

# Countercurrent Extraction of Raw Cottonseed Flakes with the Acetone-Hexane-Water Azeotrope

Vernon L. Frampton, Armand B. Pepperman, Jr., Joseph Simmons, and W. H. King

Stepwise countercurrent extraction of oil from raw cottonseed and soybean flakes at room temperature with the ternary azeotrope (b. 49° C.) composed of acetone, hexane, and water was shown to follow the dilution law, and residual crude oil in the finished meals was reduced to less than 0.5% in 3 minutes or less. Marcs and miscellas were separated by screening through a vibrating screen or by centrifugation. The oil content,  $L$ , of the marc produced at step  $P$

in the countercurrent extraction with a solvent-to-flakes ratio of  $r$ , where the centrifuge was used to separate marc and miscella at a centrifugal force  $F$ , is described by the equation,  $\log L = d - P(lr + k \log F)$ , where  $d$ ,  $l$ , and  $k$  are constants which are dependent upon the boundary conditions. Analyses of the finished cottonseed meals indicated low levels of gossypol.

Cottonseed meals are used in the United States chiefly as cattle feed, although some meals find their way into feed mixtures for broilers, laying hens, and swine. The volume of cottonseed meals of commerce used in feeding nonruminants, however, is relatively small, chiefly because their protein efficiencies are low in comparison with soybean proteins used in commercial feeds and because of the presence in the meals of gossypol and malvalic acid.

Cottonseed meals and flours of exceptionally high nutritive quality and which have good potential as protein concentrates for nonruminant animals, including man, can be prepared. Such meals have a potential outlet in the higher priced market as high quality protein supplements for swine and broiler production, especially in the cotton-producing area of the United States where other protein concentrates equivalent to 1,200,000 tons of cottonseed meal are used annually for swine production and the equivalent of 1,400,000 tons of cottonseed meal is used for broiler production. This paper is concerned with the production of such meals and flours.

Emphasis is placed here on the extraction of raw cottonseed flakes, or on raw comminuted cottonseed kernels, in contrast to cooked flakes which are normally used in oilseed processing operations. Cooking of cottonseed flakes to render the oil in conventional processing operations destroys a part of the lysine of the seed proteins and causes a portion of the lysine that escapes destruction to be bound in the meal in a manner that prevents its use by the nonruminant (Frampton, 1965). Cottonseed meals must have high levels of available lysine (available to the nonruminant) because the available lysine is the limiting factor in determining the rates of growth and efficiency of feed utilization by growing animals receiving conventional cereal-cottonseed meal rations; the rates of growth are directly proportional to the available lysine contents of the cottonseed meals. The levels of available lysine in conventional screw-press cottonseed meals range from about 2.2 to 3.5 grams per 16 grams of meal nitrogen; it ranges from about

2.9 to 3.7 in prepress solvent meals, and from about 3.1 to 3.8 in hexane-extracted meals (Frampton, 1965), with average values of 2.9, 3.4, and 3.4, respectively. Available lysine in meals prepared in the research reported here range from about 4.2 to 4.4 grams per 16 grams of meal nitrogen. Cooking of cottonseed flakes to render the oil in conventional commercial cottonseed processing operations also causes the binding of gossypol in the meal, and levels of gossypol and gossypol derivatives (reported as "total" gossypol) in conventional meals range from about 0.8 to 1.5%. Gossypol and gossypol derivatives in cottonseed meals at relatively high levels are deleterious. More recent work has revealed that gossypol from such dietary meals accumulates in the livers of swine (Smith, 1963).

The presence of crude cottonseed oil as a residuum in the meal also reduces its nutritive quality because of the presence of malvalic acid as a component of the triglycerides of cottonseed oil. Malvalic acid in sufficiently high concentrations causes a modification of fat metabolism (Reiser, 1964) and also induces abnormalities in eggs produced by hens receiving cottonseed products (Frampton *et al.*, 1966).

For some unexplained reason, rates of growth of young animals are correlated negatively with the quantity of residual crude oil in cottonseed meals ingested (Bressani, 1965).

Evidently cottonseed meals of high nutritional quality must have high levels of available lysine, low levels of gossypol and gossypol derivatives, and low levels of residual crude oil. A cottonseed meal that meets these requirements can be prepared on the extraction of raw cottonseed flakes, or comminuted kernels, with the ternary azeotrope (b. 49° C.) composed of hexane, acetone, and water (King and Frampton, 1960). Meals prepared under conditions reported in this paper have low levels of gossypol derivatives and residual crude oil. Meals prepared on extracting cottonseed flakes with the solvent mixtures are highly nutritious, having protein efficiency ratios that range from 2.5 to 2.9 (Johnston and Watts, 1964).

Moreover, oils recovered on the extraction of raw cottonseed flakes with this solvent are of excellent quality. King and Frampton (1961) showed that the oil is readily

Southern Regional Research Laboratory, New Orleans, La.

amenable to refining and to bleaching to good color by conventional refining and bleaching methods.

Of particular significance is the speed with which the oil is rendered and with which the extraction process may be carried out. The residence time of the marc in the entire operation for essentially quantitative oil extraction need not exceed 180 seconds, as demonstrated by the data reported here. Of added interest are the larger yields of neutral oil, as compared with oil yields obtained by conventional commercial operations (King *et al.*, 1961).

