[CONTRIBUTION FROM THE MULTIPLE FELLOWSHIP OF THE COTTON RESEARCH FOUNDATION, MELLON INSTITUTE]

Composition of Cottonseeds. I. Solubility of Nitrogenous Constituents

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Approximately 3,000,000 tons of cottonseeds are produced annually in the United States. At present, after removal of the hulls, the oil is recovered by pressing and the residual meal is used as a source of protein in stock foods. Depending upon the source of the seed and the efficiency of the pressing operation, the protein content of the meal varies from 45 to 55%. Commercial cottonseed meal is adjusted with hull bran to a protein content of 41% .

Inasmuch as proteins are becoming increasingly important as industrial raw materials, it appeared of interest to investigate the possibility of isolating and utilizing the proteins of the cottonseed. The work to be described is part of a general research program along these lines and deals particularly with the efficiency of extraction by aqueous solutions of salts, acids, and alkalies.

The proteins of the cottonseed were first investigated by Osborne and Vorhees.[1] Jones and Csonka[2] subsequently separated and characterized several of the fractions. There has been, however, no detailed study of their solubility behavior.

Experimental

In the procedure used for obtaining cottonseed oil commercially, the meats are first rolled, then cooked for twenty to forty minutes in an atmosphere of steam. After removal of the oil by pressing, the cake is broken and ground. The resultant cottonseed meal of commerce is not suitable for the preparation of protein. The yields are low because of denaturation of the protein by the heat treatment, and the product is dark, the color resulting from a combination of the pigments with the protein. For these reasons, most of the observations recorded refer to cottonseed meal from which the oil was removed by solvent extraction and which was never exposed to a temperature higher than 50°.

Whole cottonseed meats[3] were ground in a burr mill to 16-mesh-size particles, and extracted with ethyl ether in a large continuous extractor of the Soxhlet type for thirty-six to seventy-two hours. Approximately 10 kg. of ground meats could be accommodated in one extraction. The extraction chamber was fitted with a copper coil through which, at the end of the extraction period, warm water (45–50°) was passed to evaporate most of the entrained ether. The residual meal was air-dried, reground in the burr mill, and sieved through a 60-mesh screen to remove most of the hull particles with which the meats were originally contaminated.

One large sample of the light yellow meal so obtained was used for all the determinations. The following analytical data were obtained by the usual methods.

Constituent	Percentage
Water	9.0
Nitrogen	8.6
Lipids	0.5 (ether extraction)
Lipids	2.0 (chloroform extraction)
Ash	7.5

Five-gram portions of the meal were weighed into 8-ounce (240 cc.) centrifuge bottles. Because the meal is difficult to wet, it was stirred up thoroughly with 10 to 20 cc. of the extracting solution. The remainder (total, 200 cc.) was then added, the bottles were shaken thoroughly, and allowed to stand at room temperatures (23 to 28°).

As shown in Table I, continued agitation was unnecessary for complete extraction, and three hours was sufficient time to establish equilibrium. In some series of runs the meal suspensions were allowed to stand for twenty-four hours. The differences between these results and those obtained with the shorter experimental periods were within the limits of error.

In order to determine the amounts of nitrogenous compounds dissolved or peptized, the bottles were centrifuged and aliquots of the supernatant solutions were filtered and analyzed for nitrogen by a semimicro modification of the Kjeldahl–Gunning method (5–20 mg. nitrogen). The results, corrected to 200 cc., are expressed in terms of percentage of total nitrogen.

Table I also illustrates the effect of temperature on the efficiency of extraction by water and by 0.5 normal sodium chloride solution. The differences between the amounts

TABLE I

EFFECT OF TIME AND SHAKING ON EXTRACTION OF ETHER-EXTRACTED COTTONSEED MEAL WITH 0.5 *N* NaCl

Time, min.	% of total nitrogen extracted: Continuous agitation	No agitation
30	75.5	73.6
60	75.5	74.4
90	76.6	76.2
120	77.0	77.3
180	..	78.2
1440	..	78.3[a]

EFFECT OF TEMPERATURE

Temp., °C.	% of total nitrogen extracted: By H_2O	By 0.5 *N* NaCl
0	26.0	55.2
25	28.3	79.7
50	29.8	81.9

[a] See also footnote to Table II.

(1) T. B. Osborne and C. G. Vorhees, THIS JOURNAL, **16**, 778 (1894).

(2) D. B. Jones and F. A. Csonka, *J. Biol. Chem.*, **64**, 673 (1925).

(3) The meats were obtained through the courtesy of the Perkins Oil Company, Memphis, Tenn. They were taken from the mill after dehulling and before rolling.

extracted at 25 and at 50° were sufficiently small to indicate that attempts to carry out the extractions at constant temperature were unnecessary.

