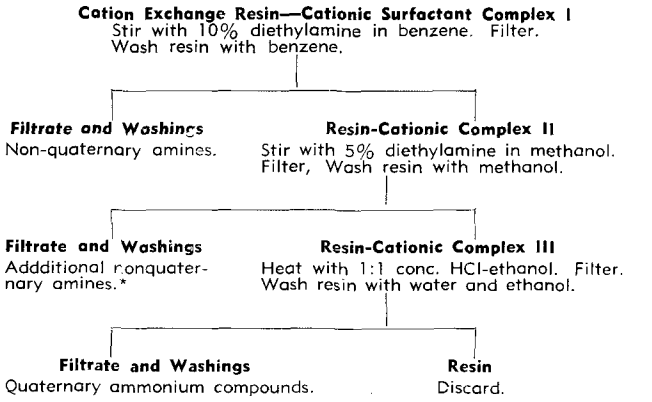

* Small amount, somewhat contaminated with quaternaries. For estimation purposes only.

FIG. 2. Separation and recovery of quaternary and nonquaternary cationics complexed with cation exchange resin.

their complex with the resin but does not remove quaternary ammonium compounds. The scheme of separation is given in Figure 2. The Cation Exchange Resin—Cationic Surfactant Complex used in Figure 2 is that obtained from the procedure for the separation of nonionics from their mixtures with cationics, above.

Results obtained by use of the procedure in Figure 2 are given in Table III. If yields of contaminated nonquaternary cationics are included for estimation purposes, the recovery of this type of cationic is substantially quantitative. Slight losses are encountered in the recovery of the quaternary cationics because of the removal of some along with the nonquaternary cationics.

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[Received November 8, 1960]

Characterization of Oils from Low-Gland and Glandless Cottonseed[1]

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New varieties of cotton which contain few or no gossypol pigment glands are being developed. Commercial production of such varieties should contribute to improving meal quality and reducing the color problems encountered with cottonseed oil. Samples of gland-containing, low-gland, and glandless seed grown in California were obtained, and the oils were extracted and characterized.

With regard to the over-all characteristics the oils from the low-gland and glandless seed were indistinguishable from regular cottonseed oil. Iodine values, contents of unsaponifiables, cloud- and pour-points, response to the Halphen test, and similar characteristics resembled those of commercial cottonseed oil. No differences were found in behavior during winterization.

Determination of the component fatty acids by gas chromatography showed the fatty acid composition of the oils to be typical. Determination of the positions of the double bonds in the unsaturated acyl groups showed no differences between the oils from gland-containing, low-gland, and glandless seed. Ultraviolet, visible, and infrared spectra of the oils revealed no differences other than the presence of gossypol in the crude oil from the gland-containing seed. The infrared spectra of the unsaponifiable fractions obtained from the oils showed some differences, which were not believed to be important.

PIGMENT GLANDS, an anatomical peculiarity of cottonseed, contain the gossypol which adversely affects the economic utility of the seed. Unless the gossypol is inactivated or removed, the seed and the meal are toxic to nonruminants when fed at a sufficiently high dietary level. Small amounts of gossypol and its derivatives in crude oils contribute to the color problems, including color reversion, encountered with such oils. Obviously the development of a variety of cotton which is free of pigment glands is of tremendous economic importance.

Hopi cotton which belongs to the same species as commercial, North American upland cotton *(Gossypium hirsutum)* was reported by Lewton (7) and later by Fulton (6) to have variable numbers of pigment glands in the boll. S.C. McMichael of the U.S. Cotton Field Station, Shafter, Calif., is carrying out further experiments in this area (9). One variety of Hopi known as "Hopi Moencopi" or "Hopi M," has been crossed by McMichael to varieties or strains of upland cotton and selections having low-gland and glandless seed were made from later segregating generations. The transfer of the glandless seed characteristic to commercial varieties has been started. According to him no difficulties in the successful completion of such a transfer are apparent.

