Measurement of Urease Activity in Soybean Oil Meal[1]

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MOIST HEATING is an essential part of soybean processing for the development of maximum nutritional value of the meal, but over-heating is detrimental. In order to obtain a highly nutritious meal for the feed industry a number of laboratory control tests have been developed for regulation of the heating operation. The most widely used of these tests are designed to measure the denaturation of protein as determined by decrease in either water-soluble nitrogen compounds (3) or urease activity (4, 6). Other control tests include an assay for thiamine (7), changes in fluorescence (2), brown color of the meal (14), inactivation of the trypsin inhibitor (5, 9, 15), determination of the change of amino nitrogen by formol titration (1), and hemagglutinating activity (11). This report deals only with assay methods for urease activity.

Urease activity is also important in the mixed-feed industry when urea and soybean oil meal are combined in cattle feeds. The urease activity in the meal must be low enough so that the urea will not be decomposed (8, 10). Some confusion surrounds the use of urease tests for compatibility of meal with urea because at low moisture content the urease is quite inactive, and its activity becomes apparent only at higher moisture levels. Reaction to give ammonia can occur when the moisture level is increased either by absorption of moisture in the meal or by mixing with other feedstuffs of high moisture content. Hence the mixed-feed industry also requires suitable methods for measurement and control of urease activity in order to determine compatibility of meal with urea.

The colorimetric and ammonia-distillation methods for determining urease activity in relatively pure systems are not reliable when assaying meal and are not suitable for routine or control analyses. The generally accepted method in the soybean industry for determining the relative urease activity in toasted meals is the modified Caskey-Knapp method (4), which measures the increase in pH of a mixture of meal, urea, and buffer solution under specified conditions. A meal which gives a pH increase of 0.02 to 0.25 at 30°C. is usually accepted by soybean processors as properly toasted for good nutrition, but a lower test value indicates the meal may have been overcooked.

When the modified Caskey-Knapp test is used to determine the compatibility of urea and soybean oil meal, a meal is considered compatible if the pH change at 30°C. is 0.1 unit or less. Such a small increase, though generally measurable with significance, is greatly affected by experimental error and allows at best a very narrow range for differentiating between low-activity meals.

A titration of the ammonia formed by the hydrolysis of urea is sometimes used as an alternative to the above method. The titration method requires more manipulation, and end-points are not sharp in the presence of the protein and buffer salts.

In feed plants where suitable laboratory equipment and technicians are not available, qualitative tests for odor of ammonia or indicator tests for alkalinity have been devised.[3]

The present investigation was undertaken at the suggestion of the Soybean Research Council of the National Soybean Processors Association to determine the relative merits of the available tests for urease activity and, if possible, to make further improvements in the testing procedure.

The Titrimetric Method

A series of studies was made, using the titrimetric method to find a means of increasing the titer. The variables investigated were size of sample and time and temperature of reaction. All meal samples, except where noted, were ground in a hammer mill to pass an 80-mesh screen. It was shown that ground meals gave higher and probably more uniform urease values than the same meal on an "as is" basis (see Curves 4 and 4a in Figures 3 and 4).

The 0.05 *M* phosphate buffer consisted of 0.025 mole K_2HPO_4 and 0.025 mole KH_2PO_4 per liter. The buffered urea solution was made up daily by dissolving 3 g. C.P. urea in 100 ml. of the above buffer.

Procedure. For each determination 10 ml. of the buffered urea solution was introduced into each of two 18 x 150-mm. test tubes contained in a constant-temperature water bath. Exactly 10 ml. of approximately 0.1 *N* HCl were also added to one of these tubes to be used as the control. Then 0.2 g. of the soybean oil meal was added to each tube except when sample weight was a variable. The tubes were stoppered, vigorously shaken, and the control set aside to cool to room temperature. The other tube was left in the water bath for the desired reaction time (30 min. except when time was a variable). The reaction was terminated by adding exactly 10 ml. of the 0.1 *N* HCl. After cooling, the sample and control were transferred quantitatively with two 5-ml. water rinses each to small beakers and titrated with 0.1 *N* NaOH to pH 4.7, using a pH meter with glass electrode. This end-point was experimentally determined as the inflection point in the titration curve of an acidified reaction product. The difference between the control and sample titrations in milliliters of 0.1 *N* NaOH is taken as the urease activity of the meal.

