ethanolamine and serine in brain lipids by a dinitrofluorobenzene technique. When their data were calculated as a phosphatidyl ethanolamine/phosphatidyl serine molar ratio, values of 2.90 for rat brain and 2.70 for sheep brain were obtained.

The technique described above appears to be suitable for the quantitative isolation of phosphatidyl ethanolamine and phosphatidyl serine for the subsequent determination of the amounts of fatty acids and fatty aldehydes present in these phospholipids. It is to be noted that the crude solid obtained by evaporation of the original extract was used for chromatography. Such a sample will allow an accurate determination of the amount of phosphatidyl ethanolamine and phosphatidyl serine in the original brain specimen, and the exact ratio of the two substances can be determined. The use of acetone precipitation or other forms of preliminary purification of the crude extract may result in variable losses.

*Addendum.* Since this work was completed, we have obtained values for phosphatidyl ethanolamine in beef brain by an independent method, using diethylaminoethyl cellulose for column chromatography. The fresh brain was found to contain 2.36% phosphatidyl ethanolamine by ion exchange cellulose chromatography compared to 2.42 and 2.67% determined for two other beef brains as described above. The close agreement of the values obtained by two independent methods on different beef brain samples is a further indication that the techniques of extraction and chromatographic separation are reproducible and quantitative.

The experience of several investigators with our method has demonstrated that a common difficulty is fragmentation of silicic acid during preliminary washing and drying. Gentle handling will assure minimum mechanical trauma. Unnecessary stirring and shaking can cause the silicic acid bed to pack so firmly that a suitable flow-rate cannot be obtained.

**Acknowledgment**

These studies were supported in part by Grants B-1847 and C-3134 from the U.S. Public Health Service.

REFERENCES

1. Lea, C.H., Rhodes, D.N., and Stoll, R.D., Biochem. J., *60*, 353–363 (1955).
2. Rouser, George, White, S.G., and Schloredt, D., Biochim. et Biophys. Acta, *28*, 71–80 (1958).
3. Hanahan, D.J., Dittmer, J.C., and Warashina, E., J. Biol. Chem., *228*, 685–700 (1957).
4. Dieckert, J.W., and Reiser, Raymond, J. Am. Oil Chemists' Soc., *33*, 535–537 (1956).
5. Marinetti, G.V., Erbland, J., and Kochen, J., Fed. Proc. *16*, 837–844 (1957).
6. Hanahan, D.J., J. Biol. Chem., *195*, 199–206 (1952).
7. Rapport, M.M., and Alonzo, N., J. Biol. Chem., *217*, 193–198 (1955).
8. Levine, C., and Chargaff, E., J. Biol. Chem., *192*, 465–479 (1951).
9. Lea, C.H., "Biochemical Problems of Lipids," Butterworths Scientific Publications, 1956, pp. 81–90.
10. Chargaff, E., Ziff, M., and Rittenberg, D., J. Biol. Chem., *144*, 343–352 (1942).
11. Collins, F.D., and Wheeldon, L.W., Biochem. J., 70, 46–49 (1958).

[Received April 7, 1960]

# Properties of Cottonseed Meals Prepared with Acetone-Petroleum Ether-Water Azeotrope [1]

**WILLIAM H. KING, JAMES C. KUCK, and VERNON L. FRAMPTON,**
**Southern Regional Research Laboratory,[2] New Orleans, Louisiana**

EVIDENCE from the most recent studies on the nutritive quality of cottonseed meals indicates that the most important factors affecting the nutritive quality of the meals as protein supplements for nonruminant feedings are the total gossypol and the lysine contents of the meals (1). The two effects are additive; the nutritive quality is directly proportional to the lysine content and inversely proportional to the total gossypol content. It is possible, on the basis of the lysine and gossypol contents of cottonseed meals, to predict, with a reasonable degree of accuracy the growth response of broilers, perhaps also of swine, receiving cottonseed meals of known total gossypol and lysine contents.

Research to test further the hypothesis that cottonseed meals may be graded in this way is continuing, and there has been included in this research effort the preparation of cottonseed meals with low total gossypol and high lysine contents.

