THE EXTRACTION OF OIL AND PROTEIN FROM COTTONSEED BY A WATER-GRINDING AND CENTRIFUGING PROCESS

A THESIS

Presented to

the Faculty of the Division of Graduate Studies

Georgia Institute of Technology

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Chemistry

by

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June 1950

222389

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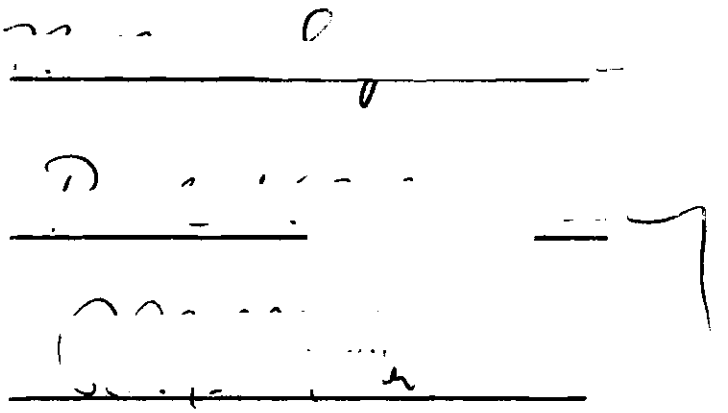

Date Approved by Chairman May 26, 1950

ACKNOWLEDGMENTS

The author would like to take this opportunity to acknowledge his indebtedness to the Georgia State Engineering Experiment Station for making this research work possible. He wishes to express his sincerest thanks to Dr. Nathan Sugarman, not only for his suggestion of the problem, but also for his most valuable aid and guidance in its prosecution. He is also grateful to Dr. P. K. Calaway, of the School of Chemistry, and Dr. R. N. Miller, of the School of Chemical Engineering, for their suggestions and comments.

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THE EXTRACTION OF OIL AND PROTEIN FROM COTTONSEED BY A WATER-GRINDING AND CENTRIFUGING PROCESS

Introduction

Development of the cottonseed industry after expansion of cotton production. The increasing population throughout the world augmented the need for cotton, In response to this need, great improvements were made in cotton spinning, weaving, and ginning, and these contributed to the expansion of the production of cotton. As a direct consequence of this expansion, there was a corresponding increase in the production of cottonseed. The problem of disposing of cottonseed, which was a nuisance to most of the cotton gins in the nineteenth century (24), stimulated efforts toward the commercial development of cottonseed processing.

The development of commercial processing of cottonseed appeared to have been somewhat retarded by the fact that most of the cottonseed had a tough hull covered by short lint fiber or fuzz. This made the seed hard to grind , reduced the quantity of oil extracted (as the lint absorbed some of the oil), and lowered the quality of the cake. This difficulty was such that early in the nineteenth

century a small bottle of cottonseed oil was produced at a cost of about $12,000 (24) in contrast to the present price of about 17 cents per pound. This difficulty, however, was overcome by the invention of the huller.

The invention of the huller and the great demand for oil by the South in the civil war created an appreciation in the American people for the usefulness of cottonseed products (29). This might be one of the factors that contributed to the wide-scale development of cottonseed processing in the United States to a greater extent than in any other cotton producing country.

Cottonseed processing. The first record of the crushing of cottonseed to obtain oil or cake apparently dates back to the early Hindu writing (34). It is also recorded that in the early stage of history the Chinese were already using the wedge press for cottonseed crushing (17). "It is not improbable that cottonseed oil as a food and cottonseed cake as a feed in some form was known to that all-wise people, the Chinese, many hundred years ago" as reported by Allison (17).

The cottonseed processing industry in the United States was started in 1892 (43) when the first successful mill for pressing oil was built at Natchez, Mississippi. Up to the present, nearly all cottonseed was processed by the hydraulic press, and the remainder by the expeller or screw press. Recently, however, a few solvent extraction

plants have been erected for the extraction of cottonseed oil, The cottonseed oil, by far the most valuable of the cottonseed products, maintained its position as the number one source of domestic vegetable oils until 1944. In that year, it was outranked by soybean oil. One of the several reasons for the increase of soybean oil utilization might be that it is more easily manipulated than cottonseed. Therefore a better method for cottonseed processing seems necessary if cottonseed is to be restored to its former supremacy.

Disadvantages of press processing method. The hydraulic press system has two intermittent operations, the cooking and the pressing, which must be coordinated. It is not flexible in operation and requires an excessive amount of manual labor. The direct labor charge has been shown to be 22 per cent of the total cost of crushing a ton of cottonseed (23).

The expeller or screw press method has the advantage of continuous operation with its resulting lower labor cost, and the elimination of press cloth (3), but it has certain inherent disadvantages that result in a lower grade of oil and meal. It is necessary to include more hull material in the expeller feed to get a free oil flow. This gives a lower protein meal and a less pure oil. Furthermore, the high temperature operation in the expeller

causes excessive denaturation of the protein. This will produce even poorer protein than the hydraulic press.

The cooking operation, a necessity with either the hydraulic or expeller pressing operation, requires considerable equipment as well as steam and power. The meats must be held in the cookers for a period of approximately one hour, which requires that the cookers be of large capacity in order to avoid a bottleneck at this point.

The prime purpose of cooking is to facilitate removal of the oil; however, cooking is known to denature the protein in such a way as to reduce the usefulness of such potentially valuable industrial raw material (31). At the same time such denatured protein also decreases the nutritive value of the meal (32). Goldovski (20) reported that in the press process the amount of protein denatured is about 28 per cent.

Disadvantages of solvent extraction method. The press process is far from effective by present day standards of industry. There is no doubt that such a process should be displaced sooner or later. It is the present trend to replace the press method with the solvent extraction method for processing cottonseed. In fact a few such extraction plants have already been established in this industry. There are many advantages over press methods. The labor cost is low. No data is available for the labor

cost of processing cottonseed by this method. However, figures for processing soybeans show that the direct labor cost is about 14.5 per cent of that of the total (5). The oil yield is high. There is only about one per cent oil left in the cake or meal from the solvent extraction process as contrasted with the 6-7 per cent oil in the press cake. The protein in the meal is only slightly denatured since no drastic heating of the seeds is necessary. *

However, there are some disadvantages in solvent extraction which should not be overlooked. First of all, the high cost of the equipment for the solvent extraction plant makes the replacement of the crushing process with solvent extraction very expensive. The products obtained from solvent extraction are oil and meal only. If theprotein is desired, further treatment of the meal for its isolation is required. This will call for additional equipment.

Although direct solvent extraction has been recommended for many years (27, 38, 39, 44, 47) as a method for processing cottonseed for oil, it has only recently been applied on an industrial scale in the United States, and does not appear to be widely practiced in other countries.

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* In the batch process for solvent extraction, however, the denaturation may amount to 40 per cent as was reported by Goldovski (20).

Examination of the literature on the subject (33, 35) indicates that solvent extraction of cottonseed presents many problems, chief among which is that involving the complex pigment system which is present in cottonseed. This unique system of pigments in the kernel of the cottonseed not only differentiates this seed from others but poses problems which are not encountered in the solvent extraction of other commercial oilseeds. Although the control of color is not the only difficulty, it is one of the principal ones encountered in application of solvent extraction to cottonseed on an industrial scale.

Furthermore, Wither and Carruth (45) discovered that gossypol, one of the principal pigments present in cottonseed, alone could produce the symptons associated with the so-called cottonseed injury produced in livestock by feeding of cottonseed in large quantity. If the solvent, as commonly employed in solvent extraction, possesses no solvent effect on those pigments, the meal would contain most of the original pigments of the seed. Such meals, therefore, could not be used as feedstuffs without further treatment.

Development of water-grinding process. In view of the disadvantages in the present processes for cottonseed as cited above, it is desirable to develop a method for processing cottonseed with the following improvements as an aim:

(1) the process msut be continuous in order to reduce the direct labor cost,

(2) the operation is conducted to comparatively low temperature in order to cause as little denaturation of the protein as possible,

(3) the operation is such that the protein can be produced simultaneously so that much additional equipment for its isolation is not necessary,

(4) the process is such that the gossypol does not present too great a problem, and

(5) the euqipment required is not too expensive, so that replacement of the present process would be feasible.

