TABLE IV

	Reichert-Meissl	Polenske	Unsaponifiable
Tallow C15	.42	.53	.73
Tallow 16	.48	.46	.88
Tallow 20	.48	.45	.82
Oil 16	.66	.10	....

to five and one-half hours for a regular alkali refined linseed oil. The color, after two hours at temperature, was 12+ and that of alkali refined linseed oil was 10+. Drying tests were made by measuring the gain in weight and the results indicate that this oil dries similarly to a high quality linseed oil.

The whole seed, the extracted meats and hulls, and the fiber of the Chinese tallow seed were analyzed. Six samples of tallow and five samples of oil were characterized as shown by the data recorded in Tables II to IV.

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Lipids of the Cottonseed
I. Some Quantitative Observations on Yield

VERNON L. FRAMPTON and HAROLD H. WEBBER
With the Technical Assistance of Frederica K. Giles, National Cotton Council of America, Research Division, Austin, Texas

EVIDENCE has been obtained that in the extraction of cottonseed with several organic solvents, the composition of the extract obtained with a given solvent will vary from seed specimen to seed specimen, and the composition of the extract obtained with a given seed specimen is dependent on the nature of the solvent used. The quantity of material extracted with a given specimen is also dependent on the nature of the solvent, and it is suggested that the selection of any particular solvent as the standard or "official" solvent to be used in the assay of oil with cottonseed is arbitrary.

At the time of the adoption of the refractometric method for oil assay with linseed oil (1) as an official method by the Association of Official Agricultural Chemists, it was suggested that the procedure might be adapted to the determination of the oil content of other oil-bearing seeds. In the course of the investigations carried out in this laboratory it has been found that the refractometric method of oil assay may be used with cottonseed, but the results obtained for the quantity of oil in any given specimen are not in agreement with those obtained on the extraction of the seed with diethyl ether or petroleum ether, as recommended in the official methods of the Association of Official Agricultural Chemists and the Cottonseed Crushers Association respectively. In turn, the determinations of the oil content of cottonseed with these solvents are not in agreement with each other.

The refractometric scheme for oil assay with vegetable oil seeds involves the grinding of a weighed quantity of the seed in the presence of a fat solvent of low volatility which differs sufficiently in its index of refraction from that of the vegetable oil that small quantities of the oil in the solvent may be determined by means of index of refraction measurements. The grinding is effected in the presence of a prescribed quantity of the solvent, washed sea sand, and a small quantity of Na_2SO_4 (anhydrous). After sufficient grinding, the ground sand, the seed residue, and the salt are removed by filtration, and the index of refraction is determined with a drop of the filtrate. The quantity of oil per unit weight of seed is then determined by reference to the proper calibration curve.

It was not feasible to use the mixture of 1-bromonaphthalene and chloro-naphthalenes, as recommended in the official method, as these substances have indices of refraction beyond the range of the instrument available to us. A mixture of 1-bromonaphthalene (Eastman) and ethyl benzoate (redistilled to constant index of refraction, $n = 1.50529^{20}_{D}$) having an index of refraction of 1.61128^{20}_{D} was adopted instead as the nonvolatile solvent for use in the determinations reported in this communication. The grinding was effected with a power-driven mortar and pestle grinder, and the requisite time of grinding, etc., were determined to yield maximum quantities of oil. The standard volume of the solvent was taken as 5 ml., the weight of seed as 0.5 g., the weight of sea sand as 2 g., the weight of sulfate as 2 g., and the time selected for the grinding was 20 minutes.

Two different procedures were used in determining the oil content of the seed specimen with the data obtained from the refractometric method of analysis. The one procedure involved the use of seed samples which were prepared by using two aliquots of seed, one of which had been exhaustively extracted with petroleum ether with a Soxhlet extraction apparatus. These aliquots were mixed in the desired proportions. The calibration curve in Figure 1 was prepared by plotting the index of refraction of the filtrates, as indicated above, from these several samples against the oil contents of the samples, as determined on extraction with petroleum ether. A linear relationship between the oil contents of the samples and the refractive index was observed. This fact in itself would indicate that the refractometric method of oil assay is applicable to cottonseed, if one assumes uniform composition of the lipid-soluble fraction from specimen to specimen. One may refer the indices of refraction obtained with his

