alkali-decolorized red-skin peanuts	None	0.04	0.06
	Stearic acid	0.03	0.16
	Oleic acid	0.03	0.47

Retention of Fatty Acid on Dispersion and Precipitation of Protein

The high free fatty acid content of the lipid fraction removed by washing peanut protein with ethanol suggested that fatty acids present in the residual lipids in the meal were dispersed as soaps when the protein was extracted from the meal and were precipitated with the protein when the dispersion was adjusted to a more acid pH value. This was verified in experiments in which fatty acids were added to ethanol-washed protein.

TABLE V.

Analytical Values Obtained on Nonlipid Fraction Removed from Protein by Washing with Ethanol.

Protein Source	Reducing Sugars (as invert sugar)	Total Sugars (as invert sugar)	Ash	Total Nitrogen	Amino Nitrogen	Amino Nitrogen After Hydrolysis
	%	%	%	%	%	%
Cottoneed	.....	12.7	8.10	4.80	.....	.....
Red-skin peanuts	30.9	32.7	4.49	5.06	0.67	3.71
Alkali-decolorized red-skin peanuts	26.9	29.0	7.64	5.25	1.10	3.76
White-skin peanuts	24.5	26.9	5.64	7.20	1.34	4.41

One-gram portions of oleic or of stearic acid dissolved in 50 ml. of commercial pentane were added to 50-gram samples of ethanol-washed and air-equilibrated protein preparations from red-skin peanuts, alkali-decolorized red-skin peanuts, and white-skin peanuts. Control samples were treated with solvent alone. The solvent was allowed to evaporate and the protein preparations were extracted with dilute sodium hydroxide solution at pH 7.8. The protein was precipitated by adjusting the extract to pH 5.0 with sulfur dioxide and separated by centrifugation. While the protein curds were still wet each was divided into two portions. One portion was given three successive washes with ethanol. Both portions of protein were vacuum dried and then allowed to air equilibrate. The proteins were analyzed for lipids by the acid hydrolysis method (7). The values given in Table IV show that fatty acids were carried along with the protein and were removed to a considerable extent by washing with ethanol in the curd stage. It also appears that oleic acid was carried along to a greater extent than was stearic acid.

Incidental to the conclusion that the lipids present in peanut protein preparations are largely fatty acids, it has been noted that the residual lipid material left

in the solvent-extracted peanut meal or cottonseed meal is very high in free fatty acids.

Summary

The nonprotein material in protein prepared by the usual methods from solvent-extracted peanut meal has been investigated. It consists of sugars, nonprotein nitrogenous substances, lipids, and ash. The sugars are practically all reducing sugars. The nonprotein nitrogenous material is amino acid in nature with some peptide linkages. Its nitrogen distribution does not differ greatly from that reported for peanut protein. The chief component of the lipid material is fatty acids possibly including some of the chain length longer than C_{18}. There are also present some phosphatides. Protein prepared similarly from cottonseed meal contains similar nonprotein materials.

The sugars, amino acid material, and lipids are removed to a considerable extent by washing the protein with alcohol in the wet curd state. This removal is more efficient if the curds have not been coalesced by dewatering treatment.

Washing with alcohol makes drying of the protein easier and seems to remove some color, but reduces solubility to some extent. Ash content is slightly lowered by washing with alcohol and phosphorus content is not appreciably changed. Since washing with alcohol removes nonnitrogenous material such as lipids and sugar, the nitrogen content of protein preparations is increased by such treatment.

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A Study of the Caustic Refining of Vegetable Oils

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CRUDE vegetable oils contain free fatty acids, phosphatides, color bodies, and other colloidal substances. The usual refining procedure is to mix the crude oil with a caustic soda solution which results in the formation of soapstock, usually in a finely divided condition, apply heat to the oil-soapstock mixture which causes the agglomeration of the soapstock, termed the "break," and then separate the soapstock from the refined oil. The refined oil thus obtained contains varying amounts of soapstock in suspension and moisture in solution. To remove this residual soapstock the refined oil is washed one or more times with hot water, with the separation of the water as far as possible between washings by centrifuging,, and the ultimate drying of the oil usually under a vacuum. Instead of washing the oil, the suspended soapstock can be agglomerated with heat in settling tanks and the thus partially clarified oil pumped through filter presses containing spent fuller's earth.

Until about 12 years ago the mixing and heating were accomplished in tanks equipped with paddle agitators and steam coils or jackets. This process is still used with some oils that are not satisfactorily refined using the present-day continuous process of refining which has largely supplanted the kettle process. In the present continuous process of refining the caustic solution is injected into a flowing stream of the oil in increments by means of a proportioning device termed a proportionometer which regulates the flow of the caustic solution in proportion to the oil flow. The resultant oil-caustic mixture is caused to flow first through a chamber containing rapidly revolving paddles, which mixes the oil and caustic solution, then through the tubes of a hot-water-jacketed heat exchanger where the mixture is heated to obtain the "break," with the temperature reached being usually about 140°F., and finally to a centrifuge where the soapstock is separated from the refined oil. This process is termed the Clayton continuous process (1).

In the kettle and the Clayton processes the oil and caustic solution are mixed as liquids. Recently, a new continuous process of refining has been developed which mixes the oil and caustic solution in the form of mists, collecting the mist-mixture on the side of a steam-jacketed tank down which the mixture flows in liquid form during which time the mixture is heated