The water-grinding process, the technology of which is to be described below, is operated chiefly with grinding mills and centrifuge. The process may be made continuous, thus reducing the labor cost. Since water is the extraction medium, explosion hazards are eliminated. The main operation is conducted at a temperature of about $60^{\circ}C.$, so that denaturation of the protein is not likely to occur. The process is designed in such a way that in one process the meal, the oil and the protein are simultaneously produced. Lastly, the equipment required, which consists chiefly of mills and centrifuges, is not as expensive as that used in solvent extraction. Therefore it seems that the development of the water-grinding process would be one possible improvement in cottonseed industry.

Technology of Water-grinding Process

In the extraction of vegetable oil with the water-grinding process, the seed is pre-treated and cleaned as in the ordinary process. The cleaned seed thus obtained is used as raw material.

It has been well known that water may displace the oil from seed. Several Russian mills are employing the Skipin process (40) at a relatively high moisture content in cooking oil seed in order to accomplish displacement of the oil. The water-grinding process, making use of the same principle, employ a relatively large volume of water as a medium for extraction of the oil. The oilseed is ground so that the surface of it will be accessible for water to displace the oil. Because of the presence of protein, the oil is usually separated out in form of an emulsion consisting of a small portion of protein and water. Pure oil is set free from this emulsion by special methods of breaking.

Thus, the water-grinding process consists of four main operations:

1. Grinding of cleaned cottonseed with water to form a slurry;
2. Clarification of the slurry, the materials produced being clarified slurry and cake (solid residue).

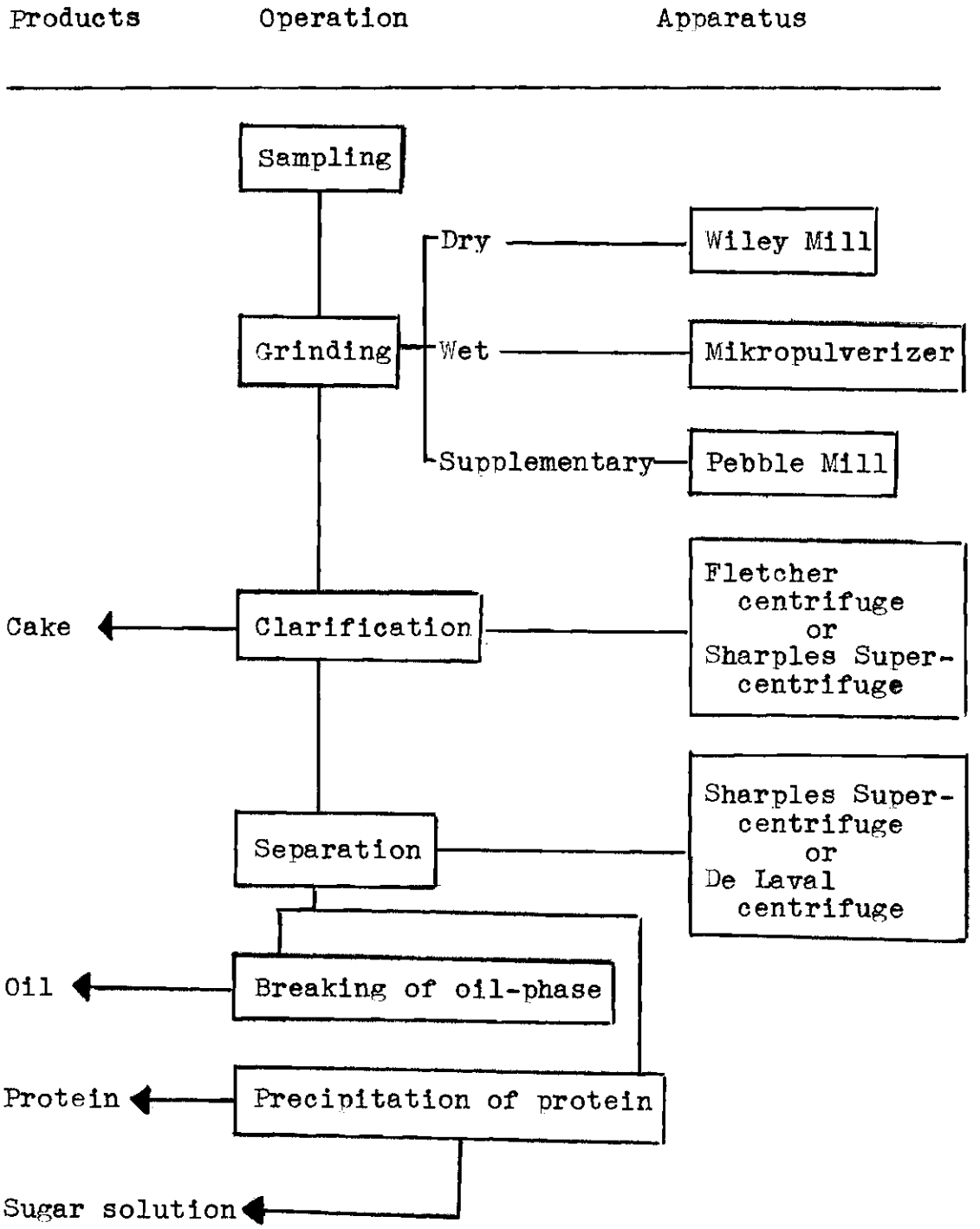
3. Separation of the clarified slurry into an oil-phase emulsion and a protein dispersion; and
4. Precipitation of the protein from the dispersion.

By the water-grinding method, four classes of products are obtained:

1. Cake, or the insoluble materials corresponding to the ordinary cake or meal, which may be used as livestock feed,
2. Protein, which is a potential industrial material for plastics (36), adhesives (4), or fibers (4),
3. Oil, which is mainly used as food, and
4. Sugar-containing liquors which may be utilized for alcohol fermentation or yeast growth (20).

A flow sheet of this process is shown in Figure 1.

Fig. 1. Flow Sheet of Water-grinding Process for Cottonseed



Experimental Work with Water-grinding Method

After a series of preliminary experiments, a general scheme of procedure was adopted as described below.

Preparation of Sample. For each experiment a certain amount of cottonseed was weighed out. With a few exceptions, each sample was first dry ground in a Wiley mill. The ground sample was then mixed with an appropriate proportion of water (usually at a ratio of 10:1 or 8:1). The desired pH value of the mixture was obtained by adding either dilute hydrochloric acid or sodium hydroxide solution. After mixing for ten minutes, the mixture was ready for wet grinding.

Grinding. The purpose of grinding is to rupture the seed cells so that the oil may be easily displaced and extracted. Several types of grinding mills have been employed for wet grinding cottonseed samples under various conditions. Both single-and multiple-stage grindings were tried. It was finally decided to employ the mikropulverizer for most single-stage wet grinding and the mikropulverizer and pebble mill for multiple-stage wet grinding. A Wiley mill was used for preliminary dry grinding.

Wiley Mill: The Wiley mill (model No. 2, manufactured by Arthur H. Thomas Co., Philadelphia) was used for dry coarse grinding as preliminary treatment for subsequent wet grinding. This machine consists of a set of

revolving knives which rotate past a set of stationary knives. The comminuting action is that of cutting. Such coarse grinding was helpful in disintegrating the hulls that might not be broken down in wet grinding.

Mikropulverizer: The mikropulverizer (manufactured by Pulverizing Machinery Co.) was used for wet grinding. This machine is essentially a high speed hammer mill. The cottonseed was wet ground at various experimental pH values. The water to seed ratio was 10:1 or sometimes 8:1. If the cottonseed sample had been preliminarily dry-ground with the Wiley mill and was to be treated with a multiple-stage grinding, it was usually ground twice in the mikropulverizer with a No. 027 screen. If the sample was to be processed with the mikropulverizer alone, it was usually necessary to pass the slurry through the machine several times to assure satisfactory refinement. This was performed by grinding the sample three times with a No. 027 screen, and another three times with a No. 020 screen (a finer mesh screen).

