melting points. A group of three fractions, chosen in the manner identical to the one indicated earlier, gave 69-69.2°C., 72.7-72.9°C., and 76-76.2°C., respectively, as their melting points.

Discussion

Chromatography over alumina was found not only to be a remarkably effective tool in bringing about resolution of hydrocarbon mixtures but also a very dependable criterion for judging the purity and homogeneity of a paraffin sample. In the light of this observation it follows beyond any reasonable doubt that the hydrocarbons obtained from the molecular distillates Nos. 1 and 3 are single hydrocarbons. Their physical constants would identify them as $\rm C_{24}$ and $\rm C_{30}$ n-paraffins, respectively. Molecular distillate No. 2 however yielded a mixture of hydrocarbons, which on the basis of chromatographic behavior and observed physical properties can be presumed to be a mixture of C_{26} and C_{28} n-paraffins. Any prediction in the case of the hydrocarbon component isolated from the molecular distillate No. 4 is not so easy as in the above other cases. However the spread noted in the case of melting points of the widely separated chromatographic fractions makes it seem likely that this particular hydrocarbon fraction may very well be a mixture of C_{32} , C_{34} , and C_{36} n-paraffins. Although there may be some doubt as to the exact identity of some of the hydrocarbons that had appeared as mixtures, there seems to be little doubt however that the first four molecular distillates collectively included n-paraffin falling continuously within the range of C24 to C36, and these, if presumed to be all of an even number carbon atom content, will be seven in number.

Summary

Solvent-fractionation used in conjunction with molecular distillation and chromatographic adsorption yielded wax fractions whose separation into their com-

ponents was successfully and easily accomplished. Without benefit of either molecular distillation or chromatography however, but merely by a solventfractionation procedure there were isolated cerotic acid and melissyl alcohol under conditions which point to their occurrence in ouricuri wax in an ester combination.

Column chromatography was found to be a remarkably successful tool in resolving the mixture of hydrocarbons which this wax contains and in establishing the homogeneity of an individual hydrocarbon. Indicated beyond a reasonable doubt is the presence of C_{24} and C_{32} n paraffins; that of all the four even homologs between these limits is strongly indicated.

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REFERENCES

- 1. Luedecke, C., Seifen, Oele, Fette, Wachse, 74, 111 (1948); C. A.,
- 1. Luedecke, C., Seifen, Uele, Fette, Wachse, 72, 111 (1920). C. A., 42, 8499.
 2. Ministry of Foreign Affairs, Brazil 1940-41. An Economic, Social, and Geographic Survey, p. 200.
 3. Piper, S. H. et al., Biochem. J., 25, 2072 (1931).
 4. Schuette, H. A., and Baldinus, J. G., J. Am. Oil Chem. Soc., 26, 651 (1949).
 5. Warth. A. H., "The Chemistry and Technology of Waxes," Reinhold Publishing Corporation, New York, 1947, pp. 110-112.

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Gossypol Material Balance, Denaturation of Protein, and Loss of Thiamine in Commercial Processing of Cottonseed

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NFORMATION on the material balance for gossy-■ pol, the denaturation of protein, and the destruction of thiamine in the commercial processing of cottonseed by hydraulic- and screw-pressing methods is considered basic to current consideration on the improvement of processing methods that may lead to the general production of meals of superior nutritive value and suitable for feeding extensively to swine and poultry (2, 7). The purpose of this report is to present data on this subject.

The yields and quality of oil have been given much attention in the development of the cottonseed processing industry. Cooking of the meats prior to pressing has become a general practice to facilitate oil extraction, to improve the quality of the oil, and to bind the gossypol and gossypol-like pigments (1), hereafter referred to as gossypol. The gossypol not bound during cooking and pressing and extractable

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with aqueous acetone is designated as free gossypol (13). The extent of gossypol binding, protein denaturation, and thiamine reduction is in each case a function of moisture content, temperature, and time of heating of the meats during cooking and pressing.

The utility of cottonseed meal in the rations of swine and poultry is influenced by the extent to which the free gossypol is reduced and by the availability of the protein. Processing conditions can be selected so that the free gossypol can be reduced to a level which does not interfere with the growth of swine and poultry (2, 9). Based on the evidence collected to date, the suggestion has been made that the free gossypol should be reduced to 0.03% or less in meals for feeding to swine and chickens (2).

