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Title: Protein Determination by Combustion		
Revision: 02	Replaces: NA	Effective: 12/26/2001

Contents

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

1. Apparatus

Equivalent instrumentation or apparatus may be substituted.

a. Robot Coupé® food processor, Robot Coupé® U.S.A. Inc., Jackson MS 39236.

b. Analytical balance capable of weighing to 0.1 mg.

c. Forced draft oven set at 101° ± 1°C.

d. Three two-stage compressed gas regulators, each set at 40 psi.

e. Ceramic combustion boats, LECO No. 529-203.

f. Foil Boat liners for liquid samples, LECO No. 502-343.

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.
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

1. Reagents

An equivalent reagent may be substituted.

a. N-Catalyst - LECO No. 502-049.

b. Anhydrone - Magnesium Perchlorate, LECO No. 501-171.

c. Lecosorb - Sodium Hydroxide on silicate carrier, LECO No. 502-174.

d. Silicone grease - LECO No. 501-241.

e. Leak detection solution - LECO No. 502-213.

g. Copper Sticks - LECO No. 502-304-500.

h. Copper Turnings - LECO No. 501-621.

i. Glass wool for furnace filter packing - LECO No. 501-081.

j. Steel wool - LECO No. 502-310.

k. Cylinder - Compressed air, medical quality.

l. Cylinder - Oxygen, 99.99% purity, Airco 4.4 grade.

m. 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.

E. SAMPLE PREPARATION

Process the sample using a commercial type food processor 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 ± 5min. 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 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.

H. HAZARD ANALYSIS

1. Method Title — Protein Determination by Combustion.

2. Required Protective Equipment — Safety glasses, heat-resistant gloves, plastic gloves, and laboratory coat.

3. Hazard

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.

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.

The Measurement Uncertainty and Method Detection Limit should be recalculated yearly or whenever a change that affects method accuracy, precision or sensitivity.

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.
Sample bucket	Empty after 49 consecutive analyses.
Reduction reagent	No more than 600 assays should be done before re-packing tube.
Steel wool	Add to the glass wool particle filter for samples containing halogens.
Glass wool particle filter	Change when any ash or other contamination is present.
Calibrate thermometer	Calibrate thermometer for the force draft oven With NIST traceable thermometer.
In-line particle filter	Change if combustion backpressure exceeds 10 psi.

3. Readiness to Perform (FSIS Training Plan)
   a. Familiarization
      i. Phase 1: Observe accredited analyst go through the procedure and run blanks and EDTA standards.
      ii. Phase II: Samples previously analyzed with known protein content.

      Note: Phases I and II may be performed concurrently.
      iii. Phase III: 36 check samples for analyst qualification:
         a. Samples provided by FSIS Accredited Laboratory Program (ALP).
         b. Report analytical findings to ALP.
         c. Notification from ALP is required to commence official analysis.

4. Intralaboratory Check Samples
   a. System, minimum contents
      i. Frequency: 1 per week, per analyst, if samples are analyzed.
      ii. Blind samples or random replicates chosen by supervisor after initial analysis.
      iii. Records are maintained by analyst and reviewed by supervisor and laboratory Quality Assurance Manager (QAM).
   b. Acceptability Criteria

      Refer to section J. l., Performance Standards

      If unacceptable values are obtained, then:
      i. Stop all official analyses for the analyst.
      ii. Investigate and identify probable cause.
      iii. Take corrective action.

5. Interlaboratory Check Sample Program
   a. System, minimum contents
      i. Frequency: 1 every other month.
      ii Samples: Provided by FSIS.
      iii Report analytical findings to provider according to their specifications.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Investigate and identify probable cause.

iii. Take corrective action.

6. Sample Acceptability and Stability

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

b. Sample receipt size, minimum: 500 g.

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

d. Sample storage:

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

7. Sensitivity

a. Method detection limit (MDL): 0.2 %.

8. Sample Set

a. 1 - 20 samples.

b. One QC sample.

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