An unaccountable variation in the results of different runs prevents the assignment of absolute accuracy within 3 to 5%. However, the duplicability of determinations in a single run was well within 1%. Thus, although the amounts of nitrogen extracted by 0.5 normal solutions of sodium sulfate and sodium sulfite do not differ by more than 0.3%, in the several runs in which they were compared directly, they were invariably efficient in the same order. For this reason, the data in each series of determinations except those of Table II) are those of a single representative run.

A portable glass electrode was used for the determination of *p*H. In the *p*H range above 9, a correction has been applied to the reading, depending upon the concentration of salt present.

Results and Discussion

The principal protein in cottonseed is a globulin. In the experiments of Jones and Csonka,[2] 84.6% of the nitrogen of cottonseed meal was soluble in 10% sodium chloride solution, and an additional 10.3% could be extracted by alkali. The non-protein nitrogen in their extracts amounted to 10.1% of the total. In the present investigation, it was found that 25 to 30% of the nitrogen was extractable with water and that, of this, a large part was present in the form of a hitherto undescribed protein. This fraction has been isolated and will be described in a later publication.

In the discussion which follows, it has been assumed that the water-soluble protein and non-protein nitrogen fractions are soluble in most of the solutions used, and that the variations in amount of nitrogen extracted may be interpreted in terms of the behavior of the globulin and glutelin fractions.

The peptizing activity of a number of inorganic salts is outlined in Table II. In the majority of cases 0.5 normal solutions extracted practically as much nitrogen as could be extracted by higher concentrations. The lyotropic series effect is less marked than that noted by Gortner and his co-workers[4] in the extraction of the proteins from wheat flour.

Sodium and potassium fluorides extracted smaller amounts of protein than did the other neutral salts. The relatively high yields obtained with calcium and magnesium halides as compared with those extracted by sodium dihydrogen phosphate suggest that, at least within a limited range, *p*H is not an important factor in salt peptization. On the alkaline side of neutrality, disodium phosphate and sodium bicarbonate were no more effective extractants than the neutral salts.

In Table III, the effect of concentration on the peptizing activity of sodium chloride and sodium sulfite is outlined in greater detail. The denaturation of cottonseed proteins which have been subjected to the cooking process is shown in the decreased yields from commercial meal.

(4) R. A. Gortner, "Outlines of Biochemistry," 2d ed., John Wiley and Sons, Inc., New York, N. Y., 1938.

TABLE II

EFFECT OF SALTS ON PEPTIZATION OF COTTONSEED PROTEINS

Salt	Percentage of Total Nitrogen Extracted 0.25 *N*	0.5 *N*	1.0 *N*	*p*H[a]
LiCl	53.4	81.2	81.8	6.4
NaF	..	57.5	73.2	7.2
NaCl	51.1	78.2	80.3	6.4
NaBr	59.1	81.5	..	6.4
NaI	68.0	81.9	..	6.4
Na_2SO_4	78.5	81.9	..	6.8
Na_2SO_3	82.8	82.8	82.1	8.3
$NaHCO_3$	..	77.7	..	8.0
Na_2HPO_4	57.9	79.4	..	8.2
NaH_2PO_4	..	28.1	35.6	5.3
Na acetate	..	65.9	78.4	7.0
Na_3 citrate	..	77.0	..	7.4
Na_2 oxalate	67.8	81.7	..	7.1
Na_2 tartrate	55.9	79.2	77.6	6.9
KF	..	54.0	74.7	7.1
KCl	..	78.1	..	6.5
KBr	..	80.5	..	6.5
KI[b]	..	81.9	81.9	6.5
$CaCl_2$	59.1	81.5	81.5	5.1
$MgCl_2$	60.9	82.3	82.9	5.5
$MgBr_2$	62.0	83.5	83.8	5.5
$MgSO_4$	58.9	82.0	82.3	5.7
$BaCl_2$	62.8	77.1	78.9	5.3

[a] *p*H for 0.5 *N* solutions; in general, the values for the other concentrations did not vary by more than 0.1 of a *p*H unit. [b] An interesting phenomenon was observed when, in preliminary trials, calculated amounts of crystalline potassium iodide were added to the meal. Although the salt dissolved immediately upon the addition of water, the amount of protein peptized was never as great as when the salt *solution* was added to the meal. The same results were observed with sodium iodide, but with the chlorides and many of the other salts no differences were detectable.

Time of standing, min.	% of total nitrogen extracted by 0.5 *N* salt solns. KI + H_2O[c]	KI solution[d]
15	67.2	80.4
30	69.6	81.0
60	70.0	81.5
120	70.1	81.9
1440	72.2	81.9

[c] 16.6 g. of KI plus 195.5 cc. of distilled water. [d] 200 cc. of 0.5 *N* KI.

