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Original Research Article

Evaluation of a rapid protein analyzer as a research tool for lean beef composition: Effects of storage time and freezing

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ABSTRACT

The Sprint Rapid Protein Analyzer (CEM Corporation) has introduced a novel method for protein content determination. Dairy and meat industries are adopting this technology, as it is easily integrated in commercial plants; however, its application as a valid tool for meat research requires further evaluation. Using the Dumas method as a reference, the Sprint Rapid Protein Analyzer was tested to evaluate the accuracy, precision and repeatability of the machine, under a variety of different beef muscle sample storage and handling conditions. The samples analysed using the Sprint Rapid Protein Analyzer showed an average within-day relative standard deviation (RSD) of 0.812% and an average inter-day RSD of 1.39%. For samples stored at 4 °C for 24, 48 or 72 h, the RSD ranged between 0.02 and 4.50%. In samples frozen at −35 and −80 °C for 2, 4, 6, 8, 10 and 12 months, the average RSD compared to fresh samples was 2.89%. Thus, the Sprint method, once properly calibrated over the compositional range encountered, may be suitable for use to determine protein content in lean beef research.

1. Introduction

To quantify the protein content of food products, the most commonly used chemical based methods are the Kjeldahl or the Dumas methods (Moore et al., 2010). Although the Kjeldahl is a highly precise reference method first introduced in the 1880s, it is a time-consuming process that requires trained technical staff, uses hazardous chemicals at high temperatures, and measures total nitrogen instead of protein directly (O'Sullivan et al., 1999; Amamcharla and Metzger, 2010; Moore et al., 2010; Moser and Herman, 2011). The alternative is the Dumas method, which uses combustion to determine protein content via total nitrogen values in a gas and has been used to determine protein levels of beef and other meat sources (King-Brink and Sebranek, 1993; O'Sullivan et al., 1999; Jung et al., 2003; Amamcharla and Metzger, 2010; Moore et al., 2010). In both methods, the average nitrogen from protein is estimated to constitute 16% of the nitrogen in the sample, hence the total nitrogen content is multiplied by a correction factor (6.25 for meat) to arrive at the value for crude protein. Variations in non-protein nitrogen amongst samples (purine and pyrimidine bases, vitamins, amino sugars, alkaloids, compound lipids etc.) may influence the accuracy.

A novel choice is the Sprint Rapid Protein Analyzer (CEM Corp., Matthews, NC, USA). This machine is based upon a dye-binding technology that measures protein content, not nitrogen, in a time and cost-efficient manner (Heller and Sherbon, 1976; Urh, 2008; Moser and Herman, 2011). It uses an iTAG™ dye that has two key properties: an aromatic nature, which provides the orange color responsible for spectral absorbance readings at 483 nm, and an acid group (Urh, 2008; Amamcharla and Metzger, 2010; Moore et al., 2010) that binds to the cationic groups of basic amino acids (Histidine, Arginine, and Lysine) and the amino terminal groups of proteins. As these specific amino acids have a high pKa (Urh, 2008), they interact with the acidic group of the iTAG™ dye, causing the formation of an insoluble dye-protein complex that precipitates out of solution (Amamcharla and Metzger, 2010). This results in the decrease of the dye in solution, resulting in a lower spectrophotometer reading. Therefore, the amount of protein present in a sample is relative to the decrease in the spectral absorbance (Amamcharla and Metzger, 2010). The advantages to

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using this new machine include its non-hazardous iTAG™ solution and waste, a self-contained and mostly self-cleaning machine, its ease of use and the ability for non-technical staff to operate this equipment (Urh, 2008; Amamcharla and Metzger, 2010). The machine's ability to measure protein content, not nitrogen, is also beneficial as it helps to reduce false positives caused by adulteration with non-protein nitrogen or by nitrogen rich additives, such as melamine in milk samples (Urh, 2008; Moore et al., 2010).

These attributes make this methodology a potential research tool for the use of measuring protein content in lean beef. This machine has been previously tested to determine protein content in milk and cream (Amamcharla and Metzger, 2010; Moore et al., 2010); however, little work outside of publications by CEM Corporation have been performed using lean beef as the food substance of interest (Moser and Herman, 2011). Therefore, studies were conducted to validate the use of the Sprint machine as an alternative protein determination method in a research application. To complete this test, the Sprint protein values obtained required confirmation by comparing to the Dumas method, which is a well-established standard with a high capacity for sample reproducibility (King-Brink and Sebranek, 1993; Jung et al., 2003). Furthermore, different storage and freezing times are usually required for meat quality research studies. It is important to understand if modifications in protein structure during storage may have an impact on the ability of the binding agent to measure protein content. Thus, the compilations of the different tests were used to confirm whether the Sprint method for protein content determination may be an effective tool in beef research.

