A Comparison of Five Commercial Solvents for Extraction of Cottonseed[1]

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OF the controllable variables influencing the yield and the qualities of the products in the solvent extraction of oilseeds the nature of the solvent is probably the most important (1). Although the literature contains numerous accounts of extraction of cottonseed with different solvents (2, 3, 4, 5) as well as studies of the effect of various solvents on cottonseed pigment glands (6), so far as is known to the authors the effect of different solvents on the yield and the qualities of the products has not previously been explored on a comparative basis in which the solvent was the only significant variable.

It is the purpose of this paper to present data which were obtained in comparative testing of five different commercial oil solvents as extractants for cottonseed by pilot-plant-scale batch extraction, showing the effect of each solvent on the yield, composition, and qualities of the products.

Experimental Conditions

Materials

Solvents. The solvents tested were hexane (which was included as a control since it is practically the standard for the industry), benzene, ethyl-ether, acetone, and butanone (ethyl-methyl-ketone). The solvents were of commercial grade, were obtained in drum lots through regular trade channels, and were employed as received. The properties of the solvents are shown in Table I.

Cottonseed and flakes. The cottonseed were prime quality, Louisiana-grown seed which had received no treatment of any kind. A supply of seed for use in the experiments was thoroughly mixed and stored in sealed drums in a cold storage locker maintained at 32° to 34° F., conditions which have been reported (8, 9) to minimize changes in stored seed. The seed were removed from storage as required for each of the extractions.

Whole meats, containing only a small amount of broken meats and 2% or less of hull material, were prepared from the seed in conventional small-scale oil mill equipment. The meats were flaked by a single pass between one-high corrugated rolls followed by a single pass between one-high, spring-loaded smooth rolls. The flakes produced ranged in thickness from .008 to .010 inch.

The freshly prepared flakes had a moisture content of 6.8 to 7.2%. To prevent dilution of the water-miscible solvents their moisture content was reduced to 4 ± 1% by drying for 3 hours at 120°F. in a circulating-air, steam-heated tray dryer.

Both meats and flakes were produced immediately prior to extraction. The partially dried flakes were loaded into the extractor and covered with solvent while still warm. Approximately 125 pounds of flakes, equivalent to about 120 pounds on a moisture-free basis, were charged to the extractor cell for extraction with each solvent. The five batches of flakes were practically identical in composition when charged to the extractor (Table III).

Procedure

Equipment. The extractions were made in an all-iron, pilot-plant batch extractor unit which was described in a previous publication (10). A diagrammatic sketch of the assembly is shown in Figure 1.

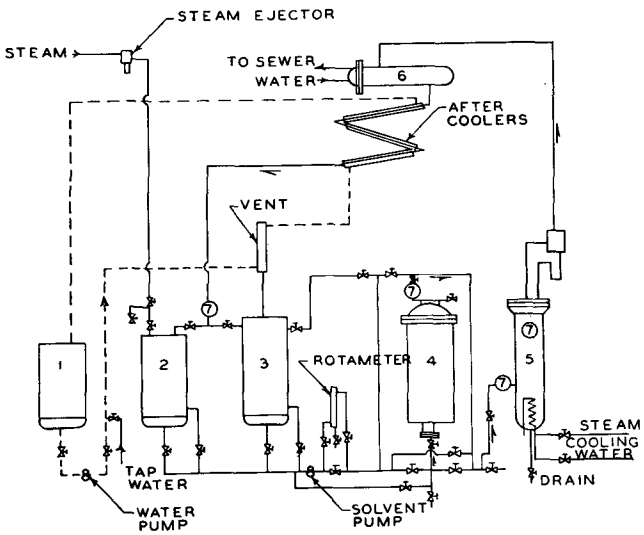

FIG. 1. Portable batch solvent extraction plant

1. Cold Water Container
2. Vacuum Distillate Tank
3. Solvent Storage Tank
4. Extractor
5. Evaporator
6. Condenser
7. Sight Glass

The unit is so designed and constructed as to enable control of solvent rate, solvent temperature, and extractor temperature. The evaporator is of the pot-type and is equipped with a totally immersed steam coil and has provisions for thermostatic control and for vacuum operation.

Extraction. To determine the maximum effect of the different solvents on the yields, composition, and qualities of the meals and crude oils, the extractions were exhaustive; a solvent-to-flake ratio of approximately 3.3 gallons of solvent per pound of flakes (moisture-free basis) was used. The heated solvent was pumped through the flakes at a rate of about 30 gallons per hour with frequent soaking periods in the early stage of extraction. When the discharge from the extractor became colorless, the rate was increased and pumping continued until the predetermined gallonage (approximately 400 gals.) had been passed through the flakes. Owing to varying lengths of time required to obtain a colorless discharge with the different solvents there was some variation in the extraction times (Table II).