Lacking specific analytical methods for laboratory evaluation of the change in nutritive or biological value of the protein induced by heat during processing, nitrogen solubility has been used as an approximate index (7, 12). Denaturation, measured by determination of nitrogen solubility, does not necessarily render the protein in the meal indigestible or nonavailable. Newer conceptions attribute the reduction in biological value of the protein of many food and feed products to the effect of heat during processing in combining essential amino acids with sugars to form complexes resistant to enzymatic action (11). The level to which thiamine is reduced has been proposed as an index to the reduction of biological value of the protein by heat applied in the processing of soybeans (8, 10). For this reason reduction of thiamine has been considered in the present investigation.

Samples and Processing Conditions

The samples used were obtained through the cooperation of five commercial cotton oil mills, designated in the tables of data as A, B, C, D, and E. The first two use hydraulic and the other three screw presses. Each mill was requested to obtain composite samples of seed, cooked meats, meal, and crude oil, each representative of a day's run, at regular two-week intervals. The composite samples were forwarded to the Southern Regional Research Laboratory for analysis.

The average conditions employed by the mills in the preparation of the meats for pressing, as approximated by the mill operators, are given in Table I.

TABLE I
Processing Conditions

Mill	Meats prepara- tion	Maximum moisture content of meats in cooker (approx.)	Cooking time (approx.)	Maximum cooking tempera- ture (approx.)	
Hydraulic Press		%	min.	°F.	
A	5-high rolls	13	90	225	
В	5-high rolls	9	68	240	
Screw Press	•	1	i		
C	5-high rolls	9	60	280	
D	5-high rolls	13	80	250	
E	5 high rolls	9	45	280	

Methods of Analysis

Moisture, oil, nitrogen, free fatty acids, and free gossypol were determined by use of the official methods of the American Oil Chemists' Society (3). The methods proposed by Pons et al. (14, 15) were used for the determination of total gossypol in the meal and oil. Nitrogen solubility was evaluated by dispersion of the meal in 0.5 M sodium chloride as proposed by Olcott and Fontaine (12). Thiamine was determined in the kernels and meals by a modification of the thiachrome method of Conner and Straub (6), which is essentially the procedure recommended by the Association of Vitamin Chemists (4).