The buffer is used to maintain the pH in the range of optimum activity of the urease, which is about 6.7 to 8.0. For raw or other highly active meals the 0.05 *M* phosphate will not hold the pH in the desired range, and considerable error may be introduced into the assay. Best results with highly active meals were obtained by holding the pH of the system in the optimum range of activity by a continuous titration of the sample.

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[2] One of the branches of the Agricultural Research Service, U. S. Department of Agriculture.

[3] Tests available through the National Soybean Processors Association, Soybean Research Council, 3818 Board of Trade Building, Chicago 4, Ill.

Modified Caskey-Knapp Method (pH Change)

Caskey and Knapp (6) first described the change in pH as a means of testing for urease in soybeans, and later Bird *et al.* (4) modified the test and compared urease values with the results obtained with chick-feeding tests.

Procedure. Ten ml. of 0.05 *M* phosphate buffer for the control and 10 ml. of a buffered urea solution for the test (same solutions as described for titrimetric method) were introduced into separate 18 x 150-mm. test tubes. The tubes were placed in a constant-temperature bath and 0.2 g. of soybean oil meal were added to each tube. The tubes were shaken every 5 min. and at the end of 30 minutes' reaction time pH values were determined, using a pH meter with glass electrode. The difference between the pH values for the control and sample is a measure of the urease activity of the meal.

Results of Above Methods

Figure 1 shows a straight-line relationship between the urease activity at 30°C. found by the titrimetric method and the weight of a commercial soybean oil meal sample when varied between 0.1 and 0.8 g. These results show that sample weight can be adjusted over a wide range to suit requirements of the assay.

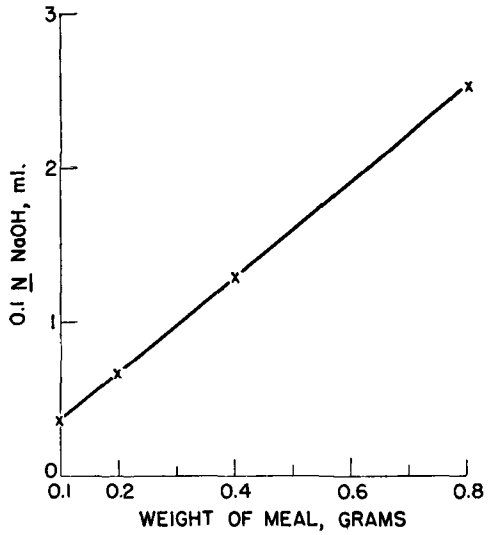

FIG. 1. The effect of meal weight on amount of ammonia titrated after reaction at 30°C. for 30 min.

Figure 2 shows effect of time of reaction between 10 and 60 min. on titration value for a soybean oil meal at 30° and 60°C. In the observed range the reaction is nearly a straight-line function of time.

Figures 3 and 4, obtained by using 0.2 g. of meal and 30-min. reaction times for the titrimetric and pH change methods, respectively, show effect of temperature on urease activity for a series of commercial soybean oil meal samples at 30°, 40°, 50°, and 60°C. This series of samples was selected from a group of regular production meals to give a wide range of urease activities. They had been rated by several processors, using their presently available method for use with urea in feeds, as follows: sample Nos. 1 and 2, unsafe; Nos. 3, 4, 5, and 6 borderline meals, and Nos. 7, 8, and 9, safe. Curves of the same number in