Several samples of low-gland and glandless cottonseed have been examined in the Southern Utilization Research and Development Division. A portion of one sample grown in 1958 was sent to Mattson and coworkers (8), who reported on the gossypol content and oil composition of the seed.

Because of the potential economic importance of this type of cottonseed, additional testing of the oil and meal from these seed is important. Breeding out the gossypol conceivably can introduce changes other than the elimination of gossypol. To conduct some of the further evaluation indicated, several new lots of seed were obtained. One was a low-gland and the other was a glandless seed, both grown in 1959. For comparison, commercial, gland-containing seed of the Acala 4-42 variety grown at the same location was obtained. Oil was extracted from these seed by cold-

[1] Presented at the fall meeting, American Oil Chemists' Society, New York, N.Y., October 17–19, 1960.

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TABLE I

Characteristics of the Cottonseed Oils

Characteristics	Crude			Refined and Bleached		
	Gland-containing	Low-gland	Glandless	Gland-containing	Low-gland	Glandless
Free fatty acids, as oleic, %	0.88	0.40	0.21	.....	.....	.....
Refining loss, %	2.9	2.3	2.3	.....	.....	.....
Color index	183.9	69.5	62.7	17.0	16.3	14.8
Photometric color	.....	.....	.....	1.38	1.42	1.09
Wesson color, Lovibond, Y/R	70/20.49	35/2.74	35/2.27	10/1.08	10/0.90	10/0.80
Iodine value, Wijs	106.6	107.4	108.0	107.8	108.0	108.6
Saponification value	192.8	192.7	192.6	193.1	196.3	196.2
Unsaponifiable matter, %	1.70	0.54	0.51	1.42	0.32	0.34
Phosphorus, %	0.00016	0.00038	0.00010	.....	.....	.....
Cloud-point, °F	.....	.....	.....	32.0	33.0	33.0
Pour-point, °F	.....	.....	.....	20.0	19.0	19.0
Yield of winterized oil, %	.....	.....	.....	80.3	78.0	76.8
Cold test of winterized oil, hr	.....	.....	.....	28.0	34.5	34.5

pressing. Portions of the oils were refined and bleached. Chemical and physical characteristics of both the crude and finished oils were determined.

Extraction and Refining

The extraction of the crude oil from each of the three samples of seed was conducted in the pilot plant. The same procedure was used in each case. An approximately 100-lb. sample of seed was ground in a Bauer mill, passed over a huller-shaker screen to separate the meats from the hulls, passed over a purifier-shaker screen to remove lighter particles, and flaked to a thickness of 0.008 to 0.010 in. in an Allis-Chalmers flaking roll. The flaked meats were pressed in a French hydraulic press at 80°F. under 5,000 p.s.i. for 1 hr. and 40 min. Approximately 55% of the oil was pressed out. The mild conditions were selected to minimize the introduction of artifacts.

To establish that the crude oils obtained by cold-pressing were representative of the oils in the seeds, the press cakes were extracted with commercial hexane and the solvent-extracted oils were characterized. No significant differences between the cold-pressed and solvent-extracted oils were found.

Each of the crude oils obtained by cold-pressing was refined according to A.O.C.S. Official Method Ca 9a-52 for Expeller or cold-pressed cottonseed oil, using 80% of maximum of 16° and 20° Bé. lye (1).

The refined oils were bleached according to A.O.C.S. Official Method Cc 8a-52 (1), using 4.67% of natural bleaching earth.

Characteristics of the Oils

The refining losses (Table I) were low for the three oils, being about half of what might be expected with commercial oils with a similar content of free fatty acids. The mild conditions under which the oils were extracted removed fewer nonoil components than do regular, commercial methods of extraction. The slightly higher loss shown in Table I for the oil from the gland-containing seed can be attributed to the slightly higher content of free fatty acids. If gossypol has an effect on the firmness of the foots obtained on refining, it was not evident in these refining tests. Firm foots were obtained in each case.