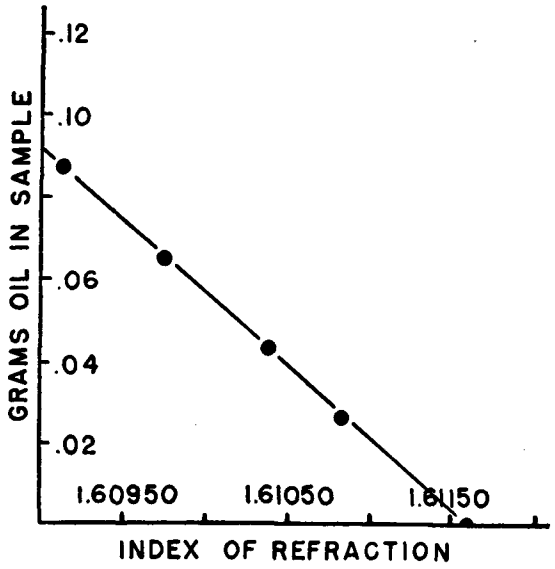

FIG. 1. Calibration curve used in the first procedure in the determination of the oil content of cottonseed by the refractometric method.

unknown samples to the calibration curve in Figure 1 in order to ascertain their oil contents.

The second procedure involved a calibration curve which was prepared by plotting the indices of refractions of solutions of crude commercial cottonseed oil (obtained from a crushing mill) against the oil contents of the solutions. A linear relationship was obtained (cf. Figure 2). In this procedure the indices of refraction of the filtrates from the unknown samples may be referred to the calibration curve in Figure 2 in order to ascertain their oil contents.

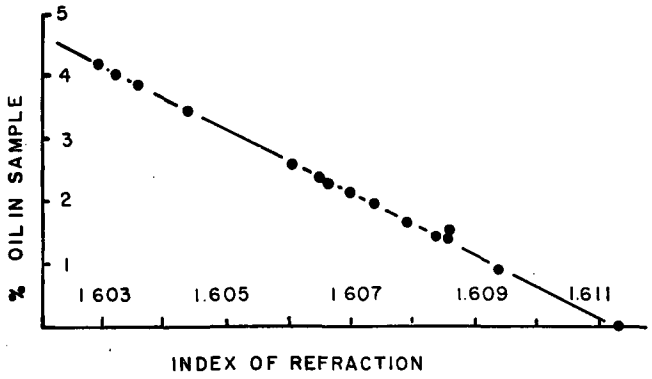

FIG. 2. Calibration curve used in the second procedure in the determination of the oil content of cottonseed by the refractometric method.

A comparison of the results obtained with the two procedures is made by including the data obtained with several cottonseed specimens (these several specimens were in reality several varieties, but their names were omitted because we do not wish to imply, without supporting evidence, that the variations observed are varietal) in Table 1. The data in column 4 were obtained by following the first procedure; those in column 5 were obtained by following the second procedure. The data for the oil contents for these several seed specimens as obtained with petroleum ether are included in column 3 of Table 1. These data do not support the assumption that the composition of the oil obtained from cottonseed on extraction with the

TABLE 1

The Quantity of Oil in Several Specimens of Delinted Cottonseed as Determined by the Refractometric Method and on Petroleum Ether Extraction

Specimen Number	Percentage of Oil in Cottonseed			
	Petroleum Ether Extraction		Refractometric Method	
	Seed Heated	Seed Not Heated	Procedure No. 1	Procedure No. 2
1	14.9	22.4	23.6	21.2
2	15.3	24.2	.....	.....
3	16.9	25.7	20.0	17.9
4	12.9	24.4	21.5	19.4
5	11.1	27.5	25.5	23.2
6	10.6	24.7	22.7	20.3
7	9.29	23.9	21.5	19.4
8	10.3	23.6	21.4	19.0
9	17.2	23.8	22.3	20.0
10	9.92	22.8	20.0	17.6
11	12.9	25.5	22.9	20.0
12	9.95	29.4	24.3	21.9
13	14.7	28.2	24.6	22.2
14	.....	26.5	23.6	21.2

mixture of α brom naphthalene and ethyl benzoate and with petroleum ether and that obtained on hydraulic crushing is the same.