Meal desolventization. The meals were desolventized by spreading them on open trays and aerating until they appeared dry. They were then heated for 4 hours

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TABLE I

Properties and Characteristics of Solvents

Solvent	Hexane	Benzene	Acetone	Ethyl-ether	Butanone[a]
Distillation Range, °F.[b]	152-160	172-230	131-135	.....	172-183
Boiling Point, °F	.....	.....	.....	94.1[b]	.....
Density (25°C.)	0.6852[b]	0.8668	0.7849	0.7183	0.8000
Refractive Index	1.385[b]	1.495	1.3741	1.3515	1.3566
Evaporation Residue, %	0.0002	0.003	0.006	0.007	0.014
Water, %[c]	.....	0.06	0.43	0.56	0.10

[a] Ethyl-methyl-ketone.
[b] From specifications of manufacturer.
[c] By the Karl Fischer method (7).

TABLE II

Extraction Data and Materials Balance

Solvent	Hexane	Benzene	Ethyl-ether	Acetone	Butanone
Extraction Conditions					
Ratio, solvent/flakes[a]	3.52	3.34	3.35	3.32	3.36
Time-extracted, hrs.	14.7	12.8	15.2	17.5	19.4
Temperature, °F. Av.	119	115	93	112	114
Maximum	131	125	105	130	124
Desolventizing Temp., °F., Max.					
Meal	120	120	120	120	120
Crude oil	135	134	130	128	140
Materials Balance					
Flakes to extractor lb.					
As charged to extractor	125.1	124.0	124.8	127.0	125.0
Moisture-free basis	120.0	119.8	119.8	120.7	118.8
Meal yield, lb.					
As removed from dryer	80.3	80.1	79.5	80.6	78.7
Moisture-free basis	76.5	75.7	73.8	75.4	73.2
Corrected for loss[b]	77.1	76.0	76.2	76.3	74.3
Percentage of flakes[c]	64.25	63.44	63.61	63.21	62.54
Crude oil yield, lb.					
Volatiles-free	42.0	41.0	42.4	41.6	43.1
Corrected for loss[b]	42.9	43.8	43.6	44.4	44.5
Percentage of flakes[c]	35.75	36.56	36.39	36.79	37.46

[a] Gallons of solvent per pound of moisture-free flakes.
[b] Corrected for mechanical loss on basis of analysis of flakes, meal, and oil.
[c] Calculated on corrected yield.

at 120°F. in a circulating-air, steam-heated tray dryer to remove the last traces of solvent. In none of the meals could the odor of the solvent be detected after this treatment.

Crude oil desolventization. The miscella from each of the extractions was fed directly from the extractor to the evaporator where it was continuously concentrated under vacuum. The concentrated miscella was filtered (through filter paper) and then reintroduced into the cleaned evaporator and further concentrated at the same conditions. The maximum temperature to which each of the miscellas was heated during the two-stage concentration is included in the data shown in Table II. Heating of the miscellas was controlled at or below 140°F. to eliminate insofar as possible any tendency to fixation of color in the oils (11, 14). The time of heating at the maximum temperature was in every case of very short duration. The miscellas were protected from contact with air and from exposure to light as much as possible during extraction and concentration.

The concentrated miscellas, as they were produced, were sealed in glass containers and stored in a light-proof cabinet until all had been accumulated. Days of storage of the various solvent miscellas prior to stripping were as follows: hexane, 35; benzene, 40; ethyl-ether, 56; acetone, 90; and butanone, 12.

Final desolventization was accomplished by stripping with nitrogen in glass laboratory apparatus under a vacuum of approximately 28 inches mercury with the temperature of the oils controlled at 131°F. Nitrogen was used in preference to steam to prevent precipitation of phospholipid material and to avoid any possibility of other physical or chemical changes in the minor oil components resulting from contact with wet steam. The oils were stripped immediately prior to refining and bleaching.

Analytical methods. The analytical methods used were, where applicable, those recommended in the Official and Tentative Methods of the American Oil Chemists' Society (12). Literature references to other methods employed are cited in the appropriate place.

Protein preparation. A 200-g. portion of each of the meals was ground to pass a 60-mesh screen and extracted with sodium hydroxide at room temperature (78°F.) with vigorous stirring throughout. A water-to-meal ratio of 15 to 1 was used, and the mix-

TABLE III

Analysis[a] of Cottonseed, Flakes, Meal, and Crude Oil[b]

Solvent	Material	Moisture & volatiles	Oil[c]	F.F.A. in Oil	Nitrogen	Protein[d]	Nitrogen solubility[e]	Ash	Phosphorus	Sugars[f]	Free[g] gossypol	Total[h] gossypol	Thiamin[i]
		%	%	%	%	%	%	%	%	%	%	%	p.p.m.
	Seed	8.38	18.66	0.35	3.34	.....	.....	.....	.....	.....	.....	.....	.....
Hexane	Flakes	4.12	35.2	0.60	6.19	38.66	.....	5.33	1.17	7.6	1.00	0.91	.....
	Meal	4.78	0.54	.....	9.63	60.19	69.5	8.26	1.77	12.0	1.10	1.23	33.41
	Crude Oil	0.13	.....	0.87	.....	.....	.....	0.06	0.032	.....	.....	0.09	.....
Benzene	Flakes	3.40	35.3	0.52	6.22	38.88	.....	5.33	1.16	7.8	0.98	0.94	.....
	Meal	6.23	0.83	.....	9.89	61.81	72.5	8.32	1.76	13.0	0.63	0.87	34.95
	Crude Oil	0.30	.....	1.10	.....	.....	.....	0.09	0.043	.....	.....	0.14	.....
Acetone	Flakes	5.00	35.0	0.68	6.21	38.84	.....	5.31	1.15	7.1	1.00	1.00	.....
	Meal	6.51	0.40	.....	9.78	61.11	74.0	8.33	1.78	11.0	0.07	0.15	33.31
	Crude Oil	0.16	.....	1.76	.....	.....	.....	0.17	0.049	.....	.....	1.42	.....
Ethyl-ether	Flakes	4.00	35.2	0.54	6.24	39.02	.....	5.34	1.16	7.8	0.99	0.98	.....
	Meal	7.17	0.18	.....	9.76	60.99	78.1	8.35	1.76	13.0	0.12	0.21	34.34
	Crude Oil	0.04	.....	2.52	.....	.....	.....	0.15	0.053	.....	.....	1.43	.....
Butanone	Flakes	4.94	35.1	0.58	6.20	38.72	.....	5.31	1.15	8.0	0.97	1.02	.....
	Meal	7.02	0.15	.....	9.83	61.45	77.2	8.45	1.78	11.0	0.06	0.11	33.89
	Crude Oil	0.15	.....	2.37	.....	.....	.....	0.15	0.075	.....	.....	1.25	.....