Oil color was determined by the use of A.O.C.S. Wesson and photometric methods, Cc 13b and Cc 13c, respectively, and by a color index method (11). Each of the oils bleached to a light color; however the oil from the gland-containing seed did not bleach quite as well as did the others.

Absorbancies at various wavelengths were measured with a Beckman spectrophotometer for each of the oils in the crude, refined, and refined and bleached states. Data obtained for the oils from the gland-containing and gland-free seed are shown in Figure 1. The oil

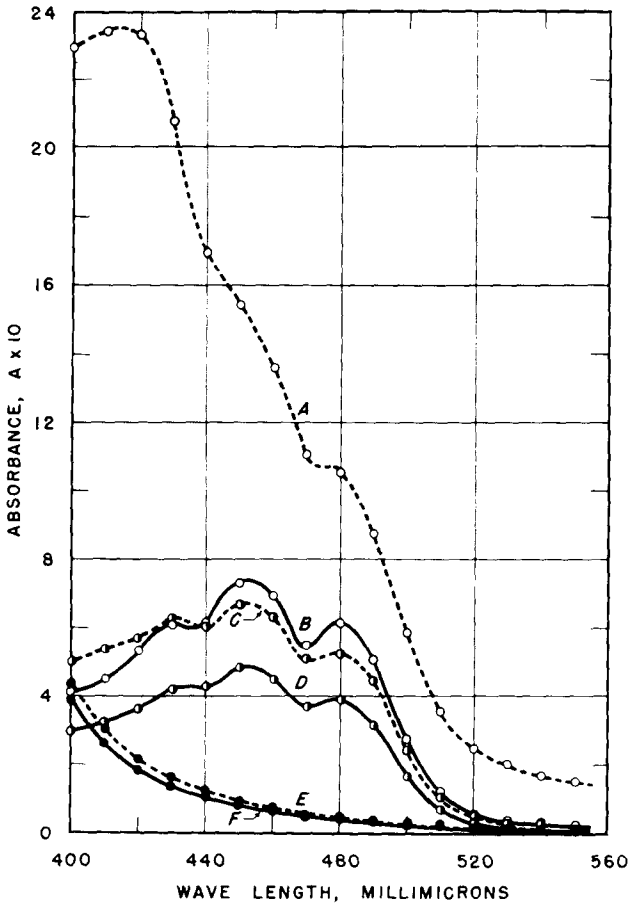

FIG. 1. Absorbance of oil from gland-containing cottonseed (*A*, crude; *C*, refined; and *E*, refined and bleached) and glandless cottonseed (*B*, crude; *D*, refined; and *F*, refined and bleached).

from the low-gland seed behaved like that from the gland-free seed. Crude oil from gland-containing seed possesses, of course, a high absorbance at the shorter wavelengths, which was caused almost entirely by the presence of gossypol. After refining, the oil from the gland-containing seed is about equivalent to the crude oil from the gland-free seed. The refined and bleached oils are similar. If the crude oil from the gland-containing seed had not been handled under mild conditions and refined and bleached shortly after being extracted from the seed, it undoubtedly would have

produced a refined and bleached oil much darker in color. Storing crude oil from gland-free seed under adverse conditions does not increase the color of the refined and bleached oil (9).

A number of other characteristics of the oils were determined. Unless stated otherwise, the Official Methods of the American Oil Chemists' Society (1) were employed in these determinations. The iodine and saponification values which were found for the three oils were within the normal range for cottonseed oils. The percentage of unsaponifiables was higher than normal for the oil from the gland-containing seed and lower than normal for the oil from the glandless and low-gland seed. The percentage of phosphorus, determined colorimetrically after ashing with the aid of alcoholic magnesium nitrate, was well below normal in all cases because the method of extracting the crude oils tended to leave the phosphatides in the press cake. Normally cottonseed oil contains about 0.02% phosphorus, corresponding to about 0.8% phosphatides.