Pebble Mill: The pebble mill employed was manufactured by Paul O. Abbe Inc., Little Fall, N. J. Pebbles of small size were used. This mill exerts a multitudinous point pressure on the seed which provides a favorable condition for crushing the oil-bearing cells completely. However, straight grinding with this machine alone was not

effective. Therefore, the pebble mill was usually used as a supplementary grinding tool. If such grinding was preceded by another preliminary grinding, especially with the mikropulverizer, the oil retention in the insoluble materials could be reduced to a great extent. Quite a few experiments were performed by such combinations of grinding.

The slurry obtained by wet grinding consisted of an emulsion of oil, solution of protein and carbohydrates, and suspension of solids. The mixture was warmed to 60°C., and adjusted to a desired pH value with dilute hydrochloric acid or sodium hydroxide solution. After stirring well for fifteen to twenty minutes, the mixture was ready for clarification.

Clarification. Clarification is the process of separating the insoluble materials from the slurry obtained in grinding. It was accomplished by using either a Fletcher centrifuge or a Sharples supercentrifuge. Sometimes a combination of the two centrifugation processes was employed. The Fletcher centrifuge is a solid basket type of 12-inch diameter, manufactured by the Fletcher Co. It rotates at a speed of 2100 rpm. The Sharples supercentrifuge is the type T41-24. The rate of rotation is adjustable. For the Fletcher centrifuge, the flow was controlled at a rate of not more than three gallons per

hour. For the supercentrifuge, the following conditions were provided:

No. of the ring..... #5
Temperature..... 60°C.
RPM..... 40,000
Nozzle..... medium

In this process, the insoluble materials were retained in the centrifuge, while the clarified slurry was drained off for further treatment. The solids in the centrifuge were washed with a small amount of warmed water (60°C.), centrifuged dry and dried in a drying oven at 110°C. This was the product called by the conventional name "cake", corresponding to the meal that might be used as feedstuff. The cake was analysed for its oil content, nitrogen content and gossypol content.

The clarified slurry consisted of the oil in emulsion and the protein together with some other soluble materials in solution. It was warmed again to 60°C. after the pH was readjusted to the desired value by adding acid or base, and well stirred for fifteen minutes. The slurry was now ready for the separation process.

Separation. The separation was performed with either a Sharples supercentrifuge or a DeLaval centrifuge. The supercentrifuge was the same as that described for the clarification process. The De Laval centrifuge was a small laboratory model. The conditions for operating the supercentrifuge were as follows:

No. of the ring	#7½ or #8
Temperature	60°C.
RPM	40,000
Nozzle	small
pH	9-10

The conditions for operating the De Laval centrifuge were as follows:

No. of the ring	#33 or #34
Temperature	60°C.
Rate	3 gal. per hr.
pH	9-10

This process was performed for the purpose of separating the oil from the protein and other soluble materials in the slurry. The oil-phase, in the form of concentrated emulsion of oil in protein solution, has a specific gravity less than that of the aqueous phase. Thus, the products of this separation process are (1) oil-phase, and (2) defatted slurry.

Treatment of oil-phase. The oil-phase which was composed of an average of 70 per cent oil, 19 per cent water and 11 per cent protein, was easily de-emulsified by heating at a temperature range of from 90°C. to 120°C. The oil so obtained usually had a dark color. In later experiments the oil-phase was de-emulsified by strong agitation with a Waring Blender at a starting temperature of 60°C. The optimum pH value for this method of de-emulsification was found to be between 5 and 6. The oil so obtained was much lighter in color. Each oil sample was analysed for its color and gossypol content.

Precipitation of protein. The defatted slurry obtained from the separation process was then carefully acidified with dilute hydrochloric acid or with sulfur dioxide gas to a pH of 4.0. At this point the protein precipitated out from the dispersion This was centrifuged and the solution was decanted off. The protein was dried in an oven at a temperature of 50°C. The protein was analysed for its oil content, nitrogen content, and gossypol content.

The remaining solution was yellowish and clear. It contained the soluble materials of cottonseed. No further study was made with this solution.

pH value in different operations. The basic principle of this water-grinding method is the separation of a colloidal solution into its components. In the preparation of this colloidal solution, optimum conditions must be provided for making these components subsequently easily separable. Since the solution is colloidal in nature, its stability is influenced by its pH. With this point of view, samples were prepared by controlling the pH value of the slurry. The influence of the pH value on the experimental results could be easily observed. This will be discussed later.

In the sequence of operations of clarification and separation the protein must be kept in dispersion so that it will not be separated out with the cake during

clarification or with the oil during separation. A knowledge of the solubility of protein is necessary, and fortunately the solubility data for cottonseed protein are available (18).

The peptization curve for cotton seed protein at various pH values is depicted in Figure 2. The pH values are varied by the addition of appropriate amounts of hydrochloric acid or sodium hydroxide.

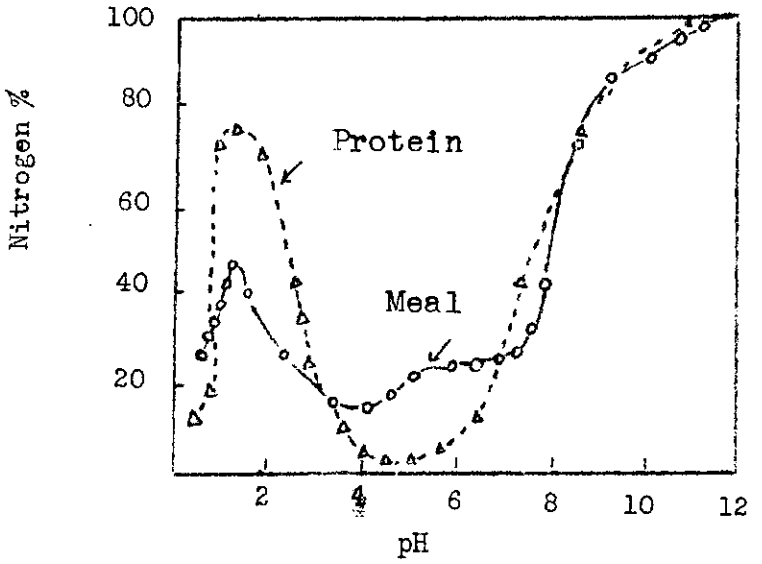


Fig. 2. Percentage of total nitrogen peptized in cottonseed meal and isolated protein at different pH values.

This curve shows that the cottonseed protein from either meal or isolated protein can be peptized both in the acid range (pH 0.75 to 2.5) and in the alkaline range (pH 7 to 12). The peptizability in the alkaline range is much greater than in the acid range. Especially is this true for cottonseed meal. Therefore, in conducting the clarification and separation processes, it was decided to adjust the pH value of the slurry to at least 8 or higher in order to keep the protein well in dispersion.

The curve in Figure 2 also reveals that the minimum peptization is in the pH range of from 3 to 5. This zone is several pH units broader in the case of cottonseed meal than for peanut meal (10). For the best isolation, the pH value of 4 was chosen as the isoelectric point for the precipitation of protein from the defatted dispersion.

Experimental Results and Discussion

The scope of this investigation is to find the optimum conditions so that cottonseed may be separated into useful products by the water-grinding process. About sixty experiments have been performed, the first fifteen of which were merely trials. Results from trials are not to be included in this discussion.

For the purpose of discussion, experiments are classified into four groups:

1. Preliminary set of experiments,
2. Experiments to reduce oil content in cake,
3. Experiments to reduce oil content in protein, and
4. Analysis of products.

Preliminary set of experiments. It has been mentioned before (p. 18), that the cottonseed protein can be peptized easily on the alkaline side of its isoelectric point. Preliminary experiments were performed with the pH values ranging from 8 to 10.5. The results from these slightly alkaline operations are shown in Table I.

