Application Note

EMPOWERING RESULTS

Instrument: CN828

Determination of Carbon and 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. Determination of carbon in flour provides information on the uniformity of the flour as well as the quality of the enrichment process. The protein content in the flour is one of the primary constituents that determines the best use for the flour, with lower-protein flour (~8%) typically being used for cakes and pastries, mid-range protein flours (~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 the nutritional or dietary value of 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 or multiplier (protein factors vary according to the sample matrix).

Instrument Model and Configuration

The LECO CN828 is a combustion carbon and nitrogen 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 non-dispersive infrared (NDIR) cell for the detection of carbon (as CO_2) and 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 CN828 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 CN828 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.

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

Sample Preparation

Samples must be of a uniform consistency to produce suitable results. Reference materials should be prepared as directed by the certificate, prior to analysis.

Note: Carbon and nitrogen results for flour samples are typically reported on a dry basis in order to avoid a reporting bias due to fluctuations in moisture levels. Therefore, either the material can be dried prior to analysis, or the moisture content can be determined and entered into the software to correct for moisture. Flour samples are typically dried at ~80 °C for 2 hours or until constant mass is achieved. The dried samples should be stored in a desiccator and must be used for analysis within 24 hours. For Flour Reference Materials, follow the sample drying instructions provided by the certificate.

Accessories

502-186 Tin Foil Cups, commercially available Reagent Grade Sucrose (finely ground), 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*

	Helium		*Argon*	
Parameter	Carbon	Nitrogen	Carbon	Nitrogen
Integration Delay	4 s	4 s	4 s	4 s
Starting Baseline	15 s	15 s	15 s	15 s
Post Baseline Delay	0 s	14 s	0 s	20 s
Use Comparator	No	No	No	No
Integration Time	18 s	50 s	25 s	65 s
Use Endline	Yes	Yes	Yes	Yes
Endline Delay	15 s	20 s	15 s	20 s
Ending Baseline	15 s	15 s	15 s	15 s
Use Profile Blank	--	--	--	Yes

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

Burn Profile

Burn Step	*Furnace Flow*	*Time*
1	5.00 L/min	40 s
2	1.00 L/min	30 s
3	5.00 L/min	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 both carbon and nitrogen when utilizing Helium as a carrier gas, and less than or equal to 0.005% (50 ppm) for both carbon and 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 (~0.1 g to ~0.3 g) of a suitable reference material into a 502-186 Tin Foil Cup and seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together.
   c. Enter reference material mass and identification into the Login screen.
   d. Transfer the tin foil cup 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 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 CN828 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 ~ 0.25 g of the flour sample into a 502-186 Tin Foil Cup and seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the tin foil cup 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.
6. Atmospheric Blank Determination.

   Note: Some atmosphere may be trapped with the sample when it is encapsulated in the tin foil cup. This may cause biased nitrogen results at low nitrogen concentrations. Therefore, an atmospheric blank should be determined and entered using the following procedure:

   a. Select the desired number of sample replicates in the Login screen (minimum of 3).
   b. Weigh a similar mass (to the mass of the samples being analyzed) of reagent grade sucrose (finely ground) into a 502-186 Tin Foil Cup and seal the cup in a manner to minimize entrapped atmosphere by twisting the top edges of the foil together.
   c. Enter the mass of the sucrose into the Login screen.
   d. Transfer the tin foil cup containing the sucrose to the appropriate position in the sample carousel.
   e. Perform steps 6b through 6d for each sucrose sample to be analyzed.
   f. Initiate the analysis sequence.
   g. The average nitrogen value obtained is considered the atmospheric blank and can be automatically compensated for using the CN828 software**.

   ***Refer to the 828 Series Operator's Instruction Manual for details regarding the setting of the atmospheric blank.*

TYPICAL RESULTS

Data was generated utilizing a linear, full regression calibration for carbon determination, and a linear, force through origin calibration for nitrogen determination, using fractional masses (~0.1 g to ~0.3 g) of 502-896 (Lot 1001) EDTA LCRM (41.00% C, 9.56% N). Flour samples were dried following the drying instructions on the certificate and stored in a desiccator until use.

