Application Note

Instrument: FP928

Determination of Nitrogen/Protein in Meat and Meat Products

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Introduction

Protein is one of the most significant nutrient components in food products. The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value in food materials, but is also the key to the economic value of these materials. Protein in meat products is most commonly calculated using the measured nitrogen content in the sample and a multiplier or conversion factor (commonly 6.25). Nitrogen determination is performed using either the classical wet chemical method (Kjeldahl), or a combustion method (Dumas). The Kjeldahl method is capable of handling macro samples sizes (~1 g) typically utilized for heterogeneous meat product samples. Physical restrictions in sample encapsulation and the difficulties of handling the ash build up within a vertical furnace combustion nitrogen instrument will often restrict the sample mass to ~500 mg or less, making the accurate analysis of heterogeneous meat products difficult. The LECO® FP928 combustion nitrogen determinator is designed to handle macro sample masses (~1 g) while maintaining a rapid analysis time with a low cost-per-analysis.

The FP928 is a macro combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a high-temperature horizontal ceramic combustion furnace, utilizing ceramic boats designed to handle macro sample masses (~1 g). 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 (10 cm^3 or 3 cm^3 volume) of the gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot of gas is carried through a heated reduction tube filled with copper to convert nitrogen oxide combustion gas species (NO_x) to nitrogen (N_2). The aliquot gas is then carried to a thermal conductivity cell (TC) for the detection of nitrogen (N_2).

Instrument Model and Configuration

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 FP928 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 FP928 offers the additional advantage of utilizing either a 10 cm^3 aliquot loop or a 3 cm^3 aliquot loop within the instrument's gas collection and handling system. The 10 cm^3 aliquot loop optimizes the system for the lowest nitrogen range and provides the best precision. The 3 cm^3 aliquot loop extends reagent life expectancy by approximately three-fold when compared to the 10 cm^3 aliquot loop, while providing the lowest cost-per-analysis with minimal impact on practical application performance for the determination of nitrogen/protein in meat and meat products (see Typical Results section).

Note: When changing carrier gas type, the flow needs to be adjusted following instructions provided in the FP928 Operator's Instruction Manual. The aliquot loop size is changed by selecting the desired aliquot loop size in the software's Method Parameters.

Method Reference

AOAC 992.15 – Crude Protein in Meat and Meat Products Including Pet Foods (Combustion Method)*

**A modified version of the AOAC 992.15 official method was utilized to generate this application note.*

Sample Preparation

Samples must be of a uniform consistency to produce suitable results. Meat samples should be prepared in accordance with the official AOAC 983.18 Method: Meat and Meat Products - Preparation of Sample. Reference materials should be prepared as directed by the certificate, prior to analysis.

Accessories

528-203 Ceramic Combustion Boats, 611-844 Spatula Flat Spoon, 604-494 Plunger, and 502-210 Tubes (disposable).

Reference Materials

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

*Method Parameters***

Gas Type	Helium or Argon
Furnace Temperature	1100 °C
Dehydration Time	0 s
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	4 s
Dose Loop Size	10 cm^3 or 3 cm^3

Element Parameters**

Parameter	Helium 10 cm^3 & 3 cm^3	Argon 10 cm^3 and 3 cm^3
Integration Delay	0 s	9 s
Starting Baseline	10 s	10 s
Post Baseline Delay	20 s	16 s
Use Comparator	No	No
Integration Time	50 s	70 s
Use Endline	Yes	Yes
Endline Delay	30 s	30 s
Ending Baseline	5 s	5 s
Use Profile Blank	--	Yes

***Refer to FP928 Operator's Instruction Manual for Parameter definitions.*

Burn Profile

Burn Step	Lance Flow	Furnace Flow	Time
1	No	Yes	5 s
2	Yes	Yes	35 s
3	Yes	No	End

Procedure

1. Prepare 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 (ceramic combustion boat is not required).
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more Blank replicates in the Login screen.
   b. Place 528-203 Ceramic Combustion Boats in the appropriate positions in the autoloader.
   c. Initiate the analysis sequence.
   d. 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) when utilizing Helium as a carrier gas, and less than or equal to 0.005% (50 ppm) 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 of a suitable reference material into a 528-203 Ceramic Combustion Boat.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the Ceramic Combustion Boat containing the reference material to the appropriate position in the autoloader.
   e. Perform steps 4b through 4d a minimum five times for each calibration/drift material used.
   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 by analyzing an appropriate mass of another suitable reference material and confirm that the results are within the acceptable tolerance range.

   Note: Typically, the LECO FP928 can be calibrated utilizing several replicates of a single mass range (nominal 0.75 g) of EDTA utilizing a single standard calibration (linear, forced through origin calibration). This is a cost effective and simple process. The calibration can be verified by analyzing different compounds such as nicotinic acid (0.15 to 0.35 g), or phenylalanine (0.15 to 0.5 g). A multi-point calibration (fractional weight or multiple calibration samples) may be used to calibrate if desired.
5. Analyze Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Weigh approximately 0.5 g to 1.0 g of the meat sample into a 528-203 Ceramic Combustion Boat utilizing a 604-494 Plunger with a 502-210 Tube.
   c. Enter sample mass and identification information into the Login screen.
   d. Transfer the Ceramic Combustion Boat containing the sample to the appropriate position in the autoloader.
   e. Perform steps 5b through 5d for each sample to be analyzed.
   f. Initiate the analysis sequence.

