

Protein Digestibility-Corrected Amino Acid Scores (PDCAAS) for Soy Protein Isolates and Concentrate: Criteria for Evaluation

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ABSTRACT: Protein quality, as determined by the PDCAAS method, is a measure of a protein's ability to provide adequate levels of essential amino acids for human needs. PDCAAS is calculated using an amino acid profile and true digestibility of a food protein. Soy protein is recognized as a high quality plant protein, but published PDCAAS values may vary based on the soy protein ingredient as well as the reproducibility and accuracy of the testing methods. Comparison of PDCAAS values for four differently processed soy ingredients, including three isolated soy proteins (ISP) and one soy protein concentrate (SPC), was made using two different laboratories with evaluation of the impact of the reproducibility and accuracy of amino acid profiles. PDCAAS calculations, using amino acid values from one laboratory, yielded a truncated PDCAAS of 1.00 for all four ingredients, while a second laboratory provided statistically significantly lower scores (0.95–1.00). We conclude that analytical method error can be a significant contributor to PDCAAS differences and can be mitigated by the application of amino acid nitrogen recovery correction factors.

KEYWORDS: PDCAAS, soy protein, amino acid, digestibility, nitrogen recovery, protein quality

INTRODUCTION

Interest in plant-based diets is increasing as evidence mounts for their enhanced health benefits and environmental sustainability. However, plant proteins vary in their ability to provide all of the essential amino acids in amounts needed to meet human requirements, with most being recognized as incomplete proteins. Soy protein is the only widely available plant protein that has been shown to be a complete protein based on numerous nitrogen balance studies using isolated soy protein (ISP) or soy protein concentrate (SPC).^{1–6}

The protein digestibility-corrected amino acid score (PDCAAS) is the most widely recognized and approved method for evaluating protein quality of food proteins. PDCAAS is required by the United States Food and Drug Administration (US-FDA) labeling regulations, which were promulgated out of the Nutrition Labeling and Education Act of 1990 (NLEA), when making claims about protein content.⁷ The method, described and recommended for use by the Food and Agriculture Organization/World Health Organization (FAO/WHO) in 1991,⁸ is based on comparing the amino acid profile of a food protein to a reference value for 2–5 year olds, to determine an amino acid score. The amino acid score is corrected through multiplying by the true digestibility of the protein, resulting in a PDCAAS value. On the basis of the rationale of the method, a PDCAAS value of 1.00 or 100% would indicate that a protein provides adequate amounts of all of the essential amino acids, when the protein is fed in nutritionally appropriate amounts, to children age two and above and adults.

PDCAAS values for a variety of plant and animal-based food proteins are shown in Figure 1.^{8–11} While most plant proteins, such as pea protein concentrate and other legumes, have lower PDCAAS values than animal proteins, soy protein has been shown to be comparable to milk, meat, and eggs. Although the PDCAAS method has been in use since the early 1990s, there are limited published PDCAAS data for soy protein. PDCAAS values have been reported for ISP ranging from 0.92 to 1.00, and 0.99

for SPC,^{8,12} while a generic reference to soy reported a value of 0.91.¹³ Additionally, little has been published on the impact of the accuracy of amino acid test results on PDCAAS values. Therefore, there is a need for the generation of accurate PDCAAS values for soy ingredients to substantiate the value of soy protein as a high-quality protein and allow for an accurate declaration of protein content in food labeling. The current study was conducted first to compare the protein quality, as measured by PDCAAS, of differently processed commercially available soy ingredients and second to evaluate the impact of methodology and associated variability of amino acid results on the PDCAAS determination. Further, we determined if it was possible to correct for inherent methodological errors in amino acid measurement to improve the accuracy and consistency of the PDCAAS values.

MATERIALS AND METHODS

Material Selection and Preparation. Four ingredients, manufactured by Solae, LLC (St. Louis, MO), were selected to include soy protein ingredients that have been processed to have different functional applications. These included three isolated soy proteins and one soy protein concentrate. Samples of each product were procured from three different, nonconsecutive, production lots, which were then composited, subsampled, and submitted for proximate analyses (protein, fat, moisture, crude fiber, and ash) and complete amino acid profile to two different independent laboratories (identified as laboratories A and B). Both laboratories offer these analyses as fee-for-service and are experienced in amino acid analyses. Blind duplicate samples of each composited product were submitted to each lab. Additionally, a subsample of each composited product was submitted to a third laboratory, experienced in this measure, for the determination of true digestibility.

