THE JOURNAL OF THE American Oil Chemists' Society

Volume 29 AUGUST, 1952 No. 8

Peptization of Soybean Meal Protein. Effect of Method of Dispersion and Age of Beans[1]

ALLAN K. SMITH, PAUL A. BELTER, and VERNON L. JOHNSEN,
Northern Regional Research Laboratory,[2] Peoria, Illinois

THE commercial processing of soybeans for meal and oil causes varying amounts of denaturation of the protein (1). One of the generally accepted methods of measuring the state of denaturation of the meal protein is to determine the relative dispersibility of its nitrogen components in water. When a high water-to-meal ratio is used for protein extraction, such as 40:1, the nitrogen dispersibility of undenatured meal will be in the range of 85 to 92%, whereas the meal which has been steamed for development of maximum nutritional value will have a nitrogen dispersion in the range of 7.0 to 16.0%.

In the selection of soybean meal for most industrial and some food uses it is essential to know the extent of protein denaturation. For example, undenaturated meal is used for making isolated protein, various types of coatings (2), adhesives, plywood glue, and for the brewing industry while controlled denaturation is practiced for the production of soy powder (soy flour) and for stock feed. In an earlier publication (1) it was shown that very little denaturation of the soybean protein occurs in commercial processing of the beans during the preparation and solvent extraction steps but that most of the denaturation is the result of heat and steam used for removing the solvent and in the "toasting" operation for the development of maximum nutritional value. Thus it is apparent that the many possible variations in processing and the need for selection of special types of meal for industrial and food uses requires an analytical method for classifying or grading the meals. Several methods of measuring the effect of heat treatment of the meal have been proposed, such as disappearance of certain vitamins, destruction of enzymes, or measuring the browning of the meal, but such procedures do not follow changes in the meal over as wide a range of steam treatment as the change in solubility of the nitrogen components in water or dispersing agents dissolved in water.

Many factors are known to influence the dispersion of the protein. Nagel, Becker, and Milner (4) were the first to investigate the water dispersion of the proteins of undenatured meal. They studied the effect of grinding the meal, time of shaking the dispersion, ratio of dispersing agent to meal, and the effect of temperature. Smith, Circle, and Brother (5), and Smith and Circle (6) also investigated the dispersion of soybean protein in water and extended the investigation to include neutral salts, acid, and alkaline solutions. Loska and Melnick (3) expanded the water dispersion test to include a laboratory procedure for evaluating the curd producing capacity of soy products; their protein extractions were carried out at 80°C.

For routine testing purposes a water extraction method was established by Smith and Circle (6) and widely used. In this method 2½ g. of meal, ground to pass a 100-mesh screen, and 100 ml. of water were placed in a 250-ml. centrifuge bottle and shaken mechanically for 30 minutes. The system was centrifuged for six minutes at about 2,000 times gravity, and a sample of the supernatant solution taken for Kjeldahl nitrogen determination. The results were reported in percentage of dispersion of the total nitrogen in the meal. Two people working in the same laboratory checked the method and obtained good agreement although repeated determinations did not give consistently close checks. Results were obtained within a range of ± 0.5% whereas similar extractions with other seed meals gave check results within the range of ± 0.2%. Unfortunately in the initial investigation the two groups of workers that checked this method used the same batch of beans, Illini variety, grown in 1936 near St. Joseph, Illinois. Recently, in attempting some temperature solubility studies on soybean meal, we found that the early work did not give a complete picture of the factors affecting nitrogen dispersion. This report presents new information on the effect of temperature of dispersion, method of stirring, pH of the system, variety, and age of the beans on the rate and amount of nitrogenous compounds dispersed. Location of growth of beans is possibly another factor which could affect rate of protein dispersion, but the present experiments could not be planned to include that variable.

