over a 13-month period. We may speculate that one of the essential components for the reaction producing the fishy flavor is a highly unsaturated fatty acid, whether supplied by linseed oil in the form of linolenic acid or by clupanodonic and the higher unsaturated fatty acids of fish oil.

The differences in unsaturation and the amounts of oil present are greater in our experimental diets than would normally be expected in practical diets. However the very large effects produced in stability (induction period) and other characteristics raises the question as to whether economically significant effects may occur as a result of smaller but similar differences in dietary fat composition which might be expected in practical commercial rations. Long-term feeding and storage experiments are under way to test this possibility.

Summary

Groups of turkeys were fed, for eight weeks prior to slaughter, diets varying in the type of animal protein concentrate (fish meal vs. meat scraps) and varying in degree of unsaturation and kind of vegetable oil present (coconut oil vs. linseed oil). Chemical and organoleptic analyses of the fresh and stored carcasses established the following points:

Differences in fatty acid composition of dietary fat of turkeys have a marked effect on the fatty-acid composition of carcass fat and correspondingly play a decisive role in the storage life of the turkey carcass.

Typically fishy flavors and odors in roasted turkey meat, which can be caused by feeding fish (oil) products, can also be produced in the absence of fish products by a highly unsaturated vegetable oil, linseed oil. The fishy flavor is present in the roasted, freshly slaughtered turkey and apparently increases very little if any in intensity during storage.

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REFERENCES

1. Asmundson, V. S., Jukes, T. H., Fyler, H. M., and Maxwell, M. L., Poultry Science, *17*, 147-151 (1938).
2. Brice, B. A., Swain, M. L., Jour. Optical Soc. Amer., *35*, 532-544 (1945), and unpublished data.
3. Cruickshank, E. M., Biochem. Jour., *28*, 965-977 (1934).
4. Kummerow, F. A., Hite, J., and Kloxin, S., Poultry Science, *27*, 689-694 (1948).
5. Marble, D. R., Hunter, J. E., Knandel, H. C., and Dutcher, R. A., Poultry Science, *17*, 49-53 (1938).
6. Pool, M. F., Hanson, H. L., and Klose, A. A., Poultry Science, *29*, 347-350 (1950).
7. U. S. Department of Agriculture, Bureau of Animal Industry, Publication No. AHD-74 (mimeo.), "Effect of feeding certain fish products on the odor and flavor of roasted turkeys" (June, 1944).

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Solvent Extraction of Cottonseeds With Hexane and Water as Co-Solvents[1]

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COTTONSEEDS are one of the major oilseeds consumed in international commerce. Their annual production in the United States is currently about 6,500,000 tons, from which a commercial yield of crude cottonseed oil is obtained amounting to about 10.5% by weight of the seed (1). It is estimated that more than 90% of this crude oil is produced by pressing methods.

Since solvent extraction processes have proven economically superior to other methods in the production of certain vegetable oils, notably soybean oil, attempts have been made continuously, since the early commercial successes with oil extraction, to apply solvent methods to cottonseed oil production (2). Fabricators of extraction processing equipment estimated that in 1949 solvent extraction offered potential savings of $5.88 per ton of cottonseed processed in a plant of 200 tons/day capacity, brought about by higher oil yield, lower labor requirements, and lower maintenance (3). Despite these advantages it is estimated that no more than 5% of the annual production of crude cottonseed oil is obtained by solvent extraction. This low percentage is probably due to inherent difficulties in the solvent extraction of cottonseed as well as to normal delays attending the introduction of new methods in an industry already heavily invested in another technique.

The chief disadvantages encountered in the solvent extraction of cottonseeds are the difficulties of filtering low oil content meal and of removing the toxic pigment, gossypol, which is protected in the meal by a resistant gland wall. If left in extracted meal, to any appreciable extent, gossypol renders the residue unfit for stock feed, thereby turning a valuable by-product into waste. The wall of the pigment gland remains unattacked in the presence of most solvents, which rapidly remove the oil. It has therefore been necessary in most cases to employ two solvents and/or two steps; one to liberate the gossypol by rupturing its protective wall; and the other to extract both oil and gossypol.

Water has been proven effective in disintegrating the pigment wall rapidly (13). This investigation undertook to study the effects of treating cottonseed meal in a single processing step, using high speed agitation with water and a suitable oil solvent immiscible with water, to obtain ready separation of resulting oil and aqueous phases. The latter is expected to contain little or no oil.

[1] This paper is based upon a thesis submitted by Donald McNeil in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering, Columbia University, June, 1950. Contribution Number 10 from the Chemical Engineering Laboratories, Engineering Center, Columbia University, New York, N. Y.

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Review of Background Material

Gossypol. A distinctive characteristic of cottonseeds is the presence of a large number of pigment glands scattered throughout all the tissue of the kernel except the radicle. These glands are strong, more or less rigid cells of ovoid form, about 100 to 400 microns long, and contain as a gelatinous suspension most of the pigments of the seed, including all the gossypol and gossypurpurin (12). The physical and chemical nature of the glands have been investigated (13). The glands have sufficient mechanical strength that they can be recovered intact from the meal by flotation on a solvent of suitable density when the meats are disintegrated by a high speed stirrer (14).

