Application Note

Instrument: FP828

Determination of Nitrogen/Protein in Whey and Powdered Dairy Products

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Introduction

Two of the main types of proteins within milk are casein and whey. Whey protein is a co-product of cheese production and represents the water-soluble protein remaining in the solution after the milk has been acidified or a coagulation agent has been added. The removal of the non-soluble casein protein portion is referred to as curds. Whey protein is a common ingredient in many dietary supplements due to its availability, solubility, and unique functional characteristics.

The accurate and precise determination of protein in whey and powdered dairy products not only plays a role in the characterization of nutritional or dietary value in dairy products but may also be key in determining the quality or category of the dairy product. Protein in whey and other dairy 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).

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 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 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 the 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 dose loop or a 3 cm^3 aliquot dose loop within the instrument's gas collection and handling system. The 10 cm^3 aliquot dose loop optimizes the system for the lowest Nitrogen range and provides the best precision. The 3 cm^3 aliquot dose loop extends reagent life expectancy by approximately three-fold when compared to the 10 cm^3 aliquot dose 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. When using the FP828 Performance model, the aliquot dose loop size is changed by selecting the desired aliquot dose loop size in the software's Method Parameters. When using the FP828 Base model, the desired dose loop is installed by the operator.

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.

Accessories

502-186 Small Tin Foil Cups, 501-614 Spatula

Reference Materials

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

Method Reference*

ISO 14891: Milk and Milk Products–Determination of Nitrogen Content (Routine method using combustion according to the Dumas principle)

**A modified version of ISO Method 14891 was utilized for the generation of data included in this application note.*

General Parameters**

Gas Type	Helium or Argon	
Combustion Temperature	950 °C	
Afterburner Temperature	850 °C	
Nominal Mass	1.0000 g	
Purge Cycles	2	
	Helium	*Argon*
Ballast Equilibrate Time	10 s	8 s
Ballast Not Filled Timeout	300 s	300 s
Aliquot Loop Fill Pressure Drop	175 mm Hg	175 mm Hg
Aliquot Loop Equilibrate Time	4 s	4 s
Dose Loop Size	10 cm^3 or 3 cm^3	10 cm^3 or 3 cm^3
Interleave Analysis	Yes	Yes
Sample Drop Detection	Disabled	Disabled

*Element Parameters***

	Helium	*Argon*
Integration Delay	3 s	5 s
Starting Baseline	15 s	15 s
Post Baseline Delay	14 s	13 s
Use Comparator	No	No
Integration Time	35 s	65 s
Use Endline	Yes	Yes
Endline Delay	25 s	30 s
Ending Baseline	15 s	15 s
Use Profile Blank	---	No

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

Burn Profile

Performance Model

Burn Step	*Furnace Flow*	*Time*
1	5.00 LPM	30 s
2	1.00 LPM	30 s
3	5.00 LPM	End

Base Model

Burn Step	*Furnace Flow*	*Time*
1	High	30 s
2	Medium	30 s
3	High	End

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Condition the System
   a. Select three to five replicates in the Login screen.
   b. Weigh ~0.15 g of a reference material or sample material into a 502-186 Tin Foil Cup and enter the conditioning sample mass and identification into the Login screen.
   c. Seal the foil cup by twisting the top edges of the foil together and transfer it to the appropriate position in the sample carousel.
   d. Perform steps 2b through 2c for each conditioning sample to be analyzed.
   e. 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 using at least five blank results 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 ~0.25 g of 502-896 EDTA (or an appropriate mass of another suitable reference material) into a 502-186 Tin Foil Cup and enter the sample mass and identification into the Login screen.
   c. Seal the foil cup by twisting the top edges of the foil together and transfer it to the appropriate position in the sample carousel.
   d. Perform steps 4b through 4c a minimum of five times.
   e. Initiate the analysis sequence.
   f. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   g. Verify the calibration or drift correction by analyzing an appropriate mass of another suitable reference material and confirm that the results are within the acceptable tolerance range.
5. Analyze Samples
   a. Select the desired number of sample replicates in the Login screen.
   b. Weigh ~0.15 g of sample into a 502-186 Tin Foil Cup and enter sample mass and identification into the Login screen.
   c. Seal the foil cup by twisting the top edges of the foil together and transfer it to the appropriate position in the sample carousel.
   d. Perform steps 5b through 5c for each sample to be analyzed.
   e. Initiate the analysis sequence.

