Absorption Measurements.—The Bausch and Lomb Littrow-type spectrograph was used with a sectorphotometer. This instrument gave simultaneous adjacent records on the photographic plate of light transmitted by solvent and solution in identical cells. The ratio of the exposures was governed by the setting of the sector wheels. Solutions of the compounds were made up to a concentration of 0.005 molar in 95% aldehyde-free ethanol.[12] This concentration was chosen because it gave reasonable absorption for camphor. The cells were 50 mm. in length. The source of illumination was an iron arc operating at about 25 amperes and all wave lengths were obtained from the iron lines themselves.

Positions of equal intensity in the adjacent spectra, transmitted by solvent and solution, were read visually on the negatives with the aid of a microprojector and a mask which obscured all but corresponding portions 1 mm. wide in the spectra, in order to prevent variations in the intensities of the lines from top to bottom from affecting the readings. The molecular extinction coefficient is defined by the equation[2] (p. 378)

$$I = I_0 10^{-\epsilon cl}$$

which may be written in the form

$$\epsilon = \frac{1}{cl} \log_{10} \frac{I_0}{I}$$

Since $\log_{10} I_0/I$ = sector setting of the instrument

$$\epsilon = \text{sector setting}/cl$$

c being concentration in moles per liter and l length of cell in centimeters.

(12) Stout and Schuette, *Ind. Eng. Chem., Anal. Ed.*, **5**, 100 (1933).

The extinction coefficients were plotted against wave length and the data obtained have been summarized in Fig. 1.

Acknowledgment.—The authors wish to express their appreciation to the University of Wisconsin for the use of the spectrograph. They are especially indebted to Dr. V. W. Meloche for his kindness and helpful suggestions during the making of the ultraviolet absorption measurements.

Summary

The ultraviolet absorption spectrum of the compound obtained by the dehydration of the methylamine salt of Reychler's acid, and that of the oxime of this acid (both of which are levorotatory) are quite similar to each other but differ markedly from the absorption spectra of *d*-camphor and Reychler's acid (both of which are dextrorotatory). These absorption data, together with the fact that bornylene showed no absorption in the same range of wave length studied, indicate that no ene-amine structure is present and that the levorotatory dehydration product is 2-(N-methylimino)-*d*-camphane-10-sulfonic acid.

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[CONTRIBUTION FROM THE U. S. REGIONAL SOYBEAN INDUSTRIAL PRODUCTS LABORATORY[1]]

Peptization of Soybean Proteins. The Effect of Neutral Salts on the Quantity of Nitrogenous Constituents Extracted from Oil-Free Meal

BY ALLAN K. SMITH, SIDNEY J. CIRCLE AND GEORGE H. BROTHER

Introduction

A review of the literature discloses that no systematic study on the salt peptization of soybean proteins has been made. Most workers[2-4] in this field have followed to a great extent the lines laid down by Osborne and Campbell,[5] who identified the principal protein in soybeans as a globulin which they called glycinin. They also found a second globulin which resembled phaseolin, about 1.5% of albumin and a small amount of proteose.

Recently Ryndin[4] studied the physical chemistry of soybean proteins. This work involved the preparation of glycinin according to Osborne's procedure, and includes data on the extraction of protein from soybean oil-free meal by sodium chloride solutions of several concentrations. He shows variation in the amount of nitrogenous matter extracted, but no general conclusions concerning salt peptization of soybean meal may be drawn, as he has studied the effect of only one salt.

In recent years it has been shown that the amount of protein extracted from seeds by neutral salts depends upon the kind and concentration of the salt used.[6-8] It was therefore decided that

(1) A coöperative organization participated in by the Bureaus of Chemistry and Soils and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

(2) Jones and Csonka, *Am. Soc. Biol. Chem. Proc.*, **26**, XXIX (1932).

(3) Hartman and Cheng, *J. Chinese Chem. Soc.*, **4**, 152 (1936).

