United States Department of Agriculture

Food Safety and Inspection Service

CLG-PRO4.05

Quantitation of Protein by Combustion

This method describes the laboratory procedure for quantitation of protein content in fresh and processed meat and poultry products.

Executive Summary

This is a food chemistry method for analysis of protein content in fresh and processed meat and poultry products. A protein analyzer is utilized to combust a sample and determine the total protein content.

Notice of Change

This method has been reformatted to a modernized design to improve accessibility and provide clarity for users. The method includes pictures that describe critical procedures for analysis.

Table of Contents

Safety Precautions

The personnel performing the analysis are to read the Safety Data Sheets for the standards and reagents used in this method. Follow all applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

Introduction

Protein in food is an important part of a balanced diet and is important for health and wellbeing. The correct determination of the protein content of meat products is important for the economic value of the food. The analysis of protein content in food products ensures that the product is compliant with labeling requirements set by the Federal Meat Inspection Act (FMIA), the Poultry Products Inspection Act (PPIA), the Egg Products Inspection Act (EPIA), and Title 9 of the Code of Federal Regulations.

Method Overview

The total protein in a sample is determined using nitrogen analysis through combustion. The sample is combusted with oxygen and the gases containing nitrogen oxides are collected. The nitrogen oxides undergo a reduction reaction with magnesium perchlorate and the resulting nitrogen is measured. The amount of nitrogen is then used as a measure of the protein content in the sample.

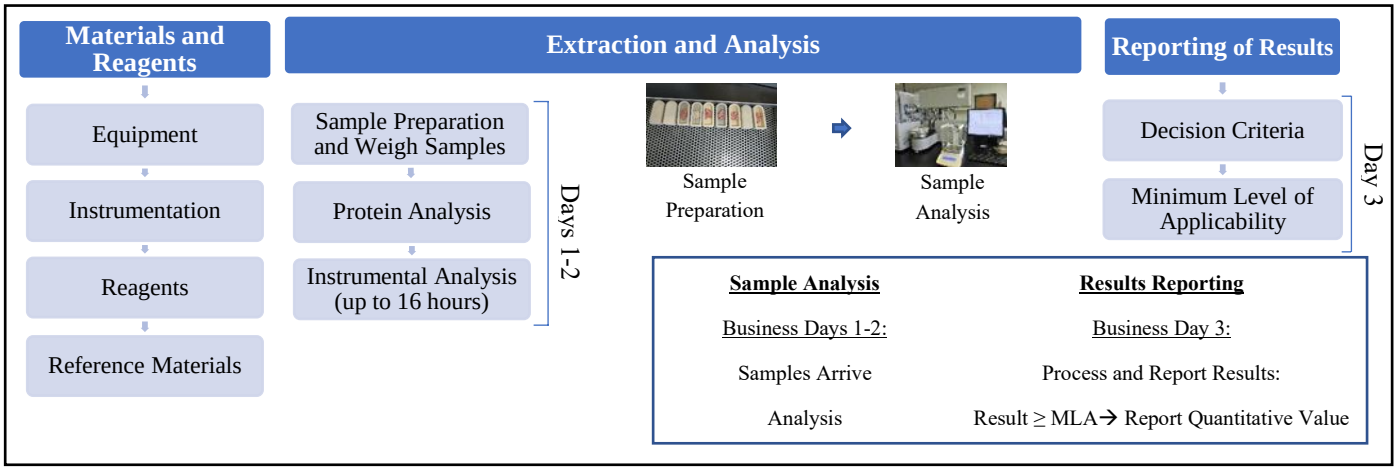

Figure 1: Overview and timeframe of protein analysis. Samples arrive at the laboratory, are weighed, and analyzed on business days 1-2. Results are reported on business day 3. This chart represents the best-case scenario, but analyses may take longer due to analytical testing circumstances. Pictures courtesy of Hue Quach and Killani Kadri, USDA-FSIS.

Decision Criteria

Quantitative results are reported for all samples. Sample results are compared to the Minimum Level of Applicability (MLA).

KEY DEFINITIONS

MLA: Lowest level at which an FSIS method has been successfully validated for a residue in each matrix. Full definition is on the CLG website here.

Disclosure Statement

The Food Safety and Inspection Service (FSIS) does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. Significant equivalence changes would require FSIS laboratory leadership approval.

