extent of geometric isomerization was not determined.

It should be pointed out that the ultraviolet method has one function that is not readily done by G.L.P.C. The presence of lard and tallow in shortenings is sometimes detectable only by the arachidonic acid content of the shortening. The quantity of acid involved is usually less than 0.3%. This amount is readily detected by the ultraviolet method, but is not easily determined quantitatively by G.L.P.C.

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[Received March 31, 1961]

Properties of Oil Extracted from Cottonseed with Acetone-Hexane-Water Solvent Mixture[1]

WILLIAM H. KING and VERNON L. FRAMPTON, Southern Regional Research Laboratory,[2] New Orleans, Louisiana

Slightly more neutral oil was obtained on the exhaustive extraction of raw cottonseed meats with the acetone-hexane-water (AHW) solvent mixture than was obtained under the same conditions with commercial hexane. Although most of the gossypol originally present in the seed is extracted with the oil when the AHW solvent mixture is used, the crude oils refined and bleached to yield oils of excellent colors. Methods of recovery of the solvent from the mixed solvent miscellas are reported, together with the refining and bleaching data for the recovered oils.

IT WAS REPORTED previously (1,2) that the extraction of raw cottonseed flakes with a solvent mixture composed of commercial hexane (boiling range 67–71°C.) 44%, acetone 53%, and water 3% yields a cottonseed meal product that is rich in available lysine (lysine with the epsilon amino groups free) as compared with cottonseed meals presently produced by conventional methods, and that is low in both "free" and "total" gossypol. The fraction of the meal nitrogen that is soluble in 0.02N aqueous NaOH is also high. Moreover the meals prepared through the use of the solvent mixture are of high nutritive quality (3).

The crude cottonseed oils obtained through the use of this solvent mixture contain the major portion of the gossypol originally present in the seed, together with other cottonseed constituents that are not normally present in oils obtained through the use of commercial hexane. It is shown in this paper that, although the crude oils obtained through the use of this solvent contain relatively large concentrations of gossypol, they may be refined and bleached to yield prime oils.

Experimental

Methods of Analysis. Free fatty acids and neutral oil contents were determined by AOCS methods (4). Color values for the refined and bleached oils were determined by the color index (area method) of Pons, Kuck, and Frampton (5). Methods of analysis for composition of the recovered solvent mixture were as follows:

Water. Twenty-five ml. of sample were pipetted into a 100-ml. A.S.T.M. oil tube graduated in 0.1 ml. steps from 0 to 3.0 ml. in the lower stem. The tube was then filled to the 100-ml. mark with with commercial hexane (petroleum ether, boiling range 67–71°C.), stoppered, and the contents mixed. After centrifuging for 5 min. at 1,000 r.p.m. the volume of the lower layer was read to obtain the tube reading. Volume per cent of water in the sample was calculated, using the following equation:

$$\text{Vol. \% of } H_2O = 4(0.56T + 0.10)$$

where T is the tube reading in ml.

Hexane. An 8-ml. sample was pipetted into a 50% (9 g.) Babcock cream bottle. Distilled water was added until the bottle was filled to the 40-ml. mark. The bottle was stoppered and the contents were mixed until all of the acetone was extracted from the hexane by the water. After centrifuging for 5 min. at 1,000 r.p.m. the scale was read between the menisci. This reading was multiplied by the factor 1.25 to obtain volume per cent of hexane.

Acetone. Volume per cent of acetone was obtained by subtracting the sum of volume percentages of hexane and water obtained by the above procedures from 100.0.

When the above determinations were made in duplicate the averaged results were found, by analyses of known mixtures, to be accurate to three significant figures. Other analytical methods used will be described later in the text.

AHW Mixed Solvent vs. Commercial Hexane in Extraction of Cottonseed Meats. The purpose of the extraction tests was to determine the comparative efficiencies of the AHW mixed solvent with commercial hexane in extraction of oil from cottonseed meats. It was also desired to compare these with the official AOCS method for determination of oil content of cottonseed meats.

[1] Presented at the 52nd Annual Meeting of the American Oil Chemists' Society, St. Louis, Missouri, May 1–3, 1961.
[2] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

Prime cottonseed were hulled. The meats were separated from most of the hulls by screening and comminuted to pass through a 20-mesh sieve. The mixed comminuted meats were used in the following extraction tests.

Duplicate 5-g. samples were extracted in Butt extraction tubes with commercial pentane (boiling range 35 to 60°C.) in accordance with the AOCS Method Ba 3-38 for determination of oil content of cottonseed meats.