Whether oil is pressed out of cottonseed or removed by extraction with an oil solvent, cottonseed meats are usually subjected to pretreatments to enhance the extractability of the oil, to reduce the "fines" problem, and to bind gossypol. In most of the pretreatments of cottonseed meats the moisture content is at least that present in the natural seed, and the temperatures are increased to 212°F. or more. The meats are usually held at these elevated temperatures for at least 30 min. While this type of pretreatment usually brings about an improvement in the extractability of the oil, it causes a part of the gossypol to become bound to the meal and it also brings about the destruction of a part of the lysine in the meal protein.

Lysine in cottonseed is heat-labile (2,3,5), and the quantities found in cottonseed meal proteins are invariably less than those found in the proteins of the raw seed. The wide variation of the lysine content (*ca.* 2–3.8 g. per 16 g. of nitrogen, as determined through the use of dinitrofluorobenzene) of the proteins in commercial cottonseed meals occurs because of the wide variation from mill to mill in the quantity of heat applied to, the moisture content of, and the temperatures reached in the seed while being processed for oil. Furthermore gossypol becomes bound in the meal while the cottonseed is being processed for

[1] Presented at the 51st Annual Meeting, American Oil Chemists' Society, Dallas, Tex., April 4–6, 1960.
[2] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

oil, and it has been demonstrated repeatedly that the quantities of bound gossypol in the meals also vary widely (0.7–1.5%) from meal to meal. This wide variation in bound gossypol may be attributed to variations in the processing conditions used, also in the quantities of gossypol in the raw seed.

The ideal pretreatment for cottonseed meats would convert them into porous solids through which the oil and the miscella percolate with ease and at the same time would avoid the binding of gossypol and the destruction of lysine. In the ideal oil-extraction procedure neither the meats nor the oil would be exposed to heat. This report is concerned with the preparation and the properties of cottonseed meals that were prepared with these ideal objectives in view by extraction of the meats with solvent mixtures.

## Experimental

*Methods of Analyses. Epsilon*-amino-free lysine was determined by the method of Conkerton and Frampton (5). Free gossypol was determined by the A.O.C.S. method (6), and total gossypol was determined by the method reported by King and Frampton (7).

*Extraction of Raw Cottonseed Meats with Dry Acetone.* Extraction of raw cottonseed flakes with dry acetone at ambient temperatures is difficult because of the spontaneous disintegration of the suspended flakes to form an extremely fine powder. Filtration under these conditions is very slow, and it is practically impossible to reduce the free gossypol below about 0.1% by this method. However the slow filtration may be circumvented by first soaking the comminuted meats in aqueous acetone (30 parts by volume of water and 70 parts of acetone), then dehydrating the mixture by adding dry acetone. This treatment tends to agglutinate the meat particles and to prevent them from becoming pulverized when the oil is extracted. Extraction of the oil, as well as pigments, is completed with dry acetone.

Decorticated cottonseed kernels (7% moisture) were flaked on smooth rolls. They were then comminuted by agitating them in a planetary type of food mixer (a flat beater was used), then the particles were classified according to size of screening. One hundred and fifty-gram lots of meats of each particle size of classification were gently agitated with 300 ml. of 70% aqueous acetone. The mixture was permitted to stand over-night, then an equal volume of acetone was added and the mixture was stirred again. The marc and miscella were separated 1 hr. later by filtration on a fritted-glass Buchner funnel. The meats were washed five times by suspending them successively in 150-ml. portions of dry acetone, then filtering the slurry. The particles of cottonseed meats retained their original shape and size and formed a compact, porous cake on the funnel.

The effect of particle size on the efficiency of extraction may be seen from the data recorded in Table I.

*Extraction of Raw Cottonseed Flakes with a Homogeneous Acetone-Petroleum Ether-Water Mixture.* Freshly decorticated Acala 4–42 cottonseed meats were adjusted to a moisture content of 15% by the addition of the requisite quantity of water, and then, in order that equilibrium might be established, the meats were stored over-night in a closed container. The moist meats were flaked to a thickness of 0.003 in. with spring-loaded, single-pair smooth rolls, and the flakes were spread out on racks and permitted to dry. In an alternate drying procedure the flakes were spread over trays in a large drying oven where they were exposed to a current of warm (less than 54°C.) air until a moisture content of 10% was reached.