TABLE II

Analysis of Mill Samples for Specified Constituents on "As Received" Basis

Sampling No.	Raw meats		Oil	Free gossypol			
	Oil	F. F. A. in oil	in meal	Raw meats	Cooked meats	Meal	Oil
	%	%	%	%	%	%	%
fill A—Hydraulic Press					İ	ĺ	
1	33.5	0.68	4.84	1.16	0.033	0.044	0.016
2	33.9	0.86	5.34	1.16	0.043	0.048	0.023
3	31.1	0.44	5.14	1.07	0.044	0.049	0.028
4	27.7	0.59	4.16	1.12	0.047	0.046	0.033
5	30.1	0.74	4.69	1.10	0.037	0.034	0.038
6	33.4	0.62	5.40	1.06	0.038	0.040	0.032
7	27.2	0.83	5.41	0.88	0.040	0.043	0.032
8	28.9	0.53	5.14	1.02	0.037	0.043	0.049
9	27.6	0.62	5.33	0.93	0.034	0.034	0.047
ill B—Hydraulic Press							***-
1	34.7	1.52	4.65	1.16	0.122	0.121	0.079
2	34.5	2.70	5.34	1.02	0.134	0.143	0.096
3	33.5	2.73	4.78	1.03	0.098	0.104	0.078
4	34.5	2.38	4.83	1.14	0.114	0.110	0.088
5	34.2	2.24	4.73	1.13	0.081	0.109	0.094
6	34.3	3.63	4.78	1.07	0.116	0.128	0.111
7	34.5	3.77	4.52	0.98	0.132	0.150	0.103
8	30.0	2.56	5.07	1.13	0.126	0.131	0.100
Iill CScrew Press					l		• • • • •
1,	31.4	0.57	4.62	0.81	0.37	0.028	0.38
2	34.9	0.78	4.86	0.91	0.32	0.031	0.38
3,	33.9	0.40	4.35	0.85	0.35	0.024	0.35
4	34.0	0.43	4.83	0.81	0.31	0.033	0.29
5	29.3	0.63	7.20	0.79	0.19	0.037	0.23
6	32.6	1.26	4.90	0.75	0.18	0.032	
7	34.1	1.62		0.72	0.16		0.19
8.	30.5	3.84	7.16			0.081	0.23
		1.80	7.75	0.61	0.23	0.062	0.21
9	35.3		5.15	0.67	0.23	0.041	0.24
10	34.5	2.64	4.58	0.74	0.28	0.030	0.27
11	32.7	0.91	4.23	0.75	0.30	0.020	0.27
12	28.4	1.05	4.25	0.81	0.22	0.027	0.19
13	31.4	1.21	4.25	0.67	0.21	0.023	0.23
14	30.5	0.99	3.97	0.75	0.25	0.023	0.23
15,	35.6	0.90	4.69	0.76	0.32	0.027	0.30
16	35.9	1.21	4.90	0.72	0.28	0.028	0.19
17	29.7	1.35	4.40	0.77	0.21	0.023	0.30
18	29.4	2.20	4.99	0.74	0.28	0.037	0.38
19	36,3	2.11	5.10	0.82	0.31	0.039	0.32
Iill D—Screw Press						0,700	0.04
1	32.5	1.59	5.15	1.15	0.21	0.039	0.28
2	33.6	1.83	4.25	1.09	0.17	0.039	$0.28 \\ 0.22$
3	36.0	3.65	4.25 5.58	1.13	0.17	0.027	0.22
ill E—Screw Press			-	l		1	
1	34.4	1.57	3.63	1.21	0.21	0.044	0.32
2	33.3	1.57	4.66	1.28	0.18	0.054	0.32
3	32.8	2.35	3.32	1.19	0.31	0.048	0.39
4	33.0	2.18	3.35	1.22	0.20	0.047	0.33
5	35.4	2.49	3.06	1.24	0.31	0.040	0.35
6,	34.9	2.84	4.54	1.17	0.21	0.052	0.33
ill Averages			2.0 =		"	····-	0.00
A	30.4	0.66	5.05	1.06	0.039	0.042	0.033
B	33.8	2.69	4.84	1.08	0.115	0.125	0.094
C	32.7	1.36	5.06	0.76	0.27	0.034	0.034
D.	34.0	2.36	4.99	1.12	0.18	0.034	0.24
E	34.0	$\begin{vmatrix} 2.30 \\ 2.17 \end{vmatrix}$	3.76	1.22	0.18	0.048	$0.24 \\ 0.34$

The analyses for the specified constituents on the original sample basis are given in Table II, and for thiamine on the same basis in Table III. The nitrogen solubility, reported in Table III, is the percentage of the total nitrogen in the meal found soluble in 0.5 M sodium chloride under the conditions of the test procedure used. The material balances for gossypol and thiamine, Tables III and IV, are given on the moisture-, oil-, and hull-free materials. These are calculated results obtained by use of determined nitrogen contents of the original meats, cooked meats, and meal, assuming that the hulls added before cooking contained no nitrogen and that there was no loss in nitrogen during cooking and pressing. The data shown in Table IV were used to calculate the distribution of gossypol percentage-wise reported in Table V.

Discussion of Results

The seed processed by the five mills were in good condition as indicated by the level of free fatty acid contents of the oil in the seed (Table II). Each mill appears to have been operated so as to give a fairly uniform degree of oil extraction, if samplings 5, 7, and 8 from Mill C are omitted from consideration. Mill E was operated to reduce the oil in the meal approximately one whole unit in percentage lower than the others.

The free gossypol content of the meals (Table II), excepting samplings 7 and 8 from Mill C, is relatively constant for each mill though each mill tends to attain a different level. Though, in general, hydraulic-pressed meals are higher in free gossypol than screw-pressed meals, it is noted that the cooking conditions maintained by hydraulic Mill A were such that an average free gossypol content of 0.042% was attained, a level approaching that found in the screw-pressed meals.