(4) Ryndin, *Colloid J. (U. S. S. R.)*, **2**, 811 (1936).

(5) Osborne and Campbell, THIS JOURNAL, **20**, 419 (1898).

(6) Gortner, Hoffman, and Sinclair, *Colloid Symposium Monograph*, **5**, 179 (1928).

(7) Staker and Gortner, *J. Phys. Chem.*, **35**, 1565 (1931).

(8) O'Hara and Saunders, THIS JOURNAL, **59**, 352 (1937).

preliminary to projected protein separation studies it would be necessary to make a systematic survey of the dispersing action of neutral salts on the oil-free soybean meal.

Experimental[9]

Materials.—The large number of varieties of soybeans,[10] their wide geographical distribution, and the variation in their composition[11] require that one variety of beans should be selected for experimental purposes. Accordingly, Illinois soybeans grown under the supervision of the U. S. Bureau of Plant Industry in 1936 near St. Joseph, Illinois, were chosen for use in the present investigation. The beans were stored in a cool dry room from the time of harvest until the samples were prepared; they were then cracked and flaked on laboratory mill rolls and the oil removed by extraction with petroleum ether (boiling point 30–60°) in a modified Soxhlet extractor[12] holding 8 kg. of beans.

Other seeds selected for comparative study were Wisconsin Pedigree spring barley, 1936 crop; Brill winter wheat, 1936 crop; and Wisconsin Pedigree rye, 1933 crop, all grown at the University of Illinois farm; Bison flax, 1936 crop raised at Crawfordsville, Indiana; and tepary beans, 1936 crop grown at Brookings, South Dakota. These were ground coarsely in a coffee mill, extracted with petroleum ether in a Soxhlet extractor, and then ground in a pebble mill for nineteen hours. Moisture, nitrogen, oil, ash, and phosphorus contents of the extracted meals are given in Table I. The salt solutions used for protein peptizations were made up on a volume normal basis from C. P. or analytical grades of salts.

TABLE I

PERCENTAGE COMPOSITION OF SEED SAMPLES AFTER OIL EXTRACTION[a]

Sample	Moisture, %	Nitrogen, %	Oil, %	Ash, %	Phosphorus, %
Soybeans	9.28	7.80	0.35	5.55	0.972
Flax	10.44	6.98	2.26	4.59	.742
Tepary	10.31	4.42	0.44	3.18	.406
Barley	10.47	1.91	.92	2.58	.177
Rye	11.70	2.31	.78	1.72	.179
Wheat	11.97	2.23	.57	1.61	.138

[a] Analytical results are the average of two or more determinations.

Meal Particle Size.—Preliminary experiments showed a marked variation in the amount of nitrogenous matter extracted with a variation in meal particle size. A close examination of the ground meal also shows a wide variation in particle size for any particular grinding procedure, due largely to the toughness of the seed coat. Since there is very little information in the literature on this subject,[7,13] it was necessary to establish a satisfactory size for the present investigation. Three different samples were obtained from flaked soybean oil-free meal by grinding in a Wiley mill through the 2, 1, and 0.5 mm. screens, respectively, and a fourth sample was ground in a quart size pebble mill for nineteen hours. A sieve analysis of each sample is given in Table II. These samples were extracted with normal solutions of sodium chloride and sodium sulfate, with half normal solutions of the four sodium halides, and with water. The results are given in Table III in terms of percentage of total nitrogen extracted for the various size gradations used. All determinations were run in duplicate by the A. O. A. C. Kjeldahl method to a precision of 1% or better for the percentage total nitrogen extracted.[14] The non-protein nitrogen for the soybean was not determined, but has been reported by Hamilton *et al.*,[15] as 5.55% of the total nitrogen.