TABLE I.
DISTRIBUTION OF OIL AMONG THE PRODUCTS IN SLIGHTLY ALKALINE OPERATION

Run No.	Operation Description: pH value			Oil Distribution among Products: Cake			Protein			Oil-phase		Total Wt. in gm.		Ratio
	Grinding	Clarification	Separation	Wt. in gm.	Oil %	Oil Wt. in gm.	Wt. in gm.	Oil %	Oil Wt. in gm.	Wt. in gm.	Oil Wt. in gm.	Oil	Non-oil	Oil/Non-oil
17	10.5	10.5	10.5	---	8.96	---	---	24.72	99.0	---	162	---	---	-----
18*	10.5	8.0	10.0	227	7.93	18.0	---	22.59	81.4	280	196	---	---	-----
19	10.5	10.5	10.0	---	9.45	---	---	28.75	----	---	---	---	---	-----
16*	10.5	8.0	8.0	219	13.49	29.6	297	18.24	54.4	210	147	231	726	0.32 : 1
21	9.0	9.0	8.5	210	11.05	23.1	351	26.66	93.3	240	168	285	801	0.36 : 1
23	10.5	10.5	8.0	210	11.05	23.1	342	27.85	94.5	244	170	288	796	0.36 : 1
25	10.5	10.5	9.0	203	12.01	24.4	324	22.15	71.8	296	206	302	822	0.37 : 1
27	10.5	10.5	10.0	214	11.59	24.8	324	25.05	81.2	279	195	301	817	0.37 : 1

(1) Weight of cottonseed sample in each experiment is 1000 grams.
(2) The cottonseed was first ground dry in Wiley mill; then wet ground in mikropulverizer.
(3) Sharples supercentrifuge was used in clarification.
(4) De Laval centrifuge was used in separation, except those marked with * , in which cases Sharples supercentrifuge was employed.

The cottonseed which was used as raw material in this study was supplied by the Buckeye Cotton Oil Co., Atlanta, Georgia. It was produced in Georgia. The analysis of the cottonseed showed the following results:

Oil content..... 31.04% (on sample received)

Moisture..... 7.81%

Protein. 31.2%

Gossypol..... 5.81 mg. per 1000 g.

Calculating on the basis of the analysis, 1000 grams of air-dry cottonseed is equivalent to 921.9 grams of moisture free cottonseed, of which 310.4 grams is oil.

From experiments such as Runs Nos. 21, 23, 25, and 27 in Table I, it is found that the total weight of oil distributed among the three products - cake, protein, and oil, - is 285-302 grams. It was also found that the loss of oil-phase in the discs of the De Laval centrifuge always amounted to 10 to 20 grams. That is to say, the oil lost in operation was about 7 to 14 grams. Such operational loss may be eliminated or reduced to a minimum in continuous operation. The sum of the oil loss plus the total weight found amount to 96 per cent of the original oil content in cottonseed. If conditions could be provided so that the extractable portion of oil could be restricted in the oil-phase instead of being widely distributed among the products, it would be possible to

have an oil yield of about 96 per cent by this process. The total weight of products in these experiments exceeded that of the raw material. Such excess was due to the inclusion of some water both in the oil and in the protein. In the last column of Table I the weight ratio of the oil to non-oil materials is fairly constant, indicating the consistency of the results.

Experiments on reduction of oil content in cake. In the preliminary set of experiments, the oil content of the cake usually was found to be high. Such high oil contents may be attributed to retention by the oil cells which had not been satisfactorily ruptured in the grinding process, or to poor clarification of the slurry. It has been claimed that satisfactory rupture of the oil cells might be attained by subjecting the material to a multitudinous point pressure effect. The pebble mill would serve for this purpose if such was the case. However, it was found that the pebble mill alone could not grind the seed satisfactorily. Therefore a set of experiments was conducted with a combination of grinding methods. Some of the samples were also clarified by double centrifugation. The results are shown in Table II.

TABLE II

OIL CONTENT OF THE CAKE UNDER EXPERIMENTAL CONDITIONS

Run No.	Cottonseed Sample @	Water Grinding			Separation			Analysis of Cake		
	Wt. in gm.	pH	1st. Mill	2nd. Mill	pH	1st. Centrifuge	2nd. Centrifuge	Wt. in gm.	Oil %	Oil Wt. in gm.
25	1000	10.5	Mikropul-verizer	---	9.0	Sharples	---	202.7	12.01	24.34
30	2000	12.0	"	Pebble	12.0	Fletcher	---	207.1	14.61	30.23
31	500	12.0	"	---	12.0	Sharple	---	110.0	13.04	14.34
32	1000	12.0	"	Pebble	12.0	"	---	151.7	5.43	8.22
33	1000	10.5	"	"	10.5	"	---	226.5	3.98	9.01
34	500	10.5	"	"	10.5	"	---	108.0	3.88	4.23
36	500	8.0	"	---	10.5	"	---	114.0	10.70	12.20
42	500	7.0	"	---	10.5	"	---	114.0	7.50	8.56
44	1000	7.0	"	Pebble	10.5	Fletcher	Sharples *	257.5	9.22	23.70
45	1000	8.0	"	"	10.5	"	Sharples	223.1	12.45	27.78
46	1000	5.0	"	"	10.5	"	Sharples	239.2	13.74	32.77

@ All the samples were first dry ground in Wiley mill.

* With two more liters of water than in Run No. 42.

From the results of this set of experiments, several remarks may be made. In the first place, when the sample was ground with a mikropulverizer and then with a pebble mill at the pH value of 10.5 to 12, the oil content in the cake was reduced to a great extent. Run No. 5, which was ground with the mikropulverizer alone at a pH value of 10.5, resulted in a cake with an oil content of 12.01 per cent. In contrast to this, a supplementary grinding with the pebble mill was in Run No. 34 resulted in an oil content of 3.88 per cent. Runs Nos. 33 and 34 show that the results were reproducible.

However, when the combination grinding process was conducted at lower pH values, the reduction of the oil content was not as effective. This is shown in Runs Nos. 44, 45 and 46. Such differences in results might be explained by the fact that the poor peptization of the protein prevented the release of oil from these insoluble materials.

Using the Fletcher centrifuge alone as in Run No. 30, the clarification was not effective even under grinding conditions satisfactory in other cases. This was due to the inefficiency of the centrifuge. The supercentrifuge alone was found suitable for clarification.

As far as the percentage of the oil lost in the cake is concerned, the highest loss was about 3 per cent

of the total oil in the cottonseed. When the sample was ground under favorable conditions, the loss was reduced to less than 1 per cent, 0.82 per cent being the lowest recorded (Run No. 34).

Experiments on reduction of oil content in the protein. In the preliminary set of experiments, the oil content in the protein was generally high. Such retention of oil in the protein is probably due to the high stability of the emulsion formed by the dispersion of the oil in the aqueous solution. The stability of the emulsion made it difficult to effect sharp separation between the oil and the protein in the course of separation. Experiments were devised to study the conditions which would be most favorable for decreasing the stability of the emulsion so that the amount of oil in the protein might be reduced to a minimum.

The degree of stability of an emulsion may be influenced by the pH value during its formation. Therefore, changing the pH value at grinding to a different value during separation may vary the results of the oil and protein separation. Both high pH value grinding and low pH value grinding were investigated, and the results were shown in Table III.

TABLE III
OIL CONTENT IN PROTEIN UNDER EXPERIMENTAL CONDITIONS

Run No.	Cottonseed Sample ¢ Wt. in gms.	Water Grinding pH	Water Grinding Mill	Clarification pH	Clarification Centrifuge	Separation pH	Separation Centrifuge	Oil % in Protein
30	2000	12.0	Micropulverizer *	12.0	Fletcher	9.0	Sharples	5.73
32	1000	12.0	" *	12.0	Sharples	10.0	"	2.18
33	1000	10.5	" *	10.5	"	9.0	"	29.10
34	500	10.5	"	10.5	"	10.5	"	25.19
42	1000	7.0	"	10.5	"	9.0	"	4.45
43	1000	7.0	"	10.5	Fletcher @	9.0	"	6.05
45	1000	5.0	" *	10.5	" @	9.0	"	4.87
46	1000	5.0	" *	10.5	" @	9.0	"	3.68

¢ All the samples were first dry ground in Wiley mill.
* Combination with second grinding in pebble mill.
@ Combination with second clarification through Sharples supercentrifuge.

From Table III one can easily find that the oil content in the protein may be greatly varied by varying the pH values during grinding. When grinding at pH value of 10.5 (Runs Nos. 33 and 34), oil retention always occurred. If the sample was ground in the nieghborhood of the pH value of 12, as in the case of Runs Nos. 30 and 32, the oil was greatly released from the protein. Similar results have been obtained in cases employing low pH grinding (i.e. Runs Nos. 42, 43, 45, and 46).

The cause of reduction of oil in the protein is not exactly known. It might be due to the destruction of some substances, for instance phosphatides, the presence of which cause the oil-protein emulsion separation difficulty. No definite explanation can be offered at present. It requires further study.

