Application Note

Instrument: TruMac® N

Nitrogen/Protein in Cheese

LECO Corporation; Saint Joseph, Michigan USA

Sample Preparation
Samples must be of uniform consistency to produce suitable results.

Accessories
528-203 Crucibles

Calibration Samples
502-092 EDTA, 502-642 Phenylalanine, Nicotinic Acid

*Analysis Parameters**

Furnace Temperature	1100°C
TE Cooler Temperature	5°C
Dehydration Time	0 seconds
Purge Cycles	2 seconds

Element Parameters

Baseline Delay Time	6 seconds
Minimum Analysis Time	35 seconds
Comparator Level	100.00
Endline Time	2 seconds
Conversion Factor	1.00
Significant Digits	5
TC Baseline Time	10 seconds

Burn Profile

Burn Cycle	Lance Flow	Purge Flow	Time (seconds)
1	Off	On	5
2	On	On	35
3	On	Off	END

Ballast Parameters

Equilibrate Time	30 seconds
Not Filled Timeout	300 seconds

Aliquot Loop

Equilibrate Pressure Time	4 seconds
High Precision	Yes
High Speed	No

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

Procedure
1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Condition the system by analyzing 3 to 5 blanks (crucible is not required).
3. Determine blank.
   a. Enter 1.0000 g mass into Sample Login (F3) using Blank as the sample name.
   b. Place a 528-203 Crucible into the appropriate position of the autoloader.
   c. Repeat steps 3a through 3b a minimum of three times.
   d. Initiate the analysis sequence (F5).
   e. Set the blank following the procedure outlined in the operator's instruction manual.
4. Calibrate.
   a. Weigh ~0.75 g of EDTA calibration sample into a 528-203 Crucible, enter mass and sample indentification into Sample Login (F3).
   b. Transfer crucible to the appropriate position of the autoloader.
   c. Repeat steps 4a through 4b a minimum of three times.
   d. Initiate the analysis sequence (F5).
   e. Calibrate the instrument following the procedure outlined in the operator's instruction manual.

   Note: Multi-point (fractional weight or multiple calibration samples) may be used to calibrate if desired. Research has shown that a properly functioning TruMac can be calibrated using several replicates of a single mass range (nominal 0.75 g) of EDTA utilizing a single standard calibration. This is a cost effective and simple process. The calibration can be verified by analyzing different compounds such as nicotinic acid (0.25 to 0.5 g) and/or phenylalanine (0.5 to 0.75 g).
5. Analyze Samples.
   a. Weigh ~0.25 to 0.3 g cheese sample into a 528-203 Crucible; enter mass and sample identification into Sample Login (F3).
   b. Transfer crucible to the appropriate position of the autoloader.
   c. Repeat steps 5a through 5b for each sample to be analyzed.
   d. Initiate the analysis sequence (F5).

   Note: If soot (carbon black) is noticed in the primary filter (steel wool filter), reduce sample mass to prevent soot build-up in this filter. Soot can be produced when larger masses of high fat samples are analyzed. The steel wool filter should be changed after analysis of ~50 cheese samples as a build-up of salts will be evident. In addition, the autoloader arm should be inspected and cleaned on a regular basis when analyzing cheese products.

*Typical Results***

Sample	*Mass g*	*% N*	*% Protein*
Parmesan	0.2634	4.78	30.5
Cheese	0.2737	4.77	30.4
	0.2545	4.71	30.0
	0.2508	4.81	30.7
	0.2561	4.71	30.0
	0.2728	4.75	30.3
	0.2557	4.69	29.9
	0.2555	4.78	30.5
	0.2563	4.79	30.6
	0.2664	4.77	30.4
	X =	***4.75***	***30.3***
	s =	***0.04***	***0.3***

Sample	*Mass g*	*% N*	*% Protein*
Fresh	0.3352	2.66	17.0
Mozzarella	0.2916	2.60	16.6
Cheese	0.3025	2.71	17.3
	0.2840	2.60	16.6
	0.2932	2.75	17.5
	0.3044	2.84	18.1
	0.2934	2.88	18.4
	0.3134	2.81	17.9
	0.3179	2.81	17.9
	0.2947	2.86	18.2
	X =	***2.75***	***17.6***
	s =	***0.10***	***0.6***

Sharp	0.2706	4.00	25.5
Cheddar	0.2718	4.08	26.0
Cheese	0.2600	4.01	25.6
	0.2760	4.02	25.7
	0.2504	4.03	25.7
	0.2614	3.99	25.5
	0.2598	4.00	25.5
	0.2657	3.98	25.4
	0.2631	4.01	25.6
	0.2652	4.00	25.5
	X =	***4.01***	***25.6***
	s =	***0.03***	***0.2***

Asiago	0.2963	4.17	26.6
Cheese	0.2562	4.19	26.7
	0.2525	4.18	26.6
	0.2612	4.12	26.3
	0.2929	4.15	26.5
	0.2512	4.22	26.9
	0.2896	4.15	26.5
	0.2774	4.15	26.5
	0.2667	4.19	26.7
	0.2542	4.19	26.7
	X =	***4.17***	***26.6***
	s =	***0.03***	***0.2***

Sharp	0.2604	4.11	26.2
Provolone	0.2685	4.18	26.6
Cheese	0.2658	4.16	26.5
	0.2594	4.08	26.0
	0.2534	4.09	26.1
	0.2524	4.12	26.3
	0.2566	4.05	25.9
	0.2633	4.07	26.0
	0.2741	4.08	26.0
	0.2711	4.09	26.1
	X =	***4.10***	***26.2***
	s =	***0.04***	***0.3***

***A protein factor of 6.38 was used to calculate the protein content. The 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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