Triplicate 6-g. portions were exhaustively extracted with commercial hexane and triplicate 6-g. portions were extracted with the AHW solvent mixture. These extractions were made at ambient temperature in Butt extraction tubes fitted with asbestos filters as used in the AOCS Method Aa 6-38 for extracting oil from cottonseed meats for FFA determination. These extractions were made by flooding the comminuted meats with 50 ml. of the respective solvents 20 times, allowing the solvent to filter through the meats into glass evaporating dishes on the steam bath, and continuously evaporating the solvent from the filtrate. Tests demonstrated that further addition of solvent did not remove any more oil from the meats. After evaporation of solvent the crude oils were dried in a vacuum desiccator and their quantities were determined. Neutral oil determinations were conducted on each residue by AOCS Method Ca 9f-575. Results are shown in Table I. These data indicate that under

TABLE I

Results of Exhaustive Extraction of Raw Cottonseed Meats with Three Solvents

Solvent used	Crude oil recovered (% of meats)				Neutral oil recovered (% of meats)			
	1	2	3	Avg.	1	2	3	Avg.
Commercial pentane (b.p. 35–60°C.)	26.4	26.5	.....	26.45	.....	.....	.....	.....
Commercial hexane (b.p. 67–71°C.)	27.1	26.4	27.1	26.9	25.4	24.8	24.0	24.7
AHW mixed solvent	34.3	33.2	31.5	33.0	26.3	26.0	24.6	25.6

the conditions used approximately 23% more nonvolatile substances and 3.6% more neutral oil are extracted from raw cottonseed meats by the AHW mixed solvent than are extracted with commercial hexane.

Recovery of Oil from the Mixed Solvent Miscellas. Two methods of recovering oil from the mixed solvent miscellas were used where excessive use of heat was avoided. The first method was by direct distillation of the mixed solvent from the crude oil in a rising film evaporator. This unit is similar to a commercial primary evaporator used in oil extraction plants for continuous recovery of commercial hexane from miscellas. The solvent mixture forms an azeotrope which is composed of 56.5% hexane, 42.1% acetone, and 1.4% water, and which boils at 49°C. However when the 44-53-3% mixture is flash-distilled in the rising film evaporator, the original mixture is recovered by continuous distillation between 48 and 52°C. Thus in the direct distillation, the solvent may be recovered at a much lower temperature than is required for commercial hexane (boiling range 67–71°C.).

The second method of oil recovery consisted of extracting the acetone and gums and other water-soluble impurities from the AHW mixed solvent-oil miscella with six portions of water. The volume of each portion of water used was 10% of the volume of the original AHW-oil miscella. This procedure yielded a hexane-oil miscella suitable for miscella refining, to which no heat has been applied.

Evaporation of the aqueous extracts of acetone from the mixed solvent-crude oil miscellas disclosed that a small quantity of dark colored solid material is extracted by the water along with the acetone. No evidence of extraction of oil in the water washings was noted. Apparently some of the gums, together with a small amount of gossypol, was extracted. To confirm this observation an experiment was performed to determine the distribution of cottonseed oil on the extraction of acetone from the mixed miscella with water. Seventy grams of refined and bleached cottonseed oil were dissolved in sufficient AHW mixed solvent to give 500 ml. of a 14% miscella. This miscella was extracted in a separatory funnel with 6 portions of 50 ml. of water each. Each portion of the aqueous acetone extracts was evaporated to dryness on the steam bath in glass evaporating dishes and dried in the oven at 105°C. for 1 hr. The weights and percentages of oil extracted by each portion of aqueous extract are shown in Table II. The amount

TABLE II

Oil Extracted by Water from AHW-Oil Miscella

Extract No.	Oil extracted (grams)	Per cent original oil extracted
1	0.0100	0.014
2	0.0050	0.007
3	0.0050	0.007
4	0.0000	0.000
5	0.0000	0.000
6	0.0000	0.000

of oil extracted with the aqueous washings may be considered insignificant since it amounts to less than 0.1 lb. of oil per ton of seed.

Refining and Bleaching of Crude AHW Extracted Oil. Amount of NaOH required to neutralize the oil-hexane miscella was determined by titrating 25 ml. with N/10 NaOH solution in the presence of phenolphthalein indicator and 100 ml. of neutralized isopropyl alcohol. Because of the dark color of the desolventized crude oil a 2-g. sample was dissolved in 50 ml. of commercial hexane and 100 ml. of neutralized isopropyl alcohol containing 1 ml. of phenolphthalein indicator solution before titrating with N/10 NaOH. The titration measures the amount of NaOH required for neutralization of the FFA and the gossypol. The equivalent weight of oleic acid is 282.5 and that of gossypol, which titrates as a dibasic acid (6), is 259. Because of the high gossypol content of the oils, the approximate weight percentage of total acidic material present in the crude oils was calculated from the equation

$$\% \text{ acidic substance} = \frac{\text{ml. N/10 NaOH} \times 0.027\,(100)}{\text{wt. of sample}}$$