As cited in the introduction, nitrogen solubility is basically a measure of the denaturation of the protein. The weighted average nitrogen solubility of the meals from the three screw-pressing mills was 12.3% as compared to the weighted average value of 31.7% for those from hydraulic-pressing mills (Table III). The greater reduction in the nitrogen solubility for screw-pressed meals is attributed to the higher temperatures employed in the cooking prior to pressing (Table I) and to the high temperatures developed in the screw press. The average nitrogen solubility for the meals from Mill A was 34.1% as compared to 29.0% for those from Mill B (Table III). The higher value for Mill A is surprising because of the prolonged cooking period employed (Table I).

Thiamine is a heat-labile substance and it is anticipated that its loss during processing may be correlated with the reduction in biological value of the protein as has been found in the processing of soybeans (8, 10). However the thiamine content of the meal itself is not necessarily an indication of the extent of the effect of heat in processing since the thiamine content of the raw cottonseed kernels varies considerably (Table III).

A better indication of the effect of heat is the percentage of reduction in thiamine, calculated from the thiamine contents of meats and meal expressed on a moisture-, oil-, and hull-free basis (Table III). The reduction of thiamine is much greater in the screw-pressed meals than in hydraulic-pressed meals and can be attributed to the high temperatures developed

TABLE III
Thiamine Content and Nitrogen Solubility of Mill Samples

Sampling	Thiamine in samples as received		Thiam oil-,	Nitrogen solubil- ity		
N_0 .	Raw meats	Meal	Raw meats	Meal	Reduc- tion	Meal
Mill A— Hydraulic Press	ppm.	ppm.	ppm.	ppm.	%	%
1	20.3	8.6	33.7	13.0	61.4	34.1
2 3	$^{20.2}_{-21.0}$	$7.6 \\ 9.3$	34.2 33.7	$\frac{10.8}{13.7}$	68.4 59.3	$\frac{31.7}{34.7}$
4	21.9	8.0	33.1	11.4	65.6	33.4
5	20.1	7.9	32.0	11.7	63.4	32.7
<u>6</u>	22.1	8.7	35.6	14.1	60.4	35.2
7	19.9	8.7	30.2 33.6	$11.5 \\ 11.2$	61.9 66.7	33.6 31.3
8 9	$\frac{21.6}{20.5}$	$\frac{8.3}{8.7}$	31.5	12.6	60.0	39.9
Mill B-	20.0	0.1	0175	12.0	00.0	00.0
Hydraulic Press						
1	19.5	12.9	35.1	18.9	46.2	28.7
2 3	$\frac{19.4}{21.2}$	$14.2 \\ 15.5$	35.5 37.7	$21.1 \\ 23.9$	40.6 36.6	26.8 34.9
4	21.5	16.7	38.5	25.3	34.3	34.6
5	18.2	12.8	31.4	18.8	40.1	31.4
<u>6</u>	17.6	13.5	31.2	19.7	36,9	30.1
7 8	$19.2 \\ 19.7$	$13.0 \\ 14.6$	$\frac{33.5}{31.9}$	$19.4 \\ 19.2$	42.1 39.8	$18.5 \\ 26.6$
Mill C—	19.7	14.0	31.5	18.4	59.8	20.0
Screw Press						
1	20.9	3.7	33.5	4.7	86.0	12.0
2	16.6	5.0	28.6 32.0	6.8	76.2	13.4
3 4	$\frac{19.0}{18.7}$	$\frac{3.9}{4.6}$	31.8	$\frac{5.6}{6.1}$	82.5 80.8	$11.1 \\ 12.4$
5	15.9	6.9	27.1	8.9	67.2	20.1
6	17.2	5.8	30.0	7.6	74.7	13.5
7	16.8	5.6	28.8	7.8	72.9	11.7
8 9	$\begin{array}{c} 14.1 \\ 13.3 \end{array}$	$\frac{3.6}{3.2}$	23.7 23.7	4.9 4.5	79.3 81.0	$11.3 \\ 10.8$
10	14.9	3.7	26.6	5.2	80.5	12.1
11	14.2	2.8	23.4	3.9	83.3	11.1
12	18.1	4.2	27.9	5.5	80.3	11.1
13	14.1	2.8	22.8	3.5	84.6	11.9
14 15	$\begin{array}{c} 17.6 \\ 16.6 \end{array}$	$\frac{2.8}{3.9}$	$\begin{array}{c c} 28.1 \\ 28.7 \end{array}$	3.5 5.4	87.5 81.2	$11.0 \\ 12.1$
16	16.1	5.5	28.1	7.6	73.0	13.6
17	19.7	4.4	30.8	5.4	82.5	10.8
18	20.6	3.9	31.8	4.9	84.6	11.2
19 Mill D	20.1	4.7	34.7	6.4	81.6	11.2
Screw Press						
1	21.7	7.6	37.0	11.2	69.7	14.5
2	21.8	4.4	39.0	7.1	81.8	8.5
3 Mill E	21.0	10.4	37.5	14.7	60.8	18.2
Screw Press						
1	19.0	8.3	32.9	12.0	63.5	12.8
2	18.8	7.1	32.5	10.0	69.2	11.2
3 4	$\frac{18.2}{18.7}$	$7.3 \\ 5.9$	$30.4 \\ 31.7$	$10.2 \\ 8.0$	66.5 74.8	$12.0 \\ 11.0$
5	18.3	5.6	32.3	8.3	74.8	10.3
6	17.6	8.3	31.5	11.9	62.2	13.8
Mill Averages	200					
A	$\frac{20.8}{19.5}$	8.4	33.1	12.2	63.0	34.1
B C	$19.5 \\ 17.1$	$\begin{array}{c} 14.2 \\ 4.3 \end{array}$	$ \begin{array}{r} 34.4 \\ 28.5 \end{array} $	$20.8 \\ 5.7$	39.6 80.0	$ \begin{array}{c c} 29.0 \\ 12.2 \end{array} $
D	21.5	7.5	37.8	11.0	70.8	13.7
E	18.4	7.1	31.9	10.1	68.4	11.9

