Determination of Nitrogen/Protein in Whey Products

LECO Corporation; Saint Joseph, Michigan USA

Instrument: FP628

Introduction

The two broad 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 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 whey product. Protein in whey 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.

Sample Preparation

Samples must be a uniform consistency to produce suitable results.

Accessories

502-186 Tin Foil Cups, 502-397 Large Tin Foil Cups, 502-040 Tin Capsules (for liquids).

Calibration Samples

502-896 EDTA, 502-642 Phenylalanine, or other suitable pure compound/Reference Material.

Reference Methods

AOAC 990.03, AOAC 992.23

Protein Factor

Protein Factor: 6.38

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).

Organic Application Note

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	5 seconds	5 seconds
Min. Analysis Time	40 seconds	40 seconds
Comparator Level	100.00	100.00
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	5 seconds	5 seconds
Min. Analysis Time	60 seconds	60 seconds
Comparator Level	100.00	100.00
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

Macro 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 502-896 EDTA reference material into a tin foil cup and seal.
   b. Enter mass and sample indentification into Sample Login (F3).
   c. Transfer sample to the appropriate position of 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 the calibration by analyzing ~0.1 g of 502-642 Phenylalanine or other suitable reference material.

   Note: 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) or nicotinic acid (~0.1 g). Multipoint (fractional weight or multiple reference materials) may also be utilized to calibrate, if desired.
4. Analyze Samples.
   a. Weigh ~0.15 g of sample into a tin foil cup or tin capsule. Seal the tin foil cup if used.

   Note: 502-040 Tin Capsules are recommended for liquid whey products. Capsules are left open for analysis.
   b. Enter mass and identification information into Sample Login (F3).
   c. Transfer sample to the appropriate position of the sample carousel.
   d. Initiate the analysis sequence (F5).

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*
Whey Protein Concentrate	0.1510	12.01	76.64	0.1505	12.03	76.72	0.1527	11.99	76.47
	0.1515	12.02	76.70	0.1516	12.04	76.80	0.1526	12.02	76.68
	0.1545	12.02	76.68	0.1527	12.03	76.75	0.1528	12.00	76.56
	0.1523	12.03	76.75	0.1518	12.03	76.73	0.1516	12.00	76.54
	0.1534	12.00	76.57	0.1513	12.04	76.80	0.1537	11.99	76.51
	Avg=	***12.02***	***76.67***	***Avg=***	***12.03***	***76.76***	***Avg=***	***12.00***	***76.55***
	s=	***0.01***	***0.07***	***s=***	***0.01***	***0.04***	***s=***	***0.01***	***0.08***
Sodium Caseinate	0.1555	14.25	90.88	0.1539	14.31	91.27	0.1532	14.28	91.13
	0.1522	14.28	91.11	0.1508	14.29	91.15	0.1551	14.29	91.20
	0.1508	14.28	91.13	0.1521	14.29	91.17	0.1526	14.30	91.21
	0.1542	14.27	91.05	0.1519	14.29	91.20	0.1506	14.31	91.31
	0.1503	14.26	91.01	0.1502	14.31	91.32	0.1543	14.29	91.14
	Avg=	***14.27***	***91.04***	***Avg=***	***14.30***	***91.22***	***Avg=***	***14.30***	***91.20***
	s=	***0.02***	***0.10***	***s=***	***0.01***	***0.07***	***s=***	***0.01***	***0.07***
Amino Acid Supplement (Liquid)	0.1534	4.82	30.75	0.1520	4.82	30.74	0.1486	4.82	30.77
	0.1585	4.82	30.77	0.1520	4.83	30.81	0.1514	4.82	30.74
	0.1633	4.83	30.82	0.1491	4.82	30.76	0.1488	4.83	30.81
	0.1507	4.82	30.76	0.1572	4.83	30.79	0.1545	4.83	30.80
	0.1533	4.82	30.77	0.1502	4.83	30.83	0.1500	4.83	30.82
	Avg=	***4.82***	***30.77***	***Avg=***	***4.83***	***30.79***	***Avg=***	***4.83***	***30.79***
	s=	***0.004***	***0.03***	***s=***	***0.005***	***0.03***	***s=***	***0.005***	***0.03***
Whey Protein Mix	0.1511	9.42	60.08	0.1504	9.44	60.23	0.1549	9.42	60.08
	0.1502	9.36	59.69	0.1530	9.42	60.12	0.1559	9.49	60.57
	0.1499	9.44	60.22	0.1517	9.45	60.29	0.1537	9.44	60.24
	0.1544	9.45	60.26	0.1521	9.43	60.13	0.1555	9.44	60.20
	0.1577	9.40	59.99	0.1540	9.42	60.12	0.1501	9.39	59.91
	Avg=	***9.41***	***60.05***	***Avg=***	***9.43***	***60.18***	***Avg=***	***9.44***	***60.20***
	s=	***0.04***	***0.23***	***s=***	***0.01***	***0.08***	***s=***	***0.04***	***0.24***