Materials and Reagents

Equipment

Table 1: Equipment Required to Perform CLG-PRO4

Equipment	Supplier and Part Number	Purpose
Food Processor	Robot Coupe USA Inc.	Homogenize sample
Cutting board and knives	General lab supplier	Preparation of sample
Analytical balance - capable of weighing to 0.1 mg.	General lab supplier	Weighing sample
Forced draft oven - Adjustable to 101 ± 1 °C.	General lab supplier	Drying sample
Three two-stage compressed gas regulators	General lab supplier	Regulating gases
Ceramic combustion boats	Cat. No. 529-203, LECO.	Holding samples
Foil Boat liners for liquid samples	Cat. No. 502-343, LECO.	Holding samples

Instrumentation

Table 2: Instrumentation

Instrument	Supplier and Model Number	Purpose
LECO TruMac N with TruMac operating software	LECO, 630-300-800	Analysis of samples

Reagents

Table 3: Reagents

Reagent	Supplier and Part Number
N-Catalyst	Cat. No. 502-049, LECO.
Anhydrone (Magnesium Perchlorate)	Cat. No. 501-171. LECO.
Lecosorb (Sodium Hydroxide on silicate carrier)	Cat. No. 502-174, LECO.
Silicone grease	Cat. No. 501-241, LECO.
Copper Sticks	Cat. No. 502-304-500, LECO.
Copper Turnings	Cat. No. 501-621, LECO.
Glass wool for furnace filter packing	Cat. No. 501-081, LECO.
Steel wool	Cat. No. 502-310, LECO.
Compressed air, medical quality	General lab supplier
Oxygen, 99.99% purity	General lab supplier
Helium, 99.99% purity	General lab supplier

Reference Materials

Table 4: Reference Materials

Standard	Supplier	Catalog Number
Ethylenediaminetetraacetic acid (EDTA)	LECO	Cat. No. 502-092

Extraction and Analysis

Sample Preparation

Samples must be kept cold and sealed from the air before and during shipping to the laboratory. Samples are used for multiple food analyses such as moisture analysis, therefore, handle samples in a way to avoid evaporation and condensation. An example of the type of sample is shown in Figure 2, with an example of a prepared sample in Figure 3.

Figure 2: Processed meat product. Photo courtesy of Getty Images.

Figure 3: Homogenized sample. Photo courtesy of Raymond Allen Williams, USDA FSIS

Protein Analysis

Samples

1) Prior to weighing, samples should be thoroughly mixed by squeezing and palpating the sample bags to ensure that the samples are homogenous. This ensures that the sample is representative of the food product being analyzed.
2) As shown in Figure 4, weigh 1.0 ± 0.2 g of sample and/or Quality Control tissue into a ceramic boat.

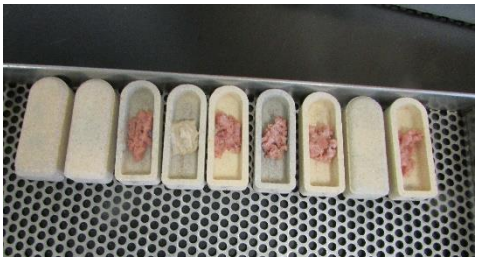

Figure 4: Samples in ceramic boat before analysis. Photo courtesy of Killani Kadri, USDA-FSIS

QUALITY CONTROL

1) Run two previously analyzed samples or samples with a known value as a recovery with each set of samples. These will be the meat recovery and meat recovery (safeguard) controls.
2) Weigh one additional portion for an intra-laboratory check sample, if necessary.

KEY DEFINITIONS

Meat Recovery: A sample with a known concentration of protein.

Reference value: The value of the known concentration of protein in the meat recovery.

Blank: A boat run without sample.

Technical Note:

If results for the % protein of a sample has previously been determined to be outside of the calibration range, reduce the weight of the sample.

Analysis

1) Dry samples in a 101 ± 1 °C convection oven for 45 ± 5 minutes. After drying, load samples into the instrument or cool and store in a desiccator until analyzed.
2) Analyze at least five blanks with boats until three consecutive blanks have a stable value with a standard deviation of less than 0.002%. Blank correct using the last three consecutive values.
3) The Manufacturer LECO TruMac N recommends to weigh four or more Ethylenediaminetetraacetic acid (EDTA) standards at 0.5000 ± 0.0625 g into ceramic boats. Run them until three consecutive values have a Relative Standard Deviation of 0.2% or less. Use the last three consecutive values to drift correct.
4) Load the set of samples into the protein analyzer.
5) Analyze samples on the instrument, as demonstrated in Figure 5.

