Protein Solubility as Quality Index for Processed Soybean

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Abstract

Protein quality of soybean meal (SBM) is linked to both the reduction of antinutritional factors (ANFs), and the optimization of protein digestibility. Both insufficient- and over-heating result in poor quality SBM. Inadequate heating fails to completely destroy the ANFs, which may have a detrimental impact on animal performance, while excessive heating reduces the availability of lysine via the Maillard reaction and possibly, to a lesser extent, of other amino acids. The objective of our study was to compare some biochemical laboratory procedures for assessing quality of SBM: urease index (UI), protein dispersibility index (PDI), KOH protein solubility (PS), and nitrogen solubility index (NSI). The experimental data reveal that UI is not useful to determine excessive heat treatment since additional heating has no effect on the urease index. KOH protein solubility remains high, during initial heat treatment. In marked contrast, the PDI and NSI decreased incrementally from 78% to 20% and from 97% to 60%, respectively, when heating 0 to 30 minutes. Combing the PDI test with the urease test could be useful to monitor soybean quality. SBM containing low UI (0.3 or below) and high PDI (40 to 45%) may indicate that the sample is definitely high quality because it has been adequately heat processed, but not overprocessed.

Keywords: soybean meal, urease index, protein dispersibility index, protein solubility, nitrogen solubility index

1. Introduction

The use of soybean products in the feed and food industry has increased steadily. Soy bean (*Glycine max)* is an important source of oil (17-25%) and protein (35-45%). It contains large amounts of vitamin B1 and B2 but it is rather low in vitamin C [1]. The crude protein of soybean meal ranges from 41 to 50% (dry matter basis) depending on the amount of hull that is removed, and the processing method used [2].

While the amino acid profile pattern is probably the main determinant of protein nutritional quality, the digestibility of a protein and bio-availability of its constituent amino acids are the next important factors [3]. This is true because proteins are digested, absorbed and utilized to different extents. Protein digestibility is defined as the percentage protein absorbed after ingestion of a certain amount of protein by humans or animals [3,4,5]. It is closely related to amino-acid availability. Protein digestibility is a major index of protein quality because a certain amount of amino-acids may be present in a food and it may not necessarily be available to the organism for nourishment. This means proteins cannot be utilized unless they are digested [3]. The differences in protein digestibility are brought about by the susceptibility of a protein to enzymatic hydrolysis in the digestive system and this is directly related to the primary, secondary and tertiary structure of the protein [6,7].

Use of soybean products in animal feeds is limited due to the presence of a number of antinutritional factors (ANF). Most critical ANFs such as protease inhibitors and lectins are heat labile and are destroyed during the manufacture of SBM. While mild heating (~90ºC) improves the nutritional value of SBM by denaturing the proteins and exposing new sites for enzymatic hydrolysis as well as inactivating heat-labile

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ANFs, overheating results in undesirable changes to the chemical structure of many essential amino acids, lowering the nutritive value of SBM. So, both insufficient- and over-heating result in poor quality SBM. Therefore, feed manufacturers require methods to distinguish adequately processed SBM from under- or over-processed meals.

During solvent processing of the soybean, lipids are removed and the meal is heated to eliminate the solvent (usually hexane) and to deactivate antinutritional factors such as trypsin inhibitors and lectin. Inadequate heating fails to completely destroy the antinutritional factors, which may have a detrimental impact on animal performance. Excessive heating reduces the availability of lysine (via the Maillard reaction) and possibly, to a lesser extent, of other amino acids [8,9]. Laboratory tests are thus needed to determine whether samples of soybean meal have received adequate, but not excessive, heat treatment following oil extraction. Of tests commonly used, the evaluation of urease activity (UI) is the easiest to perform, and is especially useful in detecting underprocessed soybean meal. It is less reliable for detecting overprocessed meal. The protein solubility (PS) test is the most commonly used assay to detect overprocessed soybean meal, although very high values are indicative of underprocessed meal.

The objective of our study was to compare some biochemical and biophysical laboratory procedures for assessing quality of SBM: urease index (UI), protein dispersibility index (PDI), KOH protein solubility (PS), nitrogen solubility index (NSI).

2. Materials and methods

The urease assay is based on the pH increase from ammonia released from urea by residual urease enzyme in a soybean meal. The urease test was conducted as following: 10 cm^3 buffered urea solution (0.07 M, pH=7.5) was added to 0.200 g finely ground SBM (test sample); 10 cm^3 phosphate buffered solution was added to 0.200 g of the same sample (blank sample). The two solutions were incubated at 30ºC for 30 minutes under stirring. In the presence of signifcant urease activity, the pH of the test solution increases due to the release of ammonia from urea. After incubation, the pH of the solutions should be determined rapidly and the degree of heating was estimated basing on the pH difference between the first and the second solution.

The protein solubility was determined according to the procedure of Araba and Dale [10]. The KOH protein solubility test is based on the solubility of soybean proteins in a dilute solution of potassium hydroxide. The procedure involves the incubation of a sample with a 0.2% KOH solution for 20 min at room temperature. Following this incubation, the sample is centrifuged and the supernatant is analyzed for the protein concentration. The solubility of the protein, expressed as a percentage, was calculated by dividing the protein content of the KOH-extracted solution by the protein content of the original soybean sample.