The solubilities of the gland wall in various solvents have been extensively investigated (13, 15, 16). The wall is unaffected by most common organic solvents, including both aliphatic and aromatic hydrocarbons and chlorohydrocarbons, and triglycerides. A few water-miscible solvents such as the lower alcohols and ketones were found in some tests to rupture the gland wall after extended contact times up to 24 hours. In all of these cases however the action was found to have been caused by residual water in the solvents as rigorously dried solvents had little effect, even at elevated temperatures. However when the glands are treated with water, they rupture rapidly and the contents stream out (13).

Gossypol, which is the principal pigment of the cottonseed, constitutes 0.4-1.2% by weight of the kernel or meal (10). It is a complex polyphenolic dicarbonyl compound having the empirical formula, $C_{30}H_{30}O_8$, whose structure has not as yet been definitely determined (16). It has only a limited solubility in most solvents except the fatty glycerides and a few other oxygenated, organic solvents such as lower ketones, glycols, and ethers (4, 16). Gossypol can be extracted from the seed by most organic solvents however, by forming first a concentrated miscella in which the gossypol is soluble, then by diluting to dissolve the residual oil (4).

Gossypol is sufficiently toxic to animals to make meal containing it unfit for feed (21). However because of the tediousness and lack of specificity of analytical tests known until recently for determination of gossypol, little work has been done to correlate the toxicity of extracted cottonseed meals with their gossypol contents (22). One investigation of this question has shown however that when the free gossypol content of cottonseed meal is reduced to 0.071%, the meal is no longer toxic to young guinea pigs (23). More recently it has been reported (45) that no correlation exists between oral toxicity of cottonseed pigments and extracted gossypol-gossypurpurin contents of samples fed to fasted rats. Greatest detoxification, of the procedures tested, was caused by heating the pigment glands in the presence of water (45).

When gossypol is heated under conditions which rupture the pigment glands, it is changed in some way to "bound gossypol," which is no longer toxic (16, 17, 21). Earlier investigators postulated that the change was the result of combination of the gossypol with amino acids in the meal, but more recent workers have found that more complex but unknown reactions are also involved (16, 24). Gossypol is also detoxified by reaction with soluble iron salts, with which it forms an inert complex (9).

Gossypol is believed to be the chief natural antioxidant in cottonseed oil. It also inhibits saponification of the oil by the Twitchell process (4, 25). High gossypol content in the oil has also been found to produce more compact foots and lower losses during caustic refining (25).

Effect of Temperature on Crude Cottonseed Oil. When crude cottonseed oil is heated above a certain temperature, its dark red color is "fixed" so that it cannot be removed by refining (25, 26). Although discussed by many workers, the mechanism of this phenomenon is not thoroughly understood. The preponderant opinion among experienced investigators attributes the cause to the presence of gossypol in the oil rather than to alteration of the extra-glandular carotenoid pigments which are also present (4). Systematic investigation has shown that color fixation is not objectionable if the oil temperature is maintained below 150° F. during evaporation of the solvent (27). Thus it is desirable that a relatively volatile solvent, such as hexane, be used for the extraction to allow for its removal from the miscella at low temperature. It has been suggested that oxidizing agents be added to the crude oil. These are claimed to increase the color of the crude but to decrease the color of the bleached oil (4).

Effect of Cooking the Meal. In hydraulic processes for recovering oil from cottonseed the meal is millrolled, then cooked with live wet steam for about an hour at temperatures up to about 240° F. before the hydraulic pressing operation. It is believed that cooking ruptures the oil cells, increases oil fluidity, coagulates proteins, detoxifies gossypol, and precipitates phosphatides to give a lower refining loss (16, 23).

When solvent extraction processes were first introduced, the meal was usually cooked either before or after extraction (2, 3, 28). The action of heat and water breaks the pigment glands so that gossypol can be extracted by the oil solvent or be decomposed thermally. While gossypol is detoxified by heating extracted meal for 90 minutes at 120° C. with 15% moisture, or 30 to 60 minutes at 100° C. with 25% moisture, cooking has definite disadvantages (23, 29). If the meal is cooked before extraction, it must be dried again so that the oil solvent can wet it. This is difficult because of the hygroscopic nature of cottonseed meal (30). It is also claimed that cooking denatures the protein of the meal, thereby decreasing the nutritional value of the meal, and probably decreases the oil yield (26).