[a] Analyses on moisture-free and volatiles-free basis except for values in column 3.
[b] Nitrogen stripped.
[c] By A.O.C.S. method Ba 3-38 with petroleum ether (12).
[d] Nitrogen × 6.25.
[e] Percentage soluble in .5N NaCl solution (15).
[f] Determined as total sugars including raffinose and reported as invert sugars (21).
[g] Determined by method of Pons and Guthrie (16).
[h] Determined by method of Pons, Hoffpauir, and O'Connor for total gossypol in meal (17) and oil (18).
[i] Determined by the modified method developed by the Analytical Division of S.R.R.L. from two published methods (19, 20).

TABLE IV

Efficiency of the Solvents for Extracting Oil[a]

Solvent	Oil in flakes	Yield of crude oil extract[b]	Oil in meal	Total oil in meal[c]	Total oil extracted[c]
	%	%	%	%	%
Hexane	35.2	35.75	0.54	0.99	99.01
Benzene	35.3	36.56	0.83	1.49	98.51
Ethyl-ether	35.2	36.39	0.18	0.33	99.67
Acetone	35.0	36.77	0.40	0.73	99.27
Butanone	35.1	37.44	0.15	0.26	99.74

[a] By A.O.C.S. method Ba 3-38, "oil" referring to the material determined by this method.
[b] Pounds of volatiles-free crude oil divided by pounds of moisture-free flakes.
[c] Calculated from data in Tables II and III.

TABLE V

Distribution of the Gossypol of the Original Flakes Between the Meal and Oil as Effected by the Solvent

Extraction solvent	Gossypol of the original flakes					
	Found in the meal as:			Found in the crude oil[a]	Accounted for	Not accounted for
	Total[a]	Free[a]	Bound[b]			
	%	%	%	%	%	%
Hexane	79.26	70.65	8.61	3.18	82.44	17.56
Benzene	56.06	40.46	15.60	5.17	61.23	38.77
Acetone	9.52	4.30	5.22	52.07	61.59	38.41
Ethyl-ether	13.54	7.53	6.01	52.71	66.24	33.76
Butanone	6.77	3.71	3.06	45.87	52.64	47.36

[a] Calculated from data in Tables II and III.
[b] By difference: Total gossypol less free gossypol.

ture was maintained at a pH of 10.0 for the 30-minute extraction period. The extract was separated from the bulk of the spent meal residue by screening and then clarified by centrifuging. The spent meal residue was not washed. The protein was precipitated from the clarified liquor by adjusting the pH of the liquor to 4.0 with gaseous sulphur dioxide and the coagulated protein reclaimed by centrifuging. The wet curd was dried, without prior washing, in a forced-air draft oven at 120° F.

Determination of protein color. The dried solid proteins were ground to pass a 20-mesh screen and their order of pigmentation (Table VI) was estimated by visual examination.

Clear dispersions of the different proteins were prepared by dissolving 0.40 g. of each in 100 ml. of 0.02 N sodium hydroxide solution and filtering through analytical-grade filter paper. The Lovibond red color of the dispersions was determined by readings in the Lovibond colorimeter using a 1-in. tube depth (see Table VI).

Spectrophotometric data on clear protein dispersions, prepared identically to those used for the Lovibond readings, were obtained and the tristimulus values for the dispersions calculated. The tristimulus coefficients were calculated and the psychophysical values, luminous transmission or brightness, dominant wave length, and purity were determined by the method and from the I.C.I. tables given in the "Handbook of Colorimetry" (13).

Refining tests. The crude oils were subjected to refining tests by two established and one non-standard method. The methods and the lye concentrations used were:

a) The method of the American Oil Chemists' Society for hydraulic-pressed cottonseed oil (Ca 9a-41). Lyes used were 12°, 14°, and 16° Bé.

b) The method of the American Oil Chemists' Society for slow-break cottonseed oil (Ca 9a-41). Lyes used were 14°, 16°, and 20° Bé.

c) The method of the American Oil Chemists' Society for hydraulic-pressed cottonseed oil but modified to require a 90-minute cold stir (14). Lyes used were 14°, 16°, and 20° Bé.