Support for the hypothesis that the several lipid components in the seed vary in an independent manner from specimen to specimen is found in a consideration of the iodine numbers and indices of refraction of the several extracts. Petroleum ether extracts were obtained from several seed specimens with the Soxhlet extraction apparatus, and the iodine numbers and indices of refraction of the individual extracts were compared. These data are presented in graphic form in Figure 3. It is evident that the ratio of those components which are responsible for the absorption of iodine and those which contribute to the index of refraction is not constant from specimen to specimen.

In the several determinations involving extraction with organic solvents, the samples of cottonseed were placed in pharmaceutical packets constructed from filter paper, and they were packed in such a manner that there was no loss of the ground seed on manipulation and extraction. The layers of the ground seed, all of which passed a 50-mesh screen, in the packets were made thin in order to facilitate the contact between the solvent and the seed. The data reported were all obtained with either the Soxhlet or the

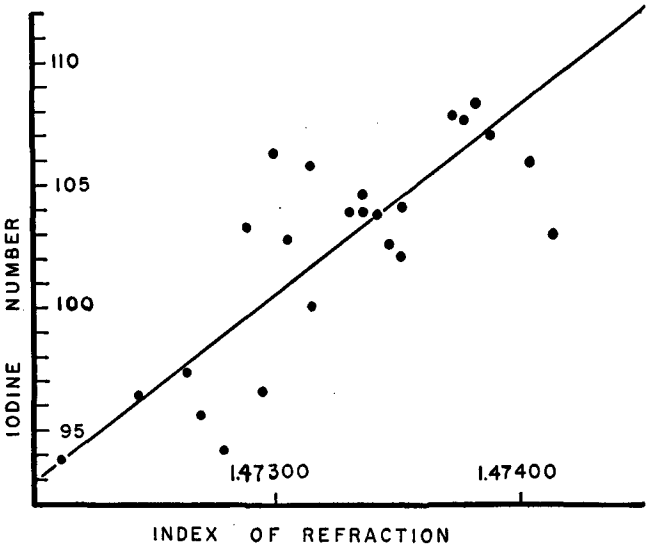

FIG. 3. Variation of the iodine number and index of refraction of the petroleum ether extracts of several cottonseed specimens.

Frampton-Giles (2) low pressure extraction apparatus. In all cases the determinations were carried out in triplicate, and the extractions were continued until the loss in weight of the packets was negligibly small during a six-hour extraction period. Aliquots were taken for moisture determinations with these several samples, and the values reported are on the moisture-free basis. The oil content of the cottonseed samples was calculated on the basis of the loss in weight of the pharmaceutical packets.

A comparison of the efficiency of several solvents in the extraction of cottonseed is included in Table 2. These data were obtained with the Soxhlet extraction apparatus. The ratio of the quantity of material extracted with any two solvents, e.g., acetone and benzene, is not constant from specimen to specimen. The implication again is that these two solvents do not extract completely identical materials from the cottonseed.

The possibility exists that alterations in the lipid fraction may be produced on exposure of the seed or the oil to the prolonged heating encountered in the Soxhlet extraction apparatus. Reference is made to the data in column 2 of Table 1. The data are for the quantity of material extractable with petroleum ether after these specimens had been dried at 110° for several hours. The nature of the mechanism which is responsible for the reduced yields in oil has not been the subject of inquiry. However, in the pursuit of the hypothesis under consideration, extractions of unheated seed specimens were effected with the Frampton-Giles low pressure extraction apparatus with a view to circumventing any of the effects of heat on the extractable material.

The apparatus evolved in this laboratory is presented diagrammatically in Figure 4. The capacity of chamber A is 60 ml.; of chamber T, 20 ml.; of reservoir R, 15 ml.; and of reservoir E, 60 ml. The inner tube of condenser H and the tube leading from chamber T to reservoir R have inner diameters of 3 mm. Condenser H is 40 cm. long.

