Determination of Nitrogen/Protein in Flour

LECO Corporation; Saint Joseph, Michigan USA

Instrument: FP628

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 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 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 FP628 is a combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a vertical quartz furnace for the sample combustion process resulting in an analysis time of 3.5 minutes with no metal oxidizer reagents in the primary or secondary furnace. 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 in the combustion gas to nitrogen. A thermal conductivity (TC) cell is used for the detection of nitrogen in the combustion gas.

Accessories

502-186 Tin Foil Cup, 502-397 Large Tin Foil Cup, 502-338 Small Gel Caps, 502-382 Medium Gel Caps, 502-810 Large Gel Caps

Calibration Samples

502-092 EDTA, 502-642 Phenylalanine or other suitable pure compound, verified with 501-563 Corn Flour, 502-274 Wheat Flour, 502-275 Rye Flour, 502-278 Rice Flour

Analysis Parameters

Combustion Temperature:	950°C
Afterburner Temperature:	850°C

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 FP628 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.

Method Selection

Both the helium and argon (10 cc aliquot only) models and aliquot loop size system configurations have the option of a High Precision method or High Throughput method. The High Precision method is optimized to deliver the best performance in terms of nitrogen results resulting in an analysis time of 4 minutes. The High Throughput method is optimized to deliver the fastest analysis time of 3.5 minutes (210 seconds) while maintaining instrument performance specifications and acceptable practical application performance (see Typical Results section).

Delivering the Right Results

Element Parameters - Helium Model

	High Precision (10 cc & 3 cc)	High Throughput (10 cc & 3 cc)
Analyze	Yes	Yes
Baseline Delay Time	4 seconds	4 seconds
Min. Analysis Time	40 seconds	40 seconds
Comparator Level	1.00	1.00
Endline Time	2 seconds	2 seconds
Conversion Factor	1	1
Significant Digits	5	5
TC Baseline Time	10 seconds	6 seconds

Element Parameters - Argon Model

	High Precision (10 cc)	High Throughput (10 cc)
Analyze	Yes	Yes
Baseline Delay Time	4 seconds	4 seconds
Min. Analysis Time	60 seconds	60 seconds
Comparator Level	1.00	1.00
Endline Time	2 seconds	2 seconds
Conversion Factor	1	1
Significant Digits	5	5
TC Baseline Time	10 seconds	6 seconds

Burn Profile

Burn Steps	*Time (seconds)*	*Furnace Flow*
1	90 seconds	High

Ballast Parameters

	High Precision	High Throughput
Equilibrate Time	30 seconds	10 seconds
Not Filled Timeout	300 seconds	300 seconds
Aliquot Loop Fill Pressure Drop	200 mm Hg	200 mm Hg
Equilibrate Pressure Time	8 seconds	4 seconds

**Refer to FP628 Operator's Instruction Manual for Method Parameter definitions.*

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Determine blank.
   a. Enter 1.0000 g mass into Sample Login (F3) using Blank as the sample name.
   b. Select 10 replicates.
   c. Initiate the analysis sequence (F5).
   d. Set the blank using at least five results following the procedure outlined in the operator's instruction manual.

   Note: Blank precision for nitrogen should be <0.001%.
3. Calibrate/Drift Correct.
   a. Weigh ~0.25 g of pure compound calibration sample (EDTA, Phenylalanine, etc) into a Tin Foil Cup/Gel Cap. Seal the Tin Foil Cup if used.
   b. Enter sample mass and identification into Sample Login (F3).
   c. Transfer sample to the appropriate position in the sample carousel.
   d. Repeat steps 3a through 3c a minimum of five times for each calibration/drift sample used.
   e. Initiate the analysis sequence (F5).
   f. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   g. Verify calibration by analyzing ~0.1 to 0.25 g of corn flour, barley, or wheat flour.
4. Analyze Samples.
   a. Weigh ~ 0.1 to 0.25 g of the unknown sample into a Tin Foil Cup/Gel Cap. Seal the Tin Foil Cup if used.
   b. Enter mass and identification information into Sample Login (F3).
   c. Transfer sample to the appropriate position of the sample carousel.
   d. Repeat steps 4a through 4c for all unknown samples.
   e. Initiate the analysis sequence (F5).
5. Atmospheric Blank.
   Some atmosphere will be trapped with the sample when it is encapsulated in the tin foil cup. Some atmosphere may also be present when using the Gel caps as well. This will cause biased nitrogen results at low nitrogen concentrations, therefore an atmospheric blank should be determined and entered using the following procedure: Analyze an inert material such as LECO 501-427 Com-Aid several times using similar weights of the Com-Aid to the weight of samples being analyzed. Enter the actual weight of the Com-Aid (Com-Aid should be baked-off in a muffle furnace at ~1000°C for 15 minutes, allowed to cool, and stored for up to 24 hours in a desiccator until used). The nitrogen value obtained is considered the atmospheric blank and can be automatically compensated using the FP628 software. Refer to the operator's instruction manual for details regarding the setting of the atmospheric blank.