Based on the analysis of cottonseed (p.21), the percentage of total oil in the sample retained by the protein may be calculated. The average values are shown below:

Grinding at pH 12
(Runs Nos 30 and 32).....average 4.00 %
Grinding at pH 7
(Runs Nos. 42 and 43).....average 5.28 %
Grinding at pH 5
(Runs Nos. 45 and 46).....average 4.29 %

Therefore the reduction of oil content in the protein may be attained by either high or low pH value grinding. In view of avoiding too much saponification of the oil at high pH value during the drastic grinding, as is the case in mikropulverizer grinding, it seems better to grind the material at rather low pH value in the neighborhood of 5 to 7. On the other hand, as it was indicated before, the reduction of oil content in the cake could be attained at a pH in the neighborhood of 12. However, such conditions may be provided when the sample is ground in the pebble mill, which is not so drastic as it would be in the mikropulverizer. Therefore, if the sample is first ground at low pH value in the mikropulverizer and then adjusted to a higher pH value in the pebble mill, the oil reduction in the cake and in the protein may be both satisfactorily attained.

Inspection of Tables II and III shows that both Runs Nos. 32 and 42 may illustrate the fact that satisfactory results of low oil content in both the protein and the cake at either high pH value or low pH value grinding may be attained. In Run No. 32, the percentage of the original oil in the cottonseed lost in the cake and in the protein were 2.64 and 2.19 respectively, making a total loss of 4.88 per cent. For Run No. 42, the respective values were 2.76 and 4.47, the total loss being 7.23 per cent.

Assuming that the oil lost in the cake and in the protein were the only loses in a continuous operation, the oil yield from this water-grinding process should approach 92 to 95 per cent.

Outline of operation. From the results obtained so far, it would be easy to visualize the appropriate conditions for operating the water-grinding process for cottonseed. Although it is outside the scope of this work, a tentative procedure for such operation may be outlined for future work.

1. The cottonseed is cleaned and delinted as in the ordinary process,
2. The cleaned cottonseed is dry ground in a coarse mill,
3. The coarse ground cottonseed is mixed with 10 parts of water and ground at a pH in the neighborhood of 7 in a mikropulverozer or some other powerful mill to produce a slurry,
4. The slurry is adjusted to a pH value in the neighborhood of 12 to produce an alkaline slurry for grinding in a pebble mill,
5. The alkaline slurry is warmed to 60°C., stirred for a period of time and clarified in a centrifuge of the basket type,
6. The insoluble materials in the basket of centrifuge

is washed with a little water, and dried at 100°C. to produce cake for animal feed,

7. The clarified slurry from the basket type centrifuge is warmed to 60°C., and passed through either a De Laval centrifuge or a Sharples supercentrifuge under proper conditions to separate oil-phase from protein dispersion,
8. The oil-phase from the separation process is adjusted to a pH value in the neighborhood of 6, warmed to 60°C., and agitated with a strong agitator to separate oil from water and protein,
9. The protein dispersion from the separation process is adjusted to a pH value of exactly 4 to precipitate the protein,
10. The protein is centrifuged to remove excess water and dried with air at a temperature of not over 50°C., and
11. The remaining solution from the precipitation of the protein may be used over again as water for grinding in the next batch, or used as a source of fermentable raw material.

Analysis of products. As mentioned previously there are three classes of products produced by this process. Although the solution remaining from the precipitation of the protein might be considered as the fourth class of product, it will not be included in this study as it could be used over again in place of fresh water. For each product no complete analysis was attempted, but a few determinations were selected for each in order to give a general idea of the quality. Only gossypol content and color of the oil were determined. For the cake and the protein, the nitrogen content and gossypol content were determined. The ash content of a few protein samples were also included.

For color determination of the oil, the method recently adopted by the American Oil Chemists' Society was followed (37) (see Appendix III).

The newly modified spectrophotometric method of Smith (41) was employed for the estimation of gossypol content in the products. Its description can be found in the Appendix (see Appendix II, A and B).

All nitrogen determinations were run by the official A.O.C.S. Kjeldahl-Cunning-Arnold method. A description of this method also can be found in the Appendix (1) (see Appendix I, C).

The ash and the moisture determinations were also run by the official A.O.C.S. methods (1) (see Appendix I).

The presence of the dark pigments in cottonseed has always constituted a problem in the utilization of this material as a seed of oil and protein. Wither and Carruth (45) discovered that gossypol, one of the principle pigments in the cottonseed, alone could produce the symptom associated with the so-called cottonseed injury produced in livestock by the feeding of cottonseed in large quantities. Following this discovery, Carruth and co-workers instituted an investigation of the chemicsl properties of gossypol. Carruth later found (14) that the reduced toxicity of cottonseed meal brought about by heat treatment could be correlated with a reduced content of extractable gossypol. Clark (15) published a series of articles on gossypol derivatives in which he showed among other things that gossypol can be extracted with aniline from heat detoxified gossypol meal. By analogy to the reaction with aniline Clark proposed (16) that the "bound" gossypol was gossypol that had combined with the protein in the cottonseed, and thus had been rendered non-toxic and unextractable with ordinary solvents.

The early investigations (9. 21, 22, 42, 46) of the anatomy of the cottonseed noted the occurrence of the pigment glands and reported observations of their reaction with water. The pigment glands, which contain all of the gossypol and its derivatives in the seed, possess a thick,

strong, resistant wall which presumably protects the gland contents from direct contact with the components of the surrounding tissue in the intact seed. These glands possesses such high mechanical strength that in seed of normal moisture content only a small fraction of them are ruptured under the pressure and shearing stresses, to which they are subjected during the rolling or grinding of seed preparatory to pressing or extraction. Consequently, the pigments glands ontaining the intraglandular pigments remain in the meal unless processing conditions are such as to rupture the gland walls. The gland walls have been shown (6, 7, 8) to be resistant to the action of most liquids except water and a few water miscible organic liquids of low molecular weight. Contact with water causes instantaneous rupture of the walls and the expulsion of the gland contents. The sensitivity of the glands to water increases as the temperature is increased (8).

From the consideration of the characteristic properties of pigment glands of the cottonseed as described above, it can be related that the pigment problem encountered in the press process is as serious as it is in the solvent extraction process. In the water-grinding process, it can be foreseen that nearly all of the gland walls will be ruptured and all of the contents will be extractable if they are not destroyed under the treatment. Thus, the meal produced by the water-grinding process will be nearly

free from gossypol. Since gossypol is not a stable compound, at least a part of it will be destroyed under the treatment of this process. Most of the undestroyed portion will be bound with the protein, and some will be dissolved in the oil. The gossypol determinations of the samples in this investigation are shown in Table IV.

TABLE IV

GOSSYPOL CONTENT IN PROTEIN AND CAKE

Run No.	% of Gossypol		Wt.of Gossypol in mg		Wt. in Protein / Wt. in Cake
	in Protein	in Cake	in Protein	in Cake	
21	0.301	0.010	1.00	0.02	50 : 1
22	0.325	0.010	0.47	0.02	24 : 1
23	0.318	0.009	1.08	0.02	54 : 1
24	0.248	0.016	---	---	---
25	0.250	0.012	0.81	0.02	40 : 1
26	---	0.013	---	---	---
27	0.300	0.010	0.96	0.02	40 : 1
28	---	0.009	000	---	---
29	0.301	0.010	0.90	0.03	30 : 1
30	0.240	0.012	0.86	0.03	29 : 1
31	0.206	0.012	0.58	0.01	58 : 1
32	0.216	0.008	0.50	0.01	48 : 1
33	0.275	0.009	1.13	0.02	57 : 1
34	0.287	0.009	0.62	0.01	62 : 1
35	0.240	0.009	0.37	0.01	37 : 1
36	0.234	0.009	0.31	0.01	31 : 1
37	0.258	0.009	0.36	0.01	36 : 1
38	0.258	0.009	---	---	---
39	0.259	0.010	---	---	---
40	0.240	0.009	---	---	---
41	0.275	0.009	---	---	---
42	0.222	0.009	0.27	0.01	27 : 1
43	0.250	0.008	0.67	0.02	33 : 1
44	0.300	0.009	0.81	0.02	40 : 1
45	0.280	0.009	0.40	0.02	20 : 1
46	0.266	0.009	0.38	0.02	19 : 1
47	0.270	0.008	0.85	0.02	48 : 1
48	0.246	0.008	0.81	0.03	27 : 1
49	0.219	0.009	0.48	0.03	16 : 1

Weight of gossypol is calculated on the basis of 1000-g. sample. 1000 g. cottonseed contain 5.81 mg. gossypol.