#### METHODS

Oil and "free" gossypol contents of the flakes and marcs were determined by methods described by the American Oil Chemists' Society (1960); "total" gossypol was determined by the methods described by King and Thurber (1953) and by Pons *et al.* (1958). Water in the marcs was determined by the toluene distillation method.

The azeotropic mixture of acetone, hexane, and water (b. 49° C., flash point -20° C.) was prepared by distilling a mixture of acetone, water, and hexane.

Prime cottonseed was hulled in conventional hulling equipment. The moisture of the kernels was adjusted to 12 to 15%, and the kernels were then flaked to 0.006- to 0.01-inch thickness by smooth spring-loaded rolls. This was the only preparation the flakes received.

Stepwise countercurrent extraction schedules were set up. The preparation of the miscellas for the several extraction schedules is illustrated in Figure 1, where the details are given for the preparation of miscellas for a five-step schedule with a solvent-to-flakes ratio of 2 to 1 by weight. Fresh flakes in step I were successively extracted five times with fresh solvent. The miscellas produced moved down one step to the left to be used in step II. The product miscella in step I had a lower oil content than the

product miscella from step II, which in turn had a lower oil content than the product from step III, etc. The fresh flakes used in the first extraction of each step moved to the right and became successively Marc 1, Marc 2, Marc 3, and finally Marc 4, which was ready for desolventization. In step II, fresh flakes were washed successively with miscellas 4, 3, 2, 1, and fresh solvent. The schedule indicated was followed until the adjusted miscellas 1, 2, 3, and 4 were obtained. The same scheme was followed in the preparation of miscellas for other extraction schedules—5<sup>2</sup> extractions were carried out in the preparation of miscellas for a five-step schedule, 10<sup>2</sup> for a 10-step schedule, and 3<sup>2</sup> for a three-step schedule.

The adjusted miscellas were used in the stepwise countercurrent extractions as illustrated in Figure 2. Note the counterflow of marc and miscella where fresh flakes are in contact with oil-rich miscella and the spent flakes are in contact with fresh solvent.

An 18-inch circular vibrating screen separator (Southwest Engineering Co.) equipped with a 165-mesh stainless steel screen was used in one phase of the investigations. Two basket centrifuges, one electrically and one air-driven, were also used in the separation of marc and miscella.

#### EXPERIMENTAL RESULTS AND DISCUSSION

**Rendering of Oil.** The speed with which oil is rendered by the azeotrope is illustrated by the results obtained from the following experiment. Eighty grams of raw cottonseed flakes (31.5% oil, 1.18% total gossypol, and 11.5% moisture) were weighed into the bowl of basket centrifuge, together with 240 grams of the azeotrope. The contents of the bowl were then commixed for 30 seconds before they were separated centrifugally at 1420 G. Ten seconds were consumed by the centrifuge in gaining the desired speed, and the centrifugation was continued for 60 seconds.

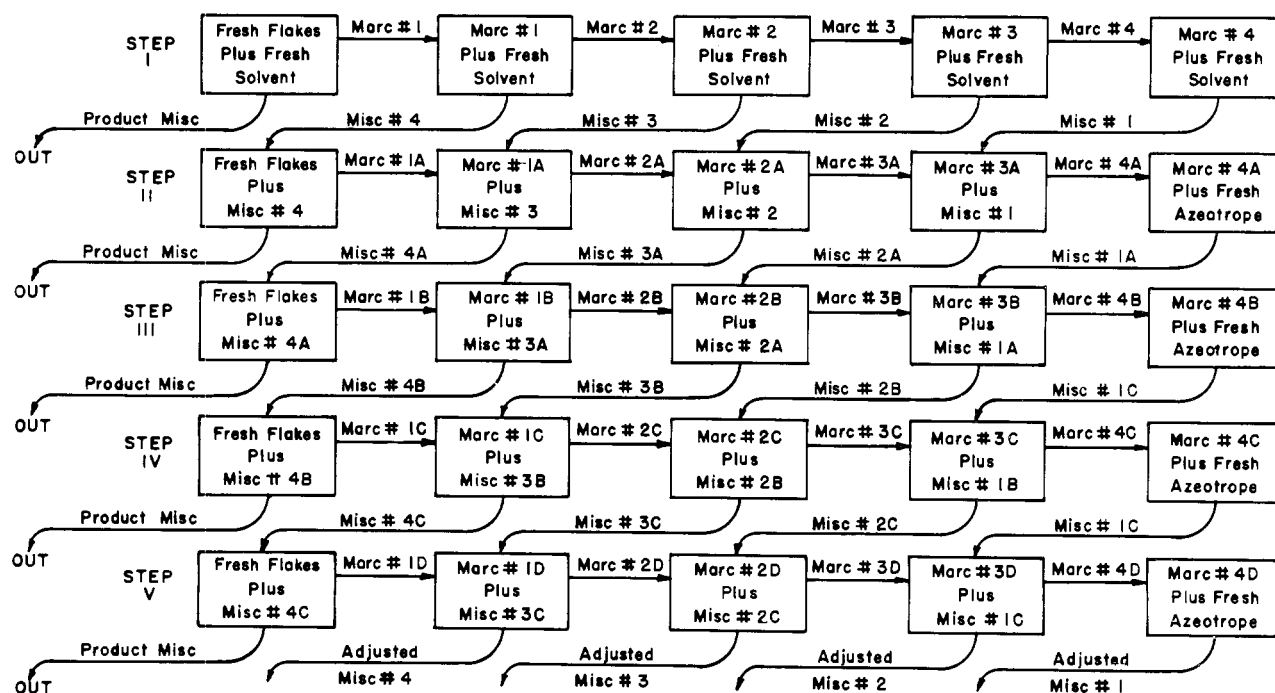


Figure 1. Method of preparation of miscella for a five-step countercurrent extraction of cottonseed flakes with the acetone-hexane-water azeotrope