Previous Extraction Processes. A number of processes for the solvent extraction of oil from cottonseeds have been described in the literature. Most of these detoxify the gossypol by cooking the meal and extract the oil with conventional oil solvents (2, 31, 32, 33). One patent claims that a light-colored oil is obtained by extraction with 85% aqueous ethanol at 85° C. (31). Another process treats the meal with dilute ethanol to extract free fatty acids and color, then removes this solution before extracting the oil (34). A mixture of methanol or ethanol with benzene has been suggested as solvent (31). The pigment glands can be separated intact from the rest of the meal by comminution and differential settling before extraction (35). On treating cottonseed meal at 160-170° F. with 91% aqueous isopropanol, it is claimed that oil, gossypol, sugars, and phosphatides are extracted. On cooling the extract, the oil separates into an al-

TABLE I

Run No.	Charge Total Wt. g.	Ratio: Solv. / Meal	Ratio: Water / Meal	Extraction Time—Hrs. Pre-soak	Extraction Time—Hrs. Stir	Stirrer Speed r.p.m.	Temp.—°C. Rise	Temp.—°C. Max.	Time to Max. Temp. hrs.	Residual Oil Soxhlet % by wt.	Residual Glands % original	Meal Characteristics: Grains	Meal Characteristics: Sticky	Meal Characteristics: Filtration	Notes
1	901	1.38:1	1:5	0	0.5	1700	—	—	—	16.7[a]	10	Coarse	No	Plugged	Cake wet with miscella when filter plugged
2	901	1.38:1	1:5	0	0.25	5000	40[a]	62	0.25	17.9	90	Coarse	Yes	Slow	Meal not rolled before extraction Solvent boiled vigorously during extraction
3	622	1.38:1	1:5	0	4	4100	33[a]	55	1.5	15.7	5	Coarse	No	Slow	Miscella cloudy
4	622	2.75:1	2:5	1.5	4	4100	41[a]	63	2.0	4.02	0	Coarse	Yes	Slow	Miscella clear, dark brown color
F5	600	2:1	2:5	4	4	4100	22	47	4.0	4.30	0	Fine	Yes	Fast	
F6	600	2:1	2:5	0	4	4100	38	62	3.5	5.19	0	Coarse	Yes	Fast	
F7	600	6:1	2:5	0	4	4100	35	67	4.0	1.90	2	Coarse	Yes	Fast	
F8	600	2:1	1:5	0	4	4100	14	38	2.0	4.80	5	Fine	No	Slow	
F9	600	2:1	1:5	4	4	4100	17	41	2.0	6.59	0	Fine	No	Slow	
F10	600	6:1	1:5	0	4	4100	33	58	3.0	1.35	0	Fine	No	Fast	
F11	600	6:1	1:5	4	4	4100	29	55	1.5	1.18	0	Coarse	No	Fast	
F12	600	6:1	2:5	4	4	4100	35	60	4.0	0.85	0	Coarse	No	Fast	
13	600	6:1	1:5	0	4	410	0	23	—	10.55	100	No Change	No Change	Fast	Stirrer speed fluctuated
14	600	6:1	0	0	4	4100	38	60	4.0	2.26	50	Dusty	No	Fast	
15	688	6:1	1:1	0	2.5	4100	40	63	0.5	17.07	0	Coarse	Very	Plugged	Meal and water mixed before extraction. Filtration incomplete when filter plugged. Solvent boiled vigorously during extraction.

[a] Approximate value.

most pure miscella of cottonseed oil and isopropanol, and a water-isopropanol layer containing sugars, gossypol, and phosphatides (26). By using water and oil-miscible solvent as isopropanol or acetone, with recycling, a reduction of free gossypol in the meal and efficient recovery of neutral oil have been obtained (46).

Meal obtained from solvent extraction of decorticated seed is frequently very friable because of its low oil content. Consequently extracted meal tends to break up into fine particles which are difficult to remove from the miscella. These fines have been a major problem of extraction processes because they tend to plug conventional filters (3, 11).

Mechanism of Oil Extraction. According to recently reported work, the amount and composition of the material extracted by an oil solvent from oil seeds cannot be considered equal either to the original content of glycerides alone or to the total original lipid content (36, 37, 41). It is instead some empirical value between them. Since the total amount of "oil" extracted is a function of solvent, temperature, moisture in the sample, and heat treatment history of the sample, there can be no established method of analysis for total extractibles (36). The rate of extraction can be described best in terms of dissolved oil on the one hand, and static hold-up on the other hand, rather than in terms of rates of diffusion alone (36, 38). The undissolved oil is said to be held in the original material by a "resistance" which is only slowly soluble (36), but which is not re-established if extracted oil be returned to the meal. Thus the rate of solution of this part of the oil is stated to be relatively independent of the concentration of the solution performing the extraction, and data are reported to indicate this to be true up to an oil concentration of 20%. It was found however that the rate of solution of undissolved oil was doubled by soaking the meal before extraction, in either solvent or dilute miscella, for a length of time equal to the extraction time.

Since the experimental data leading to the above analysis were taken from stirred, as well as percolation, extractors, they should be applicable to this investigation which employed a high speed agitation cell.

Experimental Procedure and Details

A summary of the results of this investigation is presented in Table I.

Prime cottonseeds used for this investigation were donated by the Buckeye Cotton Oil Company, Memphis, Tenn. The samples used analyzed 3.88% moisture as determined by the AOCS method (39). Solvent used was normal "Amsco" hexane donated by The American Mineral Spirits Company, New York.