Colors of the refined oils were determined in accordance with prescribed American Oil Chemists' Society methods (Cc 13b-45).

Bleaching tests. One 200-g. portion of each of the refined oils was bleached with 6% of A.O.C.S. Official Natural Bleaching Earth and one 200-g. portion with 4% of Bennett-Clark clay[3] and 1% of activated carbon. The temperatures, stirring rates, and times were as prescribed by the American Oil Chemists' Society (Cc 8a-49).

Yields of Products

Materials balance. The materials balance from extraction with each solvent is shown in Table II. The weighed yields were corrected for the unavoidable loss of material inherent in any such pilot-plant-scale experiment by the use of simultaneous equations. Calculations were based on the analyses and weights of the flakes, meals, and crude oils. All calculations were based on the corrected yield figures; and the corrected values are used throughout the discussion.

Meal yields. Meal yields from extraction with hexane are normally dependent on the percentage of oil present in the unprocessed seed and the degree of oil extraction and are not substantially affected by extraction of the relatively small amount of non-oil material soluble in hexane. In the extractions with benzene, ethyl-ether, acetone, and butanone the percentage of flakes yielded as meal, shown in Table II,

[3] The mention of trade products does not imply that they are endorsed by the Department of Agriculture over similar products not mentioned.

TABLE VI

Data on Isolated Protein from the Meals

Meal	Yield[a]		Analyses[a]		Pigmentation				
	Meal weight basis	Meal protein basis	Nitrogen	Total gossypol	Order of darkness of solid proteins[b]	Lovibond red color of dispersions[c]	Psychophysical values of dispersions[d]		
							Luminous transmittance	Dominant wave length	Purity
	%	%	%	%			%	mμ	%
Hexane-extracted	44.0	73.1	14.78	1.08	1	6.0	41.6	578.5	72.8
Benzene-extracted	40.9	66.2	15.09	0.65	2	4.5	51.6	577.4	57.8
Ethyl-ether-extracted	41.9	68.6	14.57	0.20	3	3.1	66.7	577.3	27.2
Acetone-extracted	44.0	72.1	14.79	0.12	4	2.3	72.5	576.4	26.8
Butanone-extracted	46.7	75.9	14.80	0.12	4	2.4	72.1	576.2	25.7

[a] Moisture-free basis.
[b] Arranged in the order of decreasing darkness by visual comparison.
[c] Determined on clear dispersions of 0.40 g. protein per 100 ml. of 0.02 N sodium hydroxide.
[d] Psychophysical values found from trichromatic coefficients of dispersions identical to those for Lovibond color readings.

TABLE VII

Correlation of Solvent Properties with the Crude Oil Characteristics

Extraction Solvent	Solvent Properties		Crude Oil Characteristics						
	Solubility of water[a]	Dielectric constant[b]	Density, 25°C./4°C.	Fatty acid material[c]	Neutral oil[c]	Non-oil material[c]	Gossypol-derived material[d]	Order of darkness[e]	Refining loss[f]
				%	%	%	%		%
Hexane	0.0	1.87	0.9172	1.12	96.98	1.90	0.56	1	3.5
Benzene	0.155	2.28	0.9187	1.60	95.56	2.84	1.17	2	5.3
Ethyl-ether	1.5	4.33	0.9238	1.93	94.68	3.39	2.36	3	6.8
Butanone	11.1	18.45	0.9246	1.33	93.65	5.12	2.53	4	7.3
Acetone	∞	21.40	0.9247	1.73	92.63	5.64	2.47	5	7.4

[a] From International Critical Tables, expressed as weight percentage of water soluble in solvent at extraction temperature.
[b] From data of Grimm and Patrick (35) and Hildebrand (36).
[c] By method of Wesson (37).
[d] Calculated from data in Tables II, III, and V. Pounds gossypol in oil plus pounds gossypol not accounted for divided by pounds of crude oil.
[e] Arranged in increasing order of darkness by reference to extinction curves of the crude oils.
[f] Average obtained by 9 refinings of each oil.

was measurably lower than for hexane because of the relatively large amount of non-oil material they extracted. There was however no detectable loss of nitrogen by extraction with any of the solvents and consequently no diminution in the total protein content of the meals.

Crude oil yields. The yield of crude oil with every one of the solvents actually exceeded the amount of oil[4] determined in the original flakes (Tables III and IV). This, together with the fact that some oil remained in all of the meals, as shown by the meal analyses, makes it evident that all of the solvents extracted materials other than oil. Hexane extracted comparatively little non-oil material and gave the smallest yield of crude oil while some of the solvents, notably acetone and butanone, extracted substantial amounts of such material and gave appreciably greater yields of crude oil.

A true estimate of the efficiency of the different solvents as oil extractants could be arrived at only by calculating on a weight basis the percentage of the total oil in the flake charge remaining in the meals after extraction with each solvent and determining by difference the percentage of the total oil extracted. These data are shown in Table IV, columns 5 and 6, and indicate that the solvents giving most complete oil extraction under the conditions used were butanone and ethyl-ether, in that order, followed by acetone, hexane, and benzene.

