# Challenges Associated with Determining Protein Content in Potato Products

Yang Zhou, Griffin Pole, Kent Karsjens, John Jordan, Elaine Jobgen
Eurofins Nutrition Analysis Center (ENAC), Des Moines, IA 50321

## Introduction

Protein is a biological marcomolecule consisting of sequenced amino acids. It is an essential nutrient that is required in food labelling. Potato contains rich amount of proteins. The accurate determination of protein in potato is important to assess its nutritional value. However, the current major methods for protein determination face various challenges when applied to a potato matrix. Those challenges are associated with the presence of carbohydrates and reducing sugars, non-protein nitrogen, free amino acids, and the variation of amino acids composition etc. Lowry and BCA protein assays may encounter significant interference from reducing sugars in potato. Combustion or Kjeldahl methods can be biased due to the existence of non-protein nitrogen. Bradford is a classic bioassay method, but its results are subject to the percent of basic and aromatic amino acids in samples. In order to accurately determine the protein content in potato, Eurofins Nutrition Analysis Center explored a methodology based on amino acid composition, including the total and free amino acids. Such a profile can be used to tune the assay parameters of Bradford and combustion (or Kjeldahl) methods to exclude the influence of amino acid composition and the existence of non-protein nitrogen. After the adjustment, the results from Bradford and combustion methods became consistent with each other. They were considered the true value of the protein content in the potato sample tested. This poster presents our methodology with data analysis. It provides tuned Bradford and combustion methods for laboratories to determine the protein content in potato more accurately.

## Methods and Procedures

**Combustion:** 0.5-1.5 g of potato sample is weighed into an analyzing vessel and then placed into the combustion chamber of a protein analyzer. The sample is combusted with oxygen and the gases containing nitrogen oxides are collected until a specified pressure is reached. The gas from combustion is analyzed for nitrogen content. The nitrogen content is determined and converted to protein equivalents using the standard factor 6.25.

**Bradford:** The Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 dye to proteins. When the dye binds to protein, it is converted to a stable unprotonated blue form. This blue protein-dye has an absorbance maximum at 595 nm. In this method, approximately 0.1 g sample is weighed. Next, 5 mL distilled water and 5 mL 1 N Sodium hydroxide are added to the sample. The solution is incubated for 2.5 hours at room temperature. About 1 mL Bradford dye reagent and 20 µL standard or sample are combined in each cuvette and incubated for at least 5 minutes. Bovine Serum Albumin (BSA) is used as standard to plot and calculate the protein concentration in the sample.

**Amino Acid Profile:** Tryptophan (Trp) is released from proteins by alkaline hydrolysis (4.2 M LiOH, 110 °C) under partial vacuum for 22 hours. It is then quantitated by reversed-phase LC and UV detection at 281 nm. Cystine/cysteine (Cys) and methionine (Met), whether in bound or free form, are first oxidized by performic acid to form cysteic acid and methionine sulfone, respectively. The samples are then treated with 6 N HCl for 24 hours at 110 °C to release the monomeric amino acid moiety. This digested aliquot, after further dilution, is then quantitated using cation-exchange liquid chromatography, post-column o-phthalaldehyde (OPA) derivatization, and fluorescence detection. The remaining 16 amino acids are analyzed after hydrolysis with 6 N HCl for 24 hours at 110 °C. This is followed by cation-exchange liquid chromatography. Quantitation is performed after ninhydrin derivatization and UV/Vis detection (440 nm for Pro and Hyp, 570 nm for the other amino acids). For free amino acids, the sample is extracted using 0.1 N HCl. The extracts are filtered and analyzed on the appropriate HPLC system.

**Reference:** Combustion: AOAC 979.09 Protein in Grains. Amino Acids: AOAC 994.12 (modified), AOAC 982.30 (modified), AOAC 988.15 (modified).