The hulls of the seeds were broken by passing them through a pair of power rolls set at 1/8 in. The rolled seeds were then screened through U. S. Std. 6- and 10-mesh screens. The +6 fraction, consisting largely of hulls and unbroken seeds, was discarded. The −10 mesh fraction, consisting largely of finely broken meats, was retained. The −6, +10 mesh fraction, consisting of a mixture of about equal proportions of meats and hulls, was separated by dry vanning on a flat pan. The combined fractions of meats were rolled to flakes 0.03 in. in thickness through hand rolls. Meal, thus obtained, contained a small amount of hull fragments, which darkened the oil extracted without otherwise affecting the results.

When the rolled flakes were examined under a low-power microscope with oblique lighting, the small, red, oblate pigment glands could be counted readily in the field of view as they were easily distinguished from the mass of light yellow meal. When a drop of water was placed upon the flake, the yellow contents of the pigment glands immediately started streaming out, and within five minutes the glands under view all appeared void of pigment.

In Runs No. 1-4 the extractor charge of flaked meal, "Amsco" hexane, and distilled water was measured into a one-liter Morton three-neck creased flask, fitted with a Morton high speed stirrer (47), a condenser, and a thermometer. The charges employed and the times and speeds of stirring used in the various runs are presented in Table I. Temperature was observed continuously and reported.

After extraction the contents of the flasks were filtered on paper of medium retentiveness (Whatman's No. 2) in a Buchner funnel under vacuum, and in several runs additional fresh solvent was passed through the cake while under vacuum. It is important to note that no water phase accompanied the miscella through the filter. The filter cake was soft and sticky at first, but after it had air-dried overnight it hardened to a horny, brown mass. Its oil content was determined by the Soxhlet method, and the number of residual pigment glands per unit field of view under the microscope was easily counted.

Studies of processes which involve many independent variables are usually based upon statistical techniques applied to many experimental runs. The effects however of three independent variables which interact on the dependent variables can frequently be evaluated reliably and more easily by means of a "factorial design" experiment, such as detailed in a recent paper (40). Thus, if a process can be reduced to three independent variables, for example by fixing the conditions of the other independent variables, a "factorial design" experiment can be planned. Then by choosing two limits to be tested for each independent variable, a set of eight runs is set up so that each value of every variable combines in one of the tests with both values of the other two variables.

In solvent extraction of cottonseeds using an agitation cell the rates of extraction of both oil and gossypol may be functions of a number of variables, including: solvent, time of presoak, time of extraction, stirrer speed, flake thickness, original moisture content of meal, ratio of meal to solvent, ratio of meal to water, and temperature. The solvent, time of extraction, stirrer speed, flake thickness, temperature, and original moisture in the meal were all maintained constant in our "factorial design" series of runs which are designated No. F5-F12 in Table I. The independent variables which were chosen and their limits are as follows:

Time of Presoak (in pure solvent) — zero and 4 hours
Ratio of Solvent to Meal (by weight) — 2:1 and 6:1
Ratio of Water to Meal (by weight) — 1:5 and 2:5

From the data of Run No. 4 it was known that a low value of residual oil could be obtained with no presoak by extracting four hours at 4100 r.p.m. Other work (36) has indicated that presoaking in pure solvent is as effective as extraction up to a presoak time equal to extraction time. Consequently the limits chosen for time of presoak were zero and four hours.

Limits for the ratio of solvent to meal were set at 2:1 and 6:1 in order to obtain miscella concentrations of from 5% to 15%, covering the range met in commercial practice (16).

The limits for the ratio of water to meal of 1:5 and 2:5 were based on the results of Runs No. 3-4 which show the lower limit to be adequate to rupture the pigment glands. The lower limit was doubled for an upper limit to find the effect of excess water on extraction rate and meal characteristics.

Weighed amounts of meal and "Amsco" hexane, in the desired ratios, were placed in the Morton creased flask and allowed to stand for the presoak period chosen. After the stirrer had been started slowly, distilled water was added in the desired weight ratio to the meal. The total weight was kept constant at 600 grams in order to keep agitation conditions as constant as possible. After the charge had been stirred for four hours at 4,100 r.p.m., it was filtered through Whatman's No. 2 paper on a Buchner funnel under vacuum. If the filtrate was cloudy, it was poured again through the filter cake to remove suspended fine particles of meal. After most of the miscella had been removed from the cake, the cake was washed with 100 ml. of fresh hexane. As much wash liquid as possible was recovered by pressing the filter cake. After most of the solvent had been filtered or had evaporated out of the meal, the cake was spread in a thin layer to dry overnight in the air. The air dried cake was analyzed for oil by the Soxhlet procedure and examined for pigment glands under a microscope.