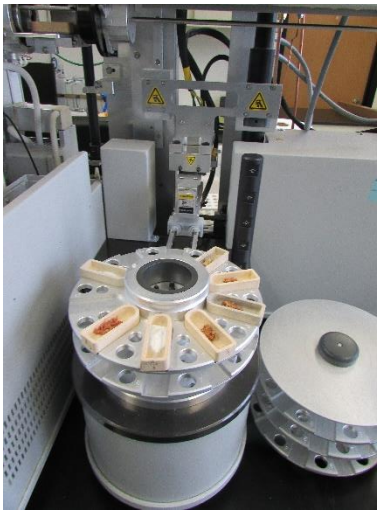

Figure 5: Samples on carousel of nitrogen analyzer. Photo courtesy of Killani Kadri, USDA-FSIS

Instrumental Analysis

Prepare instrument by following the procedure outlined in the operator's instruction manual (i.e., pack reagent tubes, perform leak checks, etc.). Optimize instrument parameters if needed to ensure system suitability.

1) Furnace temperature: 1100 °C
2) Lance flow: ~1.8 L/min
3) Purge flow: ~4.2 L/min
4) TE Cooler Temperature 5 °C
5) Dehydration Time 0 seconds(sec)
6) Purge Cycles 2
7) Element Parameters Nitrogen
8) Baseline Delay time 6 sec
9) Minimum Analysis Time 35 sec
10) TC Baseline Time 10 sec

Figure 6: Nitrogen analyzer for protein analysis. Photo courtesy of Killani Kadri, USDA-FSIS

Table 5: Burn Profile

Burn Cycle	Lance Flow	Purge Flow	Time (sec)
1	Off	On	5
2	On	On	35
3	On	Off	End

11) Ballast:

a.	Equilibrate Time	30 sec
b.	Not Filled Timeout	300 sec
c.	Aliquot Loop	
d.	Equilibrate Pressure Time	4 sec
e.	High Precision	Yes
f.	High Speed	No

Sample Set

The sequence below can be modified, as needed, but must include required controls.

1) Instrument blanks
2) EDTA standards
3) Meat recovery
4) Intra-laboratory check sample (if applicable)
5) Samples up to 20 samples
6) Meat recovery

INTRA-LABORATORY

CHECK SAMPLE

Defined on the CLG website here.

Technical Note:

Additional QCs can be run through the set to monitor reagents and instrument drift.

Reporting of Results

Decision Criteria

Calculations

Calculations are done using the instrument control software. The results are reported as % protein using the nitrogen factor of 6.25.

% Nitrogen × 6.25 = % Protein.

QUALITY CONTROL

Calibration

1) A standard curve must be established for each instrument method, and with each new lot of EDTA. The drifts of the standard curve can be corrected as often as needed by analyzing three or more EDTA standards, and using the drift correction menu in the software.
2) If the drift is off by 10% or more, re-run the EDTA calibration curve.
3) The calibration is established using a 1/x certified weighting factor and the simplest curve that minimizes the RMS error.

Quality Control Procedures

1) Three consecutive values of EDTA run before the samples must have a Relative Standard Deviation of 0.2% or less.
2) The three consecutive blanks used for blank correction shall have a standard deviation of less than 0.002%.
3) For set acceptance, the meat recovery (or check samples) prepared at the beginning of the sample set must have a ≤ 0.44% protein difference from the reference value.
4) The meat recovery (safeguard) prepared at the end of the sample set must have a ≤ 0.33% protein difference from the meat recovery prepared at the beginning of the set.

Intra-laboratory Check Samples (If applicable)

1) Acceptability criteria.
   a. The check sample must have a ≤ 0.44% protein difference from the reference value.
   b. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures when unacceptable values are obtained:
      i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Minimum Level of Applicability

Table 6: Minimum Level of Applicability

	Matrix	%
Protein	Fresh and processed meat and poultry products	0.3

References

1) Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Edition: 950.46

2) 9 CFR 319 Definitions and Standards of Identity or Composition

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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