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*
Whey Protein Concentrate	0.1510	12.01	76.65	0.1504	12.00	76.56	0.1554	12.05	76.86
	0.1506	12.03	76.72	0.1534	12.02	76.68	0.1549	11.97	76.35
	0.1502	12.01	76.64	0.1520	12.07	76.97	0.1535	12.00	76.55
	0.1512	12.03	76.77	0.1532	12.03	76.77	0.1503	12.03	76.73
	0.1517	12.04	76.80	0.1530	12.04	76.80	0.1511	12.02	76.68
	Avg=	***12.02***	***76.72***	***Avg=***	***12.03***	***76.76***	***Avg=***	***12.01***	***76.63***
	s=	***0.01***	***0.07***	***s=***	***0.02***	***0.15***	***s=***	***0.03***	***0.19***
Sodium Caseinate	0.1500	14.27	91.04	0.1500	14.32	91.36	0.1499	14.30	91.24
	0.1517	14.29	91.16	0.1527	14.30	91.25	0.1510	14.32	91.38
	0.1512	14.28	91.12	0.1505	14.27	91.02	0.1537	14.30	91.22
	0.1558	14.26	90.96	0.1503	14.29	91.18	0.1578	14.27	91.01
	0.1510	14.28	91.11	0.1543	14.29	91.16	0.1527	14.31	91.28
	Avg=	***14.28***	***91.08***	***Avg=***	***14.29***	***91.19***	***Avg=***	***14.30***	***91.23***
	s=	***0.01***	***0.08***	***s=***	***0.02***	***0.13***	***s=***	***0.02***	***0.13***
Amino Acid Supplement (Liquid)	0.1616	4.82	30.75	0.1583	4.82	30.75	0.1547	4.84	30.88
	0.1624	4.83	30.79	0.1515	4.83	30.80	0.1572	4.83	30.82
	0.1513	4.82	30.76	0.1641	4.82	30.72	0.1591	4.80	30.64
	0.1554	4.83	30.80	0.1526	4.82	30.76	0.1503	4.82	30.74
	0.1562	4.82	30.75	0.1528	4.83	30.80	0.1493	4.85	30.92
	Avg=	***4.82***	***30.77***	***Avg=***	***4.82***	***30.77***	***Avg=***	***4.83***	***30.80***
	s=	***0.004***	***0.02***	***s=***	***0.005***	***0.03***	***s=***	***0.02***	***0.11***
Whey Protein Mix	0.1521	9.45	60.30	0.1547	9.40	59.96	0.1547	9.47	60.43
	0.1538	9.38	59.85	0.1533	9.42	60.10	0.1544	9.43	60.18
	0.1549	9.44	60.21	0.1514	9.48	60.45	0.1513	9.43	60.18
	0.1576	9.42	60.11	0.1527	9.47	60.44	0.1526	9.43	60.15
	0.1542	9.36	59.70	0.1533	9.42	60.13	0.1507	9.44	60.21
	Avg=	***9.41***	***60.03***	***Avg=***	***9.44***	***60.21***	***Avg=***	***9.44***	***60.23***
	s=	***0.04***	***0.25***	***s=***	***0.03***	***0.22***	***s=***	***0.02***	***0.12***

NOTE: Protein factor 6.38

Organic Application Note

LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | 800-292-6141 | Phone: 269-985-5496
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