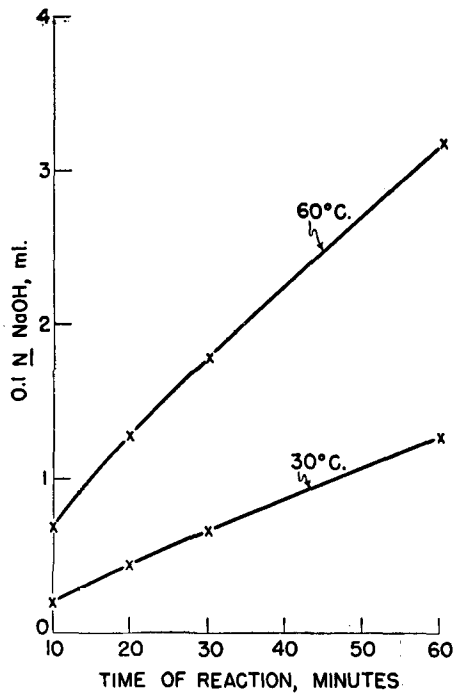

FIG. 2. The effect of reaction time on amount of ammonia titrated at reaction temperatures of 30° and 60°C.

both figures are for the same meals. These two sets of curves show the same activity trends in the temperature range of 30° to 60°C. The activity of each meal at 50°C. was about double the activity at 30°C., and

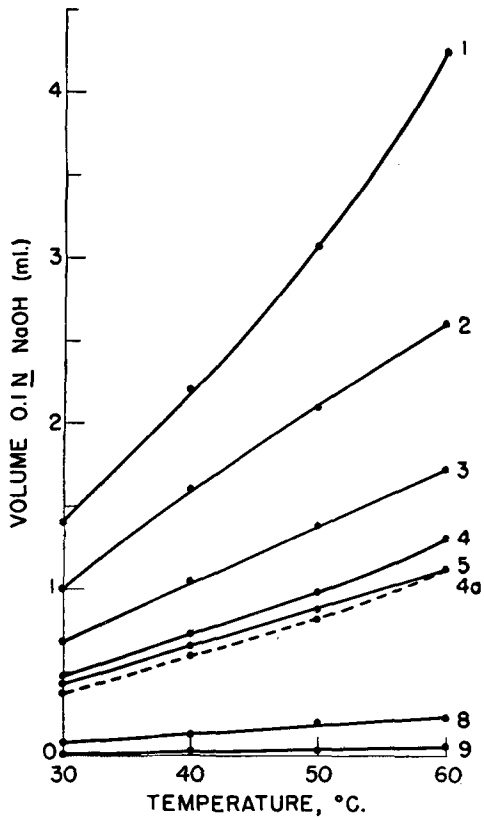

FIG. 3. The effect of temperature on activity of various meals as measured by ammonia titration method. Solid lines are for ground meals; broken line for meals on "as is" basis.

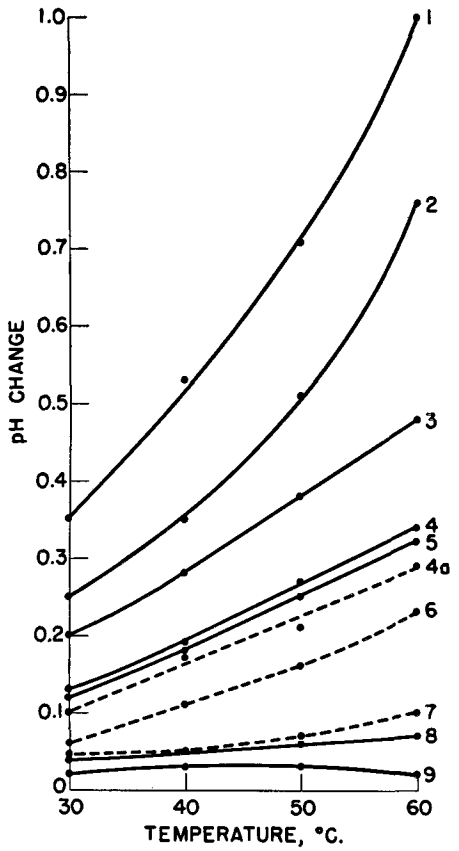

FIG. 4. The effect of temperature on activity of meals as measured by pH-change method. Solid lines are for ground meals; broken lines for meals on "as is" basis.

the activity at 60°C. was almost triple that at 30°C. Data for 70°C. showed about the same 30-min. activity as at 60°C., indicating a slow destruction of the enzyme.