Composition of Products

Distribution of flake components. The composition of the flakes used for extraction by each of the different solvents and the composition of the respective meals and crude oils are shown in Table III. The only important differences in composition between the meals extracted by the different solvents was in their content of free and total gossypol and in their nitrogen solubility. The differences in sugar content, determined as total sugars, including raffinose (21), and calculated as invert sugars, show that the meals extracted with acetone and butanone contained less sugar than did the other meals.

Of the minor constituents known to be present in cottonseed meals (22, 23, 24) only thiamine was determined. The high thiamine content of all five meals, ranging from 33.5 to 35.0 p.p.m. is of interest since the thiamine content of most commercial meals is well below this value. It has been reported recently that the thiamine content of a meal parallels its biological value (25).

The nitrogen-stripped crude oils differed in their contents of free fatty acid, gossypol, and phosphorus. Although the original flakes all had oil of substantially the same free fatty acid content, the free fatty acid values of the extracted crude oils, as determined by A.O.C.S. method Ca 5a-40, ranged from 0.87% to 2.52%. Since gossypol reacts as acid on titration with sodium hydroxide, the higher free fatty acid values for the oils containing greater percentages of gossypol is in part attributed to this component. In addition, some of the oils, as indicated by their phosphorous content, were relatively high in phospholipides material, which also reacts as acid and which contributed to the apparent high free fatty acid content of some of the oils.

Gossypol is a component of cottonseed which contributes heavily to the problems concerning oil and meal quality encountered in processing cottonseed (26, 27, 28). Since this was the flake component most markedly affected by the different solvents, the distribution of gossypol by each of the solvents was

[4] For the purposes of this discussion the material extracted from cottonseed by the A.O.C.S. method Ba 3-38 using the petroleum ether solvent specified (H 2-41) by the method will be meant when the term "oil" is used.

TABLE VIII

Refining and Bleaching Methods and Results

Crude oil[a]	Refining method	Lye Bé.°	F.F.A. of oil	Type of foots	Refined color[b]		Bleached color[b]			
							A.O.C.S. earth		B.C. clay & Nuchar	
					Y	R	Y	R	Y	R
	Modified		%							
Hexane-extracted	hydraulic[c]	16	0.87	Firm	35	4.2	15	1.9	15	1.3
Benzene-extracted	Hydraulic	16	1.10	Soft	35	11.2	35	8.8	35	8.3
Ethyl-ether-extracted	Slow break	16	2.52	Firm	35	17.4	35	16.6	35	13.9
Acetone-extracted	Hydraulic	16	1.76	Firm	35	4.5	35	2.3	35	2.7
Butanone-extracted	Hydraulic	16	2.37	Firm	35	9.2	70	8.6	70	7.3

[a] Nitrogen stripped.
[b] Colors read on 1-inch tube except for those of hexane-extracted oil, which were read on 5.25-inch tube.
[c] A.O.C.S. method for hydraulic-pressed cottonseed oil modified to employ a 90-minute cold stir.

determined from the data contained in Tables II and III and is shown in Table V.

The known instability of gossypol (29) accounts for the discrepancies shown in Table III between the free and total gossypol values for the flakes used for extraction with hexane, benzene, ethyl-ether, and acetone. The flakes had been analyzed for free gossypol soon after their preparation, but the samples were stored at room temperature for some time prior to analysis for total gossypol. Minor changes no doubt occurred during this storage period, which resulted in diminishing the total amount of gossypol in the samples. For this reason the total amount of gossypol in the batches of flakes used for extraction with these four solvents was calculated from the determined percentage of free gossypol. On the other hand, calculation of the total amount of gossypol in the flakes used for extraction with butanone was based on the total gossypol determination since both free and total gossypol had been determined in these flakes without prior storage.

As can be seen from the data in Table V, the solvents differed greatly in their effect on the gossypol content of the flakes. The more strongly polar solvents extracted most of the gossypol from the flakes along with the oil, leaving the meal relatively low in gossypol while the non-polar solvents extracted very little. It is thought that the gossypol extracted by hexane was only that which had been made accessible by mechanical rupture of the pigment glands during preparation of the flakes. The large percentage of the gossypol originally in the flakes which could not be accounted for in the meals and oils had evidently been broken down or degraded into materials no longer reacting as gossypol. It appears that the degree of gossypol breakdown can probably be attributed to the relative stability of gossypol in the different extraction solvents.

Meal Qualities

Nutritional aspects. Although conclusive evaluation of a specific cottonseed meal for nutritional purposes is impossible without feeding test data, some inferences as to its suitability for feed can be drawn from its composition as shown by analysis. The composition of the five solvent-extracted meals (Table III) shows that they differed from standard commercial meals only in the relative amounts of their components and in having higher protein solubility. They should therefore be as satisfactory protein supplement feeds as commercial meals for the types of livestock to which such meals are principally fed.

Since all of the meals were similarly produced except for the solvent used and none were heated at any time above 131°F., the variation shown in their protein solubilities (Table III) is of interest. The meals extracted with the solvents of most pronounced polar characteristics, ethyl-ether, butanone, and acetone, had the higher protein solubilities and were also lowest in both free and total gossypol.