2. Materials and methods

2.1. Meat samples and preparation

All beef samples used in this study were collected from the federally inspected research abattoir (Agriculture & Agri-Food Canada, Lacombe, AB, Canada) which operates under the guidelines of the Canadian Council on Animal Care (1993). Lean beef was taken from the *longissimus lumborum* and *semimembranosus* muscles, from both sides of the carcass and the same initial processing occurred for both the Dumas and Sprint methods. The sample was ground for 10 s, with stirring, using a Blixer® 3 Series D 3 ½ Quart grinder (Robot Coupe USA, Ridgeland, MS, USA). This ensured a homogenous sample mixture that was used in further sampling.

2.2. Instrumentation and analytical conditions – reference method (Dumas)

The instrument used for the Dumas protein determination method was the Crude Nitrogen/Protein Determinator, model CN-2000 (Leco Corp., St. Joseph, MI, USA). This method uses combustion of a dried, de-fatted lean beef sample following the Association of Official Analytical Chemists (AOAC) Official Method (992.15) (AOAC, 2005). This AOAC procedure was modified by using corn gluten, instead of EDTA, as the nitrogen standard. To prepare samples for the Dumas protocol, ground samples were dried at 102 °C for 24 h, and then crushed into a powder with a Grindomix GM200 grinder (Retsch, Haan, Germany) at 8000 rpm for 12 s. A petroleum ether fat extraction was then performed with a Foss Soxtec Extraction Unit 2050 Model 2050 (Foss, Hilleroed, Denmark), following an AOAC Official Method (960.39) (AOAC, 1995). The dried, de-fatted samples were then used in the Leco machine for the Dumas protein determination method.

2.3. Instrumentation and analytical conditions – rapid method (Sprint)

The protocol used for the Sprint rapid protein analyzer followed the method outlined in the AOAC Official Method (2011.04) for Protein in Raw and Processed Meats (AOAC, 2011). To prepare each run, homogenized lean beef (0.3150–0.3300 g) was weighed into a tared Sprint sample cup and then placed on the Sprint turntable. A filter was then added to the turntable and the run began with a homogenizer speed of 25,000 rpm for 90 s. Other equipment settings used for the run included: homogenizer – standard; filter height – normal; delay time – 0 s; settling time – 30 s; mode – standard; prehomogenizer – off. These run conditions were used in the preparation of the standards, as well as the experimental samples.

3. Calibration curve

A modification of the established protocol was required, as the original standard curve had a broad protein percentage range beyond that normally encountered in lean beef and the results were not similar to the Dumas obtained results. Therefore, a new standard curve using homogenized lean beef samples ($n = 10$) with known protein content (wet matter basis) of 18.5–24.5%, as determined using the Dumas method, was employed. The new calibration curve was develop using the internal software installed in the equipment (Sprint). For each reference point, five replicates were obtained and two outliers (>3 times SD from mean) were removed to reduce the variability at each point and strengthen linearity of the standard curve. Slope, intercept and coefficient of determination (R^2) were provided by the software based on the standard values from Leco (provided previous to the analyses) and the internal readings for variation in the iTAG™ dye absorbance.

3.1. Precision (intra-day relative standard deviation; RSD)

To test the precision of the Sprint instrument, homogenized lean beef ($n = 60$) was used to determine the intra-day RSD. Each sample was run in duplicate, and samples were from both sides of the carcass. The average protein value for each sample was determined, along with the intra-day RSD.

3.2. Repeatability (inter-day RSD)

To determine whether different Sprint Paks read protein values consistently over several days, three replicates of the same set of samples ($n = 60$) were analyzed in three consecutive days using a different Sprint Pak per day. This comparison was completed by obtaining the mean protein value, standard deviation and RSD for each sample on all three testing days (with three different Sprint Paks).

3.3. Evaluation of the effect of cold storage on protein measurements

3.3.1. Refrigerated meat

Homogenized lean beef samples ($n = 11$) were either kept fresh or stored in a 4 °C cooler. Fresh samples were processed within the day of sample homogenizing. Samples that were stored in the 4 °C cooler were held for 24, 48, and 72 h, and then analyzed with the Sprint method. Average protein values, in addition to the RSD of each ageing day vs. fresh values, were recorded.

3.4. Frozen meat

To study long term storage effects on protein content determination, samples ($n = 5$) were processed in duplicate for