	Helium 10 cm^3				**Helium 3 cm^3**				**Argon 10 cm^3**				**Argon 3 cm^{3††}**			
	Mass (g)	% C	% N	% Protein	Mass (g)	% C	% N	% Protein	Mass (g)	% C	% N	% Protein	Mass (g)	% C	% N	% Protein
Wheat Flour LCRM	0.2510	45.21	2.91	16.94	0.2543	45.36	2.90	16.93	0.2539	45.24	2.90	16.91	0.2515	45.20	2.88	16.78
LECO 502-692	0.2551	45.20	2.91	16.98	0.2541	45.40	2.91	16.99	0.2532	45.29	2.87	16.71	0.2558	45.27	2.88	16.78
Lot: 1002	0.2519	45.22	2.90	16.93	0.2512	45.32	2.91	16.98	0.2536	45.34	2.88	16.82	0.2554	45.22	2.93	17.06
% C = 45.24 ±0.27	0.2538	45.23	2.91	16.95	0.2538	45.35	2.91	16.99	0.2532	45.25	2.90	16.88	0.2513	45.22	2.87	16.72
% N = 2.88 ±0.03	0.2538	45.23	2.90	16.94	0.2523	45.33	2.89	16.88	0.2545	45.16	2.88	16.78	0.2533	45.25	2.93	17.06
Protein Factor: 5.83[†]	***Avg =***	***45.22***	***2.91***	***16.95***	***Avg =***	***45.35***	***2.91***	***16.95***	***Avg=***	***45.26***	***2.88***	***16.82***	***Avg=***	***45.23***	***2.90***	***16.88***
	s =	***0.01***	***< 0.01***	***0.02***	***s =***	***0.03***	***0.01***	***0.05***	***s=***	***0.07***	***0.01***	***0.08***	***s=***	***0.03***	***0.03***	***0.17***
Corn Flour LCRM	0.2526	44.60	1.16	7.23	0.2544	44.80	1.16	7.24	0.2555	44.73	1.12	6.97	0.2528	44.73	1.17	7.33
LECO 502-713	0.2519	44.64	1.16	7.25	0.2552	44.80	1.16	7.26	0.2523	44.65	1.14	7.10	0.2529	44.67	1.13	7.06
Lot: 1000	0.2521	44.55	1.15	7.18	0.2518	44.73	1.16	7.23	0.2528	44.71	1.14	7.09	0.2526	44.68	1.11	6.94
% N = 1.14 ±0.02	0.2538	44.60	1.16	7.26	0.2541	44.75	1.16	7.25	0.2536	44.60	1.14	7.15	0.2543	44.64	1.13	7.09
Protein Factor: 6.25[†]	0.2512	44.49	1.15	7.21	0.2545	44.70	1.16	7.27	0.2528	44.65	1.15	7.22	0.2532	44.65	1.14	7.14
	Avg =	***44.57***	***1.16***	***7.22***	***Avg =***	***44.76***	***1.16***	***7.25***	***Avg=***	***44.67***	***1.14***	***7.11***	***Avg=***	***44.67***	***1.14***	***7.11***
	s =	***0.06***	***0.01***	***0.03***	***s =***	***0.04***	***< 0.01***	***0.02***	***s=***	***0.05***	***0.01***	***0.09***	***s=***	***0.03***	***0.02***	***0.14***
Rice Flour LCRM	0.2509	44.27	1.37	8.14	0.2517	44.32	1.37	8.13	0.2542	44.36	1.36	8.10	0.2539	44.35	1.30	7.76
LECO 502-907	0.2531	44.26	1.38	8.18	0.2543	44.38	1.38	8.21	0.2542	44.32	1.34	7.95	0.2531	44.30	1.36	8.11
Lot: 1001	0.2534	44.31	1.38	8.19	0.2526	44.36	1.36	8.11	0.2533	44.31	1.35	8.01	0.2544	44.32	1.36	8.11
% C = 44.53 ±0.33	0.2519	44.29	1.36	8.11	0.2532	44.34	1.37	8.15	0.2527	44.29	1.36	8.11	0.2517	44.35	1.42	8.44
% N = 1.35 ±0.04	0.2525	44.30	1.36	8.12	0.2533	44.37	1.38	8.20	0.2527	44.30	1.36	8.08	0.2542	44.29	1.36	8.11
Protein Factor: 5.95[†]	***Avg =***	***44.29***	***1.37***	***8.15***	***Avg =***	***44.36***	***1.37***	***8.16***	***Avg=***	***44.31***	***1.35***	***8.05***	***Avg=***	***44.32***	***1.36***	***8.11***
	s =	***0.02***	***0.01***	***0.04***	***s =***	***0.02***	***0.01***	***0.04***	***s=***	***0.03***	***0.01***	***0.07***	***s=***	***0.03***	***0.04***	***0.24***

[†] *Protein factors were obtained from the United States Department of Agriculture, Circular No. 183.*

[††] *Utilizing the 3 cm^3 dose loop and argon as a carrier gas, when analyzing materials with low nitrogen concentrations, may adversely affect precision. LECO recommends utilizing the 10 cm^3 dose loop when analyzing materials with lower nitrogen concentrations if using argon as a carrier gas.*

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