

Combustion Nitrogen/Protein Determination in Food Matrices Using a Macro Sample Mass

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INTRODUCTION

One of the most significant nutritional components of food is protein. The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value in food materials but is also key to the economic value of these materials. Protein in foods and feeds is most commonly calculated using the measured nitrogen in the sample and a multiplier based on the food or feed matrix. Nitrogen determination is performed using either the classical wet chemical method (Kjeldahl) or a combustion method. The Kjeldahl method is capable of handling macro samples sizes > 1 g. Combustion nitrogen instruments typically have a vertical furnace where the technician encapsulates the samples in a tin foil or capsule for introduction into the furnace. The sample and tin ash is collected within a crucible in the furnace. Physical restrictions in sample encapsulation and realities of handling the ash build up within the furnace will often restrict the sample mass to \sim 500 mg or less. Due to sample mass limitations of combustion instruments with a vertical furnace design, there has been concern regarding the ability of combustion instruments to adequately determine protein in samples that are either heterogeneous and/or difficult to prepare, like meat products.

A new combustion nitrogen determinator has been designed to handle macro sample sizes while maintaining a rapid analysis time of 4.8 minutes with a low cost-per-analysis. This combustion instrument has a horizontal furnace where the technician weighs the sample into an open ceramic boat. An automation system loads the ceramic boat into the furnace for combustion and later removes the ceramic boat containing the samples ash. These features allow this combustion instrument to have the same macro sample mass range as the classical Kjeldahl method (>1 g).

This poster presents a variety of heterogeneous meat matrices analyzed for Total Nitrogen/Protein using a horizontal furnace combustion instrument with increasing sample mass range to the macro level (>1 g).

