Application Note

Instrument: FP828 Series

Determination of Soluble Protein in Soybean Meal

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Introduction

Soybean meal (SBM) is a primary source of protein for the feed industry worldwide, and is an important source of protein for livestock, poultry, and aquaculture diets. It has one of the highest crude protein contents of all plant-based protein sources, typically containing approximately 40% to 50% protein.

Proteins are made up of smaller units called amino acids, which are attached to one another in long polypeptide chains. When SBM is ingested by livestock, the long protein chains are broken down into smaller polypeptide chains by hydrochloric acid and the enzyme pepsin, both of which are present in the stomach (swine/bovine) or in the proventriculus and gizzard (poultry). Once these smaller polypeptide chains enter the small intestine, a variety of enzymes including trypsin, excreted by the pancreas, will then break the peptide bonds linking the amino acids together, reducing the peptide chains to tripeptides, dipeptides, or free (individual) amino acids. This allows the small intestine to absorb the smaller peptides or free amino acids, which can then be utilized by cells to build other proteins and a few other macromolecules, such as DNA. Protein that is broken down and absorbed in the small intestine is referred to as soluble protein.

Not all protein present in SBM is soluble. There are many factors during SBM processing that can affect the amount of soluble protein present. Under-processed SMB will contain trypsin inhibitors, which prevent the enzyme, trypsin, from breaking the peptide bonds linking amino acids together, therefore reducing the amount of soluble protein. Overprocessing SBM can also affect the amount of soluble protein and protein structure, reducing the nutritional value of the product. The nutritional value of protein in SBM is evaluated not only by protein structure, amino acid composition, but also by protein digestibility (or protein solubility). Currently, the analytical technique commonly used to measure SBM quality is protein solubility determination.

Complete evaluation of the protein content in SBM involves two separate analyses. A representative sample of the SBM is split into two portions. One portion is used for total (crude) protein determination and the other portion undergoes chemical treatment (typically KOH digestion) prior to the determination of soluble protein. Using this method, the % total protein is determined, the % soluble protein is determined, and the % soluble protein of the total protein content can be calculated. The % soluble protein of the total protein content is an indicator of the quality of the processing procedure used.

Instrument Model and Configuration

The LECO FP828 is a combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a vertical quartz furnace. A thermoelectric cooler removes moisture from the combustion gases before they are collected in a ballast. The gases equilibrate and mix in the ballast before a representative aliquot (3 cm^3 or 10 cm^3 volume) of the combustion gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot gas is carried to a thermal conductivity cell (TC) for the detection of nitrogen (N_2).

Reference

"Evaluation of Protein Solubility as an Indicator of Overprocessing Soybean Meal", M. Araba and N. M. Dale, Poultry Science 69: 76-83, 1990*

**Publication containing the KOH digestion procedure that was used for soluble protein determination (outlined in the Sample Preparation section).*

Accessories

502-825 Large Tin Capsules, 0.2% KOH aqueous solution (0.036% N, pH 12.5), magnetic stir plate, magnetic stir bar, centrifuge, beaker, conical centrifuge tubes, glass wool, and disposable plastic pipettes.

Reference Materials

Calibration should be performed using a glycine solution (prepared following the procedure found on the last page of this document). The calibration can be verified using an appropriate concentration of a glycine solution and/or an ammonium solution.

Sample Preparation

Samples must be of a uniform consistency to produce suitable results. Samples should be ground to pass through a 0.5 mm sieve prior to analysis. Sample results should be corrected for moisture following analysis. Glycine solutions should be prepared using the procedure found on the last page of this document.

Note: Nitrogen/Protein results for soybean meal samples are typically reported on a dry basis in order to avoid a reporting bias due to fluctuations in moisture levels. Ground soy samples are hygroscopic and will absorb moisture following drying; therefore, drying soybean meal samples prior to analysis is not recommended. Instead, soybean meal sample results should be corrected for moisture following analysis. The sample's moisture content should be determined on the day of analysis (prior to performing the KOH digestion procedure) and used to correct the results for moisture following analysis, using the instrument's software.

Following grinding, samples should be prepared using a KOH digestion procedure, following the steps outlined below.

KOH Digestion Procedure

1. Weigh 1.5 g (± 0.001 g) of a milled soybean meal sample into a beaker.
2. Add 75 mL of a 0.2% (0.036 N, pH 12.5) KOH aqueous solution to the beaker.