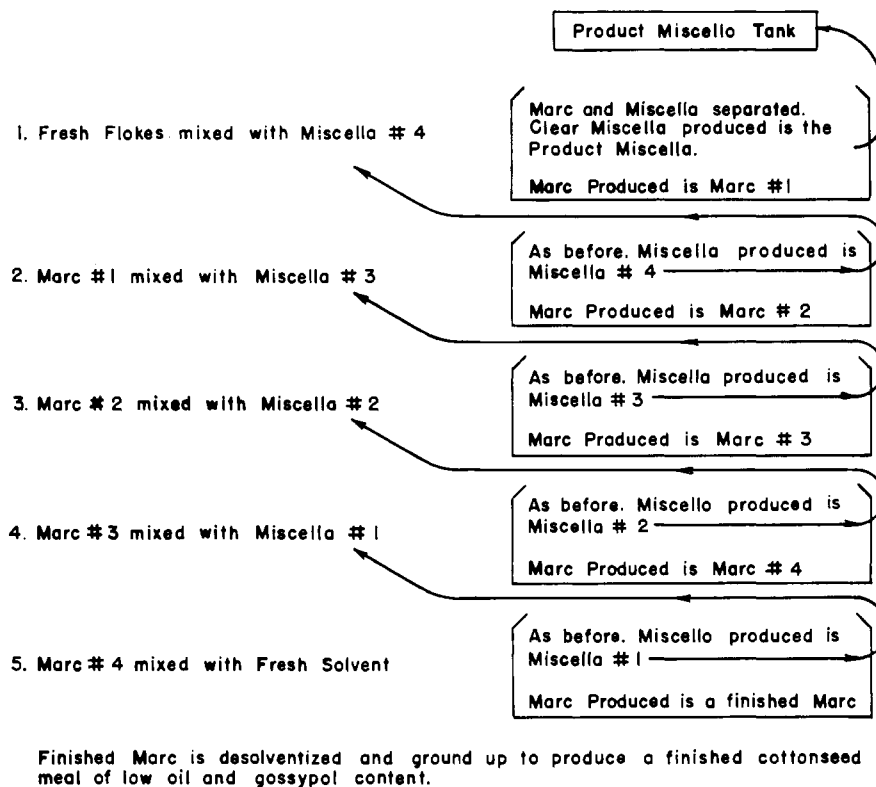


Figure 2. Stepwise countercurrent extraction of raw cottonseed flakes

The optically void supernatant liquid was decanted and the oil content of the marc (air-dry basis) was determined. This experiment was repeated, but the time of commixing was extended to 60 seconds before marc and miscella were separated. The oil content (air-dry basis) of this second marc was also determined. Additional data were obtained following this same pattern where the period of commixing of flakes and fresh solvent was extended to 2, 3, 5, 10, and 15 minutes, respectively. The oil data are plotted in Figure 3, curve *M*, against the time of commixing.

The experiment described immediately above was altered. Fresh flakes (80 grams) were commixed with 240 grams of the azeotrope for 30 seconds and the marc and miscella were separated centrifugally, as before. The optically void miscella was decanted, and a fresh lot of 240 grams of fresh azeotrope was added to and commixed with the marc for 30 seconds, and the marc and miscella were separated centrifugally. The oil content of the marc (air-dry basis) was determined. The experiment was repeated, where the two periods of commixing were extended to 60 seconds each, to 2 minutes, 3 minutes, etc. The oil contents of marcs (air-dry basis) obtained in this double exposure of fresh solvent are plotted (curve *N*) in Figure 3.

The experiment described immediately above was repeated, but the second period of commixing was limited to 10 seconds. For example, the solvent-damp marc obtained from the initial commixing period of 5 minutes was commixed with a second lot of fresh solvent for 10 seconds before marc and miscella were separated centrifugally; the solvent-damp marc obtained from the initial commixing period of 2 minutes was commixed with a second portion of

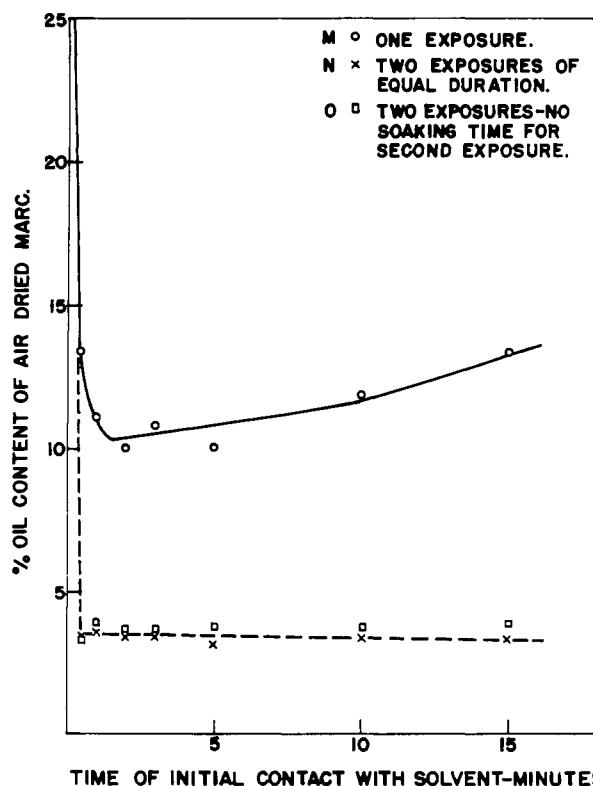


Figure 3. Relationship between oil contents of marcs and time of exposure of the fresh flakes to the azeotrope