Single experiments were also performed to find the effects of certain extraction conditions which were not changed in the factorial design experiment. In each run one change was made in the procedure of the factorial design experiment, and the results were compared with the most favorable obtained in the factorial design experiment. These runs are described in the following:

Run No. 13: Charge and extraction procedure were the same as in Run No. F10 except that the speed was held at about 410 r.p.m. Considerable difficulty was encountered in the control of stirrer speed because the motor was being operated far below its rated speed of 10,000 r.p.m.

Run No. 14: The charge consisted of a 6:1 ratio of solvent to meal and no water. Otherwise the procedure was the same as for Runs No. F7 and F10.

Run No. 15: 86 g. of meal were mixed for five minutes with 88 g. of water. Then 514 g. of hexane were added, and the mixture was stirred for one hour at 4100 r.p.m. The temperature rose rapidly to boiling. After one hour liquid had filled the closed condenser and was leaking out the top. The run was stopped at this point. Upon an attempted filtration of the mixture the sticky meal clogged the filter as soon as it was covered with meal. After washing, it was impossible to remove the miscella from the meal. The meal was then spread in a thin layer on the top of the bench to dry for two days before analysis.

In order to find the number of pigment glands left in the extracted meal, a sample was examined under a low power microscope illuminated by strong, oblique, white light. Under these conditions the orange-red pigment glands could be distinguished clearly from the yellow-brown meal. The number of glands visible under the field of view was compared with the number in a sample of untreated meal.

Gossypol analyses on cottonseed meats or air-dried, extracted meal were carried out by the method of Pons and Guthrie (20) using *p*-anisidine reagent, as tentatively adopted by the AOCS for the determination of total gossypol and gossypol-like substances in cottonseed materials.

There is no accepted method of determining the total amount of oil in a sample of cottonseed meal (36). A convenient method, which gives consistent results approximating the amount of salable crude oil in the sample, was chosen as follows:

Duplicate accurately weighed 20 gm. samples of air-dried meal were extracted for 16 hours with commercial grade hexane in a Soxhlet extractor. The extract was dried to constant weight at 105° C. to remove hexane and weighed. The result was reported as oil.

The results of this method compared favorably in a trial test with those to be obtained by the official method of the AOCS (39).

Discussion of Results

The charge for Run No. 2 consisted of cottonseed grits which were not flaked before extraction. The solvent in the extractor was boiling vigorously at 62° C. within 15 minutes, and after extraction the meal still contained 17.9% of oil (47% of original amount) and 90% of the original number of pigment glands. From these results it may be concluded that flaking of the meal before extraction is necessary because of the high residual oil, high gland concentration, and large temperature rise generated when coarse meal was used.

A ratio of water to meal of 1:5 was used in Runs No. F8 through F11. In Run No. F8, 5% of the original number of pigment glands remained unbroken, and in the other three runs all of the pigment glands were broken. In Run No. F8 the maximum temperature reached was 38° C. while in the other three runs the maximum temperature ranged from 41° C. to 58° C. From these results it is apparent that a water to meal ratio of 1:5 is adequate to break all of the pigment glands if the operating temperature is sufficient.

An increase in the ratio of water to meal in the extractor from 1:5 to 2:5 increased the stickiness and granular appearance of the meal. This was emphasized by the extremely sticky quality of the meal obtained in Run No. 15 in which a water ratio of 1:1 was used. In Runs No. 1 and 15 where the filter was covered with wet meal, the filter plugged. From these data it can be seen that any difficulties in filtration in the event of use of this process will stem from the soft and sticky character of the meal, caused by the use of an excess of moisture to break the pigment glands during extraction, rather than from the finely divided meal particles which have been encountered by other workers who use anhydrous solvents. It is apparent however that such difficulties can be avoided by employing the correct amount of water.

At water to meal ratio of 1:5 the meal, during stirring, broke into a fine powder which however was sticky enough to form a firm cake when it was filtered. At a water ratio of 2:5 the meal became soft and sticky rather than breaking; therefore the energy required for agitation, as measured by the temperature rise, increased. The drier meal was reduced to a powder during stirring in an average time of 2.1 hours and thereafter was easier to stir than the wetter meal, which was coarser and stickier. When a water ratio of 1:1 was used in Run No. 15, the temperature rose to boiling in half an hour. It also appears that meal can absorb at least an equal weight of water because the filtrate was clear in all experiments. Thus the expectation, defined in our objective given above, of obtaining an aqueous phase was eliminated with these results.

Average values from the factorial design experiment (Runs No. F5 through F12), are as follows:

Water/Meal	Residual Oil	Max. Temp. Rise
1/5	3.48%	26° C.
2/5	3.06%	33° C.

From those data it might appear on superficial examination that under the conditions of the test the addition of a moderate amount of water to the meal increased somewhat the amount of oil removed. However it is more probable that the observed increase in temperature of 33° C. as compared to 26° C. accounts for the increased oil extraction since the rate of extraction of oil is known to increase with temperature.