In the operation of the extraction apparatus, the weighed samples of ground cottonseed are prepared in the manner which has been described, and the packets are placed in chamber A. The apparatus is then evacuated with a high-vacuum pump, and stopcocks K and M are closed. Enough solvent is added through funnel O to raise the level of the liquid in H to a height of about 3 cm. above the ring seal, care being taken that the vacuum is not lost by the admission of air. Cold water is circulated through condenser C, and warm water is circulated through condenser H and through the outer jacket of chamber T. On the transfer of heat from the warm water in the outer jacket to the liquid in the inner jacket through the walls of the inner tube, the liquid boils with explosive violence. The rapid conversion of the

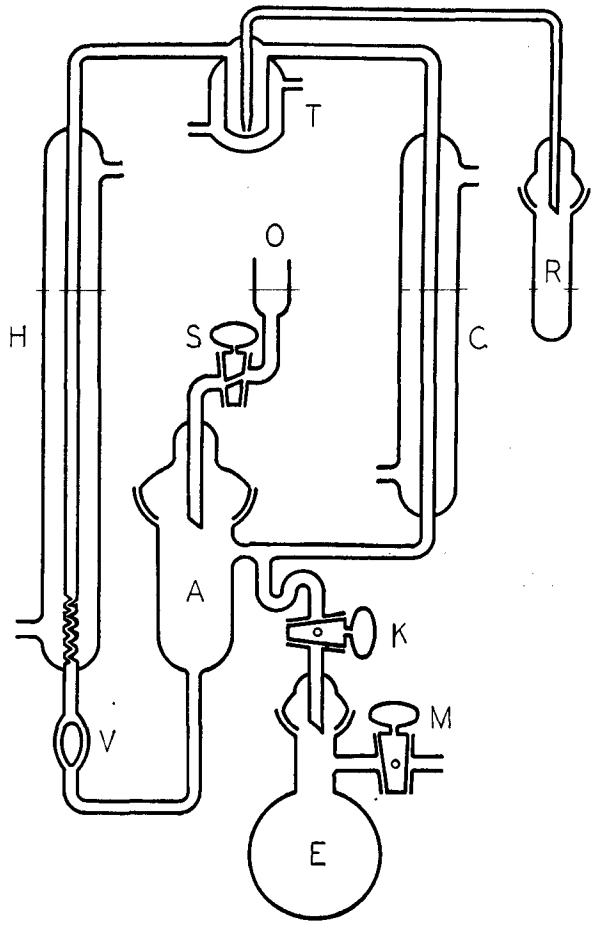

FIG. 4. The Frampton-Giles extraction apparatus.

liquid to vapor produces a region of high pressure in the condenser. Flow of the liquid below the ring seal back into chamber A is prevented by valve V, and the vapors are driven up the condenser into chamber T and finally into condenser C, where they are condensed. The condensate is then returned to chamber A. With the equalization of the pressure in the apparatus of the condensation of the vapors in C, liquid from A flows into H because of the hydrostatic head. The process is then repeated.

The data included in Tables 1 and 2 were obtained with sulfuric acid-delinted seed. The data included in Table 3, however, were obtained from specimens which had been subjected to neither heat nor acid treatment. The seed were cracked in a Wiley mill (the screens were removed so the larger fragments could pass through the mill), and no difficulty was experienced in separating the lint-covered hull fragments from the kernel fragments. The percentages of oil reported are for the kernels, and they are on the moisture-free basis. The ratios of the quantity of material extracted by any two of the solvents is also

TABLE 2

The Quantity of Oil in Several Specimens of Delinted Cottonseed as Determined With Several Solvents

Solvent	Percentage of Oil in Specimen Number						
	15	16	17	18	19	20	21
Acetone	24.1	24.6	22.9	23.5	21.6	24.4	24.5
Benzene	23.8	24.3	22.4	24.6	22.5	25.1	25.6
Chloroform	23.4	23.3	23.0	24.3	22.3	23.6	24.6
Monochlor Benzene	23.7	23.6	23.2	24.7	22.3	26.6	25.1
Ethylene Dichloride	22.5	22.1	21.4	23.8	21.3	25.5	23.5
Petroleum Ether	21.5	21.7	21.4	21.7	21.3	24.1	23.6
Diethyl Ether	21.5	23.1	21.9	23.0	22.1	24.7	24.7