The gossypol recovered in the oil, protein and cake was always found to be less than 20 per cent of the total present in cottonseed. That is to say, more than 80 per cent had been destroyed during the course of operation in the water-grinding process. The weight ratio of gossypol in the protein to that in the cake as shown in the last column of Table IV shows the concentration of pigment in the protein.

The gossypol in the cake was either very low originally or was destroyed during drying. Lyman, Holland, and Hale (25) found that cottonseed meal with 0.02 per cent gossypol would not cause injury of any kind to animals even when fed at a level of 25 per cent of the total ration for a considerable length of time. The cake produced by this water-grinding method contained always less than 0.02 per cent gossypol. Such traces of gossypol in the cake will make it suitable as feed for livestock.

The gossypol content of the oil was found to be in the range of 0.01 per cent to 0.02 per cent, as showed in Table V. This low content will not only render the oil nontoxic, but also will present no color problem as is the case with the press processes. The oil of "basic prime" quality will have a color not greater than 35 yellow and 7.6 red on the lovibond scale. Premiums are allowed on color lighter than this. The average refined

oil derived from prime crude oil, having a color or 35 yellow and 7.6 red, will yield a bleached oil having a color of 20 yellow and 2.5 red, which is the standard color for Bleachable Prime Summer Yellow cottonseed oil. All oil samples produced by the water-grinding process are within these limits. Oil produced from the oil-phase by agitation without direct heating will have a color much lighter than that produced from the press method.

TABLE V
COLOR VALUE AND GOSSYPOL CONTENT IN OIL

Run No.	Preparation from Oil-phase	Color Determination Transmittance %	Opt.Density	Red	Gossypol %
30	Rendering to 120°	62.5	0.204	8.6	0.012
32	" " "	62.8	0.202	8.5	0.016
33	" " 90°	64.0	0.194	8.1	0.015
34	" " "	63.0	0.201	8.4	0.017
42	Agitation	66.3	0.178	7.5	0.019
43	"	67.0	0.174	7.3	0.015
44	"	68.3	0.165	6.9	0.016
45	"	67.7	0.170	7.1	0.014
Combination of oil samples, by agitation		66.0	0.181	7.6	0.018

The determination of nitrogen in the cake indicated a rather low protein content. This is shown in Table VI. According to the Rule of the Association (30), the meal from cake of Prime Quality contains not less than 36 per cent protein. Samples produced in this study contained about 20 per cent or less, which would be comparable with the "Whole Pressed Cottonseed" or Expeller Cake", with protein content of 20 per cent.

TABLE VI
NITROGEN DETERMINATION IN CAKE

Run No.	Cake		Oil Content	Nitrogen Determination	
	% in Cottonseed	Wt. in gm.	%	% of Nitrogen	% of Protein
21	21.00	210	11.05	2.40	15.01
23	21.00	210	11.05	3.10	19.40
25	20.27	205	12.01	2.50	15.60
27	21.42	214	11.59	3.25	20.50
31	22.00	110	15.04	1.05	6.52
32	15.17	152	5.45	2.00	12.50
33	22.65	227	5.98	2.40	15.01
34	21.60	108	3.88	2.00	12.50
35	22.80	114	8.10	2.30	14.40
36	22.80	114	10.70	2.50	15.62
37	22.01	110	9.30	2.45	15.30
40	25.50	127	9.60	2.23	13.99
41	28.15	145	10.60	2.80	17.45
42	22.82	114	7.50	2.50	15.63
43	26.00	260	14.69	2.70	16.90
44	25.75	258	9.22	2.85	17.80
45	22.31	223	12.45	2.51	15.62
46	25.92	239	13.74	3.09	19.40
47	28.00	280	9.57	3.28	20.55
48	28.00	280	9.57	3.25	20.34
49	32.70	327	10.60	3.05	19.00
50	31.94	319	9.22	3.31	20.69

The nitrogen content in the protein is a reliable criterion of its quality. According to Arthur (2), peanut protein containing more than 16 per cent nitrogen and less than 2 per cent ash is satisfactory for the production of fibers and adhesives, which require the use of raw material containing more than 95 per cent protein (11, 12, 13, 19, 28). This will be equally applicable to the cottonseed protein. Table VII shows the nitrogen determinations carried out in this study. The low protein content together with the high gossypol content, as shown in Table IV, rendered the isolated protein from this investigation unsuitable for direct utilization. A process of purification is required.

TABLE VII

NITROGEN DETERMINATION IN PROTEIN

Run No.	Oil % Content	Nitrogen Determination		Percentage of Protein On Oil-free Basis
		Nitrogen %	Protein %	
21	26.66	11.80	74.00	
23	27.85	11.00	68.80	96.5
25	22.15	12.40	77.20	99.0
27	25.05	12.00	74.50	98.5
31	9.94	13.60	85.10	94.8
32	2.18	14.80	92.10	94.2
33	29.05	11.00	68.75	98.0
34	25.19	11.20	70.00	94.0
35	11.20	13.00	81.25	91.5
36	13.50	13.20	82.50	95.2
37	1.16	15.35	95.65	96.8
40	7.70	15.02	93.82	
42	4.45	15.02	93.82	97.5
43	6.05	14.78	92.50	98.2
44	15.20	14.82	82.53	
45	4.37	15.00	93.75	98.0
46	3.08	15.51	96.80	99.9
47	31.79	10.00	62.50	90.8
48	18.04	13.49	84.50	
49	37.11	9.25	57.82	95.2

Conclusions

1. Wet grinding under slightly alkaline conditions (pH 8 - 10.5) results in a wide distribution of the oil among the products.
2. Grinding at pH value in the neighborhood of either 7 or 12 will reduce the oil retention in the protein to only about 2 - 4 per cent.
3. Grinding at pH value in the neighborhood of 12 together with a supplementary grinding in the pebble mill will reduce the oil retention in the cake to 4.25 per cent, which is equivalent to 0.82 per cent of the total oil content in cottonseed.
4. Calculation from the results indicates that by the water-grinding method an oil yield up to 95 - 96 per cent is feasible.
5. About 80 per cent of the total gossypol in the cottonseed is destroyed in the course of this process.
6. The remaining undestroyed gossypol (about 20 per cent or less) is concentrated in the isolated protein.
7. Less than 0.02 per cent gossypol was found in either the cake or the oil. Such traces of gossypol should not give problem of toxicity of these products.

8. Since the oil produced by the water-grinding method has a color lighter than that produced from the press method, no color problem should be encountered.
9. The nitrogen content in the cake is lower than the meal or cake of Prime Quality, but is comparable with Expeller cake.
10. The analysis of the isolated protein indicates that this product is not pure enough for direct use without further purification.

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APPENDIX I

American Oil Chemists' Society
Official Method of Analysis

A. A.O.C.S. Official Method Ba 2-38: Moisture

A. Apparatus:

1. Aluminum moisture dishes, 3-gauge, 2 x 3/4 -inch tight fitting slip-over covers.
2. Forced draft oven, A.O.C.S. Specification H 1-39.
3. Descicator containing efficient descicant.
4. Jones sampler, riffle type, 6 x 6-inch.

B. Preparation of Sample:

1. Reduce the original 1000-g. sample through the riffle to a ca. 100 g. and immediately place in air-tight container. Weigh moisture sample immediately.

C. Procedure:

1. Weigh ca 5 g. of the sample into tared moisture dish.
2. Place the dish in the oven and dry at 101°C. for 2 hours.
3. Remove from the oven, cover immediately, cool in a descicator to room temperature and weigh.