3. Stir the contents of the beaker using a magnetic stir plate and a magnetic stir bar for 20 minutes.
4. Transfer the contents of the beaker into a conical centrifuge tube and centrifuge the mixture at 2,700 rpm for 15 to 25 minutes.
5. Decant the supernatant (liquid portion) from the centrifuge tube and filter it through glass wool into a beaker, being careful to avoid transferring any of the centrifugate (solids towards the bottom of the tube) into the beaker.
6. The filtered supernatant (KOH digested soy sample) will be analyzed using a 1.0 mL aliquot to determine the soluble protein content in the sample (following step 5 of the Procedure).

Note: The above procedure results in a 0.02 g sample mass (1.5 g/75 mL) per 1.0 mL aliquot of filtered supernatant. This is the sample mass that should be entered into the software and used for analysis.

Method Parameters**

Gas Type	Helium†
Furnace Temperature	950 °C
Afterburner Temperature	850 °C
Nominal Mass	1.0000 g
Purge Cycles	3
Ballast Equilibrate Time	10 s
Ballast Not Filled Timeout	300 s
Aliquot Loop Fill Pressure Drop	200 mm Hg
Aliquot Loop Equilibrate Time	6 s
Interleave Analysis	Yes
Sample Drop Detection	Disabled
Dose Loop Size	Large (10 cm^3)†

†Argon may be used as a carrier gas, and a Small (3 cm^3) dose loop may be used for analysis as well.

Element Parameters**

Integration Delay	4 s
Starting Baseline	15 s
Post Baseline Delay	14 s
Use Comparator	No
Integration Time	50 s
Use Endline	Yes
Endline Delay	20 s
Ending Baseline	15 s

Note: When utilizing Argon as a carrier gas, refer to LECO Application Note, "Determination of Nitrogen/Protein in Soybean Meal", Form No. 203-821-636, for element parameter settings.

***Refer to the 828 Series Operator's Instruction Manual for Parameter definitions.*

Burn Profile

Performance Model

Burn Step	*Furnace Flow*	*Time*
1	5.00 L/min	30 s
2	1.00 L/min	30 s
3	5.00 L/min	End

Base Model

Burn Step	*Furnace Flow*	*Time*
1	High	30 s
2	Medium	30 s
3	High	End

Note: The Burn Profile steps outlined above are for Soluble Protein determination. For Burn Profile settings used for total (crude) protein determination, refer to LECO Application Note, "Determination of Nitrogen/Protein in Soybean Meal", Form No. 203-821-636.

Procedure

1. Prepare the instrument for operation as outlined in the operator's instruction manual.
2. Condition the System.
   a. Select five or more Blank replicates in the Login screen.
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more Blank replicates in the Login screen.
   b. Initiate the analysis sequence.
   c. Set the blank following the procedure outlined in the operator's instruction manual.

   Note: The standard deviation of the last five blanks should be less than or equal to 0.001% (10 ppm) for nitrogen when utilizing Helium as a carrier gas, and less than or equal to 0.005% (50 ppm) for nitrogen when utilizing Argon as a carrier gas. Additional blanks beyond the recommended five may be required in order to achieve the recommended precision.
4. Calibrate/Drift Correct.
   a. Select the desired number of calibration/drift replicates in the Login screen (minimum of five).
   b. Weigh ~1.0 g of an appropriate concentration of a glycine solution (prepared following the procedure found on the last page of this document) into a 502-825 Large Tin Capsule. Leave the capsule open, so that atmosphere can be purged from the capsule when in the purge chamber (This will prevent biased nitrogen results due to trapped atmosphere).
   c. Enter the reference material mass and identification into the Login screen.
   d. Transfer the tin capsule containing the reference material to the appropriate position in the sample carousel.
   e. Perform steps 4b through 4d a minimum of five times.
   f. Initiate the analysis sequence.
   g. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   h. Verify the calibration/drift correction by analyzing several replicates of a different concentration of glycine solution and/or an ammonium solution, following steps 4b through 4f.
5. Analyze KOH Digested SBM Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Using a pipette, transfer 1.0 mL of the KOH digested SBM sample (filtered supernatant) into a 502-825 Large Tin Capsule. Leave the capsule open, so that atmosphere can be purged from the capsule when in the purge chamber (This will prevent biased nitrogen results due to trapped atmosphere).
   c. Enter the sample mass (0.02 g) and identification into the Login screen.
   d. Transfer the tin capsule containing the sample to the appropriate position in the sample carousel.
   e. Perform steps 5b through 5d for each sample to be analyzed.
   f. Initiate the analysis sequence.

   Note: It is important to monitor the steel wool in the primary filter tube for signs of excessive corrosion (rust). The steel wool is a sacrificial reagent, meaning that it is designed to rust before other items in the flow-path rust. However, in order for the steel wool to be effective, it must be replaced frequently.