Notes

- *Sample Drying Instructions*
  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. Any unused portion of the dried sample should be discarded.
- The FP628 can be calibrated using several replicates of a single mass (nominal 0.25 g) of EDTA utilizing a single standard calibration. The calibration can be verified by analyzing a pure compound that is different than the material used for calibration, such as phenylalanine (~0.1 g), nicotinic acid (~0.1 g), or corn flour, barley or wheat flour (~0.1 to 0.25 g). Multi-point (fractional weight or multiple calibration samples) may also be used to calibrate if desired.

TYPICAL RESULTS - High Precision Method

	3 cc Helium			10 cc Helium			10 cc Argon		
	Mass(g)	% N	% Protein	Mass(g)	% N	% Protein	Mass(g)	% N	% Protein
501-563*	0.2499	1.230	7.689	0.2504	1.231	7.696	0.2463	1.190	7.438
Lot: 1014	0.2569	1.231	7.694	0.2448	1.225	7.655	0.2478	1.212	7.573
1.21±0.02	0.2547	1.217	7.608	0.2594	1.217	7.606	0.2561	1.208	7.553
Corn Flour	0.2440	1.235	7.719	0.2537	1.230	7.686	0.2480	1.229	7.683
	0.2546	1.218	7.611	0.2433	1.229	7.683	0.2487	1.210	7.563
	Avg =	***1.226***	***7.664***	***Avg =***	***1.226***	***7.665***	***Avg =***	***1.210***	***7.562***
	s =	***0.008***	***0.051***	***s =***	***0.006***	***0.036***	***s =***	***0.014***	***0.087***
502-274**	0.2451	2.661	15.17	0.2542	2.665	15.19	0.2580	2.673	15.23
Lot: 1015	0.2514	2.669	15.22	0.2432	2.693	15.35	0.2457	2.676	15.25
2.68±0.03	0.2568	2.677	15.26	0.2441	2.670	15.22	0.2465	2.655	15.14
Wheat Flour	0.2502	2.659	15.16	0.2475	2.659	15.16	0.2432	2.659	15.16
	0.2508	2.668	15.21	0.2473	2.669	15.21	0.2573	2.687	15.31
	Avg =	***2.667***	***15.20***	***Avg =***	***2.671***	***15.23***	***Avg =***	***2.670***	***15.22***
	s =	***0.007***	***0.041***	***s =***	***0.013***	***0.073***	***s =***	***0.013***	***0.073***
502-275***	0.2465	1.726	10.06	0.2473	1.749	10.19	0.2470	1.761	10.27
Lot: 1007	0.2553	1.723	10.04	0.2463	1.747	10.19	0.2539	1.725	10.06
1.74±0.06	0.2560	1.715	10.00	0.2543	1.744	10.17	0.2460	1.688	9.84
Rye Flour	0.2545	1.720	10.03	0.2554	1.738	10.13	0.2587	1.711	9.97
	0.2475	1.715	10.00	0.2481	1.759	10.25	0.2469	1.698	9.90
	Avg =	***1.720***	***10.03***	***Avg =***	***1.747***	***10.19***	***Avg =***	***1.717***	***10.01***
	s =	***0.005***	***0.029***	***s =***	***0.008***	***0.044***	***s =***	***0.028***	***0.167***
502-278*	0.2538	1.192	7.448	0.2580	1.202	7.511	0.2434	1.147	7.17
Lot: 1013	0.2574	1.130	7.065	0.2478	1.199	7.493	0.2443	1.145	7.15
1.17±0.05	0.2440	1.183	7.394	0.2435	1.201	7.504	0.2435	1.145	7.16
Rice Four	0.2506	1.177	7.354	0.2462	1.197	7.482	0.2491	1.161	7.26
	0.2491	1.213	7.579	0.2515	1.200	7.499	0.2523	1.172	7.33
	Avg =	***1.179***	***7.368***	***Avg =***	***1.200***	***7.498***	***Avg =***	***1.154***	***7.21***
	s =	***0.030***	***0.190***	***s =***	***0.002***	***0.011***	***s =***	***0.012***	***0.08***