Experimental

Effect of Age and Time of Extraction. Two series of experiments were carried out using extraction periods of 30 and 90 minutes respectively in a re-examination of the effect of time on the amount of nitrogen dispersed. In order to cover a wide variety of conditions the tests included five varieties of beans which had been collected since 1942. The beans were cracked and flaked, the oil removed by extraction with hexane, and the meal ground to pass a 100-mesh screen.

The nitrogen dispersion results, pH values for the water extracts, nitrogen content of the defatted meal

[1] Presented at the Fall Meeting of the American Oil Chemists' Society, Oct. 8-10, 1951, in Chicago, Ill.
[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

determined within a moisture range of 7.5 to 8.5%, and the storage conditions of the beans are given in Table I.

TABLE I

Effect of Age, Variety, and Time on Water Dispersibility of Soybean Nitrogen Components

1. Numbers refer to nitrogen content of meal and letters to storage conditions of beans: CR—cold room storage at 40°F.; LH—stored at low humidity and seasonal temperature; ST—stored at seasonal temperature and humidity.
2. Nitrogen dispersed with 30 minutes shaking and pH value of dispersion.
3. Nitrogen dispersed with 90 minutes shaking and pH value.

Year	Variety				
	Lincoln	Illini	Mandarin	Dunfield	Ogden
1942					
1		8.72 CR	8.48 CR		
2		77.7-6.5	64.4-6.6		
3		82.7-6.6	72.7-6.6		
1943					
1	7.40 CR	8.26 CR	8.50 CR	7.73 CR	
2	72.5-6.5	86.5-6.5	80.8-6.5	83.8-6.5	
3	78.5-6.6	88.7-6.6	86.1-6.5	83.5-6.6	
1944					
1	8.21 ST		9.00 CR	8.05 CR	
2	76.8-6.6		81.3-6.7	82.4-6.5	
3	77.2-6.6		79.7-6.7	83.7-6.6	
1945					
1	8.18 ST	8.21 CR	8.66 CR	7.86 CR	
2	83.0-6.5	86.1-6.5	85.0-6.4	82.3-6.5	
3	82.5-6.5	89.1-6.6	84.0-6.7	86.6-6.6	
1946					
1				7.90 CR	
2				78.8-6.6	
3				85.0-6.7	
1947					
1	8.69 ST	7.78 LH		7.60 LH	8.48 CR
2	77.5-6.5	88.5-6.5		86.1-6.6	83.9-6.6
3	83.3-6.6	91.5-6.6		91.3-6.7	90.3-6.7
1948					
1	8.51 ST	7.86 LH	7.41 LH	7.52 LH	8.74 CR
2	77.6-6.6	86.2-6.6	89.3-6.5	88.9-6.6	85.4-6.8
3	84.0-6.6	91.8-6.5	91.8-6.5	89.6-6.7	92.0-6.7
1949					
1	8.14 CR	8.31 LH	8.15 LH	7.46 LH	
2	83.8-6.8	84.4-6.6	82.7-6.6	91.8-6.7	
3	89.1-6.7	92.4-6.7	90.8-6.5	93.1-6.7	
1950					
1	7.54 ST	8.07 ST		7.87 ST	7.96 ST
2	85.8-6.8	88.9-6.7		90.8-6.8	85.9-6.8
3	88.1-6.7	92.1-6.7		92.0-6.8	91.8-6.7

The beans were of good quality and, although they were not all stored under the same conditions, the storage was rated as good to excellent. It is believed that the storage conditions were a minor, although undetermined, factor in the nitrogen dispersion results.

The data for the Lincoln, Dunfield, and Illini for the 90-minute extraction period are shown graphically in Figure 1. The results presented in Figure 1 and Table I, while not entirely uniform, show for both 30- and 90-minute extraction periods a decrease in nitrogen dispersibility with age of the beans. This change is approximately 1% per year. Comparison of the results for the 30- and 90-minute extraction periods shows further that the longer extraction time gives higher as well as more uniform results, thus indicating the possibility that 30 minutes is too short a time for a satisfactory determination of water-dispersible nitrogen when the dispersion is effected by mechanical shaking.