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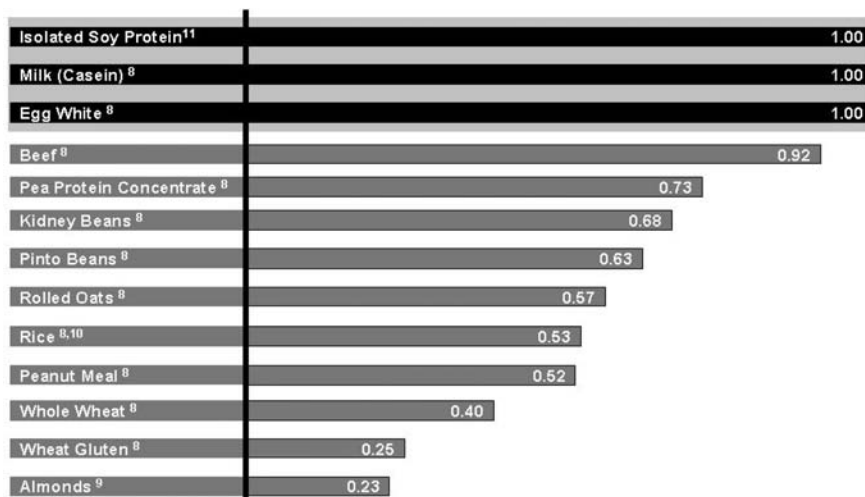


Figure 1. PDCAAS values of selected foods. PDCAAS values from published sources or calculated using publicly available amino acid and digestibility values. A score of 1.00 is the highest attainable score and is based on the amino acid reference pattern for 2–5 year olds.⁸

Protein Analysis. Both laboratories A and B analyzed protein by Kjeldahl with laboratory A using ISO 5983-1¹⁴ and laboratory B using the AOAC Method 988.05.¹⁵ Protein (%) was calculated using the commonly recognized nitrogen conversion factor of 6.25 (% protein = % nitrogen \times 6.25).^{16–18}

Amino Acid Profile Analysis. Both laboratories employed the classical amino acid methods of Moore and Stein¹⁹ (lab A²⁰ and lab B AOAC Method 994.12¹⁵). For sample hydrolysis, lab A utilized a 23 h 6 N HCl acid reflux at 110 °C with nitrogen blanketing to remove oxygen. Lab B applied a vacuum to the sample using repeated freezing and thawing steps to remove oxygen, sealed the tube, and then hydrolyzed the sample in 6 N HCl at 110 °C for 20 h.

Following hydrolysis, both laboratories employed ion exchange chromatography with post column ninhydrin derivatization for detection. For the determination of cysteine and methionine, both laboratories used a performic acid oxidation step according to Schram and Moore.²¹ Cystine and cysteine were oxidized to cysteic acid, and methionine was oxidized to methionine sulfone by treating the sample with performic acid solution for 16 h at 0 °C. Following oxidation, the sample was then hydrolyzed with 6 N HCl acid solution for 18 h at 110 °C. Cysteic acid and methionine sulfone were separated on an ion-exchange column using post-column ninhydrin derivatization and detection as described by lab B and cited as AOAC Method 985.28.¹⁵

Both laboratories used a separate analysis via an alkaline sodium hydrolysis method for the determination of tryptophan followed by ion exchange chromatography. Lab B cited the method of H. Sato²² and AOAC Method 988.15.¹⁵

Amino acid results were converted to g/100 g protein using Kjeldahl protein (6.25 conversion basis) values obtained from both laboratories. The raw data provided from both laboratories were not corrected for amino acid recoveries as received. The total amino acid nitrogen recovery (N_R) is described as the sum of each amino acid (AA) on a protein basis times the nitrogen content in each amino acid (N_{AA}) divided by 16 and is illustrated as follows:

$$\% \text{ nitrogen recovery}(N_R) = \left(\sum_{AA=1-18} \text{gAA}/16\text{gN} \cdot N_{AA} \right) / 16$$

This was calculated for all samples.