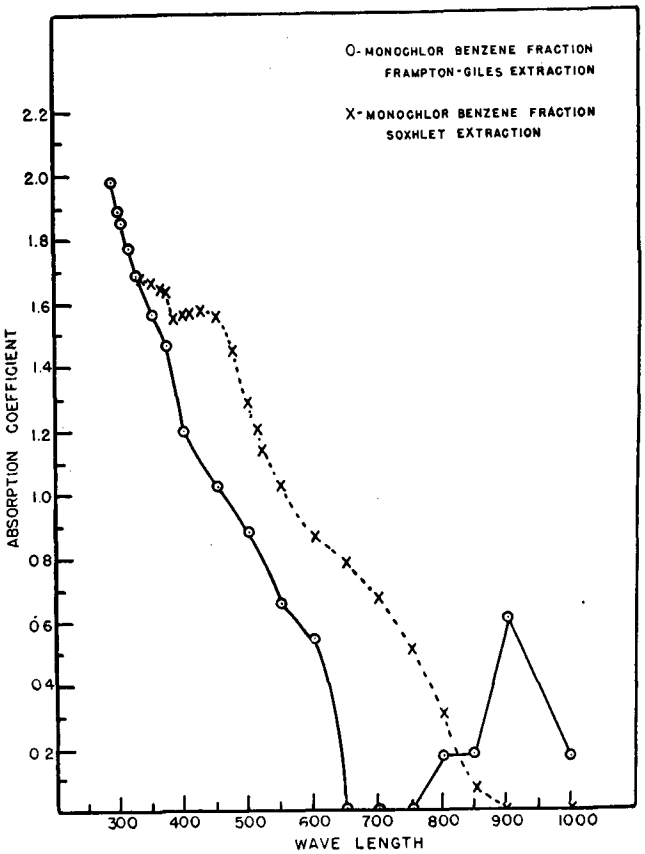

FIG. 5. Absorption spectra of the chloroform solution of the monochlor benzene extracts of a cottonseed specimen.

not constant from specimen to specimen with these extractions. The evidence seems conclusive that the quantity of material which may be obtained on solvent extraction of cottonseed is a function of the solvent used for the extraction.

Part of the effects which have been observed with the different solvents may be due to the differences in temperatures in the several extractions, although with the data reported in Table 3, this factor is not of importance. Part of the differences undoubtedly are due to differences in the solubilities of the seed constituents in the different solvents. It is probable, however, that the most important factor contributing to the differences may be specified as biological. We are dealing in all these instances with living entities which are undergoing constant change, and a comprehensive study of the lipids of the cottonseed will be required before the variations which occur and which are due to physiological processes may be distinguished from the variations which are varietal.

An effect of temperature of the qualitative properties of the oil may be demonstrated with monochlorbenzene. The absorption spectra of the chloroform solution of the monochlorbenzene extracts of cottonseed are shown in Figure 5. The one curve was obtained with the Soxhlet extraction apparatus, the other with the Frampton-Giles apparatus. The ordinate in this and the other absorption spectra curves reported herewith is the logarithm of the per cent absorption per gram of oil per liter of solution. A distorted picture is presented in plotting the data in this manner, but the justification is found in that the absorption for substances showing a weak absorption is accentuated.

TABLE 3

The Quantity of Oil in Several Specimens of Dehulled Cottonseed as Determined With Several Solvents

Solvent	Percentage of Oil in Specimen Number				
	20	22	24	25	26
Acetone	38.9	38.6	37.8	42.1	38.6
Benzene	37.2	.....	36.8	40.0	37.4
Diethyl Ether	37.4	37.5	37.0	39.4	36.7
Petroleum Ether	35.2	35.7	35.5	38.2	36.0
Monochlor Benzene	37.3	37.5	37.2	39.9	38.2
Ethylene Dichloride	35.2	36.1	35.0	38.0	34.6

There is a considerable difference in the absorption spectra in Figure 5. No attempt is made in this report to identify the various absorption maxima with specific chemical substances, but it is noteworthy that there is no absorption maximum with either of these extracts at 375 millimicrons, which corresponds to the absorption maximum of gossypol.

Additional evidence that the composition of the extracts obtained with different solvents is different is found in the absorption spectra shown in Figure 6, which includes the curves obtained with the acetone, the diethyl ether, the benzene and monochlorbenzene extracts. The concurrence of some of the absorption maxima may be noted, namely, the acetone and benzene extracts each show a maximum in the neighborhood of 560 millimicrons, the benzene and monochlorbenzene extracts each show a maximum in the neighborhood of 425 millimicrons. Only the benzene extract shows a maximum in the neighborhood of 600 millimicrons. Only the monochlorbenzene does not show a maximum at 375 millimicrons.

