Nitrogen/Protein in Starch

LECO Corporation; Saint Joseph, Michigan USA

Instrument: TruMac® N

Introduction

Nitrogen determination in starch is utilized to calculate the protein concentration using a nitrogen protein conversion factor. The protein content of starch is not only an important criterion in evaluating the quality of starch but also is an important parameter during the overall starch refining process. The protein contained in starch is a combination of water soluble and insoluble proteins from the grain used in the milling and separation process. Control and measurement of protein throughout the milling and separation process is key in the overall controlled efficient and economical operation of a milling process, ensuring the quality of the refined starch. A larger sample mass is required (>1g) for refined starch slurries, as the refined starch slurry will be typically less than 0.15% N.

The LECO TruMac N is a macro combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a ceramic horizontal furnace and large ceramic boats for the macro sample combustion process. A combustion gas collection and handling system uses helium carrier gas and a thermal conductivity cell for the detection of nitrogen.

Sample Preparation

Samples must be of uniform consistency to produce suitable results.

Accessories

528-203 Crucibles, 502-343 Nickel Boat Liners (for liquid samples)

Calibration Samples

502-092 EDTA, 502-642 Phenylalanine, 502-211 Glycine, 502-602 Ammonium Chloride Solution, Nicotinic Acid

Reference glycine solution preparation procedure on page 3.

*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 to 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. Use single standard calibration.

Note: Multi-point (fractional weight or multiple calibration samples) may be used to calibrate if desired. A 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); or with glycine solution for low concentrations.

Organic Application Note

5. Analyze Starch Powder Samples.
   a. Weigh ~1 to 2 g 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).
6. Analyze Starch Slurry Samples
   a. Place 502-343 Nickel Boat Liner into a 528-203 Crucible.
   b. Follow steps 5a through 5d.

   Note: If soot (carbon black) is noticed in the primary filter (steel wool filter), reduce sample mass to prevent build-up. Soot can be produced when larger masses of some sample types are analyzed.

Typical Results

(based on single standard calibration using 0.75 g of EDTA)

Sample	*Mass g*	*% N*	*% Protein*
Starch	1.0021	0.0398	0.249
	1.0083	0.0412	0.258
	1.0084	0.0398	0.249
	1.0048	0.0400	0.250
	1.0031	0.0407	0.254
	1.0033	0.0394	0.246
	1.0055	0.0395	0.247
	1.0049	0.0395	0.247
	1.0032	0.0397	0.248
	1.0062	0.0397	0.248
	X =	***0.0399***	***0.250***
	s =	***0.0006***	***0.004***
Starch	1.5423	0.0198	0.124
Slurry	1.5018	0.0200	0.125
	1.5805	0.0194	0.121
	1.5075	0.0198	0.124
	1.5204	0.0198	0.124
	1.5298	0.0198	0.124
	1.5071	0.0193	0.121
	1.5146	0.0193	0.121
	1.5088	0.0201	0.126
	1.5194	0.0192	0.120
	X =	***0.0197***	***0.123***
	s =	***0.0003***	***0.002***

Sample	*Mass g*	*% N*	*% Protein*
Glycine	1.0013	0.101	0.631
Solution	1.0260	0.100	0.625
@ 0.10% N	1.0194	0.100	0.625
	1.0350	0.101	0.631
	1.0217	0.099	0.619
	X =	***0.100***	***0.625***
	s =	***0.001***	***0.006***
Glycine	1.1343	0.0327	0.204
Solution	1.0303	0.0328	0.205
@ 0.0324% N	1.0442	0.0315	0.197
	1.0813	0.0315	0.197
	1.0537	0.0320	0.200
	X =	***0.0321***	***0.201***
	s =	***0.0006***	***0.004***
502-602	1.0425	0.102	0.638
@ 0.100% N	1.0427	0.100	0.625
Solution*	1.0163	0.099	0.619
	1.0401	0.100	0.625
	1.0083	0.098	0.613
	X =	***0.100***	***0.625***
	s =	***0.001***	***0.006***

**Custom CRM of ammonium chloride in water.*

GLYCINE SOLUTION PREPARATION

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

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

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

Example for 1% solution:

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

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:

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

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%

$$\frac{1.672(18.654)}{(99.824)} + 0.004 = 0.316\%\ \text{N}$$

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.*

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