Determination of True Digestibility. Digestibility testing was conducted by Eurofins Product Safety Laboratories (Dayton, NJ) using the in vivo rat assay per the FAO/WHO protocol,⁸ in compliance with all US government regulations and industry standards for animal welfare

as they apply to the testing conducted. Sprague–Dawley derived albino white rats (bodyweight 55–66 g) were fed either a diet made with a test protein, casein control, or protein-free control (4 rats per group). Each rat was provided 15 g per day of their respective diet (on a dry weight basis) for a total of 9 days. Feces were collected daily for the last 5 days of the study and at the end of the study composited, dried, and analyzed for nitrogen content. True digestibility (TD), expressed as %, was calculated as described in the FAO/WHO protocol:⁸

$$TD = \frac{I - (F - F_k)}{I} \times 100$$

where I = intake of dietary nitrogen (g) for test or casein control groups; F = fecal nitrogen (g) from the test or casein control groups; F_k = metabolic (endogenous) fecal nitrogen from the protein-free group. It is calculated as follows:

$$F_k = \text{total diet consumed by the test group (g)} \\ \times \frac{\text{mg of fecal nitrogen for the protein-free group}}{\text{g of diet consumed by the protein-free group}}$$

PDCAAS Calculation. PDCAAS was calculated using the FAO/WHO prescribed formula:⁸

$$\text{PDCAAS} = \text{amino acid score (of the most limiting AA)} \times \text{true digestibility}$$

$$\text{amino acid score (AAS)} = \frac{\text{amino acid content of test protein}}{\text{reference AA pattern}}$$

where reference AA pattern is the amino acid requirement for a preschool child (2–5 years). Values greater than 1.00 were truncated to 1.00.

Statistical Analysis. One way analysis of variance (ANOVA), using Minitab v15.1.3, was run to compare differences between laboratories and products for protein, amino acid nitrogen recovery, and PDCAAS with statistical significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Results of proximate testing, amino acid testing, and amino acid recovery calculations are presented in Table 1. The protein results were consistent with the Codex definitions for these ingredients which specify that ISPs have a dry basis protein $\geq 90\%$ and SPC $\geq 70\%$.¹⁷ However, discrepancies were noted between laboratories for protein, moisture, certain amino acids,

Table 1. Amino Acid and Proximate Composition of Four Commercially Available Soy Ingredients^a