The percentage of free gossypol and gossypol-like compounds in a cottonseed meal, particularly if present in unruptured pigment glands, has been shown to be of decisive importance in determining the amount of the meal which can be included in the diet of non-ruminant farm and experimental animals (30, 31, 32). Two of these meals, the one extracted with acetone and that extracted with butanone, were low in free gossypol, having 0.07% and 0.06%, respectively. The finding in recent work by Eagle, Hall, *et al.* (33) that acetone-extracted pigment glands containing as much as 5.0% of free gossypol were non-toxic to rats when administered orally in massive dosage, together with the finding of Ambrose and Robbins (34) that some cottonseed meals containing from .07% to .09% free gossypol were not toxic to rats, indicates that the two ketone-extracted meals may be non-toxic.

Industrial potentialities. One potential utilization of cottonseed meal is as a source of protein for industrial use. Among the factors determining the suitability of an oilseed meal as a source of industrial protein are the yields and the color of the protein obtainable. The data on protein yield given in Table VI, which were obtained at strictly comparable conditions although not representing the maximum yield of protein obtainable, indicate the relative value of the meals as sources for industrial proteins.

The order of darkness determined by visual examination of the solid proteins is given in Table VI. The air-dried proteins prepared from the meals extracted with hexane and benzene were a very dark brownish red. That from the ethyl-ether extracted meal was not quite so dark and was reddish purple. The proteins from the meals extracted with acetone and butanone were very much lighter than the others and were of a light brownish amber color.

Determinations of the color of dispersions of the proteins in sodium hydroxide solution by Lovibond colorimeter readings and spectrophotometric means enabled a precise comparison of the pigmentation of the proteins. The data obtained (Table VI) show that the order of pigmentation of cottonseed proteins parallels their total gossypol content. They show that the visual order of darkness of the solid proteins, the Lovibond red color, and the luminous transmission of their dispersions are related to the percentage of total gossypol in the proteins, which in turn is proportional to the percentage of total gossypol (Table III) in the source meal.

A plot of the percentage of total gossypol in the proteins versus the Lovibond red color of their dispersions in sodium hydroxide solution showed a straight line relationship while a similar plot of total gossypol versus luminous transmittance showed a similar but inverse relationship. It was observed that the dominant wavelength of the transmitted light shifted towards the longer wave-length range of the spectrum, and its purity with respect to the dominant wavelength tended to increase as the percentage of total gossypol in the protein increased.

Oil Qualities

Pigmentation of crude oils. All the crude oils, with the exception of that extracted with hexane, were abnormally dark in color. The hexane-extracted crude, while dark, was not abnormally so and was of a reddish tint. The crude oils extracted with benzene and ethyl-ether were very dark and of a deep red color. The butanone-extracted crude was so dark as to appear practically black while that extracted by acetone, though equally dark, had a perceptibly greenish hue. Because of their darkness, only by reference to their extinction curves (not shown in this paper), could the crude oils be arranged in the order of their increasing pigmentation. This order was obviously determined by their content of non-oil mate-

rials (Table VII, coumn 7) among which are the extracted gossypol and gossypol-like compounds as well as the deeply colored degradation products of gossypol (38). The presence of gossypol degradation products in the crude oils cannot be demonstrated by direct analytical evidence since their nature and composition are largely unknown (38, 39, 40) but must be logically inferred on the basis of the data in Table V. The high percentage of the original gossypol in each batch of flakes, which was shown (Table V) to be unaccountable, evidently had degraded or altered into a form which no longer reacted as gossypol on analysis. This alteration of gossypol, which has been reported to result in the formation of very dark pigment materials (25), could have occurred only after extraction from the protective glands in which gossypol occurs (41) and while in solution in the miscella, and become a component of the crude oil. The maximum amount of non-oil material in each of the different solvent-extracted crude oils attributable to gossypol, gossypol-like compounds, and gossypol degradation products is shown in Table VII, column 8.

It is apparent that the total non-oil material in the various crudes had a definite relation to two properties of the different solvents with which they were extracted; their degree of polarity, as measured by their dielectric constants, and the solubility of water in each. It appears that the percentage of non-oil materials in the crudes was proportional to the magnitude of the two solvent properties; i.e., as the values for the dielectric constants and the solubility of water in the different solvents increased, the non-oil content of the respective crude oils increased.

Also shown in Table VII is the correlation between several physical properties of the crudes, their order of pigmentation, their average refining losses, and their non-oil contents.

Refining and bleaching characteristics. The refining data in Table VIII, which show only the refinings giving the lightest colored oils, show clearly that the hexane-extracted crude was the only one of the oils that refined to an acceptably low color. This crude oil yielded refined oil of prime color or better by all of the methods used and had the lowest refining losses.

The refining characteristics of the crude oils extracted with the four other solvents were such that the refined oils were all much darker than that from the hexane-extracted crude, in fact, their colors could be read only on a 1-in. tube basis. The refining losses of these oils were also higher than for the hexane-extracted oil and followed the order of their non-oil content.

The refined oils, except for the hexane-extracted, did not follow the order of pigmentation of crudes. The acetone- and butanone-extracted crudes, which were those most heavily loaded with non-oil material, gave refined oils both of which were lighter in color than those from the benzene- and ethyl-ether-extracted crudes. The acetone-extracted refined oil was the lighter of the two. The ethyl-ether- and benzene-extracted crude oils both yielded very red refined oils. That from the ethyl-ether was the darkest refined oil obtained.