TABLE III

EFFECT OF SALT CONCENTRATION ON THE EXTRACTION OF PROTEINS FROM COTTONSEED MEAL BY NaCl AND Na_2SO_3

Concn. of salt, normality	Percentage of total nitrogen extracted: Ether-extracted meal with NaCl	Ether-extracted commercial meal with NaCl	Ether-extracted meal with Na_2SO_3
0.0	24.9	7.6	..
.1	28.1	8.4	..
.15	..	..	61.7
.175	..	..	68.5
.2	44.4	..	74.5
.25	..	..	82.8
.3	57.7	21.9	..
.4	71.9	..	..
.5	78.2	37.1	82.8
.75	81.5	..	..
1.0	80.3	41.6	82.1
2.0	79.3	..	..
3.0	78.9	41.2	..
4.0	76.6	..	..
5.0	73.2	38.1	..

Figure 1 illustrates the data outlined in Table IV concerning the solubility of cottonseed proteins in acid and alkali solutions. The results with ether-extracted meal are compared with those obtained in the presence of 0.5 normal sodium chloride solution, with commercial cottonseed meal, and with the behavior of soybean meal as demonstrated by Smith and Circle.[5] The cottonseed proteins differ from those of the soybean in being less soluble in water; the isoelectric range of the most abundant protein is 1.5 to 2 *p*H units higher than that of the soybean protein.

In the presence of 0.5 normal sodium chloride, the amount of protein extracted is constant throughout most of the alkaline range. Only above *p*H 11.0 does the curve rise slightly. Apparently, in the presence of salt, the solubility of the glutelin fraction is depressed. The effect of salt in depressing the *p*H of cottonseed protein solutions, especially in the alkaline range, is marked. The phenomenon also has been noted in experiments with soybean.[5,6]

At least one variable responsible for the difficulty in checking results has been traced to the effect of time on the nitrogenous constituents of cottonseed meal. Over a period of weeks and months, successive runs have demonstrated a gradual slight increase in solubility. The increase could not be correlated with changes in moisture content. A more detailed investigation of these changes is in progress. With soybean, the solubility of the proteins decreases on standing.[5,7]

TABLE IV

EFFECT OF NaOH AND H_2SO_4 ON THE EXTRACTION OF PROTEINS FROM COTTONSEED MEAL

Milli-equivalents of H_2SO_4	Percentage of total nitrogen extracted: Ether-extracted meal *p*H	%	Ether-extracted meal with 0.5 *N* NaCl *p*H	%	Ether-extracted commercial meal *p*H	%
3.60	1.9	23.6	1.5	22.9	1.9	7.2
1.80	2.3	18.1	2.0	24.9	2.3	6.4
1.17	2.9	12.9	2.9	27.1	2.8	5.4
0.81	3.8	13.6	3.8	28.9	3.7	4.8
.45	4.8	18.1	4.7	38.8	4.8	5.6
.18	5.8	22.8	5.6	72.1	5.9	6.8
0	6.9	24.9	6.4	78.2	6.8	7.6
NaOH						
0.10	7.6	32.9	6.9	78.4	7.4	14.6
.21	8.7	73.8	7.5	78.7	8.6	38.1
.31	9.6	85.6	8.8	78.4	..	..
.41	10.4	90.0	9.1	78.7	10.0	55.1
.62	10.7	91.4	9.9	79.5	10.3	60.4
.82	11.1	93.1	10.5	78.4	..	..
1.03	11.4	92.8	10.8	78.0	11.1	64.4
3.09	11.9	92.6	11.5	82.9	11.7	68.4
4.12	12.0	90.9	..	..	..	..

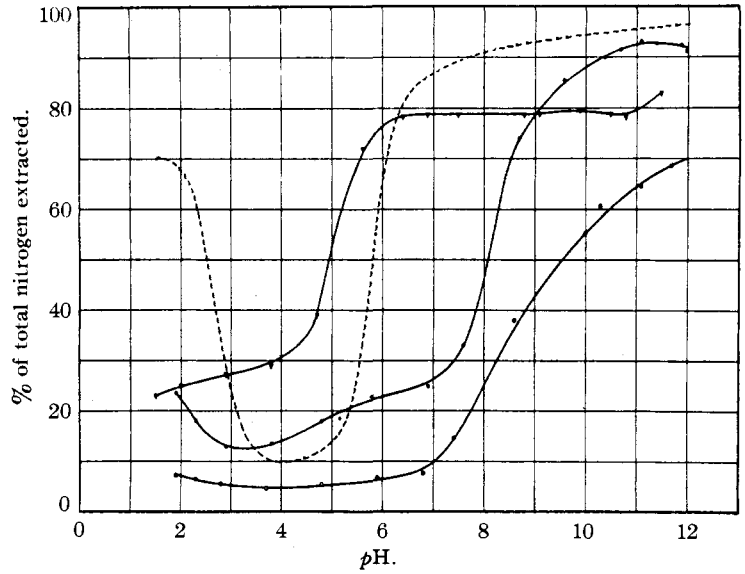

Fig. 1.—Effect of *p*H on the solubility of the nitrogenous constituents of cottonseed meal: —●—●—, ether-extracted cottonseed meal; —▽—▽—, ether-extracted cottonseed meal in 0.5 *N* NaCl; —⊙—⊙—, ether-extracted commercial cottonseed meal; ------, soybean meal.[5]

(5) A. K. Smith and S. J. Circle, *Ind. Eng. Chem.*, **30**, 1414 (1938).