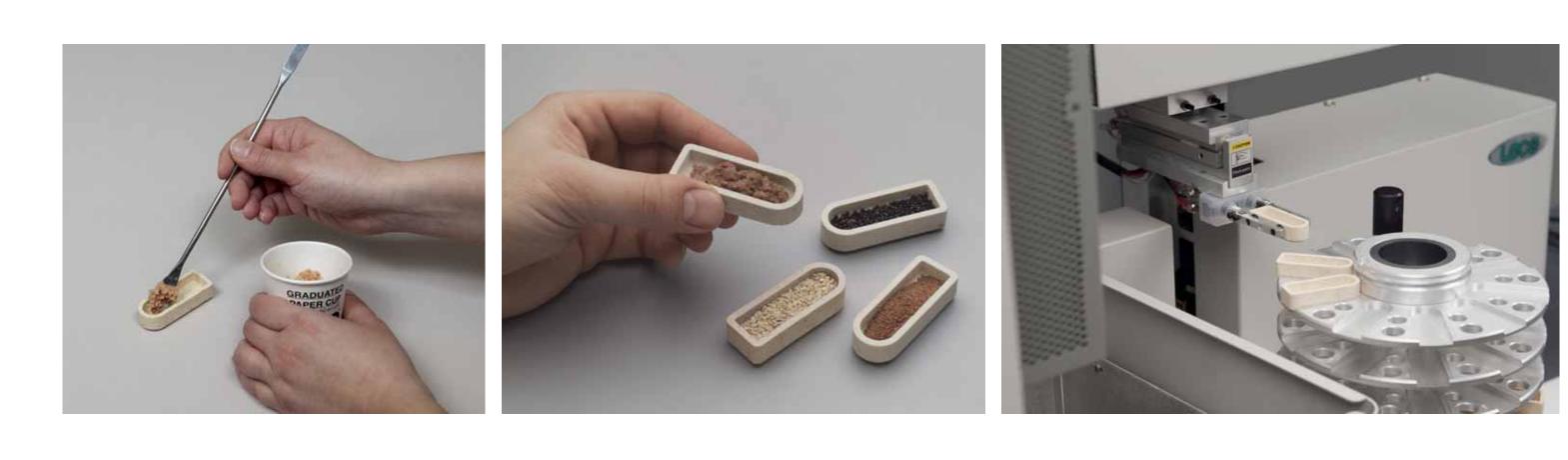
METHODOLOGY

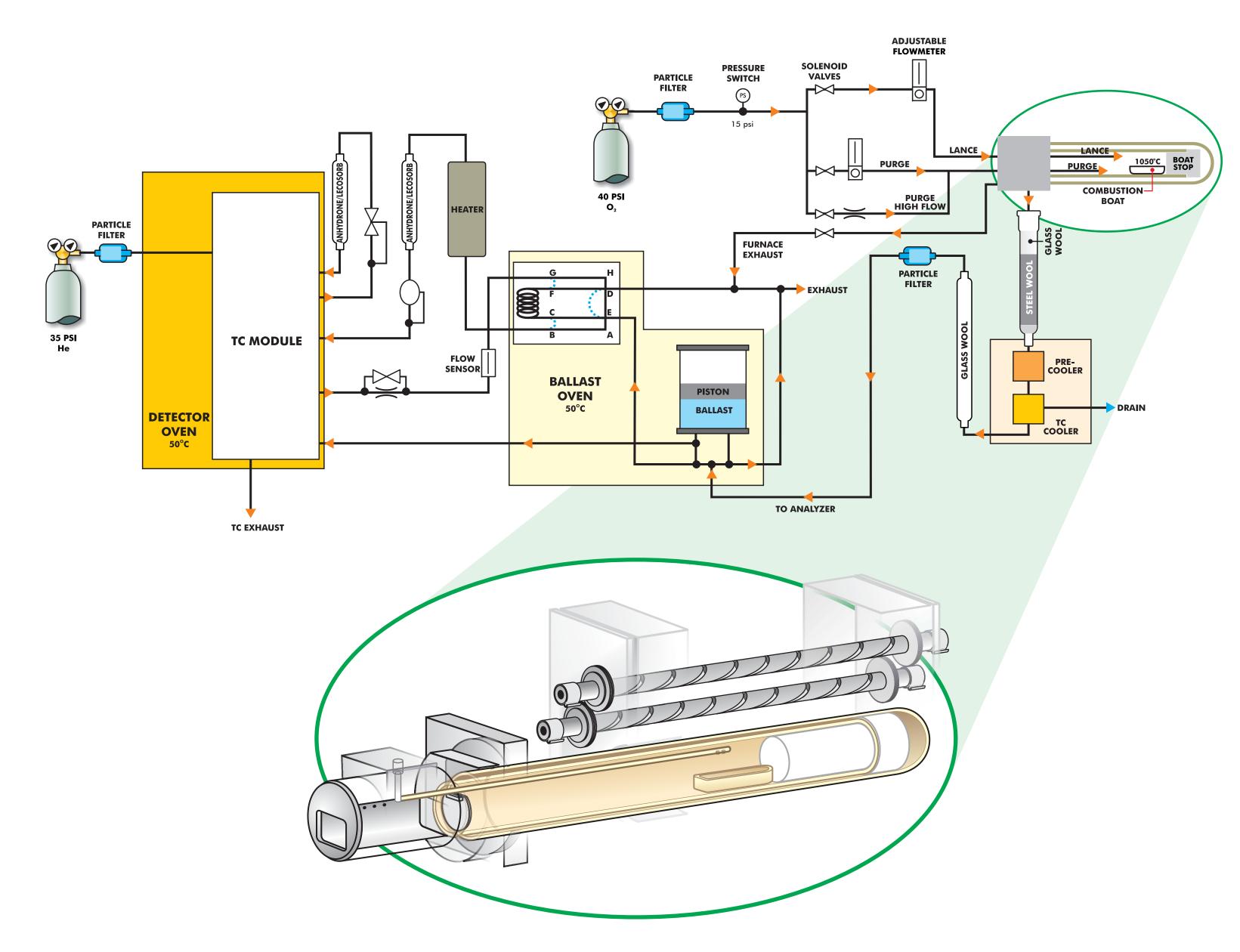


• LECO TruMac[™] N

Procedure

- Weigh ~250 mg to 2 g sample (100-200 mg) into a ceramic combustion boat
- Load the ceramic boat into the loader carousel for automated analysis