fresh solvent for 10 seconds before marc and miscella were separated, etc. The oil contents of the air-dried marcs obtained in this double exposure to the azeotrope are plotted (curve *O*) in Figure 3 against the initial commixing period.

The data reported in graphic form in Figure 3 may be regarded as strong evidence that the oil in raw cottonseed flakes is rendered by the azeotrope in 60 to 90 seconds. A soaking period of more than 2 minutes in the initial contact between fresh flakes and the solvent is not necessary to ensure efficient oil extraction, and as is obvious from these observations, the residence time of the marc in a countercurrent extraction of raw cottonseed flakes need be no longer—beyond the 60 to 90 seconds required for rendering the oil—than the time required to commix and then separate marc and miscella the requisite number of times. Stepwise countercurrent extractions have indicated that nothing is gained by commixing solvent-damp marcs and miscellas for more than ca. 7 seconds.

**Avoiding Filtration through Deep Beds.** King and Frampton (1960) established that oil and gossypol are most efficiently extracted from raw cottonseed flakes when the solvent mixture is saturated with water and the flakes contain 12% or more moisture. The moist flakes and marcs produced under these conditions have a tendency to swell and to form a plastic mass when pressure is applied. Thus, deep layers of marc, such as are encountered in some commercial basket-type extractions, pack and form a mass which is poorly penetrable by the solvent and which drains poorly. This behavior of the marcs demands a process for separation of marc and miscella where filtration through deep beds is avoided.

A vibrating screen separator which promotes the horizontal transport of the marc can be adapted for continuous operation where thin layers of marc may be handled in a moving stream. The vibrating action tends to keep the small aggregates of the marc loose and separate, and it facilitates drainage of the miscella through the screen. A circular vibrating screen (18-inch diameter) equipped with a 165-mesh stainless screen was used for this purpose. The gyrations of the screen were adjusted so the marc, fed onto the center of the screen, moved radially to the circular wall and then tangentially along the wall to the discharge port, while the miscella drained through the screen. A rubber ball tray was installed under the screen, and the bouncing action of the rubber balls striking the undersurface of the screen prevented clogging of the screen. Under these conditions it proved impossible to clog the screen regardless of the properties of the marcs or the manner in which they were fed onto the screen.

**Use of a Vibrating Screen Separator.** In the stepwise countercurrent extraction carried out with the vibrating screen separator, fresh flakes (10 pounds) were commixed with the oil-rich miscella (coming upstream in the countercurrent schedule) for 2 minutes. The mixture was then fed by hand into the screw conveyer to be transported to the vibrating screen. While the combined residence time of the marc in the conveyer and on the screen was 7 seconds, 60 seconds were consumed in processing the 10 pounds of flakes—that is, the capacity of the 18-inch screen was 10 pounds of flakes per minute.

The miscella recovered was the product miscella, and the marc discharged from the screen was commixed in the

screw conveyer with the next miscella. The marc and miscella were fed separately, but simultaneously, into the screw conveyer and were commixed as they were transported the length of the conveyer to the discharge port where the mixture was discharged onto the screen. The screw conveyer was a 6-foot trough conveyer with 6-inch ribbon flights and was driven through a gear box and belt drive adjusted to 60 r.p.m. As in the first step of the stepwise countercurrent extraction, the combined residence time of the marc in the conveyer and on the screen in this and subsequent steps was 7 seconds.

**Recycling of Fines.** As in all cottonseed extractions, a certain quantity of "fines" is produced in the preparation of the kernels and flakes for extraction, and additional fines are produced in the extraction process itself. Some of the fines were observed to drain through the screen with the miscella and to accumulate in the miscella as it moved upstream and were finally discharged with the product miscella. These fines, as contrasted with those produced on extraction with hexane, tend to aggregate and they settle rapidly and are readily recovered from the product miscella.

Investigations were carried out to determine how the fines could be recycled. Once the adjusted miscellas required for a 10-step schedule were prepared (solvent-to-flakes ratio of 4 to 1), successive 5-pound lots of fresh flakes were introduced into the system. The product miscellas were permitted to stand for the fines to settle, and the supernatant product miscella was decanted. Only a few minutes are required for them to settle, but in the experiment described, the product miscellas were permitted to stand for 30 minutes before they were decanted. The fines recovered as a sediment from the product miscellas were recycled in three experiments—namely, at steps 3, 5, and 7. Samples were taken for analyses only after the system had been in operation for many hours and the production of fines became steady (Table I).