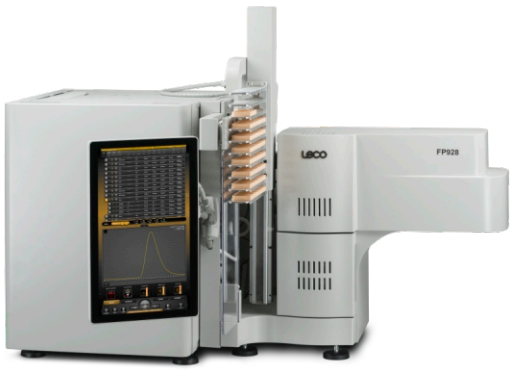
   Note: If soot (carbon black) is noticed in the primary filter (steel wool filter), reduce the sample mass to prevent soot build-up in this filter. Soot can be produced when larger masses of meat samples with a high fat content are analyzed.

TYPICAL RESULTS

Data was generated utilizing a linear force through origin calibration using ~0.75 g of 502-896 Lot #1002 EDTA LCRM (9.57% N). The calibration was verified using ~0.25 g of 502-642 Lot #1018 Phenylalanine LCRM (8.47% N) and ~0.25 g of 502-688 Lot# 1002 Nicotinic Acid LCRM (11.38% N). A protein factor of 6.25[†] was used for all samples to calculate the protein content.

	10 cm^3 Helium			3 cm^3 Helium			10 cm^3 Argon			3 cm^3 Argon		
	Mass (g)	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*
Turkey	1.0532	2.73	17.0	1.0448	2.72	17.0	1.0009	2.71	17.0	1.0619	2.65	16.6
	1.1000	2.73	17.1	1.1050	2.73	17.1	1.0574	2.70	17.0	1.0769	2.62	16.4
	1.0695	2.73	17.1	1.0342	2.74	17.1	1.0295	2.70	17.0	1.0414	2.71	16.9
	1.0340	2.73	17.1	1.1135	2.70	16.9	1.0428	2.69	17.0	1.0255	2.71	16.9
	1.0684	2.72	17.0	1.0671	2.73	17.0	1.0542	2.73	17.0	1.0916	2.68	16.7
	Avg =	***2.73***	***17.1***	***Avg =***	***2.72***	***17.0***	***Avg =***	***2.71***	***17.0***	***Avg =***	***2.67***	***16.7***
	s =	***0.01***	***<0.1***	***s =***	***0.01***	***0.1***	***s =***	***0.01***	***0.1***	***s =***	***0.04***	***0.2***
Ham	1.0432	2.87	18.0	1.0592	2.80	17.5	1.0451	2.90	18.1	1.0839	2.78	17.4
	1.0360	2.87	17.9	1.0597	2.85	17.8	1.0535	2.90	18.1	1.0271	2.85	17.8
	1.0254	2.87	18.0	1.0371	2.87	17.9	1.0399	2.82	17.6	1.0311	2.77	17.3
	1.0116	2.91	18.2	1.0463	2.89	18.1	1.0425	2.84	17.8	1.0126	2.88	18.0
	1.0236	2.87	17.9	1.0814	2.84	17.8	1.0327	2.87	17.9	1.0135	2.88	18.0
	Avg =	***2.88***	***18.0***	***Avg =***	***2.85***	***17.8***	***Avg =***	***2.86***	***17.9***	***Avg =***	***2.83***	***17.7***
	s =	***0.02***	***0.1***	***s =***	***0.03***	***0.2***	***s =***	***0.03***	***0.2***	***s =***	***0.05***	***0.3***
Hot Dog	1.0046	2.23	13.9	1.0386	2.21	13.8	1.0225	2.22	13.9	1.0369	2.20	13.7
	1.0591	2.22	13.8	1.0669	2.23	13.9	1.0395	2.21	13.8	1.0670	2.20	13.8
	1.0411	2.21	13.8	1.0256	2.18	13.6	1.0208	2.20	13.7	1.0436	2.16	13.5
	1.0579	2.20	13.7	1.0784	2.18	13.7	1.0369	2.20	13.8	1.0970	2.19	13.7
	1.0421	2.20	13.8	1.0566	2.19	13.7	1.0160	2.20	13.8	1.0732	2.24	14.0
	Avg =	***2.21***	***13.8***	***Avg =***	***2.20***	***13.7***	***Avg =***	***2.21***	***13.8***	***Avg =***	***2.20***	***13.8***
	s =	***0.01***	***0.1***	***s =***	***0.02***	***0.1***	***s =***	***0.01***	***0.1***	***s =***	***0.03***	***0.2***
Bacon	0.5429	5.34	33.4	0.5297	5.32	33.3	0.5700	5.36	33.5	0.5322	5.44	34.0
	0.5073	5.32	33.3	0.6140	5.40	33.8	0.5363	5.37	33.6	0.5325	5.25	32.8
	0.5642	5.29	33.1	0.5357	5.30	33.1	0.5595	5.31	33.2	0.5307	5.41	33.8
	0.5819	5.26	32.9	0.5019	5.25	32.8	0.5116	5.32	33.2	0.5274	5.17	32.3
	0.5554	5.34	33.4	0.5998	5.37	33.6	0.5475	5.37	33.5	0.5818	5.45	34.0
	Avg =	***5.31***	***33.2***	***Avg =***	***5.33***	***33.3***	***Avg =***	***5.35***	***33.4***	***Avg =***	***5.34***	***33.4***
	s =	***0.03***	***0.2***	***s =***	***0.06***	***0.4***	***s =***	***0.03***	***0.2***	***s =***	***0.13***	***0.8***

[†]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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