Cloud- and pour-points were determined by a test procedure devised for petroleum oils, Method D 97-57 of the American Society for Testing Materials (2), except that the sample was examined at 2° instead of 5° intervals. The values which were obtained are normal for cottonseed oils.

Composition

The composition of the crude and the refined and bleached oils in terms of simple triglycerides of saturated, oleic, and linoleic acids were calculated from iodine values and from contents of linoleic acid determined by the isomerization-spectrophotometric method. These calculated compositions (Table II) are typical of cottonseed oils. No significant differences can be detected among the oils from gland-containing, low-gland, and glandless seed.

The types and proportions of fatty acids combined as glycerides in the oils were determined by gas chromatography. For these analyses the oils and methanol were interesterified at 50°C. in the presence of sodium methylate. The reaction products were dissolved in commercial pentane and washed with water; the solvent was removed to obtain the methyl esters. These were analyzed on a diethylene glycol succinate column. The chromatograms obtained with the methyl esters of the three oils were identical in appearance. Two of the chromatograms are shown in Figure 2. The percentages of the various fatty acids determined from these curves are shown in Table II.

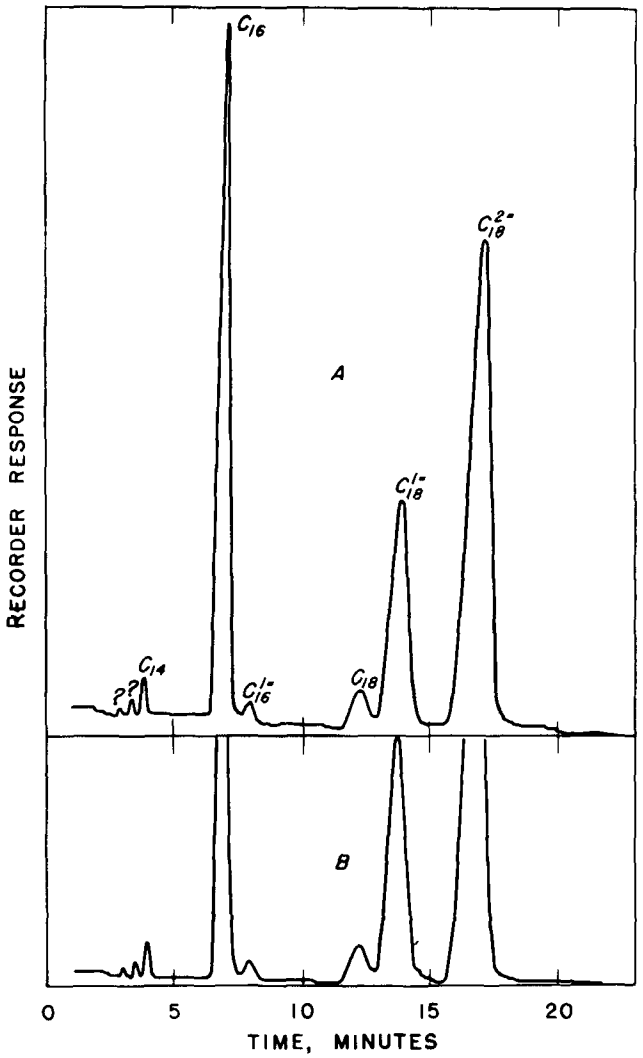

FIG. 2. Gas chromatogram of mixed methyl esters of oil from glandless *(A)* and gland-containing *(B)* cottonseed.