TYPICAL RESULTS - High Throughput Method

	3 cc Helium			10 cc Helium			10 cc Argon		
	Mass(g)	% N	% Protein	Mass(g)	% N	% Protein	Mass(g)	% N	% Protein
501-563*	0.2534	1.239	7.746	0.2505	1.233	7.708	0.2430	1.214	7.588
Lot: 1014	0.2538	1.242	7.760	0.2525	1.214	7.587	0.2542	1.206	7.538
1.21±0.02	0.2538	1.240	7.747	0.2530	1.215	7.596	0.2433	1.208	7.550
Corn Flour	0.2575	1.197	7.483	0.2474	1.210	7.561	0.2483	1.176	7.350
	0.2568	1.222	7.640	0.2480	1.216	7.597	0.2559	1.227	7.666
	Avg =	***1.228***	***7.675***	***Avg =***	***1.218***	***7.610***	***Avg =***	***1.206***	***7.538***
	s =	***0.019***	***0.118***	***s =***	***0.009***	***0.057***	***s =***	***0.019***	***0.117***
502-274**	0.2434	2.661	15.17	0.2458	2.666	15.20	0.2546	2.659	15.16
Lot: 1015	0.2520	2.674	15.24	0.2558	2.671	15.23	0.2410	2.679	15.27
2.68±0.03	0.2513	2.664	15.19	0.2467	2.676	15.25	0.2403	2.695	15.36
Wheat Flour	0.2545	2.671	15.22	0.2593	2.683	15.29	0.2437	2.710	15.45
	0.2476	2.656	15.14	0.2533	2.667	15.20	0.2523	2.655	15.13
	Avg =	***2.665***	***15.19***	***Avg =***	***2.673***	***15.23***	***Avg =***	***2.680***	***15.27***
	s =	***0.007***	***0.042***	***s =***	***0.007***	***0.0396***	***s =***	***0.023***	***0.134***
502-275***	0.2503	1.733	10.10	0.2552	1.744	10.17	0.2560	1.716	10.00
Lot: 1007	0.2427	1.738	10.13	0.2433	1.746	10.18	0.2519	1.684	9.81
1.74±0.06	0.2433	1.741	10.15	0.2422	1.749	10.20	0.2483	1.715	10.00
Rye Flour	0.2457	1.742	10.16	0.2436	1.748	10.19	0.2461	1.684	9.82
	0.2562	1.722	10.04	0.2527	1.756	10.24	0.2548	1.694	9.87
	Avg =	***1.735***	***10.12***	***Avg =***	***1.749***	***10.19***	***Avg =***	***1.699***	***9.90***
	s =	***0.008***	***0.048***	***s =***	***0.005***	***0.026***	***s =***	***0.016***	***0.092***
502-278*	0.2407	1.196	7.476	0.2541	1.203	7.517	0.2531	1.168	7.30
Lot: 1013	0.2471	1.199	7.496	0.2544	1.193	7.456	0.2530	1.163	7.27
1.17±0.05	0.2575	1.185	7.408	0.2539	1.195	7.471	0.2518	1.126	7.04
Rice Four	0.2494	1.199	7.494	0.2505	1.199	7.491	0.2574	1.158	7.24
	0.2430	1.204	7.526	0.2426	1.195	7.469	0.2543	1.180	7.38
	Avg =	***1.197***	***7.480***	***Avg =***	***1.197***	***7.481***	***Avg =***	***1.159***	***7.24***
	s =	***0.007***	***0.044***	***s =***	***0.004***	***0.024***	***s =***	***0.020***	***0.13***

Protein Factor = 6.25 Protein Factor = 5.7***Protein Factor = 5.83*

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