(6) A. K. Smith, S. J. Circle, and G. H. Brother, THIS JOURNAL, **60**, 1316 (1938).

(7) D. B. Jones and C. E. F. Gersdorff, *ibid.*, **60**, 723 (1938).

We are grateful to Dr. R. F. Nickerson for the use of some data obtained in preliminary experiments.

Summary

Cottonseed meats have been ground and extracted with ether. The effect of *p*H and of a number of salts on the extraction of the nitrogenous constituents from the meal is recorded.

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The Pressure–Area and Pressure–Temperature Relations of Expanded Monolayers of Myristic and Pentadecylic Acids

BY GEORGE C. NUTTING AND WILLIAM D. HARKINS

1. Introduction

At 25° when spread on water which contains 0.01 *N* hydrogen ion, margaric ($C_{16}H_{33}COOH$) and higher saturated fatty acids form films which condense rather abruptly at pressures of about 0.5 dyne per cm. to slightly viscous, slightly compressible liquids whose area per molecule extrapolated to zero pressure varies only from about 23 to 24.5 sq. Å.

Lower members, specifically myristic and pentadecylic acids, exhibit a widely different behavior on compression. The limiting area at zero pressure is 46 sq. Å. per molecule. The compressibility is high and the pressure–area plots instead of being linear have a marked concave curvature upward. At a pressure characteristic of the particular acid a break occurs in the pressure–area curve such that the decrement of area per unit increase in pressure increases greatly; that is, the compressibility becomes very high. The curve above the break passes finally into a form characteristic of condensed films. A film which acts in this way is designated below the kink as an expanded film, and above it, until the area per molecule is about 23 sq. Å., as a transition or intermediate film.

The higher fatty acids give similar expanded and intermediate films at higher temperatures. The existence of the expanded state was recognized and investigated by Labrouste[1] and films of this type have been investigated in great detail by Adam and others.[2]

2. Apparatus and Materials

Pressure–area measurements were made with a film balance closely similar to one described by Harkins and Myers.[3] Subsolutions were contained in a shallow trough of stainless steel equipped with a false bottom through which water from a large reservoir was circulated. The trough and film balance were contained in a metal box with a front of plate glass. The top of this box was hollow, and water from the same thermostat was circulated through it in order to keep the temperature the same as that of the trough. This box was surrounded by an air thermostat kept as closely as possible at the temperature of the inner box. Thermocouples were installed just above and just below the film and when the air and the subsolution temperatures were equal it was assumed that the film also was at the same temperature.

In all of the experiments the films were spread on 0.01 *N* sulfuric acid prepared from water redistilled from alkaline permanganate and condensed in tin.

The myristic and pentadecylic acids were generously provided by Professor E. E. Reid of the Johns Hopkins University. The preparation and purification of the acids has been described by Reid and Meyer.[4] The acids were dissolved in purified ligroin (b. p. 60–70°) and spread from a precision pipet devised by Harkins and Anderson.[5] After spreading, a period of from fifteen to forty-five minutes was allowed before compression, during which it was assumed that any micro-crystals present would spread into a monolayer.

3. Pressure–Area Measurements

The pressure–area relations of myristic acid at seven temperatures from 6.7 to 25° are exhibited in Fig. 1 and Table I. So far as is possible, all experimental points are included, except that in the area interval 26–33 sq. Å. of Curve 4 and in a few other places, one-half the points have been omitted in order to avoid too great crowding. The time interval between adjacent points was never less than one minute, and except at the highest temperatures was commonly two to five minutes in the intermediate region. Area decrements also were not uniform but their magnitude may be judged from the curves.

It will be shown later that the rate of compression has a marked influence upon the pressure of

(1) Labrouste, *Ann. Phys.*, **14**, 164 (1920).

(2) N. K. Adam, "The Physics and Chemistry of Surfaces," Clarendon Press, Oxford, England, 1938.

(3) W. D. Harkins and R. J. Myers, *J. Chem. Phys.*, **4**, 716 (1936); G. C. Nutting and W. D. Harkins, THIS JOURNAL, **61**, 1180 (1939).

(4) E. E. Reid and J. D. Meyer, *ibid.*, **55**, 1574 (1933).

(5) W. D. Harkins and T. F. Anderson, *ibid.*, **59**, 2189 (1937).