

NITROGEN / PROTEIN by COMBUSTION**PRINCIPLE**

The sample is combusted in the presence of oxygen at high temperature, whereby nitrogen-containing material is converted to molecular nitrogen and nitrogen oxides. Water vapor and other gaseous oxidation products are removed through a purification train. The oxides of nitrogen are catalytically reduced to molecular nitrogen. The total nitrogen obtained is detected in a thermal conductivity cell and quantified by integration relative to standard reference material of known nitrogen content. A measure of the protein content can be obtained by multiplying the nitrogen concentration by an appropriate factor, e.g. 6.25 for corn products.

SCOPE

This method is applicable to the determination of nitrogen in corn starch, co-products, starch slurry, and any corn product with a protein value $\geq 0.2\%$ dry solids basis.

SPECIAL APPARATUS

1. Nitrogen Combustion Analyzer and supporting equipment as per manufacturer's instructions (Note 1).
2. Analytical Balance accurate to 0.0001 grams.

REAGENTS

1. Oxygen: 99.99% purity zero grade in gas cylinder equipped with a regulator capable of delivering 40 psi followed by an in-line gas purifier (Note 2).
2. Helium: 99.99% purity zero grade in gas cylinder equipped with a regulator capable of delivering 40 psi followed by an in-line gas purifier.

STANDARDS

Nitrogen Calibration Standards: High purity EDTA (9.57% nitrogen), glycine (18.658% nitrogen), or other appropriate nitrogen standard. Solutions of these standards at lower concentrations can be prepared (Note 3).

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Optional Standards:

High Protein (Gluten Meal) Standard: A large sample of gluten meal is ground through a Wiley mill or equivalent using the 1 mm screen (to pass U.S. Standard Sieve No. 20) and blended well to insure homogeneity. Test portions are analyzed repeatedly (3 times minimum, within tolerance) for protein by the Kjeldahl method. The bulk of the ground sample is then packaged in 8 oz. screw cap bottles which are stored in a freezer. Use as the standard for meal, feed, germ, steepwater, and other high protein process samples.

Starch Standard: A large sample of unmodified corn starch is blended well and test portions are analyzed repeatedly for protein by the Kjeldahl method. The bulk of the sample is packaged in 8 oz. screw cap bottles which are stored in a freezer. Use as standard for unmodified dry starch samples only.

Modified Starch Standard: A large sample of modified corn starch is blended well and test portions are analyzed repeatedly for protein by the Kjeldahl method. The bulk of the sample is packaged in 8 oz. screw cap bottles which are stored in a freezer. Use as standard for a specific type of modified dry starch samples only. Each starch modified with a nitrogen-containing reagent will have its own standard.

CRA Check Sample Standards may be used.

PROCEDURE

Proceed according to manufacturer's instructions.

General Operating Guidelines:

A sample run is composed of a series of blanks, calibrants run as samples, calibrants, sample blanks, and samples.

General Blank Procedure:

The first several blanks (3-5) serve to flush and equilibrate the analyzer. These should not be used to evaluate the performance of the instrument. The next several blanks (3-5 more) should become consistently low in nitrogen. At this point, the blank factor can be adjusted according to manufacturer's instructions. If the samples to be analyzed are high in nitrogen, it is not necessary to adjust the blank factor at all. This adjustment, however, becomes essential for samples that are low in nitrogen.

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Select an appropriate nitrogen calibration standard to match the sample matrix and expected nitrogen level. For aqueous samples, it is better to use a liquid calibrant. For starch slurries, best results will be obtained if a starch slurry calibrant is used. This rule-of-thumb for the selection of the calibrant is especially important for samples with low levels of nitrogen.

After the blanks, a set (2-3) of calibrants run as samples should be analyzed. These serve to condition the analyzer for the testing of the calibrant. A calibrant run as a sample can also be used to check the accuracy of a daily factor.

The next several spots (4-7) in the run should be calibrants. These establish the daily factor for the determination of nitrogen in the samples. It is advisable to analyze inter-sample calibrants throughout a run to assure a robust daily factor.

General Sample Preparation:

After the calibrants, the next couple of spots (1-2) in the run should be sample blanks. Samples blanks condition the analyzer for the testing of samples. These sample blanks are especially important if there is a considerable difference in nitrogen content between the calibrant and the sample or if there are major matrix differences.