According to the mechanism for oil extraction as presented above, concentration of oil in the extracting liquid (up to 20%) should have little effect on the rate of extraction (36). The results of this investigation however indicate that when the ratio of solvent to meal was increased from 2:1 to 6:1, the percentage of oil left in the meal decreased from 5.22 to 1.32 on the average. Thus, although our miscellae had oil contents in all cases well below 15%, we found that the ratio of solvent to meal, hence the ratio of solvent to oil in the miscella, was the most significant variable in determining the amount of residual oil left in the meal, as would be predicted from the diffusion theory, employing concentration as the driving force. It is our opinion that the high speed stirring which we obtained in our agitation cell flask was sufficient to assure frequent enough contacts at the oil cell-miscella interfaces, by reason of the extremely rapid circulation and strong shearing action which existed at 4100 r.p.m., so that the thickness of the stagnant film between the oil cell and the miscella was maintained at much smaller values than those which existed in previously reported experiments. It should also be noted that each oil cell in the meal is probably surrounded by lyophobic material. In consequence, as more and more oil is extracted from the meal, the remaining oil cells must shrink and present smaller and smaller interfaces which are therefore more easily protected by neighboring lyophobic molecules at the same time that the path of penetration for the solvent-rich miscella phase is lengthened through deeper layers of the repelling, lyophobic molecules. The high degree of agitation in our experiments would more easily overcome the intramolecular repulsions than would either percolation or less highly stirred systems, allowing more frequent contacts between solvent and dissolved oil. Thus, provided the equipment is designed to produce adequate circulation and shearing forces, it is our conclusion that solvent extraction of cottonseed oil proceeds according to the normal laws of diffusion.

In the factorial design experiment, Runs No. F5-F12, the average percentage of oil in the meal from four runs in which the meal was not soaked in hexane before extraction was 3.23% while the average from four runs in which the meal was soaked for four hours in hexane was 3.31%. Thus presoaking the meal in solvent had little effect on the amount of oil or pigment glands left in the meal. It will be recalled that data obtained from experiments with much less agitation (36) indicated that soaking for half the total extraction time was found as effective as extracting with fresh solvent. Our results differ not only because the rapid agitation maintained the meal as fine particles in constant suspension, thereby keeping stagnant films thin, but also again because the extreme turbulence in the flask would tend to maintain very steep concentration gradients by overcoming the repulsive forces to the lyophobic molecules in the meal.

In view of our results under conditions of strong turbulence it is our opinion that both the data and the mechanism of extraction presented in the Review

of Background Material can be better explained in terms of the action of diffusional forces in light of the concept of intramolecular repulsion between lyophobic molecules in the meal, such as absorbed water, and solvent molecules in the surrounding liquid rather than in terms of a difficulty dissolving "resistance" (36) which protects the oil in the meal. Thus the observation that returning the oil to extracted meal does not re-establish the "resistance" can be interpreted in the new terms to mean that the returning oil molecules would tend to align themselves in the meal, probably in smaller groups, in such fashion as to avoid repulsions from the lyophobic molecules which are still present. In consequence, the solvent phase would meet less repulsion on redissolving the oil, giving the appearance of absence of the resistance originally postulated.

It was found that filtration was faster when the higher ratios of solvent to meal were uesd, presumably because of the lower viscosity of the more dilute miscella. In Runs No. F5-F12 the temperature rose 10 degrees more when a solvent to meal ratio of 6:1 was used than with a ratio of 2:1. This also is attributed in part to the lower viscosity which resulted in greater turbulence at constant stirrer speed, causing more internal friction. In addition, the charges of given weight which contained more solvent would have considerably lower heat capacities, which, added to increased internal friction accounts for the higher temperature rises.

In Runs No. 1 and 2 (extraction times ½ and ¼ hour respectively) the oil content of the meal was reduced comparatively rapidly to about 17%, even under unfavorable conditions of extraction, such as a coarsely divided sample. A comparison of the result of Run No. 2 (residual oil 17.9%) with those of Runs No. F5-F12 (extraction time 4 hours; average residual oil 3.3%) shows that the rate of extraction decreases under our conditions with increasing time, lending further support to the concept of the increasing repulsive effect of absorbed water molecules as the required depth of penetration for the solvent is increased.

The effect of speed agitation can be seen from the following data, which are based on a solvent to meal ratio of 6:1 and a water to meal ratio of 1:5:

Run No.	Stirrer Speed (r.p.m.)	Residual Oil (% by weight)	Residual Glands (% original no.)
F10	4100	1.35	0
F13	410	10.55	100

Comparing these data it is obvious that extraction in the presence of water was not effective at the low stirrer speed of 410 r.p.m. While the oil content of the meal was reduced from 30 to 10%, the glands were unaffected. Evidently in presence of an excess of oil solvent, such as hexane, water is excluded at low degrees of turbulence from the meal, but at a high degree of turbulence it is well dispersed and readily wets and adheres to the meal, leading rapidly to rupture of the pigment glands. The possibility that the higher state of turbulence may have contributed to rupture of the pigment glands is discarded in view of other work (35) which has demonstrated that they can be collected intact from hexane under high-speed stirring.