in the screw press. In hydraulic pressing the thiamine reduction observed for Mill A is considerably greater than that for Mill B and probably reflects the influence of the prolonged cooking employed by Mill A.

The gossypol balances calculated to a moisture, oil-, and hull-free basis are given in Table IV. In the raw meats, cooked meats, and meal the values may be considered either as percentages or as pounds per 100 pounds of moisture-, oil-, and hull-free material. Thus the values are expressed on a common basis without the influence of variable moisture, oil, and hull contents. It is seen that the conditions of cooking, namely, moisture content, temperature, and time, play an important role in the binding or reduction of free gossypol. Those employed by hydraulic Mill A reduced the free gossypol to a level approaching that of screw-pressed meals. However, before any such practice of more thorough cooking prior to hydraulic pressing should become general, the effects of such cooking on the nutritive value of the protein in the meal should be thoroughly investigated.

The fate of gossypol during processing is also shown in the materials balances of Table IV. A difference in

TABLE IV
Gossypol Balance in Mill Samples

	Free gossypol in moisture-, oil-, and hull-free material			Total gossypol per 100 lbs. of moisture-, oil-, and hull-free material				
Sampling No.	Raw meats	Cooked meats	Meal	Raw meats	Cooked meats	Meal	Oil	Meal and oil
	%	%	%	lbs.	lbs.	lbs.	lbs.	lbs.
Iill A—Hydraulic Press		0.004	0.000	1.00	1.04	1 50	0.01	1 5 7
1	1.93	0.064	0.066	1.86	1.64	$1.56 \\ 1.62$	0.01	1.57
2	1.96	0.079	0.068	2.00	1.65		0.01	1.63
3	1.72	0.083	0.072	1.73	1.61	1.58	0.01	1.59
4	1.69	0.085	0.066	1.66	1.48	1.48	0.01	1.49
5	1.75	0.069	0.050	1.70	1.39	1.36	0.02	1.38
6	1.71	0.077	0.064	1.60	1.50	1.53	0.01	1.54
7	1.33	0.071	0.057	1.29	1.31	1.25	0.01	1.26
8	1.59	0.068	0.058	1.48	1.37	1.31	0.01	1.32
9	1.43	0.063	0.049	1.61	1.56	1.52	0.01	1.53
fill B—Hydraulic Press					!			
1	2.09	0.25	0.18	2.05	1.93	1.93	0.04	1.97
2	1.87	0.26	0.21	1.91	1.82	1.78	0.05	1.83
3	1.83	0.20	0.16	1.95	1.96	1.91	0.04	1.95
4	$\frac{1.03}{2.04}$	0.23	0.17	2.10	2.06	1.92	0.05	1.97
5		0.16	0.16	1.85	1.86	1.75	0.05	1.80
	1.95	0.23	0.19	1.98	1.86	1.76	0.06	1.82
6	1.89		0.19	1.96	1.95	1.80	0.06	1.86
7	1.71	0.27						