TABLE II

SIEVE ANALYSIS OF 100-GRAM SAMPLES OF SOYBEAN MEAL

Wire mesh rating	Wiley mill 2 mm., g.	Wiley mill 1 mm., g.	Wiley mill 0.5 mm., g.	Pebble mill 19 hours, g.
−20	0.3			
20–40	29.2	4.4		
40–60	38.0	37.0		
60–80	10.8	18.4	12.4	
80–100	19.8	5.0	7.0	4.4
100–200		16.6	27.8	3.2
200–		17.0	51.0	89.0
Total	98.1	98.4	98.2	96.6

TABLE III

TOTAL NITROGEN EXTRACTED FROM SOYBEAN MEAL OF DIFFERENT DEGREES OF FINENESS BY WATER AND VARIOUS SALT SOLUTIONS

Type of grinding	Water, %	*N* NaCl, %	*N* Na_2SO_4, %	0.5 *N* NaF, %	0.5 *N* NaCl, %	0.5 *N* NaBr, %	0.5 *N* NaI, %
Wiley 2 mm.	83.3	75.4	73.1				
Wiley 1 mm.	84.3	79.3	76.5				
Wiley ½ mm.	89.4	84.9	81.7	63.1	84.0	86.9	87.0
Pebble mill	91.0	80.5	76.8	62.8	76.9	81.7	84.0

Extraction Procedure.—Two and one-half grams of the meal and 100 ml. of the dispersing solution were placed in a 250-ml. centrifuge bottle and shaken mechanically for thirty minutes. The bottles were centrifuged for six minutes in a centrifuge developing a maximum relative centrifugal force at the bottle tip of 1975 times gravity, and the supernatant liquid decanted into a 500-ml. volumetric flask. Second and third extractions were made likewise, but with a ten-minute shaking period. Longer shaking periods were found to be unnecessary. The combined extracts were diluted to 500 ml. and a 50-ml. aliquot taken for nitrogen analysis. No precipitation occurred on dilution, although in the case of some of the calcium chloride extracts a slight cloudiness appeared. The meal ground through the 2-mm. screen did not pack well on centrifuging; in this case the aliquot was taken for analysis after filtering the diluted extract through coarse

(9) Certain of the extraction techniques used in this investigation were adopted from the work of R. H. Nagel, H. C. Becker, and R. T. Milner on "Some Physical Factors Affecting the Dispersion of Soybean Protein in Water," *Cereal Chem.*, in press (1938).

(10) Piper and Morse, "The Soybean," Chapter IX, McGraw-Hill Book Co., Inc., New York, 1923.

(11) Csonka and Jones, *J. Agr. Research*, **46**, 51 (1933); Bailey, Capen, and LeClerc, *Cereal Chem.*, **12**, 441 (1935).

(12) Sando, *Ind. Eng. Chem.*, **16**, 1125 (1924).

(13) Bishop, *J. Inst. Brewing*, **35**, 316 (1929).

(14) We are indebted to P. Krauczunas of the analytical section of this Laboratory for many of these analyses.

(15) Hamilton, Uyei, Baker, and Grindley, THIS JOURNAL, **45**, 815 (1923).

TABLE IV

TOTAL NITROGEN EXTRACTED FROM SOYBEAN MEAL BY VARIOUS SALT SOLUTIONS[a]

Normality of soln.	Percentages											
	LiCl	NaF	NaCl	NaBr	NaI	Na_2SO_4	$Na_2C_2O_4$	$Na_2C_4H_4O_6$	KCl	$CaCl_2$	$MgCl_2$	$MgSO_4$
0.001										86.8	87.1	
.005			88.6							54.4	56.7	
.010	86.7	86.3	87.0	87.5	85.7	87.1	88.6	88.7	87.7	25.3	31.9	36.8
.0175										21.7		
.025			75.1			81.3				22.1	23.6	
.0375			57.6									
.050	46.2	51.8	49.2	56.4	58.3	62.5	76.4	62.1	56.6	24.7	24.8	27.3
.075		43.6	48.6			62.8						
.100	42.1	45.3	46.1	53.2	58.3	59.3	63.6	55.8	52.3	36.1	31.7	36.7
.175		44.6	55.0			66.7				55.6		
.250			60.7			71.8				65.1	67.4	
.375			70.9			80.6						
.500	82.2	63.1	84.0	86.9	87.0	84.4	79.0	79.6	86.0	73.4	78.2	80.8
1.0			86.0			82.0				77.5		
2.0			82.2			75.9				75.9		
3.0			78.9			67.1						
Satd.			73.6									

[a] Water extraction gives 90.1% nitrogen.

filter paper. The temperature of the extracting solutions varied from 28–33°. The temperature coefficient[9] in this range is very small and does not affect the results.