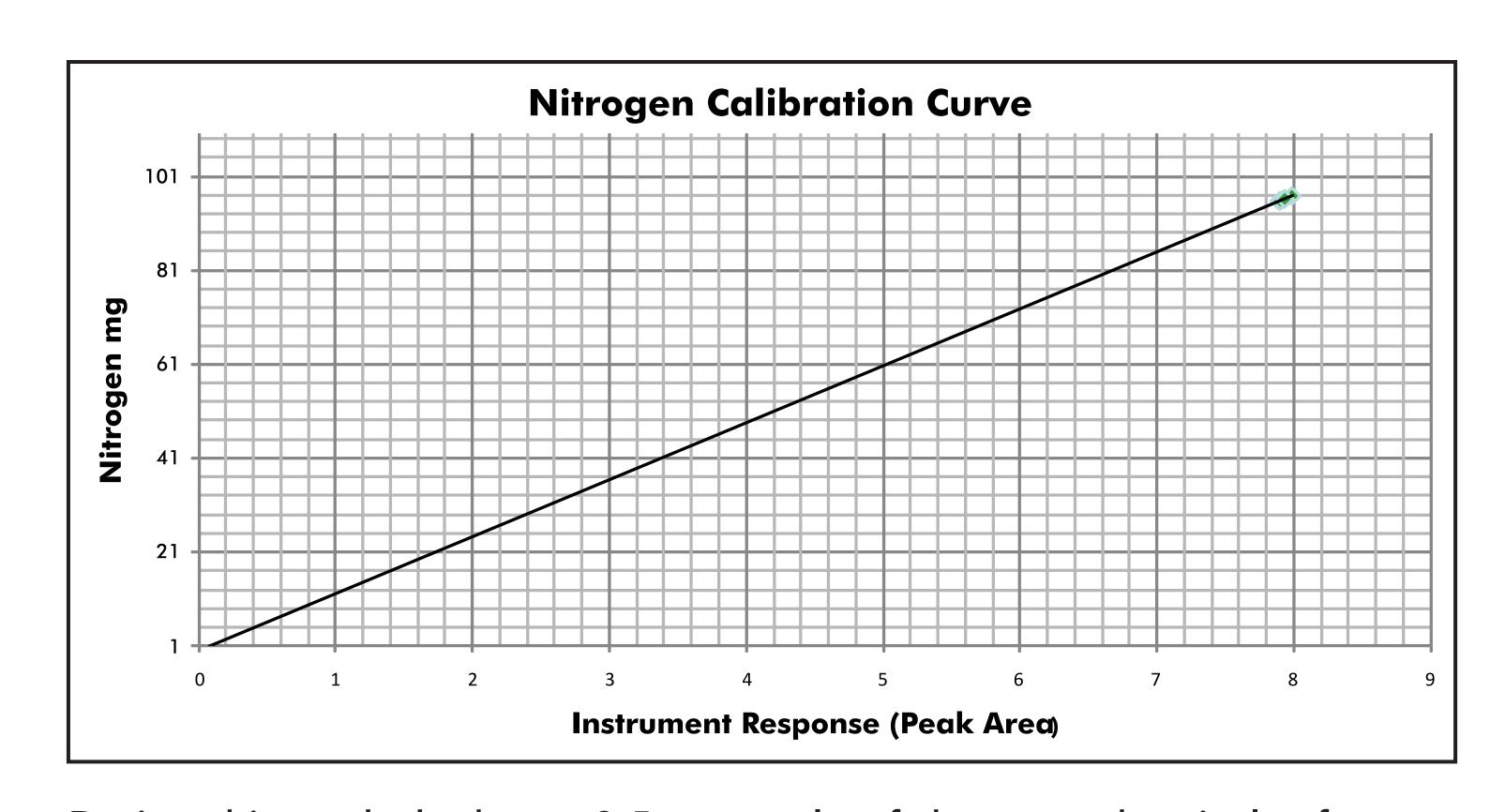
The sample in the ceramic boat is loaded into the purge chamber located in the front of the horizontal ceramic high temperature furnace. The entrained atmospheric gas is purged from the sample then the ceramic boat is introduced into the furnace nominally regulated at a temperature of 1100° C. Complete oxidation of the macro sample is ensured by the pure oxygen environment within the furnace with additional oxygen being directed onto the sample via a ceramic lance. The ceramic boat and all sample ash are removed from the furnace at the end of combustion. The combustion gases are swept from the furnace into a thermoelectric cooler where the moisture is removed. The remaining combustion gases are collected and equilibrated in a ballast where an aliquot is taken for nitrogen determination. The sample aliquot gases are swept through hot copper, removing oxygen and converting NO gas to N gas before passing through the Lecosorb and anhydrone to remove carbon dioxide and the water by-product. The remaining nitrogen gas from the sample is passed through a thermal conductivity detector to determine nitrogen.

CALIBRATION

The TruMac N was calibrated using the pure reference materials EDTA (LECO #502-092 Lot #1054). A single standard calibration was used with the mass group listed in the table 1 below. When using a nominal sample mass of 1 g the calibration covers a range of 9.57% to ~30 ppm Nitrogen (59.81 to 0.019% Protein when using a 6.25 protein multiplier).

Reference Material	Mass (g)	Certificate Conc. (%N)	Calibration Calculated (%N)	Error (%)	mg Nitrogen	Instrument Response (Peak Area)
EDTA	1.0054	9.57	9.5675	-0.02637	96.19	7.9292
EDTA	1.0003	9.57	9.5774	0.07728	95.8	7.8971
EDTA	1.0105	9.57	9.588	0.18803	96.89	7.9865
EDTA	1.0017	9.57	9.5734	0.03579	95.9	7.9049
EDTA	1.0051	9.57	9.5787	0.09125	96.28	7.9361

Table 1. Nitrogen Calibration Data



During this work, both a ~0.5 g sample of the pure chemical reference material EDTA (LECO #502-092 Lot # 1054) and a ~0.25 g sample of pure chemical reference material Nicotinic acid (99.5% Sigma Aldrich) were run as check samples interspersed within the meat matrix samples. The results obtained on these checks verify that the instrument and its calibration remained stable.

