Nitrogen/Protein in Feeds, Grains, and Oil Seeds

LECO Corporation; Saint Joseph, Michigan USA

Instrument: TruMac® N

Introduction

Protein determination is one of the most significant nutrient components. The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value in feed materials but is also key to the economic value of these materials. Protein in feed products is most commonly calculated using the measured nitrogen in the sample and a multiplier (protein factors vary according to the sample matrix). Nitrogen determination is performed using either the classical wet chemical method (Kjeldahl) or a combustion method. The Kjeldahl method is capable of handling macro samples sizes >1 g typically utilized for heterogeneous feed product samples. Physical restrictions in sample encapsulation and realities of handling the ash build up within a vertical furnace combustion nitrogen instrument will often restrict the sample mass to ~300 mg or less making the accurate analysis of heterogeneous feed products difficult. The LECO TruMac N combustion nitrogen determinator has been designed to handle macro sample sizes (~1 g) while maintaining a rapid analysis time with a low cost-per-analysis.

The LECO TruMac N is a macro combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a ceramic horizontal furnace and large ceramic boats for the macro sample combustion process. A thermoelectric cooler removes the moisture in the combustion gas without the use of chemical reagents. A 3 or 10 cc volume of combustion gas is taken using a combustion gas collection and handling system. The combustion gas collection and handling system achieves a low cost-per-analysis by reducing the amount of chemical reagents used for scrubbing and converting the nitrogen oxide combustion gas to nitrogen. A thermal conductivity (TC) cell is used for the detection of nitrogen in the combustion gas.

Sample Preparation

Samples must be of uniform consistency to produce suitable results. Typically samples should be ground to a fineness of <0.5 mm. Refer to AOAC 990.03 or AOCS Ba 4f for additional information regarding sample preparation.

Accessories

528-203 Crucibles

Calibration Samples

502-092 EDTA, 502-642 Phenylalanine,
501-050 Nicotinic Acid

*Analysis Parameters**

Furnace Temperature	1100°C
TE Cooler Temperature	5°C
Dehydration Time	0 seconds
Purge Cycles	2 seconds

Instrument Model and Configuration

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analytical gas compared to the constant thermal conductivity of the reference gas. The greater the difference between the thermal conductivity of the carrier gas and the analyte gas, the greater the sensitivity of the detector. The TruMac is available in models that support either the use of helium or argon as the instrument's carrier gas for the thermal conductivity cell.

When used as a carrier gas, helium provides the highest sensitivity, providing the best performance at the lower end of the nitrogen range. Helium models also offer the additional advantage of replacing the 10 cc aliquot loop with a 3 cc loop within the instrument's gas collection and handling system. The 10 cc aliquot loop optimizes the instrument for the lowest nitrogen range and best precision. The 3 cc aliquot loop extends reagent life expectancy by approximately three fold compared to the 10 cc aliquot loop, while providing the lowest cost-per-analysis with minimal impact on practical application performance (see Typical Results section).

Due to the recent history of low supply and general availability issues for helium gas, the argon model was developed to utilize argon as a carrier gas. Since the thermal conductivity difference between argon and nitrogen is not as great as the thermal conductivity difference between helium and nitrogen, the detector is inherently less sensitive with argon as a carrier gas. The argon model (10 cc aliquot only) has a similar practical application performance compared to the helium model, operating with equivalent instrument and method configurations (see Typical Results section).

Note: Changing carrier gas and aliquot loop size requires hardware changes within the instrument.

Element Parameters

	Helium	*Argon*
	10 cc and 3 cc	*10 cc*
Baseline Delay Time	6 seconds	6 seconds
Minimum Analysis Time	35 seconds	55 seconds
Endline Time	2 seconds	2 seconds
Conversion Factor	1.00	1.00
Significant Digits	5	5
TC Baseline Time	10 seconds	10 seconds

Organic Application Note

Burn Profile

Burn Cycle	*Lance Flow*	*Purge Flow*	*Time*
1	Off	On	5 seconds
2	On	On	35 seconds
3	On	Off	END

Ballast Parameters

Equilibrate Time	30 seconds
Not Filled Timeout	300 seconds

Aliquot Loop

Equilibrate Pressure Time	4 seconds
High Precision	Yes
High Speed	No

**Refer to TruMac Operator's Instruction Manual for Method Parameter definitions.*

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Condition the system by analyzing 3 to 5 blanks (crucible is not required).
3. Determine blank.
   a. Enter 1.0000 g mass into Sample Login (F3) using Blank as the sample name.
   b. Place a 528-203 Crucible to the appropriate position of the autoloader.
   c. Repeat steps 3a through 3b a minimum of three times.
   d. Initiate the analysis sequence (F5).
   e. Set the blank following the procedure outlined in the operator's instruction manual.
4. Calibrate.
   a. Weigh ~0.75 g of EDTA calibration sample into a 528-203 Crucible, enter mass and sample indentification into Sample Login (F3).
   b. Transfer crucible to the appropriate position of the autoloader.
   c. Repeat steps 4a through 4b a minimum of three times.
   d. Initiate the analysis sequence (F5).
   e. Calibrate the instrument following the procedure outlined in the operator's instruction manual. Use single standard calibration.