TYPICAL RESULTS

Data was generated utilizing a linear, force through origin calibration using ~0.25 g of LECO 502-896 (Lot 1007) LCRM EDTA (9.59% N). The calibration was verified using ~0.1 g of LECO 502-688 (Lot 1004) LCRM Nicotinic Acid (11.37% N). Samples were weighed and analyzed at ~0.15 grams. A protein factor of 6.38† 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*
Whey Powder Supplement	0.1453	12.48	79.6	0.1525	12.45	79.4	0.1546	12.42	79.2	0.1540	12.45	79.4
	0.1497	12.45	79.4	0.1470	12.47	79.5	0.1500	12.47	79.5	0.1579	12.46	79.5
	0.1532	12.47	79.6	0.1497	12.46	79.5	0.1540	12.46	79.5	0.1579	12.43	79.3
	0.1484	12.46	79.5	0.1489	12.45	79.4	0.1484	12.41	79.2	0.1531	12.43	79.3
	0.1424	12.50	79.7	0.1567	12.46	79.5	0.1562	12.43	79.3	0.1482	12.41	79.1
	Avg=	***12.47***	***79.6***	***Avg=***	***12.46***	***79.5***	***Avg=***	***12.44***	***79.3***	***Avg=***	***12.44***	***79.3***
	s=	***0.02***	***0.1***	***s=***	***0.01***	***<0.1***	***s=***	***0.03***	***0.2***	***s=***	***0.02***	***0.1***
Instant Breakfast Powder	0.1501	2.42	15.5	0.1458	2.42	15.4	0.1567	2.38	15.2	0.1567	2.46	15.7
	0.1588	2.42	15.5	0.1477	2.39	15.3	0.1574	2.41	15.4	0.1492	2.44	15.6
	0.1506	2.42	15.4	0.1552	2.40	15.3	0.1540	2.40	15.3	0.1517	2.42	15.5
	0.1467	2.41	15.4	0.1579	2.39	15.2	0.1527	2.41	15.4	0.1541	2.42	15.5
	0.1503	2.42	15.5	0.1536	2.35	15.0	0.1571	2.41	15.4	0.1545	2.44	15.6
	Avg=	***2.42***	***15.4***	***Avg=***	***2.39***	***15.2***	***Avg=***	***2.40***	***15.3***	***Avg=***	***2.44***	***15.5***
	s=	***0.01***	***<0.1***	***s=***	***0.03***	***0.2***	***s=***	***0.01***	***0.1***	***s=***	***0.01***	***0.1***
Powdered Milk	0.1542	5.94	37.9	0.1514	5.94	37.9	0.1515	5.92	37.8	0.1561	5.91	37.7
	0.1445	5.95	37.9	0.1520	5.95	37.9	0.1530	5.93	37.9	0.1520	5.93	37.8
	0.1552	5.95	37.9	0.1500	5.95	38.0	0.1572	5.91	37.7	0.1500	5.93	37.8
	0.1523	5.96	38.0	0.1490	5.95	38.0	0.1493	5.90	37.6	0.1417	5.92	37.7
	0.1446	5.91	37.7	0.1541	5.96	38.0	0.1473	5.90	37.7	0.1456	5.94	37.9
	Avg=	***5.94***	***37.9***	***Avg=***	***5.95***	***38.0***	***Avg=***	***5.91***	***37.7***	***Avg=***	***5.92***	***37.8***
	s=	***0.02***	***0.1***	***s=***	***0.01***	***0.1***	***s=***	***0.01***	***0.1***	***s=***	***0.01***	***0.1***
Powdered Infant Formula	0.1489	1.89	12.1	0.1493	1.84	11.8	0.1614	1.90	12.1	0.1597	1.94	12.4
	0.1466	1.89	12.0	0.1468	1.83	11.7	0.1549	1.92	12.3	0.1531	1.85	11.8
	0.1532	1.90	12.1	0.1519	1.86	11.9	0.1532	1.91	12.2	0.1533	1.90	12.1
	0.1463	1.89	12.1	0.1536	1.85	11.8	0.1552	1.89	12.0	0.1506	1.89	12.1
	0.1558	1.89	12.0	0.1561	1.87	11.9	0.1526	1.90	12.1	0.1539	1.94	12.4
	Avg=	***1.89***	***12.1***	***Avg=***	***1.85***	***11.8***	***Avg=***	***1.90***	***12.1***	***Avg=***	***1.91***	***12.2***
	s=	***<0.01***	***<0.1***	***s=***	***0.02***	***0.1***	***s=***	***0.01***	***0.1***	***s=***	***0.04***	***0.2***

†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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