There was no buildup of fines in the product miscellas, and the fines may be recycled successfully. The quantity of fines in the product miscellas was less when they were recycled at step 5 than at steps 3 or 7. Consequently, the fines were recycled at step 5 in the subsequent extractions in a 10-step countercurrent extraction schedule where lots of 10 pounds of fresh flakes were fed into the stream. Apparently, the recycling step had no material effect on the final marcs produced (Table II).

**Dilution Law.** The behavior of the system as revealed by the data reported in Figure 3 would lead one to anticipate that the stepwise countercurrent extraction may be described by the dilution law.

$$\log L = a - bP \quad (1)$$

where  $L$  is the oil content of any marc obtained for step  $P$ ,  $a$  is the logarithm of the oil content of the raw flakes, and  $b$  is the logarithm of the ratio of the oil content of one marc to that of the next succeeding marc.

The data obtained in a 10-step stepwise countercurrent extraction with a solvent-to-flakes ratio of 3 to 1 (Table III) indicate that the dilution law is followed. The equation for the regression of the oil content of the marc on the step number was

$$\log L = 1.45 - 0.094P \quad (2)$$

**Table I. Quantities and Analyses of Sediments from Final Miscellas after Recirculation at Different Steps in Countercurrent Extraction of Raw Cottonseed Flakes with Acetone-Hexane-Water Solvent**

Recirculation Step, Figure 1	Wt., Lb.	T.V.M., % <sup>a</sup>	Oil, %	Dry Meal, %	Weight T.V.M., Lb.	Weight Oil, Lb.	Weight Dry Meal, Lb.	Fines, % by Weight of Original Flakes
3	6.1	48	24	28	2.9	1.5	1.7	34
3	6.4	46	26	28	2.9	1.7	1.8	36
3	6.9	47	26	27	3.2	1.8	1.9	38
3	6.2	54	23	23	3.3	1.4	1.4	32
Av.	6.4	49	25	27	3.1	1.6	1.7	35
5	2.6	65	12	33	1.7	0.3	0.8	16
5	2.2	63	14	23	1.4	0.3	0.5	10
5	2.4	62	13	25	1.5	0.3	0.6	12
5	3.0	69	10	21	2.1	0.3	0.6	12
Av.	2.6	65	12	26	1.8	0.3	0.6	13
7	5.0	59	15	26	3.0	0.3	1.3	26
7	4.7	52	19	29	2.4	0.9	1.4	28
7	2.9	59	16	26	2.9	0.8	1.3	26
7	4.5	51	21	28	2.3	0.9	1.3	28
Av.	4.8	55	18	27	2.7	0.7	1.3	27

<sup>a</sup> Total volatile material.

**Table II. Analyses of Air-Dried Meals Produced by a 10-Step Countercurrent Extraction of Raw Cottonseed Flakes**

Recycling Step	Gossypol, %		Oil, %
	Free	Total	
Raw flakes	0.92	0.94	32.7
3	0.06	0.28	0.3
	0.08	0.30	0.7
	0.07	0.29	0.9
5	0.04	0.15	0.5
	0.04	0.16	0.7
	0.04	0.17	0.7
7	0.05	0.25	0.8
	0.06	0.23	0.9
	0.05	0.24	0.8

**Table III. Analyses of Marc and Miscella from a 10-Step Countercurrent Extraction of Raw Moist Cottonseed Flakes (30.2% Oil, 12% Moisture) with the Solvent Mixture Vibrating Screen Separator**

Step No.	Total Volatile Matter, %			Crude Oil in Air-Dried Marc, %	Total Gossypol in Meal, %
	In marc	In miscella	In air-dried marc		
Flakes	12.0	...	12.0	30.2	0.70
1	56.2	89.4	7.3	16.5	0.60
2	59.2	90.6	7.4	14.9	0.58
3	64.2	92.1	7.3	12.7	0.50
4	64.8	92.8	7.1	11.8	0.45
5	64.7	94.4	7.2	9.5	0.41
6	66.1	95.2	7.5	8.5	0.39
7	68.4	96.4	7.7	6.2	0.30
8	69.1	97.8	10.0	4.0	0.24
9	70.8	98.2	9.8	3.8	0.26
10	71.3	98.85	9.4	2.5	0.19

and the coefficient of correlation between log oil content of the marc and step number was calculated to be  $-0.98$ . The mean square owing to the regression was 0.968, while that about the regression was 0.0045. The  $F$  ratio of 215, obtained from these two mean squares for 1 and 8 degrees of freedom is highly significant, and evidently the dilution law was followed in a satisfactory manner in this extraction.

Analysis of the data in Table III shows that gossypol extraction also follows the dilution law; the regression equation was

$$\log G = -0.075 - 0.057P \quad (3)$$

where  $G$  is the gossypol content of the marc produced at step  $P$ . The mean square owing to the regression was 0.269, that about the regression 0.013, and the  $F$  ratio of 20.7 for 1 and 8 degrees of freedom is highly significant. The coefficient of correlation between log  $G$  and pass number was  $-0.98$ .