It is apparent from these results that, for both titrimetric and pH-change methods, the reaction at 50° or 60°C. gave a wider spread in urease values than heretofore obtained at room temperature, thereby reducing the effect of experimental error. However meals of low activity are still not well differentiated by either method. The following conductimetric method was developed to improve on sensitivity and on reliability of results.

Conductimetric Method

Electrical conductivity measurements are precise, and small changes are easily measured. Urease converts a urea solution of relatively low conductivity to a solution of ammonium salts of high conductivity. Sastri and Sreenivasaya (12) used the method to study urease isolated from soybeans. The temperature coefficient of conductivity is high so that such tests must be made in a constant-temperature bath controlled within $\pm$ 0.1°C. A control is necessary to correct for the conductivities of the electrolytes of the test solutions. In this method the buffer has been diluted from 0.05 *M* to 0.02 *M* and contained 3% urea.

As it is desirable to stop the action of the urease immediately after the reaction period of the test, a brief study was made of inactivators. Quinones and iodine were not very effective in the complex test systems. Shaw (13) states that silver and mercury are the best heavy metal inactivators. Trial runs showed that a small amount of mercuric chloride effectively inactivated the urease and did not result in phosphate precipitation as when silver nitrate was used.

Procedure. For each determination 10-ml. portions of the buffered urea solution were measured into 18 x 150-mm. test tubes. To one tube (control) 1 ml. of 2% mercuric chloride was also added. When the solutions were at constant temperature (30°C. bath), 0.2 g. of meal was added to each tube and the contents were vigorously shaken. After 30 min. the reaction in the sample tube was stopped by adding 1 ml. of the mercuric chloride solution. The tubes were shaken again and replaced in the bath for a few minutes to allow the coarse particles to settle. The supernatant liquid was then decanted and used for rinsing and filling the conductivity cell, which was held in the constant-temperature bath. The cell was of the fill type, holding about 2.5 ml. and having a constant of 0.68 reciprocal centimeters. The resistance was measured with a Leeds and Northrup [4] portable 60-cycle conductivity bridge to 0.5 to 1.0 ohms. Any bridge having the same or better precision can be used. The difference between specific conductance of the sample and control is a measure of relative urease activity. Specific conductance in reciprocal ohms (mhos) is found by dividing the cell constant by the measured resistance in ohms. Values for commercial soybean oil meal will be in the range of 0 to 1.2 x 10^{-3} mhos.

The conductivity method was tested on a series of soybean oil meals prepared by mixing together an inactivated meal and an untoasted meal so that any meal of given number had twice the activity of the mixture of next lower number. The results of these tests are given in Figure 5. The arrow on the graph indicates approximately the maximum activity to be

[4] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

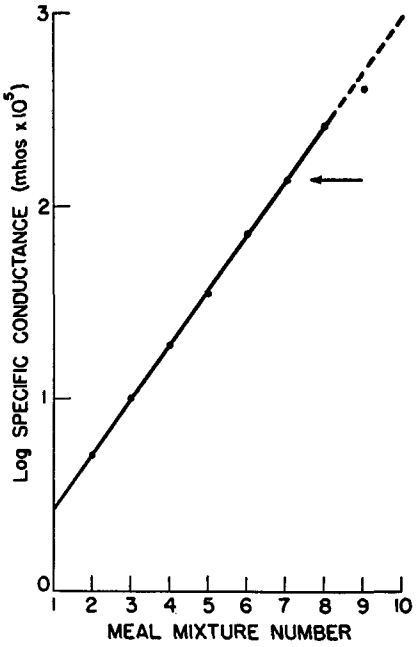

FIG. 5. The activities measured by electrical conductivity method of meal mixtures of known relative activities. Each mixture has twice the activity of mixture of next lower number.

found in commercial soybean oil meals. The straight-line arrangement of points on this graph shows the method should give excellent relative values over a 60-fold range in activities.

