Application Note

Instrument: FP828 Series

Determination of Nitrogen/Protein in Milk and Milk Products

LECO Corporation; Saint Joseph, Michigan USA

Introduction

Protein is one of the most significant nutrient components in milk products. The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value of milk products, but is also the key to determining the economic value of these materials. Protein in milk products is most commonly calculated using the measured total nitrogen content in the sample and a multiplier or conversion factor (typically 6.38). Nitrogen determination is performed using either the classical wet chemical method (Kjeldahl), or a combustion method (Dumas).

Instrument Model and Configuration

The LECO FP828 is a combustion nitrogen/protein 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 gases equilibrate and mix in the ballast before a representative aliquot (3 cm^3 or 10 cm^3 volume) of the combustion gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot gas is carried to 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 FP828 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 FP828 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. Base model instruments require manual changing of the dose loop.

Method Reference

ISO 14891: Milk and milk products - Determination of nitrogen content - Routine method using combustion according to the Dumas principle.

Sample Preparation

Milk samples should be analyzed as received. Reference materials should be prepared as directed by the certificate, prior to analysis.

Accessories

502-186 Small Tin Foil Cups, 502-040 Small Tin Capsules, 502-167 Medium Tin Capsules, 501-614 Spatula, Disposable Pipettes

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**	10 cm^3 or 3 cm^3

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

***Due to the quality of the results obtained using the 3 cm^3 dose loop and helium as a carrier gas, analysis using the 10 cm^3 dose loop and helium as a carrier gas was not performed, as it was not expected to significantly improve precision.*

Element Parameters†

	Helium	*Argon*
Integration Delay	3 s	3 s
Starting Baseline	15 s	15 s
Post Baseline Delay	14 s	14 s
Use Comparator	No	No
Integration Time	45 s	60 s
Use Endline	Yes	Yes
Endline Delay	25 s	25 s
Ending Baseline	15 s	15 s
Use Profile Blank	--	Yes

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

Burn Profile

Base Model

Burn Step	*Furnace Flow*	*Time*
1	High	End

Performance Model

Burn Step	*Furnace Flow*	*Time*
1	5.0 L/min	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.
   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% N (10 ppm) when utilizing Helium as a carrier gas, and less than or equal to 0.005% N (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 502-186 Small 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 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 and confirm that the results are within the acceptable tolerance range.

   Note: Typically, the LECO FP828 can be calibrated using several replicates of a single mass range (0.25 g) of EDTA or other suitable reference material utilizing a linear, force through origin calibration. This is a cost-effective and simple process. The calibration can be verified by analyzing a different compound such as Phenylalanine (~ 0.10 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 0.25 to 0.5 g of the milk sample into a 502-040 Small Tin Capsule or a 502-167 Medium Tin Capsule, respectively. The tin capsule should be left open and not sealed in order to avoid biased nitrogen results due to trapped atmosphere.
   c. Enter sample mass and identification information into the Login screen.
   d. Transfer the tin capsules 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.

TYPICAL RESULTS

Data was generated utilizing a linear, force through origin calibration using ~0.25 g of 502-896 LCRM EDTA Lot# 1002 (9.57% N). The calibration was verified using ~0.10 g of 502-642 LCRM Phenylalanine Lot# 1018 (8.47% N) and a prepared 1.0% N Glycine Solution[††]. A protein factor of 6.38 was used for all samples to calculate the protein content.

