Application Note

Instrument: FP928

Determination of Nitrogen/Protein in Flour

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Introduction

Flour is a fine particle powder created by milling or grinding a dry grain. The most common varieties of flour are made from wheat, although any grain can be used to make flour. Flour is typically used to make dough for a variety of bread products. The protein content in the flour is one of the primary constituents that determines the best use for the flour, with the lower-protein flour (~8%) typically being used for cakes and pastries; mid-range protein (~10%) being categorized as all-purpose; and higher-protein flours (~12%) being referred to as bread flour.

The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value in flours, but is also the key to determining the category or quality of the flour. Protein in flour and other food products is most commonly calculated using the measured nitrogen in the sample and a protein factor multiplier (protein factors vary according to the sample matrix).

The LECO FP928 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 cm^3 or 10 cm^3 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 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 a uniform consistency to produce suitable results.

Dry sample at 85 °C for two hours prior to analysis. The dried sample should be stored in a desiccator and must be used for analysis within 24 hours.

Accessories

528-203 Crucibles

Reference Materials

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

Analysis Parameters*

Gas Type	Argon or Helium
Combustion Temperature	1100 °C
Dehydration Time	0 s
Nominal Mass	1.0000 g
Purge Cycles	3

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

Instrument Model and Configuration

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analyte 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 sensitivity of the detector. The FP928 supports the use of either 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. Argon can also be used as a carrier gas, however, because 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 when using argon as a carrier gas.

The FP928 offers the additional advantage of choosing 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 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 (see Typical Results section).

Element Parameters

	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	28 s	20 s
Use Comparator	No	No
Integration Time	35 s	61 s
Use Endline	Yes	Yes
Endline Delay	30 s	30 s
Endline Baseline	5 s	5 s
Use Profile Blank	No	No

Burn Profile

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

Ballast Parameters

Equilibrate Time	10 s
Not Filled Timeout	300 s
Aliquot Loop Fill Pressure Drop	200 mm Hg
Aliquot Loop Equilibrate Time	4 s
Dose Loop Size	Large (10 cm^3) or Small (3 cm^3)

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Condition the system by analyzing three to five blanks (crucible is not required).
3. Determine Blank.
   a. Select five replicates and login Blanks.
   b. Place 528-203 Crucibles in the appropriate positions of the autoloader.
   c. Initiate the analysis sequence.
   d. Set the blank following the procedure outlined in the operator's instruction manual.

   Note: Blank Precision for nitrogen should be <0.001% utilizing the Helium carrier gas and <0.003% utilizing the Argon carrier gas.
4. Calibrate/Drift Correct.
   a. Weigh ~ 0.50 g of suitable reference material into a 528-203 Crucible.
   b. Enter sample mass and identification into Standard Login.
   c. Transfer sample to appropriate position in the autoloader.
   d. Repeat steps 4a through 4c a minimum of five times for each calibration/drift sample used.
   e. Initiate the analysis sequence.
   f. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.

   Note: Multi-point (fractional weight or multiple calibration samples) may be used to calibrate if desired. Typically, the LECO FP928 can be calibrated utilizing several replicates of a single mass range (nominal 0.50 g) of EDTA utilizing a 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 (0.10 to 0.25 g), phenylalanine (0.10 to 0.25 g), and/or (~ 1.00 g) of a known flour.
5. Analyze Samples.
   a. Weigh ~1.00 g of the unknown sample into a 528-203 Crucible.
   b. Enter mass and identification information into Sample Login.
   c. Transfer sample to the appropriate position of the autoloader.
   d. Repeat steps 5a through 5c as necessary.
   e. Initiate the analysis sequence.

   Note: If soot (carbon black) is observed in the primary filter (steel wool filter), the sample mass should be reduced to prevent further sooting. Some sample types will produce soot when analyzed at larger sample masses, and reducing the sample mass will prevent soot from building up in the primary filter.