## Results

The results are shown below in Table I and Table II. All results were derived from duplicate samples.

| Method | Result (%) |
|---|---|
| Combustion | 6.31 |
| Bradford | 4.01 |
| Total Amino Acids Profile (Added up) | 7.09 |

**Table I: Original Result of Protein Content by Different Methods**

| Amino Acid | Total (%) | Free (%) |
|---|---|---|
| Alanine | 0.247 | 0.015 |
| Arginine | 0.300 | 0.102 |
| Aspartic Acid/Asparagine | 1.567 | 0.203 |
| Cystine | 0.118 | 0.002 |
| Glutamic Acid/glutamine | 1.333 | 0.323 |
| Glycine | 0.271 | 0.004 |
| Histidine | 0.125 | 0.023 |
| Isoleucine | 0.276 | 0.021 |
| Leucine | 0.479 | 0.011 |
| Lysine | 0.468 | 0.033 |
| Methionine | 0.108 | 0.017 |
| Phenylalanine | 0.332 | 0.036 |
| Proline | 0.250 | 0.013 |
| Serine | 0.315 | 0.000 |
| Threonine | 0.271 | 0.000 |
| Tryptophan | 0.091 | 0.018 |
| Tyrosine | 0.178 | 0.021 |
| Valine | 0.365 | 0.053 |
| Total: | 7.090 | 0.893 |

**Table II: Total and Free Amino Acid Profile**

## Discussion

**Bias of combustion method** - Interference from non-protein nitrogen: Potato might contain a certain amount of non-protein nitrogen which may lead to a biased result. Non-protein nitrogen sources include nucleotides, free amino acids, inorganic or organic compounds, etc. The existence of non-protein nitrogen will be incorporated into the combustion method result. Consequently, the result will be higher than the true value of protein in potato.

The nitrogen content from combustion was determined to be 1.01%. The calculated total protein was 6.31% using the factor 6.25. It is larger than the result determined by Bradford. This suggests a large amount of non-protein nitrogen. The free amino acids content was 0.9%, which also indicates the existence of non-protein nitrogen in potato as well.

Alternatively, the conversion factor 6.25 is an estimation of the ratio of nitrogen in proteins. This value varies based on the composition of amino acids. This conversion factor can be accurately determined if amino acid profile is known. Based on the results in Table II, the actual nitrogen conversion factor for this potato would be between 5.73 – 7.51. This range relies on the ratios of Aspartic acid:Asparagine and Glutamic acid:Glutamine in the potato. Current amino acid methods cannot differentiate between them because the hydrolysis converts Asparagine and Glutamine to Aspartic Acid and Glutamic Acid respectively. The factor will be 6.32 if ratios of Aspartic acid: Asparagine and Glutamic acid: Glutamine are both 1:1.

**Bias of Bradford assay** - Bias due to amino acid composition: Development of color in Coomassie dye-based (Bradford) protein assays is associated with the presence of certain basic amino acids (primarily arginine, lysine and histidine) in the protein (Manual, Bradford assay kit).

Our investigation revealed that the content of basic amino acids in potato proteins was significantly different from the BSA standard. Table III below illustrates the differences in basic amino acids composition between the BSA standard and the potato samples we tested.

| Basic Amino Acids As % of protein | Arginine | Lysine | Histidine | Total |
|---|---|---|---|---|
| BSA* | 5.22 | 11.23 | 3.44 | 19.89 |
| Potato proteins** | 4.18 | 6.61 | 1.77 | 12.56 |

**Table III: composition of basic amino acids from BSA and potato proteins**

*:BSA composition data is from: *Amino Acids* (1995) 8: 201-208
*:Potato protein data is from total amino acids profile result of sample tested.

The BSA standard contained approximately 50% more basic amino acids than the potato proteins. Such a difference could lead to a biased result when BSA was used as a standard to create a calibration curve. The same scenario also happens if bovine γ–globulin (BGG) is used as a standard.

**Bias of total amino acids profile** – Inclusion of water (H + OH) in molecular formulas and free amino acids: When forming protein, each amino acid lost an OH from carboxyl acid group and an H atom from amine group. Adding up the percentage individual amino acid does not account for the water molecules released thus will generate a much larger result.

In our case, the total amino acids added up to 7.09%. That result contains bias from two parts: 1). From the molecular weight of water in the amino acid formulas. 2). From free amino acids, which is 0.893% in our sample. This bias can be corrected by removing weight of a water molecule from each amino acid molecular weight, and subtracting the free amino acids. Such an adjustment provides a very close estimation of protein content. This value is more accurate but there might be slight variations due to the protein composition and its synthesis mechanism (N amino acids lost N-1 water).

## Data Analysis

**1). Adjusted result from combustion by Amino Acids profile**
When non-protein nitrogen from free amino acids was excluded:
Combustion: 6.31*(6.20)/7.09=5.52%
However, that result will still have a bias due to other types of non-protein nitrogen.