A parallel set of experiments established that the fines accumulated in the product miscella in a four-step countercurrent extraction may be recycled successfully at step 3. Accordingly, a four-step countercurrent schedule was set up with a solvent-to-flakes ratio of 3 to 1 by weight, and the marc discharged at each step from the screen was pressed between canvas cloths at about 100 p.s.i. for 50 seconds to reduce the quantity of entrained miscella further. Ten-pound lots of moist raw cottonseed flakes (32% oil, 13% moisture, and 1.2% gossypol) were fed into the system. Samples were taken for analyses only after the system had become steady (Table IV). Samples of marc were taken from the discharge port of the vibrating screen separator for the data in the column headed "Marc," while samples taken from the press cake produced from the discharged marcs were taken for the data under the column headed "Press Cake." Attention is called to the low oil content of 0.4% of the final press cake in this four-

**Table IV. Analyses of Marc from a Four-Step Countercurrent Extraction of Raw Cottonseed Flakes with the Acetone-Hexane-Water Azeotrope—Solvent-to-Flakes Ratio, 3 to 1 by Weight**

Analysis	Step No.	Marc, %	Press Cake, %
Total volatile material	1	59.8	37.5
	2	66.2	38.9
	3	67.3	39.7
	4	69.6	40.2
Oil (air-dry basis)	1	18.8	5.4
	2	7.6	2.2
	3	4.1	1.1
	4	1.4	0.4
Total gossypol (air-dry basis)	1	0.78	0.56
	2	0.55	0.40
	3	0.47	0.35
	4	0.30	0.24
Water (as is basis)	1	7.5	14.6
	2	8.5	15.5
	3	10.7	16.6
	4	11.6	16.9

step extraction schedule. Values for the regression coefficient, *b*, calculated from these data were 0.37 and 0.67. Evidently the value of *b* is dependent upon the quantity of miscella entrained in the marc, and increases as the thoroughness with which the marc is drained.

The bill of materials fed into and discharged from the system for each step in the four-step countercurrent schedule is reported in Table V. Loss of solvent occurred because a part of the system was open to the atmosphere.

**Separation of Marc and Miscella.** The properties of *b* were studied in a series of extractions where marcs and miscellas were separated centrifugally. A 10-step countercurrent extraction was set up for a solvent-to-flakes ratio of 1 to 1 on a weight basis. Eighty grams of fresh flakes (11% moisture) were commixed for 60 seconds in the basket of the centrifuge with the oil-rich miscella No. 9 that was prepared for the 10-step schedule. It was necessary to add 20 grams of fresh solvent to this mixture to replace solvent lost to the system by entrainment in the final marc. Marc and miscella were separated by centrifugation at 1420 G for 1 minute, and the oil content of the air-dried marc was determined. Data for the total volatile material,

**Table VI. 10-Step Countercurrent Extraction of Raw Cottonseed Flakes with the Azeotrope—Solvent-to-Flakes Ratio, 1 to 1**

Step No.	Total Volatile Material in Marc, %	Oil Content of Marc, %	Gossypol Content of Marc, %
1	40.7	28.9	1.05
2	47.7	23.8	
3	41.1	11.5	
4	46.6	5.88	1.03
5	52.7	4.02	
6	41.1	2.48	
7	58.1	1.70	0.62
8	58.6	0.84	
9	53.9	0.59	
10	56.7	0.29	0.21

oil, and gossypol for each marc are reported in Table VI.

The data in Table VI indicate coefficient *b*, for the regression of log oil content of the marc on the step number of 0.22. The coefficient of correlation between the log oil content of the marcs and the step number was  $-0.997$ , and the ratio of the mean square owing to the regression to that about the regression was 374. This *F* ratio of 374 for 1 and 8 degrees of freedom is highly significant, and evidently the extraction followed the dilution law very closely.

A solvent-to-flakes ratio of 2 to 1 was used in five-step schedules in the extraction of raw cottonseed flakes, raw soybean flakes, and a mixture (1 to 1) of raw cottonseed and soybean flakes. Marcs and miscellas were separated at 1420 G, as in the previous extractions. The data for oil contents of the marcs and the miscellas are included in Table VII. A three-step countercurrent extraction with a solvent-to-flakes ratio of 2 to 1 was also set up, and the data are reported in Table VIII.

**Properties of Constant *b*.** Regression analyses of the data in Tables VII and VIII for marcs obtained at 1420 G with a test of homogeneity of the regression coefficients for the regression of log oil content of the marc on step number are included in Table IX. The mean square among the regression coefficients is  $0.0304/3 = 0.0101$ , while that for the pooled variance about the regressions is  $0.0301/10 = 0.00301$ . The ratio between these two mean squares is 3.37, which, for 3 and 10 degrees of freedom, is not considered significant. Evidently the differences between the several coefficients, *b*, in Table IX, may be accounted for by experimental error, and apparently the co-

**Table V. Bill of Materials Fed into and Discharged from the System at Each Step in a Four-Step Countercurrent Extraction of Raw Moist Cottonseed Flakes**

Pass No.	Solids into screw conveyor	Pounds				
		Solvent damp marc discharged from screen	Press cake	Fresh solvent into system	Miscella discharged from screen	Fines in miscella
1	10 (fresh raw flakes)	17.6	9.5	3	20.1	2.2
2	9.5	17.3	9.8	0	19.1	1.1
3	12.0	19.7	10.5	0	16.7	1.1
4	10.5	16.7	9.4	21	18.8	0.6

**Table VII. Countercurrent Extraction of Raw Cottonseed and Soybean Flakes**  
(Marc and miscella separated centrifugally at 1420 G; Solvent-to-Flakes ratio, 2 to 1)