The absorption spectra of the benzene extract and of the oils obtained on hydraulic pressing are com-

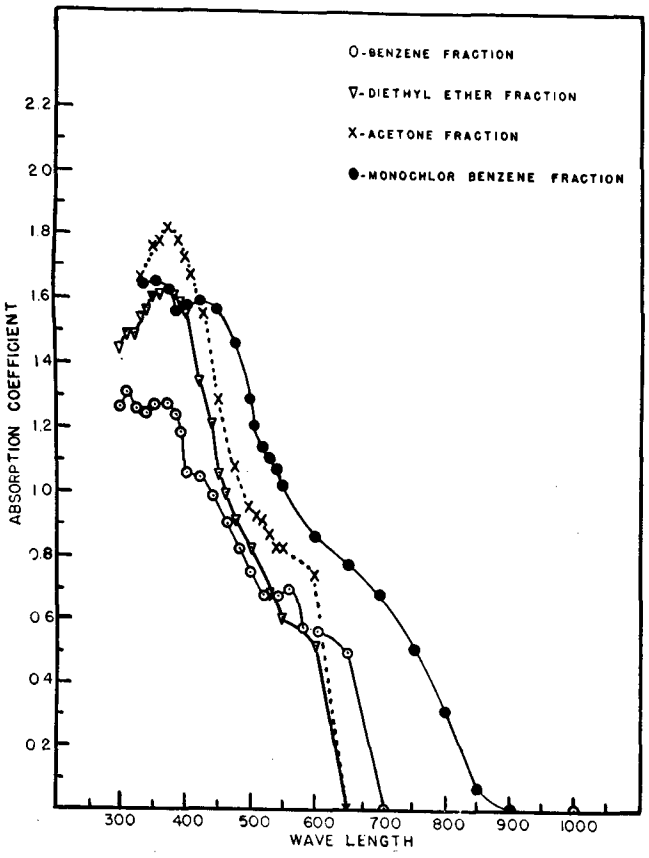

FIG. 6. Absorption spectra of the chloroform solution of cottonseed oil obtained with benzene, diethyl ether, acetone, and monochlor benzene.

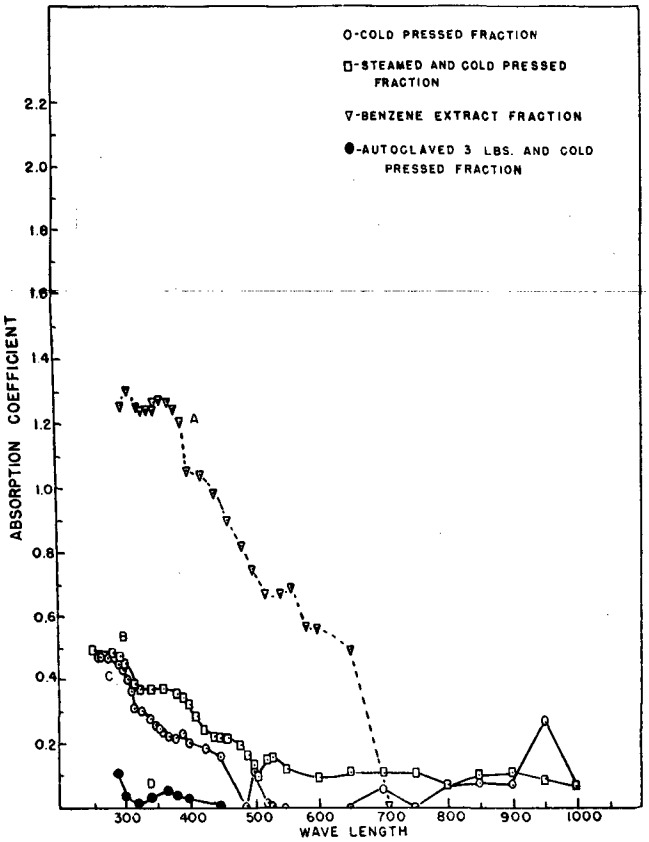

FIG. 7. Absorption spectra of the chloroform solution of cottonseed oil obtained on benzene extraction, and on hydraulic pressing of untreated seed, of steamed seed, and of autoclaved seed.

pared in Figure 7. Curve A is the benzene curve, curve B was obtained after the kernels had been steamed at atmospheric pressure in the autoclave for 20 minutes, curve C is the curve for the cold pressed oil, and curve D was obtained with a different seed specimen which had been autoclaved at 3 pounds for 5 minutes. It should be pointed out that the heights of the maxima in these various curves are not proportional to the concentration of the several constituents, since the logarithm of the per cent absorption has been plotted as the ordinate.