TABLE I

Effect of Particle Size on Extraction of Cottonseed Kernels with Aqueous Acetone of Varied Water Content [a]

| Particle size (mm. diam.) | % Oil remaining in extracted meal | % Gossypol remaining in extracted meal (oil-free basis) | | *Epsilon*-amino-free lysine [b] remaining in extracted meal (% of protein) |
|---|---|---|---|---|
| | | "Free" | "Total" | |
| 4.0 | 28.4 | 0.09 | 0.50 | 4.3 |
| 2.5 | 28.5 | 0.08 | 0.40 | 4.3 |
| 1.75 | 24.0 | 0.08 | 0.38 | 4.3 |
| 1.25 | 22.3 | 0.06 | 0.35 | 4.3 |
| 0.75 | 23.6 | 0.05 | 0.34 | 4.1 |
| 0.38 | 2.6 | 0.04 | 0.32 | 4.1 |
| 0.25 | 1.3 | 0.035 | 0.30 | 4.1 |
| Unextracted meats | 33.0 | 1.10 | 1.30 | 4.4 |

[a] Analyses on air-dry basis.
[b] Grams of lysine per 16 g. of nitrogen in the protein.

Twenty-five pound lots of either of the flakes, prepared as indicated above, were mixed with 5 gal. of a solvent mixture composed of acetone, petroleum ether (boiling range 68–71°C., known in the vegetable oil-extraction industry as commercial hexane), and water in the proportions 53:44:3. One hour later the slurry was separated on a Sweco continuous separator which was equipped with a 165-mesh screen. The marc was diverted to the original mixing vessel, and a fresh 5-gal. quantity of the acetone-petroleum ether-water mixture was added to it. The slurry was screened 30 min. later. This washing operation was repeated four more times, and finally the marc was permitted to dry at ambient temperature by spreading it out on a tray. Filtration of the miscella through paper showed that less than 1% of "fines" (collection of extremely small meal particles) was produced in the extraction procedure. Chemical data for the meal are recorded in Table II, Column 5.

The results of a more exhaustive extraction with the acetone-petroleum ether-water mixture are also recorded in Table II. In this more exhaustive extraction 150 g. of the air-dried flakes were extracted by placing them in a 4-liter filter flask, which was equipped with a bottom inlet. The flask was filled with the solvent mixture, and the resulting slurry was stirred gently but continuously by an air-driven 3-in. propeller which was located about 2 in. from the bottom of the flask. Fresh solvent was fed into the bottom of the flask at a rate of about 2 liters per hour for 24 hrs. Analysis of the effluent coming from the side neck of the flask, which was free of any suspended cottonseed meat particles, showed the effluent to be free of gossypol at the end of the extraction period.

It will be noted that meal produced in this manner contained no free gossypol.

In another experiment, to determine efficiency of the solvents studied, two parts by weight of solvent were added to one part of cottonseed flakes that had been moistened, then dried at room temperature to a moisture content of 10%. The mixture was gently stirred, and the solid material was permitted to settle. After the indicated time-intervals the mixture was again stirred, and, after the solid material settled, an aliquot of the solvent was taken for analyses. The percentage of the total oil of the flakes which was found in solution in the supernatant liquid is plotted

TABLE II

Chemical Data for Specified Cottonseed Meals [a]

| Constituent | Commercial meals | | | | Experimental "acetone petroleum ether-water" extracted | | Raw meats |
|---|---|---|---|---|---|---|---|
| | Prepress solvent-extracted | Screw-pressed | Solvent-extracted | Hydraulic-pressed | Batch-extracted | Continuous extraction | |
| Free gossypol (%) | 0.06 | 0.03 | 0.20 | 0.10 | 0.03 | 0.00 | 1.0 |
| Total gossypol (%) | 1.3 | 1.3 | 1.0 | 1.2 | 0.25 | 0.40 | 1.0 |
| *Epsilon*-amino-free lysine (g./16 g. N) | 3.7 | 3.1 | 3.8 | 3.4 | 4.3 | 4.3 | 4.2 |
| Oil (%) | 1.0 | 2.5 | 0.8 | 5.0 | 0.4 | 0.1 | 33.0 |

[a] Analyses on air-dry basis.

in Figure 1 against the time of contact between flakes and solvent. It will be noted that about 97% of the oil was found in the acetone-petroleum ether-water mixture after 4 hrs. of contact whereas only 78% was found in the acetone and about 71% in the petroleum ether.