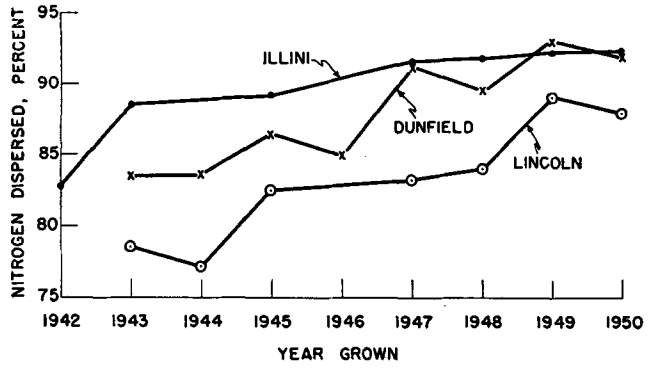

FIG. 1. The nitrogen dispersion values in water at about pH 6.6 for soybean meal derived from Illini, Dunfield, and Lincoln soybeans. Extraction time was 90 minutes.

It is interesting to note also that the data in Figure 1 indicate a consistent varietal difference, with the Illini having the highest dispersion values, followed by the Dunfield and Lincoln. If the values for the Mandarin beans were plotted on the same graph, they would give values closely corresponding to the Dunfield, and intermediate between Illini and Lincoln. The present investigation was not originally organized for varietal studies because such factors as location of growth, date of harvesting, and storage conditions have not been properly controlled. Yet the results are sufficiently consistent to suggest a varietal difference and the need for further observations to check these preliminary results.

Effect of Stirring, Temperature, and Time of Extraction. Our inability to account for certain inconsistencies in some of our dispersion results led to further re-examination of the method. Studies were made on the effect of different methods of agitating the meal-water slurry as well as of time and temperature of extraction. The hexane-extracted, undenatured meal was obtained from 1948 Hawkeye and Lincoln beans which had been stored at normal temperature and humidity and 1942 Mandarin beans stored at 40°F.

The water-to-meal ratio for these experiments was 40:1 and the extraction periods were 30, 60, 90, and 120 minutes. The results are plotted in Figure 2. The numbers on the curves refer to the methods used in dispersing the protein, which are as follows:

1. The meal-water slurry was shaken in a Precision Scientific Company mechanical shaker with a reciprocating motion at room temperature (about 25°C.).
2. The meal-water slurry was stirred slowly in a beaker by a mechanical stirrer at room temperature. This procedure gives considerably less agitation than method 1.
3. The same agitation as for No. 2 with the temperature at 50°C.
4. Very vigorous agitation at room temperature with a Lighting Type stirrer. The propeller blades were nearly equal to the diameter of the flask, and rapid rotation of the blades gave very effective shearing action.

Results and Discussion

The early work of Nagel, Becker, and Milner (4) with 1936 Illini soybean meal had shown that dispersion in a mechanical shaker was essentially complete in 30 minutes. The data for our number 1 curves in Figure 2, obtained in a similar manner to the data of Nagel *et al.*, show that the rates of dispersion for all meals are not the same and the results of Becker *et al.*, in establishing 30 minutes as giving near complete dispersion, were fortuitous in that they apparently worked with a soybean meal having an easily dispersible nitrogen. Effect of the time factor is illustrated by comparing the results for the Lincoln and Hawkeye beans by the No. 1 method where we ob-