The experiments using the three different size gradations from the Wiley mill indicate that the quantity of nitrogenous constituents extracted from the meal by all dispersing solutions used increases with a decrease in particle size. The results from the meal ground in the pebble mill, which has the smallest average particle size, agree with the results for the other meal samples in the case of water, but not for the salt solutions used which show a lower value for the quantity of nitrogen extracted. This difference amounts to 7.1% for the half-normal sodium chloride solution. This contradictory behavior may be accounted for by the different grinding action of the two mills, *i. e.*, the Wiley mill has a cutting action, while the pebble mill has a crushing or pounding action.

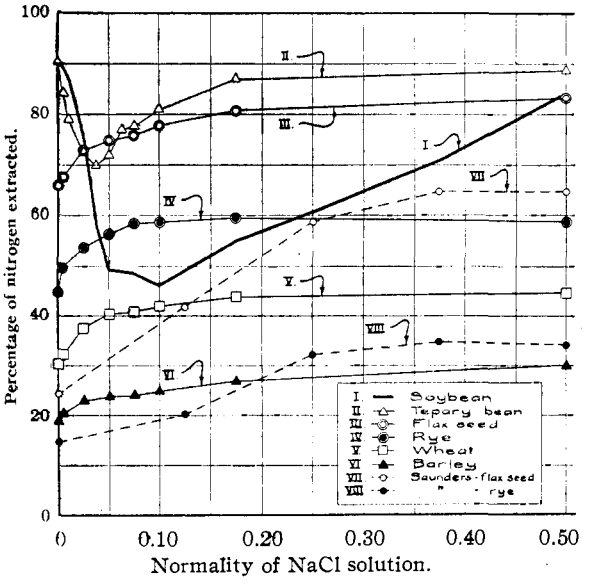

Fig. 1.

On the basis of the above results the meal selected for further study was that obtained by grinding in the Wiley mill through the 0.5-mm. screen.

Protein Peptization.—The effect of 12 different neutral salts on the peptization of nitrogenous constituents from soybean meal was determined, using the meal and the procedure outlined previously. The *p*H values of the salt dispersions were taken on a portable glass electrode *p*H meter. Results of the salt extractions are given in Table IV.

The writers know of no published study on other seeds or grains which has recorded as high a water peptization of nitrogenous constituents as is found with soybean meal. This observation led the writers to extend their salt peptization experiments to dilute as well as concentrated salt solutions. Examination of the data shows that, for soybean meal, salt solutions never peptize as much nitrogenous matter as water, and that this inhibiting action is greater in dilute solutions. A minimum dispersion for univalent cations occurs at about 0.1 *N*, and for divalent cations at about 0.02 *N*.

To determine whether any other seed exhibits the peculiar type of curve shown by the soybean, peptization studies were made on wheat, rye, and barley grains, flaxseed, and tepary beans, using sodium chloride solutions. The procedure and meals used have been described above. The results as given in Fig. 1 show that the curves for wheat, rye, barley, and flax agree in form with those found in the literature, but that the tepary bean behaves like the soybean, *i. e.*, water peptizes almost 90% of the total nitrogen, water peptization is greater than that for any salt concentration used, and a minimum occurs at about 0.04 *N* for sodium chloride.