TABLE II

Composition of the Cottonseed Oils

Constituent	Crude			Refined and Bleached		
	Gland-containing	Low-gland	Glandless	Gland-containing	Low-gland	Glandless
Glycerides[a] of						
Saturated acids, %	29.7	31.2	28.5	27.0	28.3	29.1
trans-Oleic acid, %	3.4	3.3	3.6	2.6	2.8	2.6
Total oleic acid, %	19.4	17.9	22.6	24.0	23.4	21.8
Linoleic acid, %	49.2	50.4	48.4	47.6	48.0	48.8
Fatty acid composition,[b] %						
.....	.....	.....	.....	0.1	0.1	0.1
.....	.....	.....	.....	0.2	0.3	0.2
Myristic	.....	.....	.....	0.6	1.2	0.8
Palmitic	.....	.....	.....	27.2	28.0	26.5
Palmitoleic	.....	.....	.....	0.5	0.7	0.3
Stearic	.....	.....	.....	3.1	2.2	2.9
Oleic	.....	.....	.....	20.1	15.6	18.8
Linoleic	.....	.....	.....	43.8	47.5	46.0
Conjugation[c]						
Diene, %	0.89	0.88	1.01	0	0	0
Triene, %	0.01	0.01	0.01	0.42	0.44	0.40
Unsaponifiable matter, %	1.70	0.54	0.51	1.42	0.32	0.34

[a] Calculated from iodine value and spectrophotometric data. % Saturated = 100 − (% linolein + % total olein + % unsaponifiable matter).
[b] Calculated from gas chromatographic analyses.
[c] As conugated linoleic and linolenic acids.

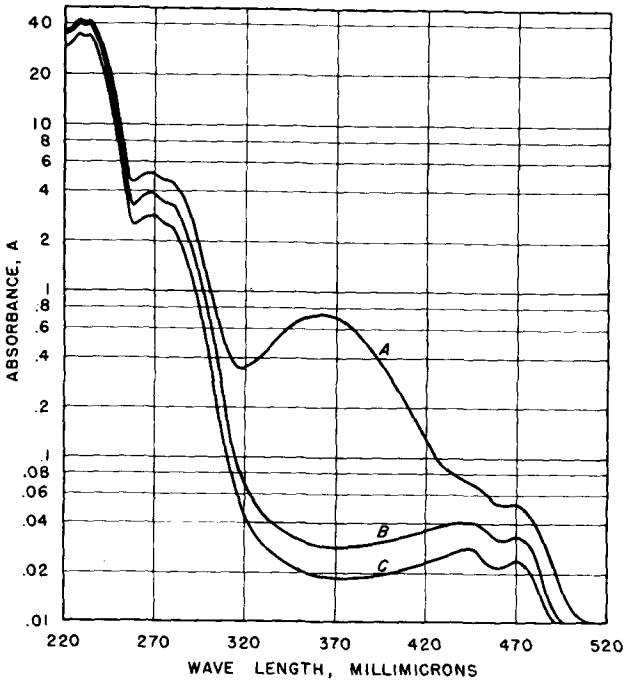

FIG. 3. Absorbances of crude oils from gland-containing *(A)*, low-gland *(B)*, and glandless *(C)* cottonseed in isoöctane solutions at concentrations of 26.400, 24.926, and 23.606 g./l., respectively.

The data obtained by gas chromatography show that the presence or absence of the gossypol pigments in cottonseed had no effect on the nature of the component fatty acids and apparently no effect on their relative proportions. The fatty acid data obtained by gas chromatography are consistent with the glyceride composition data obtained by the alkali isomerization-spectrophotometric method if the accuracy of the methods is taken into consideration as well as the fact that the recorded percentages are not calculated on the same basis.

To obtain further proof that the component fatty acids of the oils from the low-gland and glandless seed were identical with those of gland-containing seed, the positions of the double bonds closest to the ester linkages of the unsaturated acyl groups were determined. The oils were ozonized and oxidized according to the method of Bailey (3) except that the solvent employed consisted of 7 parts methanol and 3 parts ethyl acetate. Then the reaction products, free of solvent, were saponified and acidulated to obtain a mixture of mono- and dibasic acids, which were analyzed on chromatographic columns (4). Double bonds were found only in the 9-position, which was in agreement with data obtained for commercial cottonseed oils (4).

