RESEARCH ON SOME CHEMICAL ANALYSIS METHODS FOR EVALUATING THE SOYBEAN MEAL QUALITY

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Abstract
Heat treatment of soybeans can reduce the activity of trypsin inhibitors and thereby improve the digestibility of protein. Overheating, however, may destroy or reduce the availability of certain heat sensitive amino acids and reduce the nutritional value of soy protein. The objective of our study was to compare three different chemical analysis methods for evaluating the soybean meal quality, and to establish the most suitable for evaluating over processing. Commercial SBM and raw soybean (granulation 200µ) were heated in a forced air oven at 120 ºC for varying periods: 5, 10, 15, 20, 25, and 30 minutes. The urease index (UI), the KOH protein solubility and the Protein Dispersibility Index (PDI) were determined. Protein was determined by the burette method. We observed that the UI is useful to determine if the soybean meal has been heated enough to reduce the antinutritional factors, but it is not very useful for determining if soybean meal has been over-processed. A UI value of zero does not necessarily indicate over processing of SMB. KOH protein solubility is not a sensitive index for monitoring under processing of soybean meal. The KOH protein solubility changed very little up to first 10 minutes of heating and then decreased to a solubility of 65.20%. The PDI is the best method of evaluating these soybean ingredients for both under heating and over heating. PDI decreased incrementally from 78% to 20% for the heating times from 0 to 30 minutes. PDI is also the simplest measurement procedure of those that were evaluated.

Key words: urease index, protein solubility, soybean meal, protein digestibility

INTRODUCTION
Among plant protein sources, SBM has a high level of tryptophan and the highest digestible lysine content which complements the lysine deficiency of cereal grains used in animal feeds. 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 ANFs, overheating results in undesirable changes to the chemical structure of many essential amino acids lowering the nutritive value of SBM. The reduction in protein quality of soybean meal as the result of over processing is due primarily to the combination of destruction of lysine and cystine and reduced digestibility of the lysine and cystine that is not destroyed [4]. These effects on lysine may be explained by the Maillard reaction in which free amino acids bond to free carbonyl groups. Therefore, feed manufacturers require methods to distinguish adequately processed SBM from under- or over-processed meals. The destruction of the enzyme urease present in soybeans is highly correlated with the destruction of trypsin inhibitors and other ANFs. The primary purpose of the urease assay is to determine if soybean meal has been sufficiently heated to destroy most of the antinutritional factors. The objective of our study was to compare three different chemical analysis methods for evaluating the soybean meal quality, and to establish the most suitable for evaluating over processing.

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 protein solubility was determined according to the procedure of Araba and Dale [1]. 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 [2].

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.

RESULTS AND DISCUSSION

Most soybean meal is produced today by the solvent extraction process whereby the soybeans are cracked, heated and flaked before the oil is extracted with the solvent hexane. Once the oil has been removed, the flakes are toasted and ground into meal. During this production process, temperature is critical in order to deactivate the anti-nutritional factors naturally present in raw soybeans. However, to maintain optimal nutritional value, the meal must not be subjected to excessive heat, as this will denature the protein, making it less soluble and less digestible. Excessive heat or heating time reduces the availability of amino acids due to the Maillard reaction [3] and tends to destroy certain amino acids [6].

Soybean meal processors and their customers in the animal feed industry need reliable, rapid and cost-efficient methods to control the quality of their soybean meal. Protein quality of soybean meal depends on two parameters, the reduction of anti-nutritional factors and the optimization of protein digestibility [5].

Table 1 shows the effects of overheating on a commercial sample of soybean meal, to accomplish additional heat treatment. Additional heat treatment decreased KOH protein solubility, and the urease activity index rapidly approached zero.

KOH protein solubility remains high, during initial heat treatment. The urease index is useful to determine if the soybean meal has been heated enough to reduce the anti-nutritional factors, but it is not very useful for determining if soybean meal has been over-processed.

Table 2 shows the effect of heating of raw soybean on urease index, KOH protein solubility and PDI. The urease index values remained nearly constant up to 5 minutes of heating and then dropped to 0.02 and less. The KOH protein solubility changed very little up to 10 minutes of heating and then decreased to a solubility of 65.20%.

The urease index is approaching to zero after 15 minutes of heating, so supplementary heating time has no effect on the urease index, showing that this test is unuseful in detecting over processing. The urease index value of zero does not necessarily indicate over processing of SMB. In marked contrast the PDI index decreased incrementally from 78% to 20% for the heating times from 0 to 30 minutes.

These results show that KOH protein solubility usually remains high during initial heat treatment. 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.
Table 1
Effect of heating time on UI and KOH protein solubility

<table>
<thead>
<tr>
<th>Heating time (minutes)</th>
<th>Urease index, units of pH increase</th>
<th>KOH protein solubility %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
<td>87.40</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>78.20</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>71.20</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>69.95</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>59.95</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>58.19</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>53.20</td>
</tr>
</tbody>
</table>

Table 2
Effect of heating time on UI, KOH protein solubility and PDI

<table>
<thead>
<tr>
<th>Heating time (minutes)</th>
<th>Urease index, units of pH increase</th>
<th>KOH protein solubility %</th>
<th>PDI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.0</td>
<td>89.65</td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>87.40</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>0.02</td>
<td>87.00</td>
<td>62</td>
</tr>
<tr>
<td>15</td>
<td>0.01</td>
<td>80.15</td>
<td>51</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>72.20</td>
<td>38</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>68.25</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>65.20</td>
<td>20</td>
</tr>
</tbody>
</table>

The experimental data suggest that PDI is a better indicator of minimum adequate heating of soybean meal than either the urease index or the KOH solubility index.

The results of the three methods of chemical analysis are graphed in Figure 1.

![Figure 1. Variation of UI, PDI and KOH protein solubility with the heating time](image)

**CONCLUSIONS**

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 and KOH protein solubility.
The urease index is useful to determine if the soybean meal has been heated enough to reduce the antinutritional factors, but it is not very useful for determining if soybean meal has been over-processed.
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.
The Protein Dispersibility Index (PDI) is the best method of evaluating these soybean ingredients for both under heating and over heating. The PDI is also the simplest measurement tool of those that were evaluated.

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REFERENCES
Journal articles