Action of Salts on Water Extract.—Soybean meal was extracted with water in the manner outlined above, but the combined extracts were diluted with enough water and calcium chloride to make the 500 ml. of the final solution

0.0175 *N* with respect to the calcium ion. A white flocculent precipitate formed immediately. The mixture was shaken and filtered, and an aliquot was taken for analysis. While water peptizes 90.1% total nitrogen, this filtered solution contained only 20.7% of the total nitrogen in the amount of meal used. This value is in close agreement with the 21.7% total nitrogen peptized by 0.0175 *N* calcium chloride in the regular procedure.

In the same manner when sodium chloride is added to a water-peptized soybean meal solution to make it 0.1 *N*, a similar white flocculent precipitate forms, but at a much slower rate than with calcium chloride. The filtered solution contained 53.1% total nitrogen as compared with 46.1% total nitrogen peptized by 0.1 *N* sodium chloride in the regular procedure.

Table V contains the above data as well as the percentage of total nitrogen peptized by three separate successive extractions of a soybean meal sample by 0 1 *N* sodium chloride, by 0.0175 *N* calcium chloride, and by water. Also given in the table is the percentage of total nitrogen left in solution after flocculation of three separate successive water extracts of a meal sample, by addition of enough calcium chloride to make the water extracts 0.0175 *N* in respect to this salt, and by addition of enough sodium chloride to make them 0.1 *N*.

TABLE V

PERCENTAGE OF NITROGEN EXTRACTED BEFORE AND AFTER FLOCCULATION

Number and volume of extract, ml.	Water extract before flocculation, %	Addn. of $CaCl_2$ to water extract until 0.0175 *N*, %	Extracted with 0.0175 *N*, $CaCl_2$, %	Addition of NaCl to water extract until 0.1 *N*, %	Extracted with 0.1 *N* NaCl, %
First, 93	79.0	16.3	16.0	51.2	38.2
Second, 100	8.8	1.8	2.5	5.2	4.4
Third, 100	1.6	0.5	1.0	1.4	2.2
Sum of three separate	89.4	18.6	19.5	57.8	44.8
Combined, dil. to 500 ml.	90.1[a]	20.7	21.7[a]	53.1	46.1[a]

[a] Placed in table for comparison.

Discussion

In Fig. 1 are included dispersion curves for flaxseed and rye taken from the data of O'Hara and Saunders.[8] These curves are consistently much lower than the corresponding curves plotted from the results of this study, *e. g.*, for water extraction of flaxseed Saunders found 24.27% of the total nitrogen, whereas 65.6% total nitrogen was obtained in the present investigation. Gortner,[7] on the other hand, found 64.07% total nitrogen for water extraction of flaxseed. Considering differences in variety and technique, the results reported herein agree well with those of Gortner. It was noted that Gortner ground the entire seed to pass a hundred mesh screen and used the resulting whole meal. This procedure is very similar to the one used in this study. An examination of Saunders' papers disclosed the fact that after the seed was ground in a Wiley mill, and the meal was sifted, a particular sieve fraction was selected for extraction experiments. For flaxseed the 80–100 mesh portion was chosen,[8] and for orange seed meal either the 60–80,[8] 40–60,[16] or 20–40[17] sieve fraction. The portion selected for rye is not mentioned.

On examination of the various fractions of sifted soybean oil-free meal, the major part of the hulls is found in the coarser fractions. For the 1-mm. meal, which contains 7.80% nitrogen, the coarsest fraction, 20–40 mesh, has 6.59% nitrogen, whereas the finest, passing through the 200 mesh screen, has 8.78%. It is also true that the nitrogenous matter of the hull is extracted much less easily by water[9] and neutral salt solutions than is that of the remaining seed parts. These facts and the results of the particle size experiments may largely account for the disagreement noted above.

From the biochemical and industrial viewpoints, a study of the chemical and physical differences between the various seed parts, such as hull, endosperm, and embryo, would be very valuable. Work of this nature is in progress at this Laboratory. It is believed, however, that mechanical separation of these seed parts is not accomplished well enough by ordinary grinding and screening processes to be of material value. In the present study it was deemed advisable to use the entire meal sample rather than any particular sieve fraction thereof.