The hexane-extracted refined oil was the only one which bleached satisfactorily. While bleaching did reduce the colors of the other refined oils (Table VIII), the reductions were not great enough to render the oils suitable for the customary uses of vegetable oils. The bleached oil colors followed the order of the refined oil colors.

The fact that the amount of pigmentation in the crude did not determine the amount of pigmentation in the refined oils suggests that the pigments in the refined oils resulted from the relative stability of gossypol in the extraction solvents. The pigments in the refined oils were evidently non-acidic and could not be removed by refining with alkali; in addition they were of the oil-soluble, non-polar types which are not adsorbed strongly enough to be removed by customary percentages of standard bleaching media.

Summary

Five different oil solvents of commercial grade were tested as extractants for cottonseed by making comparable pilot-plant-scale batch extractions with each. The flakes were prepared by replicate procedures from the same lot of prime seed, and the only variable between the different extractions was the solvent employed. The solvents—hexane, benzene, ethyl-ether, acetone, and butanone—were compared as to their effect on the yield, composition, properties, and processing characteristics of the meals and the crude oils produced.

The yields and qualities of the crude oils and meals were chiefly determined by the amount and nature of the non-oil materials extracted by the respective solvents. The solvents which were more polar and in which water was more soluble yielded crude oils which were correspondingly higher in non-oil content and in gossypol and gossypol-degradation products and were more deeply pigmented and had higher refining losses.

The color of the refined and of the refined and bleached oils was not proportional to the non-oil content and extent of pigmentation of the crudes, except for the hexane-extracted oil, which had the lowest non-oil content. The other refined and bleached oils all had exceptionally dark colors, owing to the fact that some of the non-oil material, which included gossypol and gossypol-derived products, could not be removed by the refining and bleaching methods employed.

The solvents which yielded the more heavily pigmented crude oils yielded meals which were all lower in free gossypol content than the meal extracted with hexane. These same meals were of higher protein solubility and yielded lighter-colored proteins. The latter characteristic is apparently due to their lower total gossypol content.

Acetone and butanone yielded the darkest crude oils, but next to hexane the lightest refined and refined and bleached oils, although the refined and the refined and bleached oils were still very dark. These two solvents were excellent oil extractants and the meals obtained by their use were very low in free gossypol, high in protein solubility, and yielded proteins of exceptionally light color for cottonseed protein. Application of these two solvents for processing cottonseed would appear to depend on the development of more effective methods of decolorizing the oils.

Since any improvements in meal quality with the experimental solvents were achieved at the expense of oil quality and the differences in yields of oil were not such as to offset this loss in quality, it may be

concluded, on the basis of the data on this lot of prime cottonseed, that none of the experimental solvents compare favorably with hexane as extractants for cottonseed.