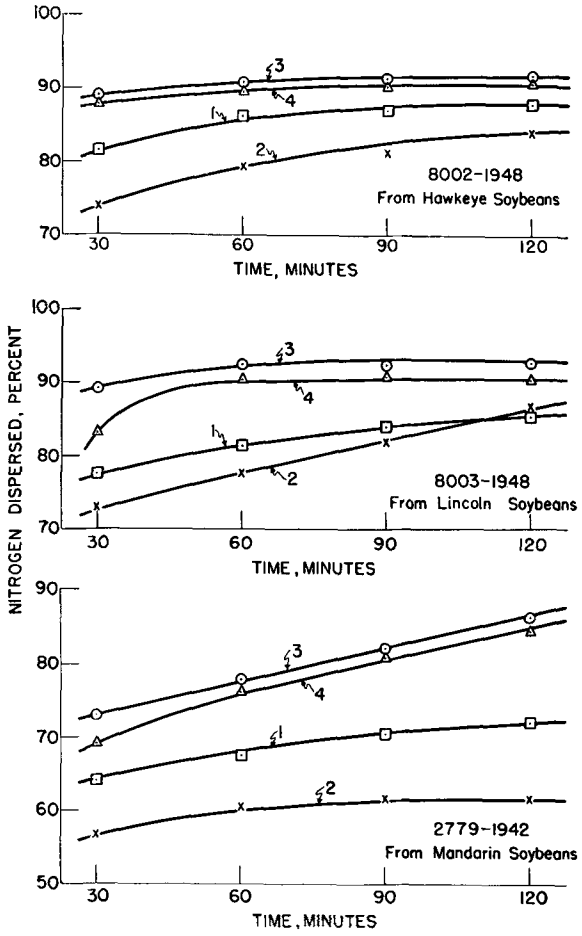

FIG. 2. The nitrogen dispersion values in water at pH 6.6 for soybean meal derived from 1948 Lincoln, 1948 Hawkeye, and 1942 Mandarin beans. The numbers on the curves refer to method of agitating the slurry, as described in the text.

tained 77.5% and 81.5% at 30 minutes, and 85.5% and 86.0%, respectively, at 120 minutes. These values are substantially lower for the 30-minute shaking period than those obtained for other 1948 beans reported in Table I.

The respective lower dispersion values of 64.4% and 72.7% for the 1942 Mandarin at 60 and 120 minutes are attributed primarily to the longer storage period of these beans.

Curves 1, 2, 3, and 4 in Figure 2 show that method of agitation as well as time are major factors in the rate and amount of nitrogen dispersed. The mild agitation at 50°C., Method 3, and vigorous agitation at 25°C., Method 4, give nearly the same results, with small but consistently higher values at 50°C. The consistently lower values obtained at room temperature with mild stirring, Method 2, serve to emphasize the importance of controlling the mechanical operations. Also dispersion experiments were conducted in a Waring blendor; this means of agitation gave near-maximum dispersion in five minutes, but the rapid rise in temperature from such vigorous stirring confused the results, and the details of the work are not reported here.

The results show that increasing the temperature from 25°C. to 50°C. has essentially the same effect on nitrogen dispersion as vigorous stirring. However the higher results at 50°C. are not attributed to a temperature solubility coefficient effect. The high ratio of water-to-meal used in these dispersion systems should suffice to disperse all the protein at 25°C. The 2.5 g. of meal is approximately 50% protein and if all were dispersed, the solution would have a protein concentration of 1.25%, which is insufficient for saturation. Other experiments in our laboratory at room temperature and at pH 6.6 have shown that countercurrent extraction of soybean meal with water will build up a protein concentration in excess of 15% and a considerable lowering of the temperature of this solution is necessary to precipitate an appreciable quantity of the protein.

The data for the 1942 Mandarin beans, Figure 2, show that storage has modified the protein to give a slow rate of dispersion; i.e., after 120 minutes of vigorous stirring at room temperature the dispersed nitrogen is 84.5% whereas the 1948 Lincoln and Hawkeye are at 91.0% and 90.5%, respectively. The aging effect is further illustrated by comparing the above results for 1942 Mandarin with the 1948 Mandarin value shown in Table I, where 90 minutes of shaking gave a dispersion value of 91.8%.