The refining experiments were performed by using various amounts of alkali in excess over the amount required for neutralization of the crude oils. All refining tests were conducted within a period of one week after extraction. The oils obtained in oil-hexane miscella form by washing the AHW-oil miscellas with water were refined in the miscella form. This was accomplished by washing the hexane-oil miscella with aqueous alkali solution. Twenty per cent (W/V) aqueous NaOH solutions were used for the alkali washes. This solution is equivalent to 23°Bé lye con-

taining 17.1% NaOH by weight. Refining was accomplished in each instance by shaking 200 ml. of the miscella or 50 g. of the crude oil with the stated amount of aqueous alkali for 2 min. by hand in a 250-ml. centrifuge bottle. After centrifuging for 5 min. at 1,000 r.p.m. 25 ml. of the refined oil-hexane miscella were transferred by pipette to a weighed dish, the solvent removed by distillation on the steam bath, and the refined oil dried at 105°C. for 1 hr. and weighed. Concentration of crude oil in the original oil-hexane miscella was determined in the same way and yield of refined oil was calculated from these data. Data are reported in Table III.

The remainder of the refined miscella was decanted from the semiliquid foots through a paper filter into a side-neck filter flask, desolventized and stripped *en vacuo* on the steam bath, and then filtered through paper. The color was determined and then a 10-g. portion was bleached with the standard amount of AOCS official bleaching earth. The bleached oil was filtered and the color index was determined.

From the refining data in Table III it can be seen that an excess of alkali of 0.4 g. per 100 ml. of miscella was required to refine the oil to acceptable refined and bleached colors. This is equivalent to 3.1 g. of NaOH for each 100 g. of crude oil. Refining losses of 7,5,7,8, and 4%, respectively, obtained by the method previously described indicate that these are essentially equivalent to the total acidic substances originally present in the crude oils.

The crude oil obtained by direct distillation of solvent from the AHW miscella was refined with the same caustic solution. The quantities used are listed in Table IV. Yields of refined oil obtained from the desolventized crude oils were obtained by decanting the refined oil from the compact foots in the centrifuge bottle into a tared dish and weighing.

The data in Table IV show that 0.5 g. of alkali in excess of that required for neutralization of acidic substances is required to refine 100 g. of crude oil to acceptable color. The yield of refined oil under these conditions is 98% of the neutral oil content of the crude oil.

The FFA contents of all refined oils with a color index of 90 or below were found to be below 0.1%.

TABLE IV

Refining Data for Crude Oil Obtained by Direct Distillation of Solvent from AHW Mixed Solvent-Oil Miscellas

NaOH required for 100 g. of oil (g.)	Total acidic substances in crude oil (%)	Excess NaOH used per 100 g. of crude oil (g.)	Yield of refined oil (%)	Neutral oil content (%)	Color index of refined and bleached oils (5)	
					Refined	Bleached
0.660	4.5	....	crude	89.3	.....	.....
0.660	4.5	1.5	86.3	.....	73	12
0.660	4.5	0.5	87.2	.....	80	17
0.660	4.5	0.0	.....[1]	.....	190	100

[1] Liquid foots impossible to separate quantitatively.

The refined oil from the crude oil obtained by distillation of the solvent was bleached in the same way as with the oil refined in miscella. The color indices are also reported in Table IV.

Discussion

The foregoing data show that the AHW mixture is an efficient solvent for extraction of oil and gossypol from raw cottonseed. While the crude oils obtained with this solvent contain more impurities than oils obtained by extraction with commercial hexane, they may be refined and bleached to oils of excellent quality if handled promptly. The yields of refined oil are excellent. Yields equivalent to 98 to 100% of the neutral oil are obtainable. There appears to be no problem due to fixation of color in the oils due to heating upon recovery of the solvent by direct distillation in the presence of gossypol. It is believed that this is due to the relatively low boiling point of the mixed solvent, and the ease with which it can be recovered in standard solvent recovery equipment.

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[Received May 12, 1961]

TABLE III

Refining Data for Water-Washed Oil-Hexane Miscellas Obtained by Extraction of Raw Cottonseed Meats with AHW Mixed Solvent

Lot No.	Oil per 100 ml. miscella (g.)	Acidic substances in oil (%)	NaOH required for 100 ml. miscella	Grams excess NaOH used		Refined oil, yield from crude	Color index of oil (5)	
				For 100 ml. miscella	For 100 g. oil		Refined	Bleached
			(g.)	(g.)	(g.)	(%)		
1	14.3	7.0	0.947	0.8	5.6	93	117	25
2	12.8	5.6	1.406	1.3	10.2	95	103	20
3	13.7	7.8	0.758	0.6	4.4	93	83	13
4	12.8	8.4	0.558	0.4	3.1	92	96	18
5	12.8	4.3	0.281	0.2	1.6	96	273	218