Marc No.	Oil Content of Marc, %			Miscella No.	Oil Content of Miscella, %		
	Cottonseed	Soybean	Mixed cottonseed soybean		Cottonseed	Soybean	Mixed cottonseed soybean
1	19.8	13.9	18.6	Product	16.1	10.4	13.2
2	12.2	6.90	8.90	1	10.7	5.80	8.02
3	5.74	3.10	4.22	2	4.91	2.90	3.37
4	2.58	1.48	1.72	3	2.01	1.10	1.40
5	1.22	0.46	0.58	4	0.65	0.05	0.46

**Table VIII. Oil Contents of Cottonseed Marcs Produced at Different Centrifugal Speed in a Three-Step Countercurrent Extraction Schedule—Solvent-to-flakes-ratio 2 to 1**

Pass No.	Oil Content of Air-Dried Marcs	
	1420 G	6400 G
1	20.67	7.40
2	10.58	1.88
3	4.15	0.48

efficient is not a function of the quantity of oil in the original flakes or in the number of steps in the countercurrent extraction schedule.

The dependence of  $b$  on the solvent-to-flakes ratio is illustrated by the data plotted in Figure 4, where the regression coefficient is plotted against the solvent-to-flakes ratio. A centrifugal force of 1420 G was used in the separation of marc and miscella in securing the data in the several countercurrent extractions carried out. The confidence limits of  $b$  at the 5% level of probability were determined and are indicated by the lengths of the bars drawn through the experimental points. The indications are that  $b$  is a linear function of the solvent-to-flakes ratio.

The influence of the centrifugal force on the oil content of the marcs is illustrated in Table VIII. Two countercurrent extractions were carried out with a solvent-to-flakes ratio of 2 to 1 where centrifugal forces of 1420 and 6400 G applied for 60 seconds were used in the separation of marc and miscella.

Single extractions with fresh azeotrope (solvent-to-flakes ratio of 2 to 1) were carried out where marc and miscella were separated in each instance under a different centrifugal force. The ratio of the oil content of the raw flakes

to the oil content of the marc is plotted against the centrifugal force in Figure 5. The mean square owing to the regression was determined to be 5.2518, that about the regression 0.0379. The  $F$  ratio of 138 for 1 and 5 degrees of freedom is highly significant. Evidently the ratio of the oil in the original flakes to that of the air-dried marc is a linear function of the centrifugal force used in separating marc and miscella.

**Relationship of Oil Content of Marcs to Step Number, Solvent-to-Flakes Ratio, and Centrifugal Force.** The ratio of oil in the fresh flakes to that of the marc obtained in the extractions immediately above is approximately equal to  $\text{antilog } b$ , and if the assumption is made that the two ratios have the same properties, it must follow that  $b$  is directly proportional to  $\log$  centrifugal force. Figure 4 shows that  $b$  is a linear function of the solvent-to-flakes ratio. On the basis of these linear relationships, the general relationship relating the oil content,  $L$ , of a marc at step  $P$  obtained in an extraction with a solvent-to-flakes ratio of  $r$  and centrifugal force  $F$  used in separating marc and miscella is

$$\log L = d - P[lr + k \log F] \quad (4)$$

The validity of the equation in describing the relationship between the oil content of the air-dried marcs, the step number in the stepwise countercurrent extraction, the solvent-to-flakes ratio, and the centrifugal force used to separate marc and miscella was tested by using the experimental data in Table X in estimating the constants  $d$ ,  $l$ , and  $k$ . The method of least squares was used. Included in Table X are the calculated values for the oil contents of the several marcs.

**Table IX. Regression Analyses and Test of Homogeneity of Regression Coefficients of Data Reported in Tables VII and VIII**

Sample	No. of Steps	Sum of Squares		Sum of Products	$b$	Sum of Squares Due to Regression	Degrees of Freedom Due to Regression	Sum of Squares about Regression	Degrees of Freedom about Regression
		For pass No.	For oil content of marcs						
Raw cottonseed flakes	3	2.00	0.2458	0.6981	0.349	0.2436	1	0.0022	1
Raw cottonseed flakes	5	10.00	0.9646	3.096	0.310	0.9610	1	0.0036	3
50:50 mixture raw cottonseed and soybean flakes	5	10.00	1.3991	3.727	0.373	1.3910	1	0.0081	3
Raw soybean flakes	5	10.00	1.3262	3.623	0.362	1.3100	1	0.0162	3
Sum		32	3.9357	11.440		3.9056	4	0.0301	10
Pooled					0.348	3.8752	1		
						0.0304	3		

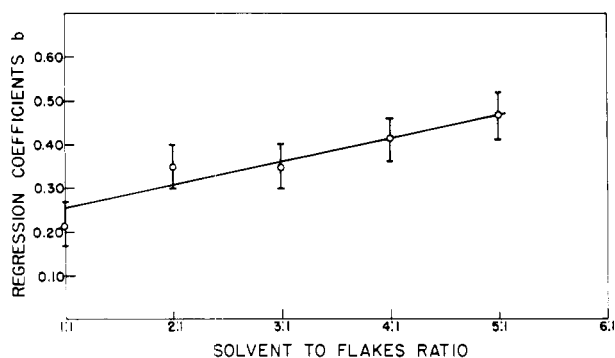


Figure 4. Relationship between solvent-to-flakes ratio and regression coefficient  $b$