A true explanation of the higher values obtained by vigorous stirring or an increase in temperature is not readily apparent, but some of the following factors may contribute to the observed results: a) The cell structure of the beans may not have been well enough destroyed by the hammer mill for easy liberation of the protein. b) A part of the protein may be attached to insoluble carbohydrate particles, and vigorous stirring or heating is required to bring the protein into dispersion. c) The heating or stirring may be supplying the energy necessary for dispersing coarse protein particles or complexes. The action of the hammer mill may weld some otherwise soluble protein into insoluble meal particles. d) Another factor retarding the protein dispersion and common to the first three suggestions may be the formation of a hydrated shell around each particle of protein, which gives a "case hardening" effect that retards penetration of water and rate of dispersion; this phenomena is observed in reverse in the drying of protein.

In addition to the effect of physical factors noted above on protein dispersions, the influence of pH of the system should be considered. The work of Smith and Circle (6) showed that on the alkaline side of the isoelectric point of soybean protein the greatest effect of pH on dispersion is between pH values of 5.0 and 6.7. For the shorter range of pH 6.0-6.7 the nitrogen dispersion values increase from 54 to 85% or an average change of 4.4% nitrogen for each 0.1 pH unit. This sensitivity of protein dispersion to pH suggests that minor pH difference may account for the difficulty in checking duplicate experiments as well as the loss of dispersible nitrogen during storage; however the pH measurements were not carried out with sufficient exactness to clearly support these possibilities.

Despite the many variables influencing the dispersion of the soybean meal nitrogen in water, experience has shown that the 30-minute extraction method can be made to work satisfactorily for the differentiation of meals in the higher ranges of nitrogen solubility. High precision is not required in many practical measurements, and the 30-minute extraction time can be extended to 90 minutes or longer when higher precision is required. However steam treatment of the meal as practiced in soybean processing lowers nitrogen dis-

persibility very rapidly, and after 15 minutes at atmospheric pressure the dispersibility of the nitrogen is reduced to such a low value that further treatment produces only minor changes in the solubility of the nitrogen and increases the difficulty of differentiating between samples steamed for longer periods. In Figure 3 is shown the change in nitrogen dispersion with

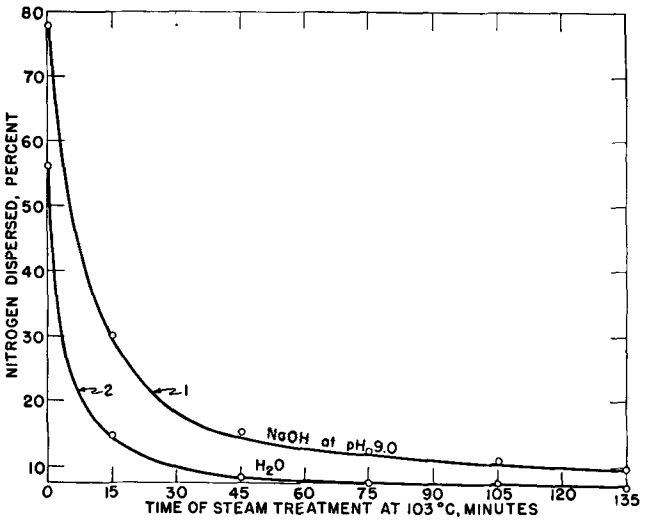

FIG. 3. The relation of nitrogen dispersibility of soybean meal in water at pH 6.6 and 9.0 with steam treatment of the meal at 103°C.

steam treatment at 103°C. The meal used in these experiments had been slightly denatured in the solvent extraction operation so that its initial solubility in water was only 56.9%. The data for laboratory steamed meal, having an initial nitrogen dispersion of 92.5%, was reported earlier (1).

It can be seen that an ideal dispersion curve for measuring the effect of steam would be a straight line between the dispersion value at zero time of about 90% and the 8% which may be obtained after 60 minutes. A search was made to find a dispersion medium which would more nearly approach the ideal situation. Curve 1 in Figure 3 represents a dispersion at pH 9.0 and is an improvement in the right direction but still not satisfactory.

