

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

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Title: Protein Determination by Combustion		
Revision: 03	Replaces: CLG-PRO4.02	Effective: 07/27/2009

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A. INTRODUCTION

1. Theory

Total protein is determined using nitrogen analysis. The sample is combusted with oxygen and the gases containing nitrogen oxides are collected in a ballast tank until a specified pressure is reached. Helium is used as a carrier and an aliquot of combustion gas containing nitrogen oxides is reduced to nitrogen. It is then passed through a tube containing magnesium perchlorate and sodium hydroxide on a silicate carrier to remove water and carbon dioxide. The nitrogen is measured with a thermal conductivity detector using helium as a reference. Nitrogen is then converted to protein using a conversion factor.

Note: This method is not an endorsement by the Food Safety and Inspection Service (FSIS) of the LECO FP-2000[®] over other commercially available instruments.

It may be necessary to use operating procedures and/or follow manufacturer's instructions for equivalent instruments from other manufacturers.

2. Applicability

This procedure is applicable to the determination of protein content in fresh and processed meat and poultry products.

B. EQUIPMENT

Note: Equivalent instrumentation or apparatus may be substituted.

1. Apparatus

- a. Robot Coupé food processor - Robot Coupé U.S.A. Inc.
- b. Analytical balance - capable of weighing to 0.1 mg.
- c. Forced draft oven - Adjustable to 101 ± 1 °C.
- d. Three two-stage compressed gas regulators - Each set at 40 psi.
- e. Ceramic combustion boats - Cat. No. 529-203, LECO.
- f. Foil Boat liners for liquid samples - Cat. No. 502-343, LECO.

2. Instrumentation

- a. LECO FP-2000 Protein Analyzer - Version 4.08 or equivalent software, and Autoloader. (Instrument parameters must be optimized for specific instrumentation used). The following are examples of settings for the LECO FP-2000:

Furnace temperature: 1150 °C.

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- Lance flow: 1.0 L/min.
- Purge flow: 4.5 L/min.
- TC cell sensitivity: 1500.
- Nitrogen conversion factor: 6.25 for meat and meat products.
- b. Printer: 9-pin, Okidata Microline 320.

C. REAGENTS AND SOLUTIONS

Note: An equivalent reagent or supply may be substituted.

3. Reagents and supplies

- a. N-Catalyst - Cat. No. 502-049, LECO.
- b. Anhydron (Magnesium Perchlorate) - Cat. No. 501-171, LECO.
- c. Lecosorb (Sodium Hydroxide on silicate carrier) - Cat. No. 502-174, LECO.
- d. Silicone grease - Cat. No. 501-241, LECO.
- e. Leak detection solution - Cat. No. 502-213, LECO.
- f. Copper Sticks - Cat. No. 502-304-500, LECO.
- g. Copper Turnings - Cat. No. 501-621, LECO.
- h. Glass wool for furnace filter packing - Cat. No. 501-081, LECO.
- i. Steel wool - Cat. No. 502-310, LECO.
- j. Cylinder - Compressed air, medical quality.
- k. Cylinder - Oxygen, 99.99% purity, Airco 4.4 grade.
- l. Cylinder - Helium, 99.99% purity, Airco 5.0 grade.

D. STANDARDS

1. Combustion Calibration EDTA

Approximately 99.5% Pure, Cat. No. 25, 404-5, Aldrich Chemical Company, or other suitable organic material of high purity and known nitrogen content.

Determine the % meat protein equivalent of the standard by multiplying the % purity by 59.91 (6.25 x %N in EDTA (9.586%).

Note: A standard curve must be established for each method (see LECO manual). The drift of the curve can be corrected as often as needed by analyzing three or more EDTA standards, and using the drift correction icon.

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E. SAMPLE PREPARATION

Process the sample until a homogeneous mixture is obtained.

F. ANALYTICAL PROCEDURE

1. For the operation of LECO FP-2000

Prepare instrument by following the procedure outlined in the operator's instruction manual (i.e. pack reagent tubes, perform leak checks, etc.).

- a. Weigh 1.0 ± 0.2 g of sample into a ceramic boat.
- b. Dry samples in a 101 ± 1 °C convection oven for 45 ± 5 min. After drying, place in desiccator to cool and/or hold until ready to load into the instrument.

Note: Sample may be stored in desiccator until analyzed.

2. LECO FP-2000 setup

- a. Run 5 or more blanks until values are reproducible and lower than 0.375% protein. Drift correct for the blank using the last three consecutive values.
- b. Run 4 or more EDTA standards until three consecutive values agree within < 0.15% protein of each other. Use the last three consecutive values to drift correct for the EDTA .