The results on the effect of meal particle size show that the quantity of nitrogenous constituents extracted from soybean meal increases with an increase in number of successive extractions and with a decrease in particle size. There is also an indication that the method of grinding affects the results. The importance of further studies in this phase of protein investigation and the necessity of standardizing procedures and techniques is evident.

The *p*H of the water extract of soybean meal is 6.7; for the salt solution extracts the values are somewhat lower, the lowest being 5.2 for the 1 *N* calcium chloride solution. The *p*H values of the salt extracts decrease with an increase in salt concentration; this change is largest for the calcium and magnesium chloride solutions. However, the *p*H change does not correlate with a change in the nitrogen extracted.

(16) Saunders, THIS JOURNAL, **53**, 696 (1931).
(17) Rotha and Saunders, *ibid.*, **54**, 342 (1932).

Since the salt solutions are made up of neutral salts, the *p*H change that follows the addition of meal to the solution probably is the result of some reaction between the meal and the salt solution. A more exact evaluation of the relative influence of the salt ions and the hydrogen ion will be published soon.

The peptization by water and salt solutions of the nitrogenous constituents from soybean and tepary bean meals is unusual in three ways: the amount of nitrogen extracted is greater for water than for any of the salts studied; a very high percentage of their nitrogenous constituents is extracted by water (about 90%); and in dilute solutions there is a sharp minimum in the peptization curve varying with the kind of salt used.

This minimum on the curves for soybean spreads over a wider range of concentration for the univalent cations than for the divalent cations. For the sodium salts that have been studied in greatest detail, namely, sodium chloride, sodium fluoride, and sodium sulfate, there is indication of a leveling off or even a slight rise between 0.05 and 0.1 *N*. This does not appear to be true for the salts of divalent cations. This is correlated with the fact that divalent cations flocculate the protein in an aqueous extract more completely, at a much faster rate, and at a lower concentration than do univalent cations, in accordance with the Hardy–Schulze rule.[18] An examination of Table IV shows lyotropic effects of both anions and cations.

The curve in the present study for sodium chloride extraction of soybean meal agrees well with that of Ryndin,[4] except that he did not investigate the concentration range between 0.0 and 0.4 *N*. Consequently his curve does not show as low a minimum. His report does not contain a satisfactory description of the technique used, so that a close agreement may be fortuitous. He also gives a sodium chloride dispersion curve for dialyzed soybean meal which is of the same type as the flax and cereal curves shown here. From these two curves he concludes that the high percentage of nitrogenous constituents dispersed from soybean meal by water is due to the presence of dialyzable salts in the seed. However, this conclusion is not convincing for the reason that he gives no consideration to the *p*H changes occurring during dialysis. It is known from work in this Laboratory that electrodialysis will lower the *p*H of a water dispersed soybean meal extract as much as three units long before the salts are removed. It may be worth while to point out also that the theory that salts normally present in the meal are largely responsible for the protein dispersion, does not agree with the dispersion curves for soybean and tepary bean meals, since added salts up to a limited concentration (minimum on the curves) inhibit the dispersion of the protein.

(18) Taylor, "Treatise on Physical Chemistry," Vol. II, D. Van Nostrand Co., New York, N. Y., 1931 p. 1696.

Summary

1. The dispersion of the nitrogenous constituents of soybean meal by 12 different neutral salts has been studied.

2. Neutral salts disperse less of the nitrogenous constituents from soybean meal and tepary bean meal than is dispersed by water.

3. A minimum occurs in the salt dispersion curves for soybean meal at about 0.1 *N* for univalent cations, and at about 0.02 *N* for divalent cations. The sodium chloride dispersion curve for the tepary bean has a minimum at 0.0375 *N*.

4. The amount of protein extracted from soybean meal increases with a decrease in particle size and with an increase in the number of extractions.

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