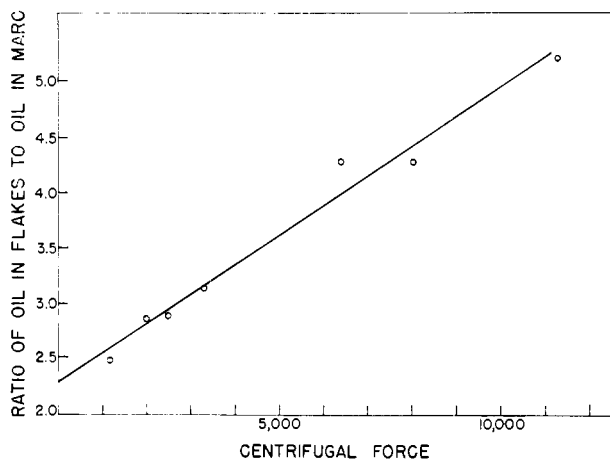


Figure 5. Relationship between ratio of oil in fresh flakes to oil in marc and centrifugal force used in separating marc and miscella

The agreement between observed and calculated oil contents is good. Analysis of the data in Table X indicates that the mean square between observed and calculated oil contents of the marcs is 0.53, while that for the error variance is 0.27. The ratio between these two mean squares of 0.96 for 1 and 8 degrees of freedom is not significant, and the differences between calculated and observed oil contents of the marcs in the table are accounted for by the accumulated experimental error. Statistically, the observed and calculated values are identical, and Equation 4 describes the extraction.

The constant  $d$  in the Equation 4 is not the log of the oil content of the original flakes, since it contains constants from the regression of  $b$  on the solvent-to-flakes ratio and of  $b$  on the logarithm of the centrifugal force.

**Reduction of Gossypol.** While the work reported is concerned chiefly with oil extraction, some data on gossypol are included. The significance of the gossypol data is not clear, since the nature of the gossypol derivatives which are accounted for in the analyses is not known. However, the azeotrope reduces the gossypol and gossypol derivatives to levels that are very much less than those found in conventional cottonseed meals.

**Off-Flavors.** The authors detected no off-flavors in the meals (attributable to condensation products of acetone) when the azeotrope was prepared by distillation from glass equipment and the extraction was carried out at room temperature where the total exposure of flakes to solvent was of only a few minutes' duration.

Table X. Calculated and Observed Oil Contents of Air-Dried Marcs and Oil Contents Calculated for Equation  $\log L = d - P(lr + k \log F)$

Pass No.	$r$	$F$	Oil Content, %	
			Obsd.	Calcd.
1	2	1420	21	21
2	2	1420	10	9.4
3	2	1420	5.4	5.1
1	2	6400	7.4	6.9
2	2	6400	1.9	1.5
3	2	6400	0.50	0.26
1	3	1420	18	16
2	3	1420	7.5	8.0
3	3	1420	3.5	4.0

**Advantages.** The advantages gained in using the azeotrope as a solvent in oilseed processing may be summarized:

The solvent is a low boiling azeotrope (b.  $49^\circ \text{C}$ ).

The residence time of the marc in the extraction procedure need not exceed 3 minutes and, as illustrated in Table VIII, oil levels in the finished meals may be reduced in this time interval to less than 0.5% in a three-step stepwise countercurrent extraction.

Cooking of the comminuted oilseed kernels or oilseed flakes is eliminated, together with the cost of cooking.

In contrast with conventional commercial cottonseed meals, meals prepared through the use of the azeotrope are characterized by a high level of available lysine, and low levels of gossypol and of residual crude oil, and high nutritive quality.

Yield of refinable and bleachable oil is higher than is obtainable through conventional processing operations.

Operation costs and capital investment are probably less than those encountered in conventional screw press or prepress solvent operations, especially if either the vibrating screen or centrifuge is used in separating marc and miscella.

#### LITERATURE CITED

- American Oil Chemists' Society, Chicago, Ill., 2nd ed. revised to 1960.  
 Bressani, Ricardo, *U. S. Dept. Agr., ARS 72-38*, 112-29 (1965).  
 Frampton, V. L., *Cereal Sci. Today* **10**, 577-82 (1965).  
 Frampton, V. L., Kuck, J. C., Pepperman, A. B., Jr., Pons, W. A., Jr., Watts, A. B., Johnston, Charles, *Poultry Sci.* **45**, 527-35 (1966).  
 Johnston, Charles, Watts, A. B., *Poultry Sci.* **43**, 957 (1964).  
 King, W. H., Frampton, V. L., *J. Am. Oil Chemists' Soc.* **38**, 487-99 (1961).  
 King, W. H., Frampton, V. L. (to Secretary of Agriculture), U. S. Patent **2,950,198** (August 23, 1960).  
 King, W. H., Kuck, J. C., Frampton, V. L., *J. Am. Oil Chemists' Soc.* **38**, 19-21 (1961).  
 King, W. H., Thurber, Francis, *J. Am. Oil Chemists' Soc.* **33**, 169-71 (1953).  
 Pons, W. A., Jr., Pittman, R. A., Hoffpauir, C. L., *J. Am. Oil Chemists' Soc.* **35**, 93-97 (1958).  
 Reiser, Raymond, *Biochem. Biophys. Res. Commun.* **17**, 8-11 (1964).  
 Smith, F. H., *J. Am. Oil Chemists' Soc.* **40**, 60-1 (1963).

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