Reference Material	EDTA (LECO # 502-092)	Nicotinic Acid (99.5% Sigma Aldrich)	
Average (%N)	9.586	11.399	
Standard Deviation	0.007	0.016	
RSD (%)	0.07	0.14	
Expected (%N)	9.57	11.38	
Recovery (%)	100.2	100.2	
# of Runs	8	12	

Table 2. Nitrogen Check Sample Data

RESULTS

Three characteristically heterogeneous meat matrix samples were chosen for this study to demonstrate the performance and capability of the instrument for the total nitrogen/protein determination in the meat sample matrix over a broad sample mass range. The samples represent some of the most difficult meat materials to work with on a combustion nitrogen instrument in terms of sample handling, high fat content, and general sample preparation for the proper sampling of a heterogeneous material. The sample group for this study includes a beef sample from an advanced meat recovery (AMR) system, a raw bacon sample, and a cooked bacon sample. A protein factor of 6.25 was used for all of the meat samples.

Mass Range		% N	% Protein
	Average	2.16	13.52
~0.25 g n=5	Std Dev	0.06	0.36
	RSD (%)	2.63	2.63
	Average	2.12	13.27
~0.5 g n=5	Std Dev	0.03	0.2
	RSD (%)	1.51	1.51
	Average	2.11	13.16
~1 g n=5	Std Dev	0.02	0.13
	RSD (%)	1	1
	Average	2.21	13.81
~0.25 g n=5	Std Dev	0.05	0.31
	RSD (%)	2.21	2.21
	Average	2.23	13.92
~0.5 g n=5	Std Dev	0.04	0.24
	RSD (%)	1.7	1.7
	Average	2.23	13.95
~1 g n=5	Std Dev	0.03	0.21
	RSD (%)	1.48	1.48
	Average	6.38	39.9
~0.25 g n=5	Std Dev	0.07	0.49
	RSD (%)	1.23	1.24
	Average	6.33	39.59
~0.5 g n=5	Std Dev	0.05	0.28
	RSD (%)	0.72	0.72
	Average	6.36	39.73
~1 g n=5	Std Dev	0.03	0.17
	RSD (%)	0.42	0.42
	~0.25 g n=5 ~0.5 g n=5 ~0.25 g n=5 ~0.5 g n=5 ~0.5 g n=5 ~0.5 g n=5	Average	Average 2.16 Std Dev 0.06 RSD (%) 2.63 Average 2.12 -0.5 g

CONCLUSIONS

This work has two objectives:

- Demonstrate improvements in precision when sample mass is increased in a characteristically heterogeneous meat matrix material
- Demonstrate the capability of a combustion nitrogen/protein instrument with a horizontal furnace to analyze macro sample mass (~1 g)

The total nitrogen/protein determined for all of the sample mass groups within the individual meat samples had generally acceptable precision with all of the relative standard deviations (RSD) values being less than ~2.65%. Both the AMR Beef and Cooked Bacon had >60% reduction in RSD (2.36 to 1.00 and 1.24 to 0.49 respectively) when the sample mass was increased from 0.25 to 1 g. The Raw Bacon also had a reduction of ~33% in RSD (2.21 to 1.48) as the sample mass was increased from 0.25 to 1 g. All of the data was within the expected nitrogen /protein ranges for the meat samples and all of the individual meat matrices displayed improved precision as the sample mass was increased with the best precision resulting from the 1 g sample mass groups. All of the samples had less than ~1.50% RSD levels at the 1 g sample mass.

No issues were experienced with physical restrictions for the introduction of the macro meat samples into the furnace, incomplete combustion (soot build up) or ash/residue build-up within the furnace of the macro horizontal combustion instrument. The macro horizontal combustion instrument utilizes large open ceramic boats facilitating the sample weighing and furnace introduction process as well as allowing for ash removal post analysis. The use of 100% oxygen within the horizontal furnace ensures complete combustion of the macro meat samples.

The horizontal macro nitrogen/protein combustion instrument offered to the market by LECO delivers additional benefits to laboratories.

High Throughput and Efficiency

- Rapid analysis time of 4.8 minutes concurrent
- 50-sample instrument autoloader
- Simple sample pretreatment and, handling requirements
- Flexibility in sample mass and nitrogen/protein range

Low Cost/Analysis*

~\$0.70

*cost represents operational costs only calculated using LECO list pricing and average US compressed gas pricing for Helium and Oxygen