TYPICAL RESULTS

Soluble protein data was generated utilizing a linear, force through origin calibration using a 0.2% N glycine solution. The calibration was verified using LECO 502-602 Ammonium Solution (0.1% N), a 0.1% N glycine solution, and a 0.01% N glycine solution. Glycine solutions were prepared following the procedure on the last page of this document. All reference materials were weighed and analyzed at ~1.0 gram. All KOH digested soybean meal samples were measured accurately to 1.0 mL using a pipette, then 0.02 g was entered into the sample Login screen as the sample mass. Samples were ground to pass through a 0.5 mm sieve using a cyclone mill prior to analysis and KOH treatment. Crude protein data was generated following the method outlined in LECO Application Note, "Determination of Nitrogen/Protein in Soybean Meal", Form No. 203-821-636. All sample results were corrected for moisture following analysis using the instrument's software. The moisture content of the sample was determined on the same day as analysis utilizing the LECO TGM800 set to 80°C with a 2-hour hold time. Results are reported on a dry basis. A protein factor of 5.71[††] was used to calculate the protein content.

	Crude (Total) Protein		
	Mass (g)	*% N*	*% Protein*
Soybean Meal (untreated)	0.1513	8.14	46.48
Moisture: 9.45%	0.1513	8.15	46.52
	0.1510	8.13	46.42
	0.1523	8.14	46.46
	0.1515	8.12	46.39
	0.1529	8.12	46.36
	0.1516	8.12	46.35
	0.1525	8.11	46.30
	0.1534	8.13	46.42
	0.1537	8.14	46.48
	Avg =	***8.13***	***46.42***
	s =	***0.01***	***0.07***

	KOH Digested (Soluble) Protein		
	Mass (g)	*% N*	*% Protein[‡]*
Soybean Meal	0.0200	6.93	39.60
(treated with KOH)	0.0200	6.94	39.64
Moisture: 9.45%	0.0200	6.96	39.73
	0.0200	6.88	39.26
	0.0200	7.00	39.98
	0.0200	6.96	39.75
	0.0200	7.07	40.35
	0.0200	6.94	39.60
	0.0200	6.99	39.89
	0.0200	6.92	39.49
	Avg =	***6.96***	***39.73***
	s =	***0.05***	***0.30***

The following equation was used to determine the % Soluble Protein[‡‡] content:

$$\%\ \text{Soluble Protein}^{‡‡} = \frac{\text{Avg \% KOH Digested Protein}^{‡}}{\text{Avg \% Crude Protein}} \times 100$$

Results: $\frac{39.73\ \%}{46.42\ \%} \times 100$ **= 85.59 % Soluble Protein[‡‡]**

[††]Protein factor was obtained from the United States Department of Agriculture, Circular No. 183.
[‡]Percent soluble protein present in the sample (% KOH Digested Protein).
[‡‡]Percent of the total protein content in the sample that is soluble.

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GLYCINE SOLUTION PREPARATION

1. The following formula can be used to make a specific concentration:

$$G = \frac{C}{(0.99^{\dagger} * 0.18658)}$$

where: C = desired nitrogen concentration as percent
G = grams of glycine powder

Example for 1% solution:

$$G = \frac{1}{(0.99^{\dagger} * 0.18658)} = 5.414$$

NOTE: A quick reference chart, shown below, shows the grams of glycine powder needed to reach given concentrations.

2. Place a flask on the balance and tare. The flask should be large enough to hold 100 ml (where 100 g = 100 ml).
3. Add the amount of glycine calculated in step 1 and record the mass.
4. Add distilled water until the total mass equals 100 g, then record the mass (W).
5. Seal the flask and mix the contents.
6. To figure the exact concentration:

$$\% \text{ Nitrogen} = \frac{G\,(18.658 * 0.99^{\dagger})}{W}$$

where: G = mass in grams of glycine recorded in step 3
W = mass in grams of water and glycine powder recorded in step 4

7. If the distilled water is not pure, determining the nitrogen concentration may be necessary.
   a. Analyze five samples of distilled water.
   b. Average the nitrogen content of the five samples (A).
   c. Add this average to % nitrogen calculated for the calibration solution.

Example: To make a calibration solution of approximately 0.3% nitrogen:
where: G = 1.672 g
W = 99.824 g
A = 0.004%

$$\frac{1.672(18.471)}{(99.824)} + 0.004 = 0.313\%\ \text{N}$$

QUICK REFERENCE CONCENTRATION TABLE

Nitrogen Concentration	Grams of Glycine[†]
0.10%	0.541
0.30%	1.624
0.50%	2.707
0.75%	4.060
1.00%	5.414

[†]*Assuming 99.0% purity of glycine powder.*