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REFERENCES

1. MacGee, A. E., Oil Mill Gazetteer, *52*, No. 2, 17-43 (1947).
2. Sievers, A. F., McIntyre, J. D., Cotton Oil Press, *4*, No. 10, 44-8 (1944).
3. Shrader, J. H., Cotton Oil Press, *5*, No. 12, 29-32 (1922).
4. Boatner, C. H., Hall, C. M., O'Conner, R. T., Castillon, L. E., Curet, M. C., J. Am. Oil Chem. Soc., *24*, 276-283 (1947).
5. Harris, W. D., Oil Mill Gazetteer, *50*, No. 5, 14-21 (1945).
6. Boatner, C. H., Hall, C. M., Oil and Soap, *23*, No. 4, 123-138 (1946).
7. Fischer, Karl, Angew. Chem., *48*, 394-396 (1935).
8. Stansbury, M. F., Guthrie, J. D., J. Agr. Research, *75*, No. 2, 49-61 (1947).
9. Pons, W. A. Jr., Murray, M. D., O'Connor, R. T., Guthrie, J. D., J. Am. Oil Chem. Soc., *25*, 308-313 (1948).
10. Pominski, J., Molaison, L. J., Crovetto, A. J., Westbrook, R. D., D'Aquin, E. L., Guilbeau, W. F., Oil Mill Gazetteer, *51*, No. 12, 33-39 (1947).
11. Olcott, H. S., Ind. Eng. Chem., *33*, 611 (1941).
12. "Official and Tentative Methods of the American Oil Chemists' Society," ed. by V. C. Mehlenbacher, 2nd Ed., Chicago (Rev. to 1950).
13. "Handbook of Colorimetry," Massachusetts Inst. Tech., Cambridge, Mass. (1936).
14. Vix, H. L. E., Pollard, E. F., Spadaro, J. J., Gastrock, E. A., Ind. Eng. Chem., *38*, 635-642 (1946).
15. Olcott, H. S., Fontaine, T. D., J. Am. Chem. Soc., *61*, 2037-2040 (1939).
16. Pons, W. A. Jr., Guthrie, J. D., J. Am. Oil Chem. Soc., *26*, 671-676 (1949).
17. Pons, W. A. Jr., Hoffpauir, C. L., O'Connor, R. T., J. Am. Oil Chem. Soc., *27*, 390-393 (1950).
18. Pons, W. A. Jr., Hoffpauir, C. L., O'Connor, R. T., J. Am. Oil Chem. Soc., *28*, 8-12 (1951).
19. Conner, R. T., Straub, G. I., Ind. Eng. Chem., Anal. Ed., *13*, 380 (1941).
20. "Methods of Vitamin Assay," The Association of Vitamin Chemists, Interscience Publishers Inc., N. Y. (1947).
21. Stansbury, M. F., Guthrie, J. D., J. Agr. Research, *75*, 49 (1947).
22. Cheldelin, V. H., Lane, R. L., Soc. Expt. Biol. and Med. Proc., *54*, 53-55 (1943).
23. Fraps, G. S., Tex. Agr. Expt. Sta. Bull. 128 (1910).
24. Rewald, B., Biochem. J., *36*, 822-824 (1942).
25. Thurber, F. H., private communication.
26. Goldovskii, A. M., Podol'skaya, M. Z., Vsesoyuz. Nauch.-Issledovatel. Inst. Zhirov, Cottonseed-Oil Production *1936*, 55-61 (in English 61).
27. Goldovskii, A. M., Podol'skaya, M. Z., Mirer, E. A., *ibid.*, 71-77 (in English 77).
28. "Cottonseed and Cottonseed Products," ed. by Bailey, A. E., Interscience Publishers Inc., N. Y., 1948, p. 343.
29. *Ibid.*, p. 239.
30. Withers, W. A., Carruth, F. E., J. Agr. Research, *5*, No. 7, 261-288 (1915).
31. Hale, F., Texas Agr. Expt. Sta. Bull., 410 (1930).
32. Lyman, C. M., Holland, B. R., Hale, F., Ind. Eng. Chem., Anal. Ed., *15*, 489-491 (1943).
33. Eagle, E., Hall, C. M., Castillon, L. E., Boatner, C. H., J. Am. Oil Chem. Soc., *27*, 300-303 (1950).
34. Ambrose, A. M., Robbins, D. J., J. Nutrition, *43*, No. 3, 357-370 (1951).
35. Grimm, F. V., Patrick, W. A., J. Am. Chem. Soc., *45*, 2794-2802 (1923).
36. "Solubility of Non-electrolytes," Hildebrand, Joel H., Reinhold Publishing Corp., N. Y., 2nd Ed. (1936).
37. Jamieson, G. S., "Vegetable Fats and Oils," Monograph Series No. 58, The Chemical Catalog Co., N. Y. (1932), pp. 400-403.
38. Boatner, C. H., Oil and Soap, *21*, 10-15 (1944).
39. Boatner, C. H., Samuels, C. S., Hall, C. M., Curet, M. C., J. Am. Chem. Soc., *69*, 668 (1947).
40. *Ibid.*, p. 1268.
41. Boatner, C. H., Botan. Gaz., *108*, 484 (1947).

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Melting Points and Solubilities of Ternary Mixtures Containing Oleic Acid

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THE literature contains very few references to the melting or setting points of ternary mixtures of the naturally occurring fatty acids. Heintz (4) almost 100 years ago prepared a few of them and determined their melting points. His data appear to be in good agreement with the facts. Recently Paquot (5) has reopened the subject. Shriner *et al.* (8) have studied the behavior of a ternary mixture containing a component margaric acid, which is not found in fatty oils.

It may be concluded from the few publications cited above that the melting-point behavior of fatty acid mixtures is complicated and unpredictable. This is not surprising inasmuch as irregularities due to eutectics are to be expected. This circumstance is of still greater importance in this case than in that affecting binary mixtures. It is well known (1, 3, 7) that, in the latter case, characteristic depressions appear in the respective curves but that their positions are not a matter of prediction. There is one exception however, and that pertains to binary mixtures containing oleic acid. In this case the irregularities are confined to the extremities of the curves probably because of the fact that oleic acid has a low melting point. The curve is therefore on the whole regular, and values obtained by interpolation agree well with those determined experimentally. The general character of such a curve up to about 50% oleic content is illustrated by the line for mixtures of palmitic-oleic acid, designated "0% stearic" in Figure 1. The eutectic existing for very low contents of oleic acid (9) has been neglected as being of no importance for our purposes.

Parallel to the curve marked "0% stearic acid," there have been plotted others for various mixtures of this acid with palmitic acid, using as starting points the melting points of binary mixtures of these two. It can be seen that this procedure leads to a very complete presentation of the melting points of the ternary system palmitic-stearic-oleic acids. They are accurate within the range where the oleic acid content is of commercial importance, or 10 to 50%.

Each line therein (Fig. 1) refers to a well defined palmitic-stearic acid mixture containing increasing quantities of oleic acid, the amount of which is revealed on the abscissa of the graph. For example, the melting point of a ternary mixture of 27 parts stearic acid, 63 parts palmitic acids, and 10 parts oleic acid can be determined by assuming that the saturated part of the mixture contains 30% stearic acid. In that case the reference curve bearing this name will furnish the needed information.

The data of Carlinfanti and Levi-Malvano (1) were found valuable in checking the accuracy of our graphic method (Fig. 1). The authors reported the

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