8	1.83	0.23	0.17	1.83	1.72	1.70	0.05	1.75
ill C—Screw Press								
1	1.30	0.66	0.035	1.28	1.26	0.99	0.20	1.19
2	1.57	0.61	0.042	1.59	1.35	1.08	0.21	1.29
3	1.43	0.66	0.035	1.40	1.34	1.08	0.20	1.28
4	1.38	0.56	0.044	1.43	1.34	1.08	0.16	1.24
5	1.35	0.33	0.048	1.30	1.21	1.18	0.11	1.29
6	1.31	0.35	0.042	1.26	1.25	1.09	0.11	1.20
7	1.23	0.38	0.112	1.25	1.17	1.01	0.12	1.13
8	1.02	0.42	0.084	1.34	1.15	0.93	0.10	1.03
9	1.19	0.49	0.058	1.41	1.20	0.94	0.14	1.08
10		0.49	0.038	1.44	1,32	1.05	0.16	1.21
	1.32		0.048	1.10	1.14	0.91	0.16	1.07
[1	1.23	0.58						
12	1.25	0.41	0.035	1.13	1.08	0.94	0.10	1.04
13	1.09	0.42	0.029	1.05	1.04	0.76	0.14	0.90
14	1.20	0.45	0.029	0.97	0.91	0.72	0.12	0.84
15	1.32	0.63	0.038	1.18	1.21	0.78	0.18	0.96
16	1.26	0.57	0.039	1.24	1.29	1.05	0.12	1.17
17	1.21	0.37	0.028	1.24	1.23	0.92	0.17	1.09
18	1.14	0.48	0.046	1.28	1.16	1.07	0.18	1.25
19	1.42	0.60	0.053	1.49	1.29	1.05	0.19	1.24
ill D—Screw Press								
1	1.96	0.43	0.058	1.91	1.66	1.50	0.15	1.65
2	1.95	0.36	0.043	1.89	1,78	1.58	0.13	1.71
3	2.02	0.30	0.067	1.79	1.58	1.37	0.12	1,49
ill E—Screw Press	2,04				1			
1	2.10	0.44	0.064	2.18	2.02	1.78	0.20	1.98
2	$\frac{2.10}{2.21}$	0.44	0.076	$\frac{2.18}{2.19}$	2.02	1.87	0.20	2.05
3	1.99			$\frac{2.19}{2.04}$		1.70		1.95
4		0.64	0.067		2.05		0.25	
	2.07	0.41	0.064	2.04	1.97	1.72	0.21	1.93
5	2.19	0.66	0.059	2.24	2.20	1.80	0.24	2.04
6	2.10	0.42	0.075	2.15	2.11	1.89	0.18	2.07
ill Averages						l I		
<u>A</u>	1.68	0.073	0.061	1.66	1.50	1.47	0.01	1.48
<u>B</u>	1.90	0.23	0.18	1.95	1.90	1.82	0.05	1.87
<u>C</u>	1.27	0.50	0.046	1.28	1.21	0.98	0.15	1.13
D	1.98	0.36	0.056	1.86	1.67	1.48	0.13	1.62
E	2.11	0.49	0.068	2.14	2.07	1.79	$0.\bar{21}$	2.00

the average total gossypol content of the raw meats is noted as varying from 1.28 to 2.14 pounds. There appears to be a small loss of gossypol during the cooking operations for both hydraulic- and screw-press methods and little, if any, loss in the pressing operations. On adding the total gossypol in the meal and in the corresponding oil, the results seem to indicate that very little gossypol is lost or destroyed by either the hydraulic- or screw-pressing step of processing. The variations in the values for total gossypol in the cooked meats and in the meal and oil are well within the usual errors of sampling and analysis.