*TYPICAL RESULTS***

	10 cm³ Helium			3 cm³ Helium			10 cm³ Argon			3 cm³ Argon		
	Mass (g)	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*
Corn Flour LRM	1.0011	1.067	6.67	1.0003	1.069	6.68	0.9968	1.076	6.73	1.0067	1.067	6.67
LECO 501-563-150[†]	1.0002	1.067	6.67	1.0005	1.072	6.70	1.0012	1.059	6.62	1.0065	1.052	6.58
Lot 1016	1.0001	1.065	6.66	1.0005	1.073	6.70	1.0038	1.070	6.69	1.0041	1.054	6.59
% N = 1.06 ±0.03	0.9999	1.065	6.66	1.0002	1.061	6.63	1.0062	1.069	6.68	0.9963	1.059	6.62
[†]**Protein Factor = 6.25**	1.0012	1.065	6.65	1.0003	1.063	6.64	1.0105	1.063	6.65	1.0038	1.061	6.63
	Avg =	***1.066***	***6.66***	***Avg =***	***1.068***	***6.67***	***Avg =***	***1.068***	***6.67***	***Avg =***	***1.059***	***6.62***
	s =	***0.001***	***0.01***	***s =***	***0.005***	***0.03***	***s =***	***0.007***	***0.04***	***s =***	***0.006***	***0.04***
Wheat Flour LCRM	1.0002	2.682	15.3	1.0004	2.681	15.3	0.9975	2.631	15.0	0.9976	2.636	15.0
LECO 502-692[††]	1.0007	2.675	15.2	1.0002	2.685	15.3	0.9979	2.682	15.3	0.9907	2.617	14.9
Lot 1000	1.0015	2.684	15.3	1.0009	2.680	15.3	0.9915	2.629	15.0	0.9703	2.614	14.9
% N = 2.66 ±0.05	1.0004	2.677	15.3	1.0008	2.673	15.2	1.0019	2.635	15.0	0.9808	2.620	14.9
[†]**Protein Factor = 5.70**	1.0002	2.665	15.2	1.0014	2.690	15.3	1.0047	2.643	15.1	1.0071	2.651	15.1
	Avg =	***2.676***	***15.3***	***Avg =***	***2.682***	***15.3***	***Avg =***	***2.644***	***15.1***	***Avg =***	***2.627***	***15.0***
	s =	***0.007***	***0.04***	***s =***	***0.006***	***0.04***	***s =***	***0.022***	***0.12***	***s =***	***0.015***	***0.09***
Rice Flour LCRM	1.0002	1.569	9.34	1.0007	1.578	9.39	1.0043	1.573	9.36	1.0088	1.577	9.38
LECO 502-907[‡]	1.0009	1.572	9.35	1.0010	1.577	9.38	0.9997	1.569	9.33	1.0036	1.579	9.40
Lot 1000	1.0014	1.573	9.36	1.0015	1.573	9.36	0.9979	1.568	9.33	0.9971	1.569	9.34
% N = 1.56 ±0.05	1.0002	1.570	9.34	1.0013	1.576	9.38	1.0094	1.572	9.35	1.0084	1.575	9.37
[†]**Protein Factor = 5.95**	1.0006	1.566	9.32	1.0007	1.573	9.36	0.9971	1.585	9.43	1.0003	1.566	9.32
	Avg =	***1.570***	***9.34***	***Avg =***	***1.575***	***9.37***	***Avg =***	***1.573***	***9.36***	***Avg =***	***1.573***	***9.36***
	s =	***0.003***	***0.02***	***s =***	***0.002***	***0.01***	***s =***	***0.007***	***0.04***	***s =***	***0.006***	***0.03***
Barley Flour LCRM	1.0007	2.011	11.7	1.0007	2.007	11.7	1.0051	2.009	11.7	1.0056	2.001	11.7
LECO 502-906[‡‡]	1.0009	2.009	11.7	1.0005	2.005	11.7	1.0194	2.005	11.7	1.0091	2.000	11.7
Lot 1000	1.0015	2.005	11.7	1.0002	2.002	11.7	1.0093	2.006	11.7	0.9965	1.999	11.7
% N = 2.01 ±0.05	1.0009	2.003	11.7	1.0001	2.006	11.7	1.0037	2.007	11.7	0.9992	1.992	11.6
[†]**Protein Factor = 5.83**	1.0010	2.012	11.7	1.0008	2.016	11.8	1.0035	2.002	11.7	1.0100	2.003	11.7
	Avg =	***2.008***	***11.7***	***Avg =***	***2.007***	***11.7***	***Avg =***	***2.006***	***11.7***	***Avg =***	***1.999***	***11.7***
	s =	***0.004***	***0.02***	***s =***	***0.005***	***0.03***	***s =***	***0.003***	***0.02***	***s =***	***0.004***	***0.02***

**LECO and NIST standard reference materials were used for calibration utilizing a linear force through origin calibration curve.
[†]Protein factors are provided by the United States Department of Agricultures, circular #183.

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