D. Calculation:

$$\text{Moisture } \% = \frac{\text{Loss in weight x 100}}{\text{Weight of sample}}$$

APPENDIX I (continued)

B. A.O.C.S. Official Method Ba 3-38: Oil

A. Apparatus:

1. Butt type extraction apparatus.
2. Filter paper, 150 mm.
3. Absorbent cotton free of petroleum ether extract.
4. Porcelain mortar and pestle, The mortar must be at least 4 inches i.d. at the top, the pestle handle must be large enough to afford a firm hand grip. The inner surface of the mortar is kept rough by occassionally grinding sand in it.
5. Sieve, U.S. No. 20.
6. Laboratory mill suitable for grinding the sample to a max. particle size of U.S. No. 20 sieve.

B. Reagent:
1. Petrleum ether, A.O.C.S. Specification H 2-41.

C. Preparation of sample:

1. Grind the 100-g. portion from A.O.C.S. Official Method Ba-2-38, Section B, through the laboratory mill to a uniform fineness, ca 20-mesh. Return immediately to an air-tight container. Oil, ground moisture, and ammonia are determined on this portion.

D. Procedure: (a) Meal and ground cake.

1. Weigh ca 5 g. of the ground sample into a filter paper and enclosed in a second filter paper folded in such a fashion as to prevent escape of the meal. The second paper is left open at the top like a thimble. A piece of absorbent cotton may be placed in the top of the thimble to distribute the solvent as it drops on the sample.
2. Place wrapped sample in the Butt extraction tube and assemble the apparatus. Put ca 25 ml. of petroleum ether into the tared extraction flask before attaching to the tube.

3. Heat on a water bath or electric hot plate at such a rate that the solvent will drop from the condenser on the center of the thimble at the rate of at least 150 drops per minute.
4. Keep the volume of solvent fairly constant by adding enough to make up for any that may be lost due to evaporation. Continue extraction for 3 hours.

5. Cool and disconnect the extraction flask. Evaporate the ether on a steam or water bath until no odor of ether remains, A gentle stream of clean, dry air may be used to facilitate removal of the solvent. Cool to room temperature, carefully remove amy moisture or dirt from the outside of the flask and weigh. Repeat heating until constant weight is obtained.

6. Determine the moisture in the ground sample as directed in A.O.C.S. Official Method Ba 2-38.

D. Procedure;(b) Cottonseed meats.

1. Weigh accurately ca 2 g. of the ground sample and proceed as directed in (a) above, continuing the extraction for 2 hours only.
2. Remove the thimble from the Butt tube, allow the ether to evaporate from the filter paper and sample at room temperature. Then carefully transfer the sample to the mortar so that there will be no loss. Grind the sample in the mortar with the pestle for at least 1 minute or with 100 vigorous strokes. Use no abrasive.
3. Return the reground sample to the same filter paper and continue the extraction as before for 2 additional hours. From here on, proceed as directed in (a), paragraphs 4, 5, and 6.

E. Calculation;

$$\text{Oil, \%} = \frac{\text{Weight of oil} \times 100}{\text{Weight of sample}}$$

APPENDIX I (continued)

C. A.O.C.S. Official Method Ba 4-38
Nitrogen-ammonia-protein

A. Apparatus:

1. Kjeldahl digestion and distillation apparatus, complete with heat source, traps, and block-tin or equivalent non-corrosive tubing containers.
2. Kjeldahl flask, 800 ml.
3. Distillate receiving flasks, 500 ml. or any convenient size.

B. Reagents:

1. Murcury or mercuric oxide, A.C.S. grade.
2. Sulfric acid, sp. gr. 1.84.
3. Zinc metal, granular, 20-mesh.
4. Potassium or sodium sulfate, A.C.S. grade.
5. Potassium or sodium sulfide, 4 % solution in water.
6. Sodium hydroxide solution, sp. gr. 1.50.
7. Sodium hydroxide solution, o.25 N, accurately standardized.
8. Sulfric acid, 0.5 N. accurately standardized.
9. Mthyl red indicator solution, 0.1 % in ethyl alcohol or alizarin red S, 0.3 % in distilled water.

C. Procedure:

1. Use a sample prepared as directed in A.O.C.S. Official Method,Ba 3-38, Section C.
2. Weigh 1.7032 g. of sample into Kjeldahl flask. If calculation are carried out in term of N, weigh 1.4008 g. Add ca 0.5 g. mercury (0.7 g. mercury oxide), 10 g. of K_2SO_4 or Na_2SO_4 and 25 ml. of H_2SO_4.
3. Place the flask on the digestion rack (in an inclined position) and heat, below the boiling point of the acid, for 5 to 15 minutes, or until frothing ceases.
4. Increase the temperature and digest until digestion is complete. A good indication of this is

when the liquid becomes clear and colorless, but to be certain, heating should be continued for at least 30 minutes beyond this point.

5. Cool, add ca 300 ml. of water, a few granules of zinc to prevent bumping, and sufficient sodium sulfide solution (usually ca 25 ml.) to precipitate all of the mercury.
6. Transfer accurately a sufficient quantity of the standard acid into receiving flask so that there will be an excess of at least 0.5 ml. of 0.5 N acid. Add sufficient distilled water to cover the end of the outlet tube and attach to outlet end of condenser tube. The distillate should discharge through a glass tube at the bottom of the receiving flask.
7. Mix thoroughly and add sufficient alkali solution (usually 60 ml.) to make strongly alkaline. Pour alkali down the side of the Kjeldahl flask so that it does not mix with the acid at once.
8. Connect the Kjeldahl flaks to the other end of the condenser tube and miz the contents by shaking. Apply heat and distill until at least 150 ml. of distillate have been collected.
9. Titrate the content of the receiving flask with 0.25 N NaOH solution using 3 or 4 drops of indicator.
10. Conduct a blank determination on the reagents simultaneously with the sample and similar in all respects.
11. Determine moisture in the ground sample as directed in A.O.C.S. Official Method Ba 2-38 Section C.

D. Calculation:

$$\text{Nitrogen, \%} = \frac{(B-S) \times N \times 0.014008 \times 100}{\text{Weigh of sample}}$$

B = ml. of alkali back titration of blank
S = ml. of alkali back titration of sample

% nitrogen x 6.25 = % protein

APPENDIX I (continued)

D. A.O.C.S. Official Method Ba 5-47: Ash

A. Apparatus:

1. Porcelain combustion capsule, Coors No. 170, size No. 3 35-ml. capacity.
2. Electric muffle furnace with automatic pyrometer control to regulate at a temperature of 600° C. 15° C.
3. Desicator containing an efficient desicant.
4. Air-tight sample containers of convenient size.

B. Preparation of samples:

1. Use sample prepared as directed in A.O.C.S. Official Method Ba 3-38, Section C, paragraph 1.

C. Procedure:

1. Weigh 2 g. of well mixed sample into the previously heated and tared combustion capsule, place in muffle furnace previously heated to 600°C. and maintain at this temperature (15°C.) for 2 hours.
2. Transfer capsule to a desicator, cool to room temperature and weigh immediately thereafter.

D. Calculation : (Report to nearest 0.1%).

$$\text{Ash, \%} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

APPENDIXII

A. Estimation of Gossypol in Cottonseed Meal and Cottonseed Meats (F. H. Smith: Ind. Eng. Chem. 18 43, 1946)

A. Procedure:

The extraction is carried out with the Waring Blender using the small-size container, No. 17244, Central Scientific Company. The cardboard washer is removed from the screw cap and replaced with a washer cut from a sheet of rubber packing.

To a 2-gram charge of cottonseed meal placed in the Waring Blender container, add 20 ml. of 30 % (by weight) alcohol (384 ml. of 95 % alcohol diluted to 1000 ml.) and allow to stand for 10 minutes. Rotate the jar two or three times by hand during this period, Add 55 ml. of 72 % (by weight) alcohol (830 ml. of 95 % alcohol diluted to 1000 ml.) to give a mixture having an alcoholic content of 60 % by weight. After adding 15 ml. of peroxide-free ethyl ether, blend for 5 minutes. Stop the blender and rinse down the walls of the container by swirling once during the period of blending. The cap should be loose enough to permit the expanding vapor to escape.

After blending, remove the cap, swirl the jar to suspend the redisue, and pour into a 250-ml. beaker. Rinse the cap and jar with a stream of the alcohol-ether mixture (1000 ml. of 72 % alcohol by weight to 70 ml. of ether), used for washing and making dilutions, from a washing bottle and transfer the washing to a second beaker to be used to wash the first beaker and residue after filtering through a filter tube (Corning 9480). Insert the filter tube and a bent tube for the application of suction in a two-holed rubber stopper placed in the top of a bell jar.