Present hydraulic- and screw-pressing methods differ somewhat in the stage of processing during which the free gossypol in the raw meats is bound and in the distribution of the gossypol in the products. To demonstrate this, the analytical data from Table IV were used to calculate the percentage of the total amount of gossypol present in the raw meats which was bound in both cooking and pressing operations, left in the meal as free gossypol, and removed in the crude oil. These data are given in Table V. With respect to hydraulic pressing it is apparent that the major reduction in free gossypol takes place in the cooker, and little, if any, reduction is realized in the press. Of the free gossypol which remains after cook-

ing and pressing the greater portion is left in the meal, and very little is expressed in the crude oil.

In screw pressing, reduction of free gossypol takes place in both the cooking and pressing steps and, for these commercial mills, the gossypol binding is greater in the cooker than in the press. The levels of gossypol binding in the cookers were not uniform; for Mill C variations from 47 to 74% were noted. The major portion of the free gossypol which remains after cooking and pressing was found in the crude oil while a consistently small amount was left in the meal.

The variations in the binding of gossypol in the cooking are attributed to variations in the combinations of moisture content, time, and temperature of cooking, and to variations in the effectiveness of the mechanical preparation of the meats in rupturing the pigment glands. This observation agrees with the results recently reported by Batson *et al.* (5).

It is noted in Table V that when the level of gossypol binding in the cooker is low, the amount of gossypol present in the crude oil is correspondingly high. This relationship is demonstrated by the scatter diagram for the data of the screw-pressing mills shown in Figure 1. The equation for the relationship of the gossypol in the oil on the gossypol bound in

TABLE V Distribution of Gossypol

	Bound	Bound	In meal	Removed	
Sampling No.	in cooker	in press	as free gossypol	in crude oil	
Mill A—Hydraulic Press	%	%	%	%	
1	96.6		3.5	0.5	
2	96.1	0.1	3.4	0.5	
3	94.9	0.1	4.2	0.6	
4	94.9	0.5	4.0	0.6	
5	95.9		2.9	1.2	
6 7	$95.2 \\ 94.5$	0.2 0.3	4.0 4.4	0.6	
8	95.4	0.5	3.9	0.8	
9	96.1	0.3	3.0	0.6	
Mill B—Hydraulic Press	30.1	0.0	0.0	0.0	
1	87.8	1.5	8.8	2.0	
2	86.4		11.0	2.6	
3	89.7		8.2	2.1	
4	89.0	0.5	8.1	2.4	
5	91.4		8.6	2.7	
<u>6</u>	88.4	•••••	9.6	3.0	
7	86.2		11.2	3.1	
8 Mill C—Screw Press	87.4	0.6	9.3	2.7	
1	48.4	32.8	2.7	15.6	
2	61.6	22.6	2.6	13.2	
3	52.9	30.0	2.5	14.3	
4 5	60.8	25.2	3.1	11.2	
6	$74.6 \\ 72.2$	$12.3 \\ 15.9$	3.7 3.3	8.5 8.7	
7	69.6	12.0	9.0	9.6	
8	68.6	17.9	6.3	7.5	
,9	65.2	20.6	4.1	9.9	
10	60.4	25.7	3.0	11.1	
11	47.3	35.5	2.5	14.5	
12	63.7	23.9	3.1	8.8	
13	60.0	23.8	2.8	13.3	
14	53.6	30.9	3.0	12.4	
15	46.6	34.7	3.2	15.3	
16	54.0	33.1	3.1	9.7	
17 18	$70.2 \\ 62.5$	13.7 19.5	2.3	13.7	
19	59.7	24.2	3.6 3.6	14.1	
Mill D—Screw Press	33.1	24.2	5.0	12.8	
1	77.5	11.5	3.0	7.9	
2	81.0	10.1	2.3	6.9	
3 Mill E—Screw Press	83.2	6.1	3.7	6.7	
1	79.8	8.3	2.9	9.2	
2	84.0	4.1	3.5	8.2	
3	68.6	15.7	3.3	12.3	
<u>4</u>	79.9	6.9	3.1	10.3	
5	70.5	16.1	2.6	10.7	
6 Mill Averages	80.5	7.4	3.5	8.4	
A	95.5	0.2	3.7	0.7	
B	88.3	0.3	9.4	2.6	
<u>c</u>	60.6	23.9	3.6	12.3	
D	80.6	9.2	3.0	7.2	
E	77.2	9.8	3.2	9.9	

cooking, both expressed as percentages of the amount of gossypol present in the seed, was found to be:

% Gossypol in oil = 23.89 - 0.196 (% gossypol bound in cooking).