The relative efficiency in the extraction of unbound gossypol is shown in Figure 2, where the aliquots

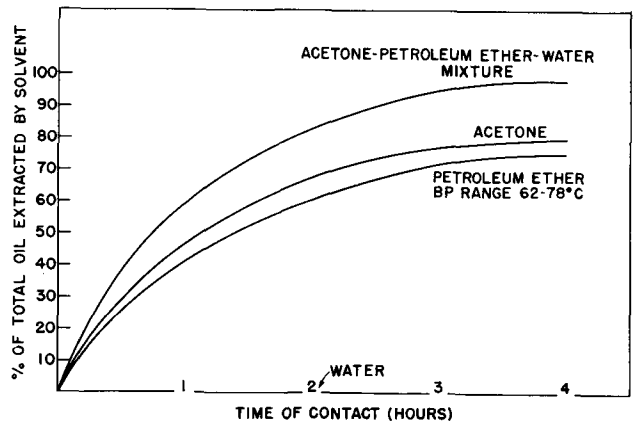


FIG. 1. Total oil of the flakes found in the supernatant miscella plotted against time of contact between flakes and solvent. Zero extraction with water.

described immediately above were analyzed for gossypol. Virtually all of the unbound gossypol is removed into the acetone-petroleum ether-water mixture in 1 hr. whereas only about 60% is removed into acetone in 4 hrs. None of it appears in hexane.

## Discussion

Two distinct advantages are derived from the use of the acetone-petroleum ether-water mixture in extracting raw cottonseed meats. These are the preservation of lysine and the reduction of the total gossypol. A comparison of the data obtained in this investigation and data which are typical of current commercially-produced cottonseed meals may be found in Table II where analyses for free gossypol, total gossypol,

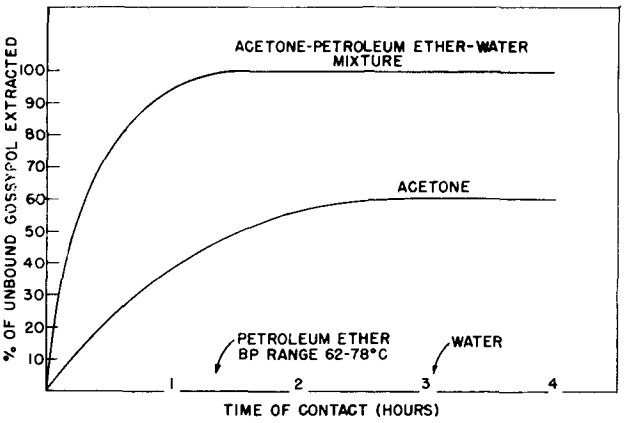


FIG. 2. Relative efficiency of solvents in extracting gossypol from raw flakes. Zero extraction with petroleum ether and water.

lysine (*epsilon*-amino-free lysine, percentage of protein), and oil are recorded. The extracted and ground meals were tan-colored and relatively free from dustiness.

The properties of the solvent mixture permit the processing of relatively moist flakes. Moreover the raw cottonseed flakes can be extracted without the accompaniment of a large quantity of fines.

Solvent recovery from oil or marc does not present a serious problem since the mixture composed of acetone, petroleum ether (boiling range 67–71°C.), and water forms a constant boiling mixture with the composition 42.1–56.5–1.4% by volume, respectively. However practical problems that may arise in the commercial use of the solvent mixture remain to be worked out.

REFERENCES

1. Unpublished data, Southern Regional Research Laboratory.
2. Martinez, W. H., and Frampton, Vernon L., J. Agr. Food Chem., *6*, 312 (1958).
3. Conkerton, E. J., Martinez, W. H., Mann, G. E., and Frampton, V. L., J. Agr. Food Chem., *5*, 460–463 (1957).
4. Martinez, W. H., Frampton, Vernon L., and Cabell, C. H., J. Agr. Food Chem., in press.
5. Conkerton, E. J., and Frampton, Vernon L., Arch. Biochem. and Biophysics, *81*, 130–134 (1959).
6. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed., rev. to 1959, Chicago, 1946–59.
7. King, W. H., Frampton, Vernon L., and Altschul, A. M., J. Am. Oil Chemists' Soc., *35*, 358–360 (1958).

[Received April 28, 1960]