Determinations of the oil contents of two samples by the soxhlet and the official AOCS (39) procedures gave the following results:

Sample	Soxhlet	AOCS
Unextracted flakes	31.7%	33.9%
Extracted Meal of Run No. F11	1.18%	0.66%

From these data it is our opinion that the Soxhlet results were close enough to those obtained by the official method to make the former method acceptable for our work.

Determinations of pigments, glands and of gossypol may be summarized as follows:

Sample	Glands (% of original)	Gossypol (% by weight)
Unextracted Flakes	100	1.23
Extracted Meal of Run No. F8	5	0.48

From these data it is apparent that after 95% of the pigment glands were broken, 26% of the original gossypol-like substances remained on the meal, and thus gossypol adsorbed on the tissue of the meal sample was about 0.41% of the air-dry weight of the extracted meal. Since this analytical method is so new, no studies have been published relating its results to toxicity of extracted meal. However hydraulic-pressed meal containing 0.2% gossypol, as determined by this test, is sold as stock food (42) Thus the tolerable concentration of gossypol and related substances is at least 0.2%.

Since gossypol is soluble in cottonseed oil, though not in hexane, it was assumed at the beginning of the investigation that once the pigment glands were broken, the gossypol would dissolve in the miscella. That this did not occur completely is probably due to the low concentration of oil in the miscella (5-15%).

No difficulty was encountered in removing the fines from the miscella by filtration. The addition to the meal of 1 part of water per 5 parts of meal caused the fine particles of meal to cling together, yet to provide a filterable cake. Some fine particles usually came through the filter at the beginning of filtration, but these were always removed when the filtrate was poured through the filter cake again after the filter was coated with meal. Thus one of the major difficulties of present solvent extraction processes was avoided.

In concluding the discussion, some appraisal is offered of industrial applications of the findings of this investigation. While our work was exploratory in nature, the importance of a high degree of turbulence and finely divided meal, produced in our case by high speed stirring, is apparent in our results. Industrially, this might be provided by one or more stages of a properly selected colloid mill, disk mill, or rotary pump, to mix intimately meal, water, and hexane. In accordance with our findings the meal would be slurried in the solvent, and the slurry and water would be proportioned on feeding to shearing-mixing stages. Obviously the four-hour period arbitrarily used in our work is unsatisfactory for industrial application, but further work with improved equipment is expected to yield substantial improvement in this factor. The virtual absence of pigment glands in meal properly treated with water and the improvement in filtering and handling char-

acteristics of such meal indicate strong industrial potential for greater use of water in solvent extraction of cottonseeds. The absence of the glands moreover indicates that any gossypol remaining in the extracted meal is only adsorbed and thus in all probability is readily removable either by washing the meal with concentrated miscella or with crude cottonseed oil while caked in a filter press, or by designing the extraction stages to employ the more concentrated miscella for the latter stages.

Also, although the extracted meal was wet with both water and hexane after filtration, the water would not decrease the vapor pressure of hexane since the two liquids are not miscible. Hence the presence of water on the meal should cause no difficulty in the recovery of the solvent from the meal by evaporation. If some water evaporates with the hexane, it can be separated easily from the latter by decantation. And, assuming that the adsorbed gossypol in the meal can be removed as noted above, it would not be necessary to dry the meal beyond the drying accomplished during removal of the hexane as it handles easily and would be directly suitable as a stock feed.

Finally, in view of the discussion regarding intramolecular repulsions due to lyophobic molecules and of the applicability of the laws of diffusion to cottonseed oil extraction, if equipment design encompasses this obstacle, our work is believed to be applicable in its generalized aspects to oil solvents other than hexane.

Further experimental work is currently in progress which proposes to test or to extend the conclusions and the conjectures presented. This work will be reported at a later date.

Summary

By stirring violently for four hours at 4,100 r.p.m. a mixture of dehulled and flaked cottonseed meal, hexane, and water, with respective hexane/meal and water/meal weight ratios of 6:1 and 1:5, all the pigment glands of the seed are broken, the oil content of the meal is reduced to about 1%, and a miscella phase only, free of water, is produced as all the water added remains with the meal. Extracted meal thus obtained is easily filtered from the miscella and air-dries to an easily handled, non-sticky cake. If the water is increased in the mixture to a water/meal ratio of 2:5, other results remain about the same, but the meal is excessively sticky and is difficult to filter from the miscella. Pre-soaking the meal in solvent before extraction has little or no effect, under our conditions of extraction, on the amount of oil left in the meal.

It is proposed that solvent extraction of cottonseed oil proceeds according to the normal laws of diffusion. Rapid solvent action is believed to be hindered not by presence of a difficulty dissolving "resistance" proposed in other work but rather by presence in the meal of lyophobic molecules, such as absorbed water, which offer strong intra-molecular repulsion to solvent penetration. In consequence it is necessary to provide strong shearing action and turbulence to promote frequent contacts between the oil cell and the miscella.

Commercial applications of results are discussed.