The correlation coefficient of this relation is -0.81. It is highly significant.

Summary

The processing of cottonseed by five commercial mills has been systematically examined with reference to free gossypol reduction, nitrogen solubility, thiamine reduction, material balances of total gossypol, and the distribution of gossypol in processing.

One hydraulic mill reduced the free gossypol in the meal to a low level, approximating the level obtained in screw pressing. For a given mill the free gossypol contents of the meals were found to be fairly uniform.

Low free gossypol content of hydraulic-pressed meals depends on the thoroughness with which the gossypol is bound in the cooking. Gossypol is bound in both the cooking and pressing in the production of screw-pressed meals.

A relatively small amount of total gossypol is lost or destroyed in processing cottonseed by either hydraulic- or screw-pressing methods. This small loss occurs while the meats are being prepared for press-

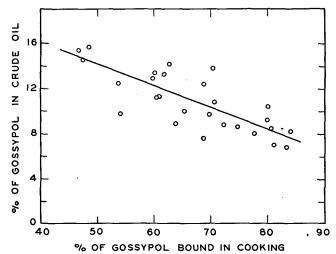


Fig. 1. Relation between percentage of gossypol in crude oil and percentage of gossypol bound in cooking in screw pressing cottonseed.

ing. No significant loss was found which could be attributed to the pressing operations.

Screw-pressed oils appear to contain several times as much gossypol as hydraulic-pressed oils, with the amount dependent on the extent of the binding of gossypol in the cooking and mechanical preparation of the meats for pressing.

The high temperatures developed in screw pressing contributed to a higher reduction in thiamine and nitrogen solubility than was observed for hydraulic pressing.

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REFERENCES

1. Alderks, O. H., in A. E. Bailey (ed.), "Cottonseed and Cottonseed Products," p. 618, New York, Interscience, 1948.
2. Altschul, A. M., Official Proc. 55th Ann. Convention Natl. Cottonseed Products Assoc. 1951, 32-34, 36.
3. American Oil Chemists' Society, "Official and Tentative Methods," 2nd edition, rev. to 1950, Chicago, 1946-50.
4. Association of Vitamin Chemists Inc., "Methods of Vitamin Assay," 2nd edition, pp. 107-127, New York, Interscience, 1951.
5. Batson, D. M., Thurber, F. H., and Altschul, A. M., J. Am. Oil Chem. Soc., 28, 468-72 (1951).
6. Conner, R. T., and Straub, G. J., Ind. Eng. Chem., Anal. Ed., 13, 380-84 (1941).
7. Haddon, R., Schwartz, A. K., Williams, P. A. Thurber, F. H.

7. Haddon, R., Schwartz, A. K., Williams, P. A., Thurber, F. H., Karon, M. L., Dechary, J., Guice, W., Kupperman, R., O'Connor, R., and Altschul, A. M., Cotton Gin and Oil Mill Press, 52, No. 9, 18-20 (1950).

(1950).

8. Hafner, F. H., Soybean Digest, 11, No. 8, 18-20 (1951).

9. Hale, F., and Lyman, C. M., in A. E. Bailey (ed.), "Cottonseed and Cottonseed Products," pp. 830-33, New York, Interscience, 1948.

10. Hayward, J. W., Feedstuffs, 22, No. 30, 30-35, 38, 40-42 (1950).

11. National Research Council, Food and Nutrition Board, Reprint and Circ. Ser. No. 131, 19 pp. (June 1950).

12. Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., 34, 714-16 (1942).

(1942).

13. Pons, W. A. Jr., and Guthrie, J. D., J. Am. Oil Chem. Soc., 26, 671-76 (1949).

14. Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chem. Soc., 27, 390-93 (1950).

15. Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chem. Soc., 28, 8-12 (1951).

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