Application Note

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Instrument: FP928

Determination of Nitrogen/Protein in Cheese

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Introduction

Protein is one of the most significant nutrient components in cheese. The accurate and precise determination of protein not only plays a role in the characterization of the nutritional value of cheese but is also key in determining the economic value of different cheeses. A rapid and precise method for the determination of total protein in cheese provides a vital quality and process control procedure.

Protein in cheese is most commonly calculated using the measured total Nitrogen content in the sample and a multiplier or protein conversion factor (typically 6.38). Protein conversion factors vary depending on the sample matrix. Nitrogen determination in organic materials is performed using either a classical wet chemical method (Kjeldahl), or a combustion method (Dumas). The Kjeldahl method involves sample digestion, distillation, and ammonia determination typically by titration. This method involves time-consuming sample preparation and the use of hazardous materials. The LECO FP928 is a Nitrogen determinator that utilizes an automated combustion (Dumas) method and provides accurate and precise results in approximately five minutes. This eliminates involved sample preparation and the use of hazardous materials resulting in a rapid, cost-effective method for the quality control of cheese production.

Instrument Model and Configuration

The FP928 is a macro combustion Nitrogen/Protein determinator that utilizes a pure Oxygen environment in a high-temperature horizontal ceramic combustion furnace, utilizing ceramic boats designed to handle macro sample masses (~1.0 g). 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 gas is extracted and introduced into a flowing stream of inert carrier gas (Helium or Argon) for analysis. The aliquot of gas is carried through a heated reduction tube, filled with Copper, to convert Nitrogen Oxide combustion gas species (NO_x) to Nitrogen (N_2). The aliquot gas is then 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 FP928 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 FP928 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 with minimal impact on practical application performance for the determination of Nitrogen/Protein in cheese.

Note: When changing carrier gas type, the flow needs to be adjusted following instructions provided in the 928 Series Operator's Instruction Manual. 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.

Accessories

528-203 Ceramic Combustion Boats, 611-844 Spatula Flat Spoon

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
Furnace Temperature	1100 °C
Ramp Rate	12 °C/min
Dehydration Time	0 s
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	4 s
Dose Loop Size	Large (10 cm^3) or Small (3 cm^3)

Element Parameters**

	Helium	Argon
	(3 cm³ and 10 cm³)	
Integration Delay	0 s	9 s
Starting Baseline	10 s	10 s
Post Baseline Delay	20 s	20 s
Use Comparator	No	No
Integration Time	50 s	61 s
Use Endline	Yes	Yes
Endline Delay	30 s	30 s
Ending Baseline	5 s	5 s
Use Profile Blank	--	Yes

***Refer to 928 Series Operator's Instruction Manual for Parameter definitions.*

Burn Profile

Burn Step	Lance Flow	Furnace Flow	Time
1	No	Yes	5 s
2	Yes	Yes	35 s
3	Yes	No	End

Analysis Considerations

The combustion of cheese samples with a high-fat content can result in some degree of sooting. The primary filter tube (glass and steel wool) should be monitored for signs of sooting. If soot is observed in the glass and steel wool within the primary filter tube, reduce the sample mass to prevent this soot build-up, and change the glass and steel wool as necessary.

The combustion of cheese samples can also result in the formation of salts; therefore, it is important to monitor the primary filter tube for signs of excessive corrosion of the steel wool and change it as necessary (approximately every 50 cheese samples). In addition, the autoloader arm should be inspected and cleaned on a regular basis when analyzing cheese products.

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 (ceramic combustion boat is not required).
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more blank replicates in the Login screen.
   b. Place 528-203 Ceramic Combustion Boats in the appropriate positions in the autoloader.
   c. Initiate the analysis sequence.
   d. 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) 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 ~0.75 g of 502-896 EDTA (or an appropriate mass of another suitable reference material) into a 528-203 Ceramic Combustion Boat.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the ceramic combustion boat containing the reference material to the appropriate position in the autoloader.
   e. Perform steps 4b through 4d a minimum of five times for each calibration/drift material used.
   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 by analyzing an appropriate mass of another suitable reference material and confirm that the results are within the acceptable tolerance range.