The Halphen test, which is employed to detect qualitatively the presence of cottonseed oil in other fats and oils, was made on the oils from the low-gland and glandless cottonseed. Typical red colors developed, indicating positive tests. From the intensities of the colors it was concluded that these oils were comparable to commercial cottonseed oils with regard to the content of the color-producing component, which is believed to be a fatty acid containing a propene ring.

Ultraviolet, visible, and infrared absorption spectra were obtained for each of the three oils, both in the crude and in the refined and bleached state. All of the infrared spectra were alike. Also no differences were noted in the spectra for the visible region. No significant absorption bands were found. The crude oil from the gland-containing seed had three absorption bands in the ultraviolet region (Figure 3); an intense band at 360 mμ, attributed to gossypol, and bands at 234 and 268 mμ, attributed to diene and triene conjugation, respectively. The crude oils from the low-gland and glandless seed had no absorption bands at 360 mμ but did have the bands at 234 and 268 mμ. On refining and bleaching the crude oils, the gossypol was removed, of course, from the one oil which contained it.

The unsaponifiable fractions were isolated from samples of the crude oils and examined spectrophotometrically. Differences appeared only in the infrared spectra. The infrared spectrum of the unsaponifiable fraction from the gland-containing cottonseed differed from the other two corresponding spectra, one of which is shown in Figure 4. The latter spectra had twin

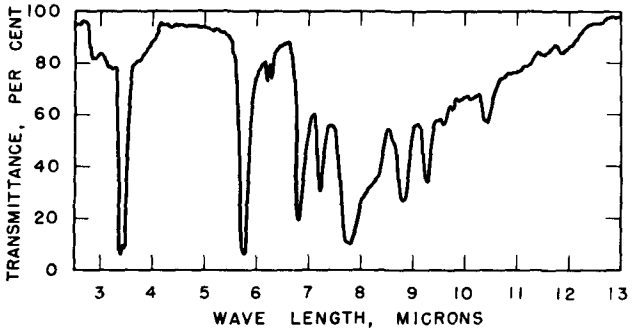

FIG. 4. Infrared spectrum of unsaponifiable fraction of crude cottonseed oil from glandless seed. Fraction dissolved in chloroform.

absorption bands at 6.2–6.3 μ and a band at 10.5 μ whereas the spectrum of the unsaponifiable fraction from the gland-containing cottonseed oil had only a single band at 6.2 μ and slight indications of bands at 6.3 and 10.5 μ. The reasons for the observed differences cannot be explained at this time.

Oxidative Stability

The contents of total tocopherols of the crude and of the refined and bleached oils were determined by a modification of the Emmerie-Engel procedure (5), using α-tocopherol as the standard and the method of Parker and McFarlane (10) to remove pigments from the oil. An Evelyn colorimeter set at 100% transmission and a 520 filter were used. The amounts of tocopherols which were found (Table III) were only about one-fourth as large as expected. The values are uniformly low, and there is no real difference between the oils from the three types of seed. The reason for these low values is not known definitely. However the most probable explanation is that each of the crude oils was relatively heavily contaminated with metals, and the tocopherols were largely destroyed before the tests were made. The cold-pressing operations in which the oils were obtained were carried out in freshly scrubbed and cleaned equipment. Such cleaning is known to remove protective films of polymerized oils and to expose the oil being processed to

TABLE III

Stability of the Cottonseed Oils

Characteristic	Crude			Refined and Bleached		
	Gland-containing	Low-gland	Glandless	Gland containing	Low-gland	Glandless
Total tocopherols, %	0.032	0.029	0.028	0.026	0.021	0.023
Peroxide values, m.e.	2.3	17.6	14.3	13.1	11.7	11.8
Stability, A.O.M., hr.[a]	18.7	8.2	7.8	6.7	4.8	5.8

[a] Time necessary to reach peroxide value of 100 m.e./kg. at 97.7°C.

a relatively reactive surface. The initial peroxide values of the oils bear out this supposition.