3. Sample Analysis

- a. Load the set of samples into autoloader. (For quality control, an EDTA standard should be placed for every eight samples. If the instrument drifts during the run, these EDTA standards can be used to drift correct and samples can be recalculated.)
- b. Enter standard and meat sample weights in the order in which they are in the rack. Weights can be entered manually, from an interfaced balance, or electronically (from floppy disc).
- c. Analyze samples.

G. CALCULATIONS

Calculations are done automatically by the data system. The results will be reported as % nitrogen unless a nitrogen factor of 6.25 (for meat) was entered into the method setup initially. If an EDTA sample within the run is more than ± 0.2 from its calculated protein equivalent, a drift correction is performed. The 4 samples preceding and following the corrected EDTA must be recalculated.

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H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Safety glasses, heat-resistant gloves, plastic gloves, laboratory coat.
2. Hazards

Procedure Step	Hazard	Recommended Safe Procedures
Unit operates at 220 volts AC and has a high voltage power supply	Can cause severe burns/electric shock	Turn instrument off and remove metal objects from hands and arms before reaching into the instrument cabinet.
Crucible combustion tube and reduction tube	Extremely hot (700 - 1150 °C)	Allow to cool or use suitable tool when they are hot
Pure Oxygen	Explosive	Remove all ignition sources from the laboratory area
Compressed gas cylinder	Explosive	Mount cylinders firmly and have two stage regulators attached before cylinder valves are opened.
Magnesium Perchlorate	Strong oxidizer, contact with flammable materials may cause ignition. Causes irritation to skin, eyes, and respiratory tract	Use in a fume hood.
Sodium Hydroxide	Causes burns to all body tissue. Corrosive. Reacts with some metals to form H ₂	Use in a fume hood.

3. Disposal Procedures

<i>Procedure Step</i>	<i>Recommended Safe Procedures</i>
Copper	Dispose in accordance with local, state, and Federal regulations.
Sodium Hydroxide	Collect waste in tightly sealed container and store away from non-compatibles in a cool, storage area/cabinet for disposal in accordance with local, state, and Federal regulations.

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Magnesium Perchlorate Collect waste in tightly sealed container and store away from non-compatibles in a cool, storage area/cabinet for disposal in accordance with local, state, and Federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	Analytical Range %	Repeatability	Reproducibility
Protein	¹	< 0.24 ²	< 0.32 ²

¹ Limit may vary due to sample aliquot size and sample type.

² One Standard Deviation based on historical data.

2. Critical Control Points and Specifications

Record	Acceptable Control
Sample Condition	Sample must be dried before loading in the autoloader sample rack.
Forced Draft Oven	101 ± 1 °C.
Sample weight	1.0 ± 0.2 g. Note: Weigh less sample if % total protein is out of calibration range.
Reduction reagent	No more than 600 assays should be done before re-packing tube.

3. Readiness To Perform

a. Familiarization

- i. Phase I: Standards- Not Applicable.
- ii. Phase II: Fortified samples- Run a set of 5 -10 previously analyzed samples in duplicate. Repeat on two additional days. (Different samples may be used on each day).
- iii. Phase III: Check samples for analyst accreditation.
 - (a) 15 check samples for initial analyst qualification.
 - (b) Samples submitted by the Quality Assurance Manager (QAM),

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Accredited Laboratory Program (ALP), or supervisor

(c) Authorization from the Quality Assurance Manager (QAM) and Supervisor are required to commence official analysis

b. Acceptability criteria.

Refer to I. 1.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: 1 per week, per analyst, if samples are analyzed.

ii. Records are maintained by analyst and reviewed by supervisor and laboratory Quality Assurance Manager (QAM).

b. Acceptability criteria.

Refer to section I.1., Performance Standards

If unacceptable values are obtained, then:

i. Stop all official analyses for the analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrix: Fresh and processed meat and poultry products.

b. Sample receipt size, minimum: 1 lb.

c. Condition upon receipt: Unspoiled and sealed from the air.

d. Sample storage

Time and Condition: 24 months frozen or 1 - 3 weeks refrigerated.

6. Sample Set

a. EDTA

b. Meat recovery

c. Samples

7. Sensitivity

Method detection limit (MDL): 0.2 %.

J. WORKSHEET

None

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K. APPROVALS AND AUTHORITIES

1. Approvals on file.
2. Issuing Authority: Director, Laboratory Quality Assurance Division.