Figure 4 shows additional dispersion data on the same meal used for obtaining Figure 3. The dispersing solutions for the three curves in Figure 4 are 2.5% Duponol (Duponal C, sodium lauryl sulfate equivalent to the meal), adjusted as indicated to pH values of 8.0, 9.0, and 10.0, with sodium hydroxide. Of these three sets of data the one at pH 8.0 gives the most desirable results for measuring protein differences. Data for a different meal at pH 7.1 obtained later was not as satisfactory as the one at pH 8.0, indicating no advantage in further lowering of the pH value. The data in Figure 4 show an average change in nitrogen dispersibility of 0.7% nitrogen per minute for the first 60 minutes of steam treatment. For the next 45 minutes the change in dispersibility is only 0.21% nitrogen per minute. In the commercial processing of soybeans the meal is never intentionally steamed longer than 60 minutes so that beyond that time the curve has no practical significance. The use of detergent solutions appears to offer an advantage over

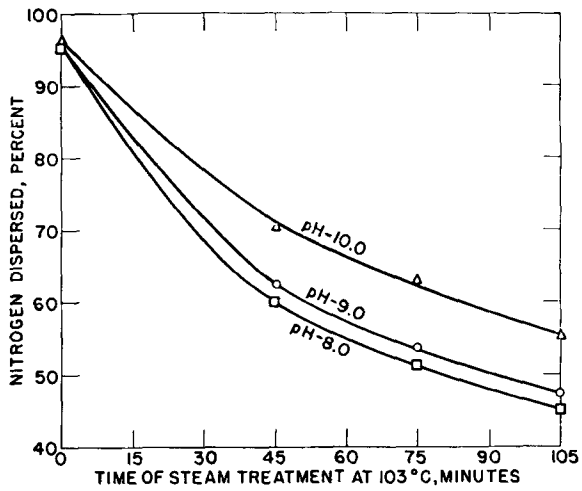

FIG. 4. The relation of nitrogen dispersibility of soybean meal in Duponol solutions at pH values of 8.0, 9.0, and 10.0 with steam treatment of the meal at 103°C.

water in comparing highly denatured meals; however the accumulation of more data is necessary before a routine test procedure can be developed.

Conclusions

From the data collected thus far on the dispersion of the nitrogen compounds of soybean meal, it is not feasible to recommend a procedure that will be equally suitable for all analytical situations.

The several factors influencing the dispersion of soybean meal protein in water, such as time, temperature, method of stirring, and pH value of the solution, emphasize the empirical nature of the determination and the need for selecting a standard procedure to suit a given need.

A 30-minute water extraction at room temperature and at a water-to-meal ratio of 40:1 will serve to differentiate between soybean meals that have had comparatively little denaturing treatment and in cases in which highly precise values are not required. When better check results are required, they can be obtained by using a longer extraction period.

The drastically denatured meals can be differentiated better by dispersion in sodium lauryl sulfate solution at pH 8.0 than by dispersion in water. The nature of the results indicate that further research with protein dispersing agents will lead to a better method than is now available.

Acknowledgment

The authors are indebted to Charles Blessin and Don DeLong of this laboratory for assistance in a substantial portion of the dispersion determinations.

REFERENCES

1. Belter, Paul A., and Smith, Allan K., J. Am. Oil Chem. Soc., *29*, 170-174 (1952).
2. Davidson, Glenn, and Cagle, James H., The Paper Industry and Paper World, June 1947.
3. Loska, Stephen J. Jr., and Melnick, Daniel, Cereal Chem., *27*, 127 (1950).
4. Nagel, R. H., Becker, H. C., and Milner, R. T., Cereal Chem., *15*, 463 (1938).
5. Smith, Allan K., Circle, Sidney J., and Brother, George H., J. Am. Chem. Soc., *60*, 1316 (1938).
6. Smith, Allan K., and Circle, Sidney J., Ind. Eng. Chem., *30*, 1414 (1938).

[Received October 29, 1951]