Comparison of the Methods

The results for the three methods are compared in Figure 6. Seven commercial samples of soybean oil meal having a wide range of activity were used for comparison. The numerical values for the three methods were brought into the same range by multiplying the values obtained by the pH method by 4 and the conductance values by 0.85. The results by the three methods check each other very well.

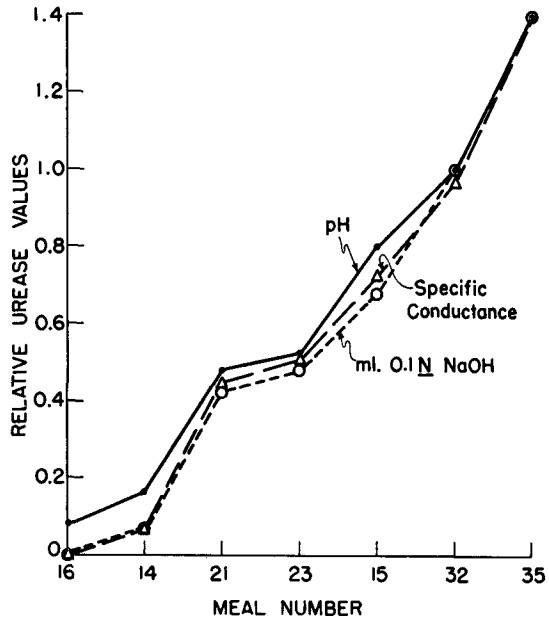

FIG. 6. Comparison of values obtained by the three methods of assay for meals of varying urease activity.

The differences in the three methods of assay are found principally in the operational techniques. The titrimetric method has the advantage of giving a direct measure of urease activity, but the end-point is not sharp and good results require extreme care in titration. The titration method requires appreciably more time than the others. The pH-change method is simple in operation, but the recorded differences between samples at room temperature are very small and frequent checking of the meter is required to prevent substantial errors. The sensitivity of the titrimetric and pH-change methods can be improved by using 50° or 60°C. reaction temperatures, and a further spread in determined values can be obtained by increasing the size of sample or reaction time. For a final determination of the relative adaptability of the three methods for the control of toasting, they must be compared under practical operating conditions. However from laboratory experience it is the opinion of the authors that maximum precision is obtained with the least skill by the conductimetric method.

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Materials Balance in a Tung Oil Mill

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TUNG OIL is produced from seed of the tung tree (*Aleurites fordii* and *A. montana*), a member of the Euphorbia family. The tung fruit is about 2 in. in diameter and normally contains four or five seeds. The entire fruit is covered by a hull about a quarter of an inch thick. Each seed is coated by a hard shell; in it is the kernel, which constitutes about a third of the weight of the fruit and contains about 65% oil. About 39,000,000 pounds of tung oil were produced in the southeastern states from the 1953 crop.

In the United States tung fruit is processed by pressing in continuous screw presses. A materials balance on a tung mill has never been published, however. Such a balance is needed to show the losses that occur at different stages of the milling process so that more intelligent efforts can be made to lower the losses. It is also needed to check the accuracy of the methods of analysis used for oil in tung products by determining if the weights of oil shown by analysis of the fruit are actually equal to the oil produced plus that left in by-products. This paper reports a materials balance made in a commercial mill under its normal operating conditions.

The filter cake from tung mills contains 40% or more of tung oil. A much-discussed question in the

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