   Note: Typically, the LECO FP928 can be calibrated utilizing several replicates of a single mass range (nominal 0.75 g) of EDTA utilizing a single standard calibration (linear, force through origin calibration). This is a cost effective and simple process. The calibration can be verified by analyzing different compounds such as nicotinic acid (0.1 to 0.25 g), and/or phenylalanine (0.1 to 0.25 g). A multi-point calibration (fractional masses 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 0.3 g of the cheese sample into a 528-203 Ceramic Combustion Boat.
   c. Enter sample mass and identification information into the Login screen.
   d. Transfer the ceramic combustion boat containing the sample to the appropriate position in the autoloader.
   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 ~0.75 g of LECO 502-896 (Lot 1003) LCRM EDTA (9.58% 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 ~0.25 to 0.3 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*
Parmesan	0.2772	4.69	29.9	0.2804	4.72	30.1	0.2483	4.74	30.2	0.2824	4.70	30.0
Cheese	0.2640	4.73	30.2	0.2686	4.72	30.1	0.2460	4.78	30.5	0.2764	4.73	30.2
	0.2617	4.74	30.2	0.2793	4.75	30.3	0.2552	4.77	30.5	0.2736	4.70	30.0
	0.2795	4.71	30.0	0.2725	4.71	30.1	0.2458	4.76	30.3	0.2853	4.71	30.1
	0.2766	4.69	29.9	0.2837	4.75	30.3	0.2522	4.73	30.2	0.2746	4.72	30.1
	Avg=	***4.71***	***30.1***	***Avg=***	***4.73***	***30.2***	***Avg=***	***4.76***	***30.3***	***Avg=***	***4.71***	***30.1***
	s=	***0.02***	***0.1***	***s=***	***0.02***	***0.1***	***s=***	***0.02***	***0.1***	***s=***	***0.01***	***0.1***
Swiss Cheese	0.2691	3.01	19.2	0.2726	3.02	19.3	0.2807	3.01	19.2	0.2801	3.00	19.1
	0.2768	3.01	19.2	0.2704	3.03	19.3	0.2660	3.03	19.3	0.2956	3.00	19.1
	0.2649	3.01	19.2	0.2785	3.03	19.3	0.2568	3.04	19.4	0.2671	3.01	19.2
	0.2769	3.01	19.2	0.2807	3.05	19.4	0.2611	3.03	19.4	0.2820	3.04	19.4
	0.2822	3.02	19.3	0.2982	3.07	19.6	0.2776	3.05	19.5	0.2817	3.06	19.5
	Avg=	***3.01***	***19.2***	***Avg=***	***3.04***	***19.4***	***Avg=***	***3.03***	***19.4***	***Avg=***	***3.02***	***19.3***
	s=	***0.01***	***<0.1***	***s=***	***0.02***	***0.1***	***s=***	***0.01***	***0.1***	***s=***	***0.03***	***0.2***
Pepperjack	0.2882	3.68	23.5	0.2910	3.74	23.9	0.2514	3.65	23.3	0.2840	3.75	23.9
Cheese	0.2835	3.69	23.6	0.2943	3.75	23.9	0.2788	3.66	23.4	0.2819	3.73	23.8
	0.2719	3.67	23.4	0.2765	3.74	23.9	0.2713	3.66	23.4	0.2738	3.73	23.8
	0.2804	3.69	23.5	0.2756	3.70	23.6	0.2680	3.68	23.5	0.2826	3.68	23.5
	0.2765	3.69	23.5	0.2790	3.73	23.8	0.2899	3.67	23.4	0.2782	3.71	23.7
	Avg=	***3.68***	***23.5***	***Avg=***	***3.73***	***23.8***	***Avg=***	***3.67***	***23.4***	***Avg=***	***3.72***	***23.7***
	s=	***0.01***	***0.1***	***s=***	***0.02***	***0.1***	***s=***	***0.01***	***0.1***	***s=***	***0.02***	***0.2***
Sharp Cheddar	0.2703	3.53	22.5	0.2842	3.54	22.6	0.2820	3.55	22.7	0.2718	3.61	23.0
Cheese	0.2712	3.53	22.5	0.2698	3.51	22.4	0.2859	3.57	22.8	0.2861	3.64	23.2
	0.2660	3.54	22.6	0.2820	3.50	22.3	0.2900	3.54	22.6	0.2872	3.54	22.6
	0.2810	3.52	22.5	0.2839	3.52	22.5	0.2805	3.53	22.5	0.2892	3.47	22.2
	0.2699	3.52	22.5	0.2894	3.52	22.5	0.2774	3.56	22.7	0.2873	3.55	22.6
	Avg=	***3.53***	***22.5***	***Avg=***	***3.52***	***22.4***	***Avg=***	***3.55***	***22.6***	***Avg=***	***3.56***	***22.7***
	s=	***0.01***	***0.1***	***s=***	***0.01***	***0.1***	***s=***	***0.02***	***0.1***	***s=***	***0.06***	***0.4***

†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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