Receive the filtrate in a 100-ml. volumetric flask, containing 5 ml. of ether, placed under the bell jar. This replaces that lost during the filtration and prevents a slight turbidity due to the separation of oil from the mixture. Wash the first beaker and residue with the washing from the blender jar and then a second time

with the alcohol-ether mixture from the was-bottle. Allow to cool and make to volume with the alcohol-ether mixture.

Use a charge of 0.2500 gram of cottonseed meats (or the protein) for the determination and mix the 20 ml. of 30 % and the 55 ml. of the 72 % alcohol before adding; otherwise a sticky paste is obtained which is not readily extracted. The 15 ml. of ether may be added directly to the jar. Blend the meats immediately, after which proceed as with cottonseed meal.

Transfer two 5-ml. aliquots to 25-ml. volumetric falsk. Dilute one of the aliquots to volume with 72 % alcohol containing ether (1000 to 70 ml.) to be used as the blank in reading the transmittance of the gossypol with the spectrophotometer. Add to the other aliquot 0.5 ml. of freshly distilled aniline and heat on the metal top of the steam bath for 40 minutes. Adjust the steam so that only a small amount of steam is escaping. (This heating may be done in a water bath at about 60°C.). Remove from the steam bath, add 5 to 10 ml. of the 72 % alcohol-ether mixture, and allow to cool. Then make to volume with the alcohol-ether mixture. Mix and read the intensity of color as per cent transmittance on the spectrophotometer at 445 mμ using the blank prepared from the extract. Take care to avoid contaminating the blank with aniline.

The weight of gossypol in milligram in 25 ml. may be scaled from the standard transmittance-concentration curve or read from the prepared conversion table.

B. Calculation:

$$\text{Concentration in mg. of gossypol/25 ml.} = \frac{2.000 - \log T}{4.846}$$

in which T is the per cent transmittance. A conversion table giving the milligrams of gossypol in 25 ml. corresponding to the per cent transmittance may be calculated from the equation.

The value obtained is the per cent of gossypol in cottonseed meal. In the case of cottonseed meats (or the protein) the milligram of gossypol found per 25 ml. times 8 gives the per cent of gossypol.

APPENDIX II (continued)

B. Spectrophotometric Method for Estimation Gossypol in Crude Cottonseed Oil (F. H. Smith: Ind. Eng. Chem. 18 41, 1946)

A. Procedure:

Filter the crude cottonseed oil under reduced pressure through a layer of washed Hyflo Super-Cel about 2 mm. thick placed over a disk of filter paper in a Hirsch funnel. (It is necessary to wash the Hyflo Super-Cel with hydrochloric acid to remove the traces of iron present, as iron destroys the gossypol. Boil 100 grams of Hyflo Super-Cel with 600 ml. of distilled water and 50 ml. of concentrated hydrochloric acid for 10 to 15 minutes, filter through a large Buchner funnel, and wash well with distilled water. Repeat the process and dry). Prepare the layer of Hyflo Super-Cel by pouring a suspension of the Super-Cel in Skellysolve F or B over the paper disk while suction is applied. Discard the first few milliliters of oil filtered. The oil may be collected in a test tube placed in the suction flask. Pipet 5 ml. of the filtered crude oil into a 100-ml. volumetric flask, wiping the outside of the pipet with a clean cloth before adjusting to the mark. After draining, rinse the pipet into the volumetric flask with a stream of Skellysolve B from a wash bottle. Make to volume with Skellysolve B. Transfer two 3-ml. aliquots to 25-ml. volumetric flasks. Dilute one of the aliquots to volume with Skellysolve B to be used as the blank in the spectrophotometric determination of gossypol. Dilute the other aliquot to 6 ml. with Skellysolve B and add 0.5 ml. of freshly distilled aniline (water white); then heat for 40 minutes on the metal top of the steam bath. Adjust the heating so only a small amount of steam is escaping from the bath. This cause only slight loss of Skellysolve B during the heating. This heating may also be done in a water bath at 60°to 65°C.

As the flasks are removed from the steam bath, add about 10 ml. Skellysolve B to prevent the aniline from separating. Allow to cool, then make to volume with Skellysolve B and mix. Read the percentage of transmittance on the Cloeman double monochrometer spectrophotometer at 440 mμ , using the aliquot diluted with Skellysolve B as a blank. Scale the weight of gossypol in the 25 ml. from

the standar curve or read it from the prepared conversion table. This value represents the weight of gossypol in the aliquot taken. The 3-ml. aliquot is equivalent to 0.15 ml. of oil. The specific gravity, 0.925, of cottonseed oil times the volume gives the weight of oil used in the determination (0.925 x 0.15 0.1388 gram).

B. Calculation:

$$\text{Concentration of gossypol in mg./25 ml.} = \frac{2.000 - \log T}{5.171}$$

in which T is the per cent transmittance. A conversion table giving the milligram of gossypol in 25 ml. corresponding to the per cent transmittance may be calculated from the equation.

The 3-ml. aliquot is equivalent to 0.1388 gram of oil. The per cent gossypol is found by multiplying the weight of gossypol found in the aliquot by 100 and dividing by the weight of oil used.

APPENDIX III

Determination of Color of Vegetable Oil
A Spectrophotometric Method
(Report of the Color Committee, A.O.C.S., May 1949)

A. Apparatus

1. Spectrophotometer
   a. Coleman Model 6-4 junior, or any other spectrophotometer with the same band width at the wavelength where the color measurement is to be made.
   b. The instrument must be capable of adjustment to give the following reading on the standardized nickel sulfate solution (3) after setting the zero point and after adjusting the (zero density) 100 % transmittance point to (zero density) 100 % transmittance against carbon tetrachloride in a type "B" 25 mm. cuvette.

400	millimicrons	less than 4 % transmittance
470	"	38.8 ± 2%
510	"	73.4 ± 2%
525	"	69.5 ± 2%
550	"	54.4 ± 2%
700	"	less than 2 %

2. Matched glass cuvettes, approximately 21.8 mm. inside diameter. Coleman type B 25 mm. cuvettes meets this requirement. All cuvettes should check within ±0.6 % transmittance at the 50 % transmittance level. The cuvettes should be kept clean and free from scratches.
3. Standardizing filter and standardizing nickel sulfate solution.
4. Filter paper, fine porosity such as E & D No. 192, Whatman No. 12, Reeve-Angel No. 871, or S & S No. 596.

B. Reagents

1. C.P. Carbon tetrachloride. If not water white, it should be redistilled.

C. Procedure

1. The sample must be absolutely clear. If not, filter through a speciied paper at a temperature of at least 10°C. above the melting point of the fat. The sample should not be held melted longer than necessary, since darkening may occur.
2. Turn on the spectrophotometer and allow at least a 20 minutes warm-up period before standardizing or making any measurement. Adjust the instrument to read the zero and 100 % transmittance points correctly at the calibration wavelength. Read the calibrating filter. If the correct reading for the filter is not obtained, adjust the instrument to give the exact reading for the filter following the manufacturer's instructions.
3. Set the wavelength scale to the desired wavelength.
4. Recheck the zero reading of the instrument, and with a cuvette filled with carbon tetrachloride in the instrument, set the 100 % transmission point exactly.
5. Fill a cuvette with the standardizing nickel sulfate solution and read the transmittance at 400, 470, 510, 550, and 700 millimicrons. The readings must fall within the reading prescribed.
6. Fill a cuvette with the sample using a sufficient amount of oil to insure a full column in the light beam.
7. Place the filled tube in the instrument and read the optical density to the nearest 0.001 from the scale at 525 millimicrons.
8. If the reading is above 0.700, dilute 10 ml. of the sample to 100 ml. with carbon tetrachloride, mix thoroughly, filter if necessary, and reread. Multiply the reading by 10.
9. If the dilute sample still has a reading above 0.700, dilute 10 ml. of the sample (CCl_4 mixture) to 100 ml. with more CCl_4. Mix thoroughly and reread. Multioly the reading obtained by 100.

D. Calculation

Red color = Density x factor

Factors: 77 for 550 millimicrons
42 for 525 millimicrons