Application Note

Instrument: FP828 Series

Determination of Nitrogen/Protein in Soybean Meal

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Introduction

Soybean meal is the primary source of protein for the feed industry worldwide, and is an important source of protein for livestock, poultry, and aquaculture diets. Soybean meal has the highest crude protein content of all plant-based protein sources, typically containing approximately 40% to 50% protein. Soybean meal is characterized as being derived either from dehulled beans or beans having hulls. Dehulled meal has a higher content of crude protein, amino acids, and metabolizable energy than meal produced from soybeans having hulls. Soybeans are also utilized to produce soy flour, an alternative non-wheat, gluten-free flour made from dehulled, roasted soybeans that are finely ground.

The accurate and precise determination of protein plays a key role in the characterization of the nutritional or dietary value of soybean meal and soy flour, and is therefore important in determining the quality of the meal or flour. Protein in soybean meal and flour, along with other feeds and food products, is commonly calculated using the measured nitrogen in the sample and a protein factor or multiplier (protein factors vary according to the sample matrix).

Instrument Model and Configuration

The LECO FP828 is a combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a vertical quartz furnace, ensuring complete combustion and superior analyte recovery. A thermoelectric cooler removes moisture from the combustion gases before they are collected in a ballast. The combustion gases equilibrate and mix in the ballast before a representative aliquot (3 cm^3 or 10 cm^3 volume) of the 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).

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analyte gas compared to a reference/carrier gas. The greater the difference between the thermal conductivity of the carrier gas and the analyte gas, the greater sensitivity of the detector. The FP828 supports either the use of helium or argon as the instrument's carrier gas. When used as a carrier gas, helium provides the highest sensitivity, and the best performance at the lower limit of the nitrogen range. The thermal conductivity difference between argon and nitrogen is not as great as the thermal conductivity difference between helium and nitrogen, therefore the detector is inherently less sensitive when using argon as a carrier gas.

The FP828 offers the additional advantage of utilizing either a 10 cm^3 aliquot dose loop or a 3 cm^3 aliquot dose loop within the instrument's gas collection and handling system. The 10 cm^3 aliquot dose loop optimizes the system for the lowest nitrogen range and provides the best precision. The 3 cm^3 aliquot dose loop extends reagent life expectancy by approximately three-fold when compared to the 10 cm^3 aliquot dose loop, while providing the lowest cost-per-analysis.

Note: When changing carrier gas type, refer to the 828 Series Operator's Instruction Manual for the procedure on setting the gas flow rate. When using the FP828 Performance model, the aliquot dose loop size is changed by selecting the desired aliquot dose loop size in the software's Method Parameters. When using the FP828 Base model, the desired dose loop is installed by the operator.

Method Reference

AOAC 990.03: Protein (Crude) in Animal Feed, Combustion Method;
AOCS BA4F-00: Combustion Method for Determination of Crude Protein in Soybean Meal

Sample Preparation

Samples must be of a uniform consistency to produce suitable results. Soy samples should be prepared in accordance with AOAC Official Method 950.02 - Animal Feed, Preparation of Sample. Samples should be ground to pass through a 1 mm sieve prior to analysis. All soy sample results should be corrected for moisture following analysis. Reference materials should be prepared as directed by the certificate, prior to analysis.

Note: Nitrogen/Protein results for soy 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 soy samples prior to analysis is not recommended. Instead, soy samples should be corrected for moisture following analysis. The sample's moisture content should be determined on the day of analysis and used to correct the results for moisture following analysis, using the instrument's software.

Accessories

502-186 Small Tin Foil Cups or 502-338 Small Quik-Cap Capsules, and 501-614 Spatula

Reference Materials

LCRM®, LRM®, NIST, or other suitable reference materials.

*Method Parameters**

Gas Type	Helium or Argon
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) or Small (3 cm^3)

*Element Parameters**

Parameter	*Helium* Nitrogen	*Argon* Nitrogen
Integration Delay	4 s	4 s
Starting Baseline	15 s	15 s
Post Baseline Delay	14 s	18 s
Use Comparator	No	No
Integration Time	50 s	70 s
Use Endline	Yes	Yes
Endline Delay	20 s	20 s
Ending Baseline	15 s	15 s

**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	End

Base Model

Burn Step	*Furnace Flow*	*Time*
1	High	End

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 an appropriate mass (~0.15 g) of a suitable reference material into a 502-186 Tin Foil Cup or a 502-338 Small Quik-Cap Capsule. If using a tin foil cup, seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together. If using a quik-cap capsule, leave the capsule open so that atmosphere can be purged from the capsule when in the purge chamber.
   c. Enter reference material mass and identification into the Login screen.
   d. Transfer the tin foil cup or quik-cap 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 an appropriate mass of another/different suitable reference material, following steps 4b through 4f, and confirm that the results are within the acceptable tolerance range.

   Note: Typically, the FP828 can be calibrated using several replicates of a single mass range of a suitable reference material utilizing a linear, force through origin calibration. This is a cost-effective and simple process. A multi-point calibration (fractional mass or multiple calibration materials) may be used to calibrate if desired.
5. Analyze Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Weigh ~ 0.15 g of soy sample into a 502-186 Tin Foil Cup or a 502-338 Small Quik-Cap Capsule. If using a tin foil cup, seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together. If using a quik-cap capsule, leave the capsule open so that atmosphere can be purged from the capsule when in the purge chamber.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the tin foil cup or quik-cap 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.