Absorption spectra for three fractions obtained on extracting two different specimens of cottonseed with chloroform are shown in Figure 8. In each case the second fraction represents the bulk of the oil, and in both cases the third fraction was tacky and semisolid. Data for the iodine numbers of these fractions are presented in Table 4.

TABLE 4

Iodine Numbers of Three Fractions of Chloroform-Extracted Oil From Three Varieties of Dehulled Cottonseed

	Fraction 1	Fraction 2	Fraction 3
Variety A	109	102	86.8
Variety B	102	102	89.4
Variety C	103	100	86.8

Some data obtained for the iodine number and index of refraction with the extracts of a seed specimen with several solvents support the general thesis of this communication. These data are tabulated in Table 5.

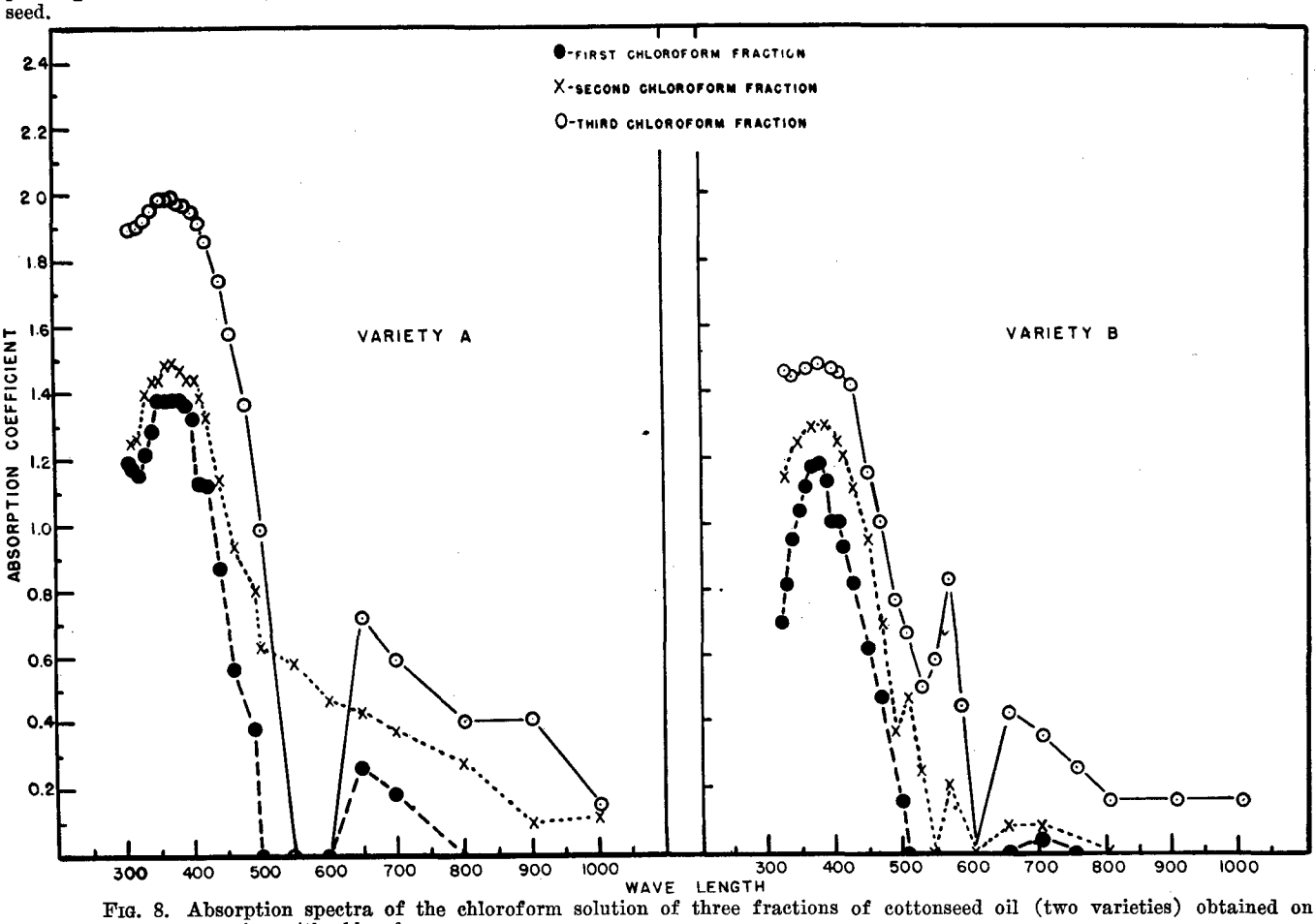

FIG. 8. Absorption spectra of the chloroform solution of three fractions of cottonseed oil (two varieties) obtained on fractional extraction with chloroform.