Probably because of the presence of pro-oxidants and oxidation prior to the tests, the stabilities of all except one sample were low. The crude oil obtained from the gland-containing seed had a normal keeping-quality. This crude oil undoubtedly was more resistant to oxidation because of the presence of gossypol, which is a powerful antioxidant (12). In this one respect gossypol is a desirable component.

Winterizability

Because a large percentage of the cottonseed oil production is winterized to make salad oil, the performance of any new cottonseed oils in this operation is important. Therefore the experimental oils were winterized by a procedure similar to that employed commercially. The temperature of each refined and bleached oil was lowered from 86°F. to 55°F. during an 8-hr. period and then held at 55°F. for 4 hrs. Subsequently the temperature was lowered successively to 52°, 49°, 46°, 44°, and 42°F. The holding time at each temperature was 12 hrs. The oil was filtered under gravity at 42 °F. during a period of 24 hrs. Yields and the cold tests on the winterized oils are recorded in Table I.

The oils from the low-gland and glandless seed behaved like ordinary, commercial cottonseed oils, indicating that the glyceride configurations and the proportions of the various glycerides were the same. Indirectly these findings are in agreement with the report by Mattson *et al.* (8) that practically all of the saturated acyl groups in the glycerides from the glandless seed are found in the 1- and 3-positions, which are the same positions in which they occur in the oil from gland-containing seed.

Acknowledgments

The authors wish to express their appreciation to J.H. Turner, U.S. Cotton Field Station, Shafter, Calif., for supplying samples of the seed; to A.J. Crovetto for assistance in the pressing operations; to V.O. Cirino and J.A. Harris for some of the chemical analyses; to R.T. O'Connor, E.R. McCall, R.A. Pittman, and Dorothy C. Heinzelman for the spectrophotometric measurements; and to H.D. Royce, Wesson Division, Hunt Foods and Industries Inc., New Orleans, La., for the gas chromatographic analyses.

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[Received November 21, 1960]

Report of the Uniform Methods Committee, 1960

THE MEETING of the Uniform Methods Committee was held at the Hotel New Yorker in New York on October 18, 1960. K.E. Holt, R.J. Houle, R.A. Marmor, L.D. Metcalf, Endre Sipos, E.M. Sallee, editor *ex-officio*, and Dan L. Henry were present. Visitors were Edward Handschumaker, T.H. Hopper, and Tom Parks.

The following matters were discussed and decisions made, as indicated.

1. **Report of the Seed and Meal Analysis Committee, M.H. Fowler, chairman**

   The Seed and Meal Analysis Committee recommended changes in the fuming equipment and the use of the Micro-Samplmill in Method AO 4-38. Their report was accepted by the Uniform Methods Committee. Some editorial changes were discussed pertaining to the treatment of the statistical data and the inclusion of a drawing of the Henry oven. The changes are recommended for adoption.

2. **Report of the Soap and Synthetic Detergent Analysis Committee (Joint with A.S.T.M.), J.C. Harris, chairman**

   Statistical data had been requested by the Uniform Methods Committee at the spring meeting. These data were presented in this report, and it is recommended that they be adopted and be included in the 1960 revisions.

3. **Report of the Spectroscopy Committee, R.T. O'Connor, chairman**

   The report on a method for the determination of Isolated *trans* Isomers was presented. This made use of the methyl ester derivatives of the fatty acids, and, although the method is satisfactory for use after the esters were obtained, full agreement had not been reached on their preparation.

   In line with the above report it is recommended that the spectroscopic method be adopted and that the preparation of the methyl esters be referred to another committee.

4. **Report of the Bleaching Methods Committee, T.C. Smith, chairman**

   This report shows that 3,000 cans of new natural bleaching earth are ready for canning and are then to be supplied for distribution. The Chemists' Committee, N.C.P.A., and the Technical Committee, N.S.P.A., have approved this earth. It is recommended that this earth be accepted as the official natural bleaching earth.