Nitrogen Determination in Beer

LECO Corporation; Saint Joseph, Michigan USA

Instrument: TruMac® N

Introduction

Nitrogen determination in Beer is utilized to calculate the protein concentration using a nitrogen protein conversion factor. The protein content of beer is not only an important criterion in evaluating the quality of beer but also is an important parameter during the brewing process. The protein contained in beer is primarily water soluble proteins from the grains used in the malting and brewing process. Control and measurement of protein throughout the brewing process is important in order to ensure the survival, growth, and productivity of the yeast utilized to convert sugars to ethanol and carbon dioxide. The yeast organisms depend on a variety of conditions including the availability of amino groups derived from enzymatic hydrolysis of protein during the brewing process.

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, followed by a combustion gas collection and handling system using helium carrier gas and a thermal conductivity cell for the detection of nitrogen.

Sample Preparation

Remove CO_2 from beer samples by following procedure outlined in AOAC 920.49—"Remove CO_2 by transferring sample to a large flask and shaking, gently at first and then vigorously, keeping temperature of beer at 20-25°C."

Accessories

528-203 Crucibles, 502-343 Nickel Boat Liner

Calibration Samples

Glycine solutions prepared using the procedure found on the last page of this document.

*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 seconds
2	On	On	35 seconds
3	On	Off	END

Ballast Parameters

Ballast	
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 502-343 Nickel Boat Liner into a 528-203 Crucible and transfer crucible to the appropriate position of the autoloader.
   c. Repeat steps 3a through 3c 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/Drift Correct.
   a. Place a 502-343 Nickel Boat Liner into a 528-203 Crucible.
   b. Weigh ~ 1 g of glycine solution calibration sample into the Nickel Boat Liner, enter mass and sample indentification into Sample Login (F3).
   c. Transfer crucible to the appropriate position of the autoloader.
   d. Repeat steps 4a through 4c a minimum of three 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. Use single standard or linear calibrations only.
5. Analyze Samples.
   a. Place a 502-343 Nickel Boat Liner into a 528-203 Crucible.
   b. Weigh ~ 1 g beer sample into the Nickel Boat Liner; enter mass and sample identification into Sample Login (F3).
   c. Transfer crucible to the appropriate position of the autoloader.
   d. Repeat steps 5a through 5c for each beer sample to be analyzed. Initiate the analysis sequence (F5).

Organic Application Note

Delivering the Right Results

Typical Results

Sample	*Mass g*	*% N*
Lager Beer	1.0200	0.0559
	1.0132	0.0552
	1.0030	0.0545
	1.0133	0.0552
	1.0212	0.0552
	1.0177	0.0541
	1.0235	0.0541
	1.0148	0.0543
	1.0082	0.0547
	1.0007	0.0550
	1.0102	0.0545
	1.0243	0.0552
	1.0243	0.0553
	1.0121	0.0546
	1.0024	0.0549
	1.0243	0.0552
	***X* =**	***0.0549***
	***s* =**	***0.0005***

Sample	*Mass g*	*% N*
Stout	1.0234	0.0576
	1.0012	0.0575
	1.0100	0.0576
	1.0258	0.0575
	1.0101	0.0570
	1.0101	0.0566
	1.0040	0.0568
	1.0028	0.0573
	1.0112	0.0574
	1.0157	0.0570
	1.0113	0.0577
	1.0034	0.0572
	1.0168	0.0572
	1.0009	0.0571
	1.0148	0.0572
	1.0235	0.0581
	***X* =**	***0.0573***
	***s* =**	***0.0004***

Golden Ale	1.0001	0.0630
	1.0122	0.0631
	1.0019	0.0627
	1.0002	0.0635
	1.0111	0.0635
	1.0089	0.0638
	1.0159	0.0624
	1.0182	0.0628
	1.0001	0.0626
	1.0297	0.0678
	1.0202	0.0636
	1.0207	0.0634
	1.0237	0.0637
	1.0255	0.0640
	1.0035	0.0643
	1.0007	0.0627
	***X* =**	***0.0636***
	***s* =**	***0.0012***

Glycine	1.0089	0.0704
Solution	1.0105	0.0682
0.07% N	1.0051	0.0699
	1.0161	0.0704
	1.0135	0.0705
	1.0007	0.0705
	1.0066	0.0696
	1.0122	0.0703
	1.0032	0.0703
	1.0049	0.0694
	1.0026	0.0686
	1.0069	0.0694
	1.0125	0.0699
	1.0111	0.0697
	1.0265	0.0711
	1.0106	0.0707
	***X* =**	***0.0699***
	***s* =**	***0.0008***

**Results based on single standard calibration using 0.1% nitrogen glycine solution. Results were obtained over a two-day period; instrument blank and calibration were not changed during this time period.*

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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Organic Application Note