TABLE 5

Iodine Numbers and Indices of Refraction of the Several Oil Specimens Obtained From a Given Lot of Dehulled Cottonseed

Fraction	Iodine No.	Refractive Index
Diethyl Ether	84.4	1.47601
Chloroform	99.0	1.47523
Acetone	98.8	1.47476
Carbon Tetrachloride	.....	1.47414
Petroleum Ether (40-55°)	103.1	1.47087
Autoclaved + cold pressed	104.5	1.47039
Cold pressed	104.5	1.47029

It should perhaps be reported that some of these several extracts are not homogeneous; crystalline material separates from the chloroform and acetone extracts, and with petroleum ether it has been noted that a brown substance, presumably a polymer, is deposited on the upper part of the stripping column of the Frampton-Giles extraction apparatus.

The results which have been obtained in this study are in keeping with those which have been reported for other oil-bearing seeds (3, 4, 5, 6, 7, 8, 9, 10, 11).

Summary

1. Consistent results are obtained with the refractometric method of oil assay, but they are not in agreement with those obtained by the official methods involving extraction with diethyl ether or petroleum ether.

2. The composition of the extract obtained with cottonseed using petroleum ether varies from specimen to specimen.

3. The yield of lipid-soluble materials obtained on extraction of cottonseed with several solvents is different with each solvent.

4. Heat treatment of cottonseed reduced the quantity of lipids which could be extracted with petroleum ether.

5. Absorption spectra of chloroform solutions of cottonseed extracts obtained with several solvents show qualitative differences in the material obtained with the different solvents. Iodine numbers and indices of refraction are also different.

6. Distinct qualitative differences are noted in the different fractions obtained on the extraction of cottonseed with chloroform. These differences are apparent in the absorption spectra and iodine numbers of the fractions.

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The Spectrophotometric Determination of Glycerol*

LAURENCE K. WHYTE
Colgate-Palmolive-Peet Co., Kansas City, Kansas

A SPECTROPHOTOMETRIC method based upon the measurement of the characteristic blue color of the sodium cupri-glycerol complex is presented for the determination of glycerol in various glycerol-containing materials.

A simple, rapid, and reliable method for the estimation of glycerol has continued to be a much desired development, especially by most producers and processors of this commodity. The two officially accepted analytical procedures at the present time are the international acetin and dichromate oxidation methods. Neither of these methods is entirely desirable since they both lack a satisfactory degree of specificity, the former method constituting a measure of the reactive hydroxyl radicles and the latter of the total oxidizable material, expressed in terms of glycerol. Glycerol values obtained by the acetin method tend to be low due to the following inherent and varying stoichiometrical errors:

1. Failure of the acetylation equilibrium to attain 100%.
2. Partial premature saponification of the ester during the neutralization phase.

The applicability of the acetin method is also limited to glycerol concentrations in excess of about 60%. Other methods exist involving the oxidation of glycerol with periodic acid (1, 2), based upon the work of Malprade (3). The use of this reagent provides a higher degree of specificity and therefore is useful to the organic analyst.

A fourth method, that of Bertram and Rutgers (4), depends upon the formation of a sodium cupri-glycerol compound and its subsequent estimation by iodimetry. The reagent employed forms complexes only with compounds possessing hydroxyl groups attached to adjacent carbon atoms, and, therefore the method is characterized by a degree of specificity rendering its application to most glycerol-containing materials very satisfactory. The method is extremely rapid and relatively simple.

In presenting this spectrophotometric development founded upon the aforementioned procedure of Bertram and Rutgers, the author feels that previously encountered anomalies manifested in the original method have been eliminated, and the rapidity and simplicity of the determination very considerably enhanced.

* Presented at the 37th Annual Meeting of the American Oil Chemists' Society in New Orleans, La., on May 15-17, 1946.