The Protein Dispersibility Index assay is also based on the solubility of soybean protein. For this test the solubility is in water. The PDI method uses ten minutes of high speed mixing in distilled water at 8,500 rpm [11].

Nitrogen Solubility Index uses a slow stirring technique. Nitrogen is extracted from the ground flour by placing approximately 1.5 g into a 200 ml beaker and adding 75 ml of 0.5% KOH. The sample is stirred 20 minutes at 120 rpm.

SBM (granulation 200μ) was heated in a forced air oven at 120 ºC for varying periods of time: 5, 10, 15, 20, 25 and 30 minutes. Protein was determined by the biuret method [12].

3. Results and discussion

Protein quality of soybean meal is linked to both the reduction of antinutritional factors, and the optimization of protein digestibility. Direct analysis of both specifications is difficult in routine operations. It is therefore replaced with indirect tests such as urease index, PDI, NSI and KOH protein solubility. The most used quality index in our country is the urease index. Although the urease test is routinely done, the results do not correlate well with animal performance [13].

The results of the experiment are presented in Table 1.

The urease activity detected in the SMB sample was 2.0 pH units and approached zero by 20 minutes heat treatment. Additional heating has no effect on the urease index, showing that this test is unusefull in detecting overprocessing. An urease

index value of zero does not necessarily indicate overprocessing of SMB.

The solubility of soybean protein in potassium hydroxide solution is inversely related to degree of heat treatment. The PS of raw soybean flour approaches 100%, while meals which have been heated to a dark brown color may have PS as low as 30 or 40%. Values lower than 78, and especially lower than 74%, reflect an incremental decrease in lysine availability for all animals [14]. In our experiment KOH protein solubility remains high, during initial heat treatment. In marked contrast, the PDI index decreased incrementally from 78% to 20% for the heating times from 0 to 30 minutes. NSI index decreased incrementally from 97% to 60% for the heating times from 0 to 30 minutes.

Table 1. Effect of heating time on UI, PS, PDI and NSI

Heating time (minutes)	Urease index (units of pH increase)	KOH protein solubility %	PDI (%)	NSI (%)
0	2.0	89.65	78	97
5	1.8	87.40	65	90
10	0.5	87.00	58	80
15	0.02	80.15	51	72
20	0	72.20	38	70
25	0	68.25	25	65
30	0	65.20	20	60

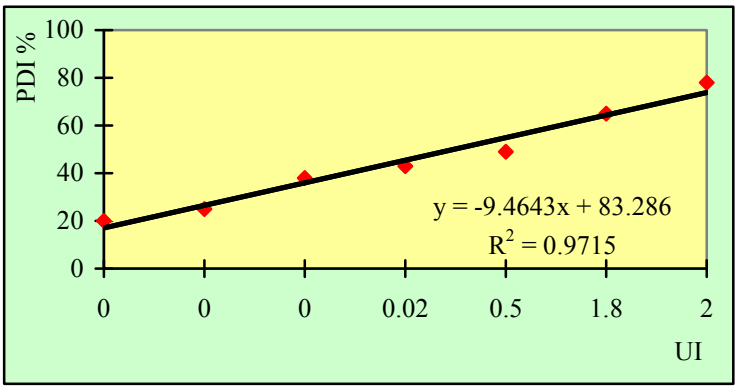

Figure 1. Correlation between PDI and PS

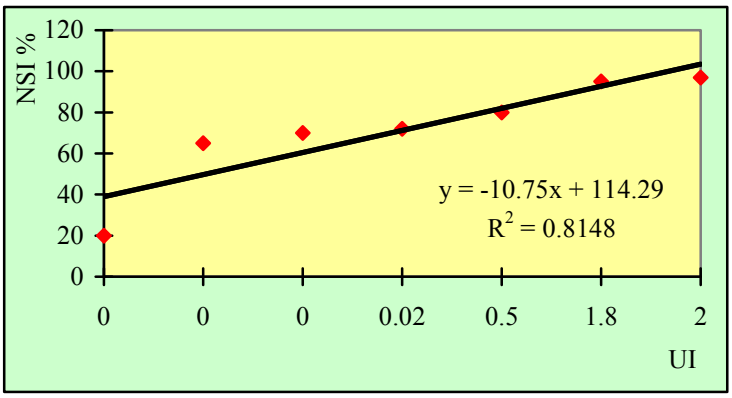

Figure 2. Correlation between NSI and PS

Therefore, PDI and NSI are a more consistent and sensitive indicator for monitoring both under-heating and over-heating of SBM. PDI values below 45% indicate adequate heating to destroy ANFs.

We observed a positive correlation (Figure 1) between PDI and UI values (r = 0.9857), and NSI and UI values, respectively (r = 0.9713).

4. Conclusions

While KOH solubility is a good index for determining over-processing of soybean meal, KOH protein solubility is not a sensitive index for monitoring under-processing of soybean meal.
Combing the PDI test with the urease test could be useful to soybean processors and poultry nutritionists for better monitoring of soybean meal quality. For example, a soybean meal containing low urease (0.3 or below) and high PDI (40 to 45%) may indicate that the sample is definitely high quality because it has been adequately heat processed, but not over-processed.

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