   Note: Multi-point (fractional weight or multiple calibration samples) may be used to calibrate if desired. Research has shown that a properly functioning TruMac can be calibrated using several replicates of a single mass range (nominal 0.75 g) of EDTA utilizing a single standard calibration. This is a cost-effective and simple process. The calibration can be verified by analyzing different compounds such as nicotinic acid (0.25 to 0.5 g) and/or phenylalanine (0.5 to 0.75 g).
5. Analyze Samples.
   a. Weigh ~0.5 to 1 g sample into a 528-203 Crucible; enter mass and sample identification into Sample Login (F3).
   b. Transfer crucible to the appropriate position of the autoloader.
   c. Repeat steps 5a through 5b for each sample to be analyzed.
   d. Initiate the analysis sequence (F5).

Notes

- *If soot (carbon black) is noticed in the primary filter (steel wool filter), reduce sample mass to prevent soot build-up in this filter. Soot can be produced when larger masses of high-fat samples are analyzed.*

TYPICAL RESULTS

	3 cc Helium			10 cc Helium			10 cc Argon		
	Mass(g)	*% N*	*% Protein*	*Mass(g)*	*% N*	*% Protein*	*Mass(g)*	*% N*	*% Protein*
Pet Food #1	1.0751	4.31	26.9	1.0496	4.29	26.8	1.089	4.27	26.7
	1.0454	4.26	26.6	1.0885	4.20	26.3	1.0689	4.28	26.8
	1.0489	4.28	26.8	1.0793	4.23	26.4	1.08	4.23	26.4
	1.0309	4.28	26.8	1.0647	4.28	26.8	1.105	4.30	26.9
	1.0831	4.31	26.9	1.0847	4.28	26.8	0.975	4.21	26.3
	Avg =	***4.29***	***26.8***	***Avg =***	***4.26***	***26.6***	***Avg =***	***4.26***	***26.6***
	s =	***0.02***	***0.1***	***s =***	***0.04***	***0.2***	***s =***	***0.04***	***0.3***
Pet Food #2	1.0568	5.49	34.3	1.002	5.57	34.8	1.0361	5.58	34.9
	1.0175	5.56	34.8	1.0235	5.54	34.6	0.9906	5.60	35.0
	1.0204	5.52	34.5	1.0395	5.53	34.6	1.021	5.56	34.8
	1.0288	5.48	34.3	1.0735	5.56	34.8	1.0386	5.55	34.7
	1.0623	5.53	34.6	1.0401	5.55	34.7	1.0443	5.53	34.6
	Avg =	***5.52***	***34.5***	***Avg =***	***5.55***	***34.7***	***Avg =***	***5.56***	***34.8***
	s =	***0.03***	***0.2***	***s =***	***0.02***	***0.1***	***s =***	***0.03***	***0.2***
Grain #1	1.0411	6.05	37.8	1.0166	6.08	38.0	1.0895	6.05	37.8
	1.0112	6.07	37.9	1.0393	6.09	38.1	1.0538	6.06	37.9
	1.0107	6.04	37.8	1.0238	6.08	38.0	1.0393	6.04	37.8
	1.0388	6.06	37.9	1.011	6.07	37.9	1.0617	6.07	37.9
	1.0116	6.07	37.9	1.0375	6.08	38.0	1.0354	6.05	37.8
	Avg =	***6.06***	***37.9***	***Avg =***	***6.08***	***38.0***	***Avg =***	***6.05***	***37.8***
	s =	***0.01***	***0.1***	***s =***	***0.01***	***0.1***	***s =***	***0.01***	***0.1***
Grain #2	1.0261	4.55	28.4	1.0154	4.56	28.5	1.0597	4.55	28.4
	1.0047	4.59	28.7	1.0084	4.57	28.6	1.0236	4.56	28.5
	1.0116	4.56	28.5	1.0207	4.55	28.4	1.0125	4.53	28.3
	1.0179	4.57	28.6	1.0159	4.57	28.6	1.0026	4.56	28.5
	1.0179	4.56	28.5	1.0506	4.55	28.4	1.0766	4.57	28.6
	Avg =	***4.57***	***28.5***	***Avg =***	***4.56***	***28.5***	***Avg =***	***4.55***	***28.5***
	s =	***0.02***	***0.1***	***s =***	***0.01***	***0.1***	***s =***	***0.02***	***0.1***

Note: A protein factor of 6.25 was used to calculate the % Protein.

Organic Application Note

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