	3 cm³ Helium			10 cm³ Argon			3 cm³ Argon		
	Mass (g)	*% N*	*% Protein*	*Mass (g)[‡]*	*% N*	*% Protein*	*Mass (g)[‡]*	*% N*	*% Protein*
Whole Milk	0.2785	0.529	3.38	0.5091	0.513	3.28	0.5176	0.519	3.31
	0.2431	0.547	3.49	0.4949	0.520	3.32	0.5207	0.534	3.41
	0.2728	0.537	3.43	0.5104	0.529	3.38	0.5419	0.562	3.59
	0.2610	0.544	3.47	0.5113	0.526	3.36	0.5050	0.525	3.35
	0.2478	0.539	3.44	0.5325	0.510	3.25	0.4902	0.504	3.22
	Avg =	***0.539***	***3.44***	***Avg =***	***0.520***	***3.32***	***Avg =***	***0.529***	***3.37***
	s =	***0.007***	***0.04***	***s =***	***0.008***	***0.05***	***s =***	***0.022***	***0.14***
Skim Milk	0.2639	0.571	3.64	0.5213	0.551	3.52	0.4954	0.531	3.39
	0.2650	0.557	3.55	0.5063	0.546	3.48	0.5355	0.555	3.54
	0.2631	0.565	3.61	0.4988	0.547	3.49	0.5353	0.552	3.52
	0.2784	0.556	3.55	0.5337	0.544	3.47	0.5182	0.541	3.45
	0.2548	0.557	3.55	0.5293	0.549	3.50	0.5524	0.528	3.37
	Avg =	***0.561***	***3.58***	***Avg =***	***0.547***	***3.49***	***Avg =***	***0.541***	***3.45***
	s =	***0.006***	***0.04***	***s =***	***0.003***	***0.02***	***s =***	***0.012***	***0.08***
Half and Half	0.2468	0.486	3.10	0.4953	0.462	2.95	0.5004	0.430	2.75
	0.2402	0.491	3.13	0.5143	0.470	3.00	0.5215	0.484	3.09
	0.2751	0.479	3.06	0.5207	0.462	2.95	0.4971	0.511	3.26
	0.2718	0.481	3.07	0.5152	0.468	2.99	0.4971	0.462	2.95
	0.2558	0.480	3.06	0.4954	0.472	3.01	0.4975	0.468	2.98
	Avg =	***0.484***	***3.08***	***Avg =***	***0.467***	***2.98***	***Avg =***	***0.471***	***3.01***
	s =	***0.005***	***0.03***	***s =***	***0.005***	***0.03***	***s =***	***0.030***	***0.19***

[††]Glycine solutions should be prepared using the procedure found on the last page of this document.

[‡]Due to the decreased sensitivity of the TC cell when using argon as a carrier gas, a sample mass of ~0.5 g is required for optimal accuracy and precision.

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GLYCINE SOLUTION PREPARATION

1. The following formula can be used to make a specific concentration:

$$G = \frac{C}{(0.99^{\dagger} * 0.18658)}$$

where: C = desired nitrogen concentration as percent
G = grams of glycine powder

Example for 1% solution:

$$G = \frac{1}{(0.99^{\dagger} * 0.18658)} = 5.414$$

NOTE: A quick reference chart, shown below, shows the grams of glycine powder needed to reach given concentrations.

2. Place a flask on the balance and tare. The flask should be large enough to hold 100 ml (where 100 g = 100 ml).
3. Add the amount of glycine calculated in step 1 and record the mass.
4. Add distilled water until the total mass equals 100 g, then record the mass (W).
5. Seal the flask and mix the contents.
6. To figure the exact concentration:

$$\% \text{ Nitrogen} = \frac{G\,(18.658 * 0.99^{\dagger})}{W}$$

where: G = mass in grams of glycine recorded in step 3
W = mass in grams of water and glycine powder recorded in step 4

7. If the distilled water is not pure, determining the nitrogen concentration may be necessary.
   a. Analyze five samples of distilled water.
   b. Average the nitrogen content of the five samples (A).
   c. Add this average to % nitrogen calculated for the calibration solution.

Example: To make a calibration solution of approximately 0.3% nitrogen:
where: G = 1.672 g
W = 99.824 g
A = 0.004%

$$\frac{1.672(18.471)}{(99.824)} + 0.004 = 0.313\%\ \text{N}$$

QUICK REFERENCE CONCENTRATION TABLE

Nitrogen Concentration	Grams of Glycine[†]
0.10%	0.541
0.30%	1.624
0.50%	2.707
0.75%	4.060
1.00%	5.414

[†]*Assuming 99.0% purity of glycine powder.*