Use a sample preparation procedure appropriate to the matrix being analyzed to ensure a homogenous sample. Weigh sample according to table below. Ensure instrument response is within the response for the calibration standards (Note 4).

| Sample | Weight Range, g | Check Sample | Expected Nitrogen Level, %, dsb |
|------------------|------------------------|---------------------|--|
| Corn | 0.6000-1.0000 | High Protein | 1.28 – 2.00 |
| Dry Starch | 1.0000-1.6000 | Starch | 0.015 – 0.130 |
| Cationic Starch | 0.5000-0.8000 | Cationic Starch | 0.100 – 0.500 |
| Corn Gluten Meal | 0.3000-0.7000 | High Protein | 5.5 – 10.5 |
| Corn Gluten Feed | 0.6000-1.0000 | High Protein | 2.5 – 4.0 |
| Heavy Steepwater | 0.4000-0.6000 | High protein | 0.5 – 1.5 |
| Dorrclone Starch | 1.8000-2.5000 | Starch | 0.015 – 0.130 |
| Gluten Slurry | 0.3000-0.7000 | High Protein | 5.5 – 10.5 |
| Corn Germ Meal | 0.6000-1.0000 | High Protein | 2.5 – 6.0 |

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The next spots in the run should be samples. It is advisable to include a calibrant after a fixed interval of samples, for example, after every five or ten samples. For better accuracy, run samples in duplicate if possible.

Finally, some blanks (1-2) are included at the very end of the run to clean out the instrument.

CALCULATIONS

The instrument will provide a printed table of results as either % nitrogen or % protein. For manual calculations, use the following formula.

Percent Crude Protein “as is” = % N x 6.25 (for corn products).

NOTES AND SAFETY PRECAUTIONS

1. Follow manufacturer’s recommendations for safe operation of the instrument. Be cautious of hot surfaces and electrical hazards during routine maintenance.

Nitrogen Analyzer: Leco Model FP-528 or FP-2000 Nitrogen Determinator equipped with automatic sampler or autoloader, and printer
http://www.leco.com/products/organic/fp_528/fp_528.htm; or Elementar Vario MAX CN – Carbon/Nitrogen Analyzer; or equivalent. Elementar Americas help line is 856-787-0022 or <http://www.elementar-inc.com>.

2. Many materials are very flammable in the presence of oxygen. Keep away from flame or sparks.
3. Glycine Solution Standard: Dissolve glycine (aminoacetic acid, $\text{H}_2\text{NCH}_2\text{COOH}$) in water to obtain a nitrogen concentration just above that of the samples to be analyzed. Calculate amount of glycine to be used on the basis of a theoretical nitrogen contents of 18.658%. Weigh the calculated amount of glycine powder in a dry, tared 125 mL Erlenmeyer flask with a rubber stopper. Add 100 mL of purified water and reweigh. Seal the flask and mix to dissolve. Recalculate the exact concentration taking into account the actual weights measured. The solution(s) may be stored in a refrigerator and discarded after 2 days, or may be kept frozen indefinitely.

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4. Care must be taken in weighing samples. Samples which are high in carbohydrates may not combust completely if the sample weights are too high. Liquid slurry samples should be shaken immediately before being weighed to insure homogeneity. For samples which are high in nitrogen, sample weights should be low but not so low as to introduce balance errors into the analysis (minimum weight should be 0.1g).

REFERENCES

1. AOAC Official Method 990.03, Protein (Crude) in Animal Feed, Combustion Method. First Action 1990. *AOAC Official Methods of Analysis (Current Edition)*.
2. Comparison of Kjeldahl Method for Determination of Crude Protein in Cereal Grains and Oilseeds with Generic Combustion Method: Collaborative Study. Bicsak, R. C., *Journal of AOAC International*, Vol. 76, No. 4, (780- 786) 1993.
3. Performance of an Automated High Temperature Combustion-Thermal Conductivity Method for Measurement of Protein Content of Food Products. King-Brink, M, & Sebranek, J. G. Paper presented March 1993 Pittsburgh Conference and latter published in *The Analyzer*, Vol. IV, Leco Corporation.
4. Protein-Nitrogen Combustion Study Results, Brown. J. S, *INFORM*, Vol. 5 No. 5, May 1994.
5. Rose A. Sweeney, Generic Combustion Method for Determination of Crude Protein in Feeds: Collaborative Study, *J. Assoc. Off. Anal. Chem.* 72, no. 5, 770-774 (1989).
6. Robert W. Sachen and Nancy J. Thiex, Effect of Sample Introduction and Atmospheric Blank on Determination of Nitrogen (Crude Protein) by Combustion, *J. of AOAC Int'l* 80, no. 1, 14-19 (1997).