TYPICAL RESULTS

Data was generated utilizing a linear, force through origin calibration using ~0.15 g of 502-896 (Lot 1001) EDTA LCRM (9.56% N). The calibration was verified using ~0.15 g of LECO 502-688 (Lot 1002) Nicotinic Acid LCRM (11.38% N), and ~0.15 g of LECO 502-642 (Lot 1018) Phenylalanine LCRM (8.47% N). All soy samples were analyzed at ~0.15 grams. The Soybean and Soybean Meal samples were ground to pass through a 1 mm sieve, using a cyclone mill, prior to analysis. All soy samples were analyzed as received and results were corrected for moisture following analysis. The Moisture content for each sample was determined utilizing the LECO TGM800 on the same day as analysis. Samples were dried at 80 °C, using a 2-hour hold time. The moisture values obtained were used to correct the results for moisture following analysis, using the instrument's software. Results are reported on a dry basis. Reference materials were prepared as directed by the certificate, prior to analysis. A protein factor of 5.71† was used for all soy samples to calculate the protein content.

	Helium 10 cm³			***Helium 3 cm³***			***Argon 10 cm³***			***Argon 3 cm³***		
	Mass (g)	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*
Soy Flour #1	0.1537	8.46	48.29	0.1524	8.46	48.31	0.1536	8.46	48.31	0.1541	8.38	47.86
Moisture (Helium): 5.15%	0.1515	8.45	48.28	0.1524	8.47	48.35	0.1539	8.43	48.13	0.1532	8.54	48.79
Moisture (Argon): 5.11%	0.1533	8.47	48.37	0.1521	8.46	48.31	0.1532	8.41	47.99	0.1552	8.40	47.97
	0.1541	8.47	48.38	0.1529	8.46	48.29	0.1529	8.45	48.25	0.1555	8.53	48.69
	0.1537	8.47	48.34	0.1530	8.42	48.09	0.1529	8.45	48.27	0.1555	8.50	48.54
	Avg =	***8.46***	***48.33***	***Avg =***	***8.45***	***48.27***	***Avg =***	***8.44***	***48.19***	***Avg =***	***8.47***	***48.37***
	s =	***0.01***	***0.05***	***s =***	***0.02***	***0.10***	***s =***	***0.02***	***0.13***	***s =***	***0.07***	***0.43***
Soy Flour #2	0.1505	8.63	49.30	0.1531	8.63	49.27	0.1551	8.59	49.07	0.1515	8.62	49.24
Moisture (Helium): 4.84%	0.1523	8.64	49.33	0.1522	8.61	49.17	0.1542	8.61	49.16	0.1513	8.61	49.14
Moisture (Argon): 4.76%	0.1542	8.64	49.32	0.1533	8.61	49.18	0.1556	8.59	49.06	0.1515	8.55	48.81
	0.1530	8.63	49.27	0.1532	8.61	49.16	0.1551	8.59	49.03	0.1527	8.64	49.33
	0.1521	8.64	49.34	0.1513	8.58	49.02	0.1550	8.61	49.16	0.1524	8.65	49.42
	Avg =	***8.64***	***49.31***	***Avg =***	***8.61***	***49.16***	***Avg =***	***8.60***	***49.10***	***Avg =***	***8.61***	***49.19***
	s =	***< 0.01***	***0.03***	***s =***	***0.02***	***0.09***	***s =***	***0.01***	***0.06***	***s =***	***0.04***	***0.24***
Soybeans	0.1540	6.84	39.04	0.1510	6.80	38.83	0.1527	6.78	38.70	0.1523	6.80	38.86
Whole Beans, Ground	0.1537	6.85	39.09	0.1510	6.81	38.90	0.1523	6.80	38.81	0.1538	6.80	38.82
Moisture (Helium): 6.08%	0.1535	6.82	38.94	0.1507	6.82	38.93	0.1524	6.80	38.85	0.1540	6.85	39.14
Moisture (Argon): 6.08%	0.1528	6.84	39.07	0.1528	6.81	38.89	0.1541	6.79	38.79	0.1536	6.81	38.90
	0.1529	6.82	38.94	0.1532	6.78	38.71	0.1543	6.80	38.81	0.1530	6.71	38.33
	Avg =	***6.83***	***39.01***	***Avg =***	***6.80***	***38.85***	***Avg =***	***6.79***	***38.79***	***Avg =***	***6.80***	***38.81***
	s =	***0.01***	***0.07***	***s =***	***0.02***	***0.09***	***s =***	***0.01***	***0.06***	***s =***	***0.05***	***0.29***
Soybean Meal	0.1534	8.14	46.47	0.1520	8.17	46.63	0.1552	8.15	46.57	0.1544	8.13	46.40
Ground	0.1545	8.13	46.45	0.1523	8.17	46.66	0.1552	8.16	46.60	0.1534	8.16	46.60
Moisture (Helium): 9.64%	0.1541	8.13	46.41	0.1519	8.13	46.40	0.1545	8.15	46.56	0.1549	8.11	46.31
Moisture (Argon): 9.68%	0.1543	8.14	46.48	0.1533	8.12	46.39	0.1549	8.16	46.57	0.1532	8.16	46.58
	0.1546	8.14	46.47	0.1536	8.11	46.29	0.1552	8.11	46.33	0.1542	8.07	46.06
	Avg =	***8.14***	***46.45***	***Avg =***	***8.14***	***46.47***	***Avg =***	***8.15***	***46.52***	***Avg =***	***8.12***	***46.39***
	s =	***0.01***	***0.03***	***s =***	***0.03***	***0.16***	***s =***	***0.02***	***0.11***	***s =***	***0.04***	***0.22***

†Protein factor was obtained from the United States Department of Agriculture, Circular No. 183. The choice of protein factor to be used for determining protein content in different materials is the subject of some debate. As a result, if being used for commerce, the value of this conversion factor should be part of the contractual agreement between buyer and seller.

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