**2). Adjusted result from Bradford based on Amino Acids composition**
Step 1: The adjusted protein concentration based on composition difference in Table I is:
Result = (4.011%*19.89)/12.56 = 6.35%

Step 2: Exclude free basic amino acids:
Result = 6.35% *(0.893-0.158)/0.893 = 5.23%.

**3). Adjusted protein result directly from Amino Acids profile**
After adjustment, the result of protein is 5.33% by adding up all amino acids (Table IV).

**4). Result summary after adjustment**
In summary, we concluded that the content of protein in this potato sample should be approximately 5.23 – 5.33 %. The combustion result may still include other minor sources of nitrogen.

| Amino Acids (%) | Total | Free | In-Protein | MW | Exclude Water | Adjusted |
|---|---|---|---|---|---|---|
| Alanine | 0.247 | 0.015 | 0.232 | 89 | 71 | 0.185 |
| Arginine | 0.300 | 0.102 | 0.198 | 174 | 156 | 0.177 |
| Aspartic Acid | 1.567 | 0.203 | 1.365 | 133 | 115 | 1.180 |
| Cystine | 0.118 | 0.002 | 0.116 | 121 | 103 | 0.099 |
| Glutamic Acid | 1.333 | 0.323 | 1.010 | 147 | 129 | 0.886 |
| Glycine | 0.271 | 0.004 | 0.267 | 75 | 57 | 0.203 |
| Histidine | 0.125 | 0.023 | 0.103 | 155 | 137 | 0.091 |
| Isoleucine | 0.276 | 0.021 | 0.255 | 131 | 113 | 0.220 |
| Leucine | 0.479 | 0.011 | 0.469 | 131 | 113 | 0.404 |
| Lysine | 0.468 | 0.033 | 0.435 | 146 | 128 | 0.381 |
| Methionine | 0.108 | 0.017 | 0.091 | 149 | 131 | 0.080 |
| Phenylalanine | 0.332 | 0.036 | 0.296 | 165 | 147 | 0.263 |
| Proline | 0.250 | 0.013 | 0.237 | 115 | 97 | 0.199 |
| Serine | 0.315 | 0.000 | 0.315 | 105 | 87 | 0.261 |
| Threonine | 0.271 | 0.000 | 0.271 | 119 | 101 | 0.230 |
| Tryptophan | 0.091 | 0.018 | 0.073 | 204 | 186 | 0.067 |
| Tyrosine | 0.178 | 0.021 | 0.157 | 181 | 163 | 0.141 |
| Valine | 0.365 | 0.053 | 0.313 | 117 | 99 | 0.264 |
| **Total (%)** | **7.090** | **0.893** | **6.198** | | | **5.330** |

**Table IV: Adjusted percentage of amino acids in sample**

| Method | Result (%) |
|---|---|
| Combustion | 5.52 |
| Bradford | 5.23 |
| Total Amino Acids Profile (Calculated) | 5.33 |

**Table V: Adjusted Result of Protein Content by Different Methods**

**5). Estimated non-protein nitrogen by combustion, Bradford and amino acids profile**

Estimated non-protein nitrogen from free amino acids:
Amount = (6.31%-5.52%)/6.25 = 0.126%.
Percentage of total nitrogen = 0.126/1.01% = 12.5 %

Estimated non-protein nitrogen from other sources:
The adjusted content of protein in potato was ~5.3 %, then part of the result of 5.52% was from non-protein nitrogen. Thus ~0.22% of calculated protein was from nucleotides, or inorganic compounds.
Amount = 0.22%/6.25 = 0.0352%.
Percentage of total nitrogen = 0.0352%/1.01% = 3.49%.

Thus, the non-protein nitrogen from free amino acids was 12.5% of the total nitrogen. About 3.49% of the total nitrogen was non-protein nitrogen from nucleotides, organic or inorganic compounds, etc.

## Conclusion

In order to accurately determine protein content in potato or similar matrices, adjustments are recommended for each method:

1). For Combustion method: Choose a customized conversion factor and exclude free amino acids based on the protein profile.
2). For Bradford method: Use a conversion factor to account for the difference in amino acid distribution in the standard and samples. Alternatively, use purified potato proteins as standards for calibration.
3). For Amino Acid profile: Subtract the free amino acids and the weight of water molecule released in condensation.

We consider the Bradford method with adjustment to be the best approach. For more information, please contact Eurofins Nutrition Analysis Center.

For 129th AOAC Meeting & Exposition, Sept 2015, Los Angeles. Contacts: Dr. Yang Zhou - yangzhou@eurofinsus.com