REFERENCES

1. U. S. Dept. of Agriculture, Annual Report (1949).
2. Moore, N. H., Food Industries, *19*, 471 (1947).
3. Bilbe, Oil Mill Gazetteer, *51*, No. 1, 18 (1949).
4. Boatner, C. H., *et al.*, J. Am. Oil Chemists' Soc., *24*, 276 (1947).
5. Carlin: "Ultrasonics," New York, McGraw-Hill Publishing Co. Inc., 1949.
6. Rayleigh, J. W., Phil, Mag., VI, *34*, 94 (1917).
7. Sollner, K., Chem. Reviews, *34*, 371 (1944).
8. Shaw, J. L., (Development Dept., International Nickel Co. Inc.) private communication, Nov. 7, 1949.
9. Olcott, H. S., J. Am. Oil Chemists' Soc., *25*, 125 (1948).
10. Jamieson, G. S., "Vegetable Fats and Oils," 2nd Ed., New York, Reinhold Publishing Corp., 1943.
11. Cole, L. U., Oil Mill Gazetteer, *51*, No. 1, 16 (1949).
12. Boatner, C. H., and Hall, C. M., Oil & Soap, *23*, 123 (1946).
13. Boatner, C. H., *et al.*, Botan. Gaz., *108*, 484 (1947).
14. Gastrock, E. A., *et al.*, J. Am. Oil Chem. Soc., *24*, 228 (1947).
15. Boatner, C. H., Botan. Gaz., *109*, 76 (1947).
16. Bailey, A. E., "Cottonseed and Cottonseed Products," New York, Interscience Publishers, 1948.
17. Boatner, C. H., *et al.*, J. Am. Oil Chem. Soc., *24*, 97 (1947).
18. Kirschenbauer, H., "Fats and Oils," New York, Reinhold Publishing Corp., 1944.
19. Halverson, J. O., and Smith, F. H., Ind. Eng. Chem., (Anal. Ed.), *13*, 46 (1941).
20. Pons, W. A., and Guthrie, J. D., J. Am. Oil Chem. Soc., *26*, 671 (1949).
21. Gastrock, E. A., *et al.*, *Ibid.*, *25*, 345 (1948).
22. Gossypol Analysis Committee, American Oil Chemists' Society: *Ibid.*, *25*, 249 (1948).
23. Lyman, C. M., Holland, B. R., Hale, F., Ind. Eng. Chem., *36*, 188 (1944).
24. Boatner, C. H., *et al.*, J. Am. Oil Chem. Soc., *26*, 28 (1949).
25. Royce, H. D., and Lindsey, F. A., Ind. Eng. Chem., *25*, 1047 (1933).
26. Bishop, F. F., *et al.*, J. Am. Oil Chem. Soc., *24*, 370 (1947).
27. Gastrock, E. A., *et al.*, Ind. Eng. Chem., *38*, 635 (1946).
28. Goldovski, A. M., and Podolskaga, M. Z., Masloboino Zhirovoe Delo, *14*, 45, 9 (1938), [Chem. Abs., *33*, 5688 (1939)].
29. Adamova, A. A., and Lebedeva, M. A., Gigiena i Sanit., *12*, No. 7, 33 (1947), [Chem. Abs., *42*, 2033 (1948)].
30. McInnes, C. C., (American Mineral Spirits Co.), private communication, Nov. 3, 1949.
31. Rewald, B. A., U. S. Patent 1,917,734 (1933).
32. Rosenthal, Henry, U. S. Patent 2,254,245 (1941).
33. Duncan, I. J., J. Am. Oil Chem. Soc., *25*, 277 (1948).
34. Bollman, H., U. S. Patent 1,653,201 (1927).
35. Vix, H. L., *et al.*, J. Am. Oil Chem. Soc., *25*, 526 (1949).
36. Coats, H. B., and Karnofsky, G., *Ibid.*, *27*, 51 (1950).
37. Karnofsky, G., *Ibid.*, *26*, 564 (1949).
38. Osborn, J. O., and Katz, D. F., Trans. Am. Inst. Chem. Engrs., *40*, 511 (1944).
39. American Oil Chemists' Society, "Official and Tentative Methods of Analysis," 2nd Ed., Chicago, (revised to 1949).
40. Gore, W. L., Ind. Eng. Chem., *42*, 320 (1950).
41. Ayers, A. L., and Dooley, J. J., J. Am. Oil Chem. Soc., *25*, 372 (1948).
42. Lauro, M. F. (chief chemist, New York Produce Exchange), private communication, April 13, 1950.
43. Richards, P., (Enjay Company Inc., New York City), private communication, Nov. 7, 1949.
44. Anon: Business Week, November 19, 1949.
45. Eagle, E., *et al.*, J. Am. Oil Chem. Soc., *27*, 300 (1950).
46. Harris, W. D., and Hayward, J. W., *Ibid.*, 273 (1950).
47. Morton, A. A., and Redman, L. M., Ind. and Eng. Chem., *40*, 1190 (1948).

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