| analysis | isolated soy protein 1 ^a | | isolated soy protein 2 ^a | | isolated soy protein 3 ^a | | soy protein concentrate ^a | |
|--------------------|-------------------------------------|--------------------|-------------------------------------|-------|-------------------------------------|-------|--------------------------------------|-------|
| | lab A ^b | lab B ^b | lab A | lab B | lab A | lab B | lab A | lab B |
| protein, as is | 86.5 | 87.5 | 85.9 | 87.0 | 85.7 | 86.7 | 74.6 | 74.7 |
| protein, dry basis | 90.8 | 92.5 | 90.3 | 91.9 | 90.4 | 92.1 | 78.4 | 78.9 |
| moisture | 4.8 | 5.4 | 4.9 | 5.4 | 5.3 | 5.8 | 4.8 | 5.3 |
| fat | 4.1 | 5.2 | 4.4 | 5.5 | 4.3 | 5.1 | 1.9 | 3.0 |
| ash | 4.0 | 3.9 | 4.4 | 4.3 | 5.2 | 5.0 | 7.3 | 6.6 |
| fiber | <1.0 | 0.7 | <1.0 | 0.2 | <1.0 | <0.2 | <1.0 | 0 |
| alanine | 4.25 | 4.18 | 4.26 | 4.16 | 4.27 | 4.18 | 4.03 | 3.97 |
| arginine | 8.44 | 7.31 | 8.56 | 7.36 | 8.41 | 7.31 | 8.58 | 7.45 |
| aspartic acid | 12.37 | 11.26 | 12.52 | 11.26 | 12.38 | 11.23 | 12.94 | 11.76 |
| cysteine | 1.31 | 1.19 | 1.33 | 1.21 | 1.28 | 1.22 | 1.56 | 1.41 |
| glutamic acid | 19.42 | 18.86 | 19.63 | 18.98 | 19.61 | 18.97 | 20.24 | 19.83 |
| glycine | 4.20 | 3.95 | 4.23 | 3.97 | 4.23 | 3.96 | 4.22 | 4.00 |
| histidine | 2.60 | 2.29 | 2.59 | 2.31 | 2.66 | 2.30 | 2.64 | 2.34 |
| isoleucine | 4.89 | 4.51 | 4.88 | 4.47 | 4.93 | 4.46 | 4.66 | 4.32 |
| leucine | 8.15 | 7.88 | 8.21 | 7.81 | 8.17 | 7.81 | 7.71 | 7.43 |
| lysine | 6.30 | 6.05 | 6.46 | 6.06 | 6.42 | 6.07 | 6.32 | 6.12 |
| methionine | 1.28 | 1.24 | 1.30 | 1.27 | 1.33 | 1.29 | 1.31 | 1.29 |
| phenylalanine | 5.19 | 5.02 | 5.28 | 4.94 | 5.24 | 4.97 | 5.08 | 4.86 |
| proline | 5.24 | 5.26 | 5.18 | 5.22 | 5.32 | 5.24 | 5.23 | 5.32 |
| serine | 5.09 | 4.87 | 5.11 | 4.88 | 5.08 | 4.85 | 5.13 | 4.94 |
| threonine | 3.73 | 3.56 | 3.75 | 3.57 | 3.76 | 3.59 | 3.69 | 3.56 |
| tryptophan | 1.34 | 1.17 | 1.39 | 1.20 | 1.39 | 1.15 | 1.33 | 1.15 |
| tyrosine | 3.88 | 3.71 | 3.94 | 3.69 | 3.93 | 3.68 | 3.76 | 3.56 |
| valine | 5.10 | 4.94 | 5.08 | 4.92 | 5.13 | 4.92 | 4.77 | 4.66 |
| % N recovery | 88.63 | 82.82 | 89.47 | 82.95 | 89.21 | 82.82 | 89.07 | 83.54 |

^aISP 1 is a composite of production lots M340027555, P440019641, and G010019600. ISP 2 is a composite of production lots M330023493, M330023707, and P220013698. ISP 3 is a composite of production lots M350024215, M350024950, and M350025617. SPC is a composite of production lots M320003212, M320003255, and M320003297. ^bValues are expressed as mean value of 2 replicates. Proximates are g/100g product; AA are g/100g protein.

Table 2. PDCAAS Calculations for Four Commercially Available Soy Ingredients

| essential amino acid | FAO/WHO amino acid reference pattern ^a | isolated soy protein 1 | | isolated soy protein 2 | | isolated soy protein 3 | | soy protein concentrate | |
|--------------------------|---------------------------------------------------|------------------------|--------------------|------------------------|-----------|------------------------|-----------|-------------------------|-------|
| | | lab A ^b | lab B ^b | lab A | lab B | lab A | lab B | lab B | lab B |
| histidine | 19 | 26.0 | 22.9 | 25.9 | 23.1 | 26.6 | 23.0 | 26.4 | 23.4 |
| isoleucine | 28 | 48.9 | 45.1 | 48.8 | 44.7 | 49.3 | 44.6 | 46.6 | 43.2 |
| leucine | 66 | 81.5 | 78.8 | 82.1 | 78.1 | 81.7 | 78.1 | 77.1 | 74.3 |
| lysine | 58 | 63.0 | 60.5 | 64.6 | 60.6 | 64.2 | 60.7 | 63.2 | 61.2 |
| methionine + cysteine | 25 | 26.0 | 24.3 | 26.3 | 24.7 | 26.1 | 25.0 | 28.8 | 26.9 |
| phenylalanine + tyrosine | 63 | 90.8 | 87.3 | 92.1 | 86.3 | 91.7 | 86.5 | 88.4 | 84.1 |
| threonine | 34 | 37.3 | 35.6 | 37.5 | 35.7 | 37.6 | 35.9 | 36.9 | 35.6 |
| tryptophan | 11 | 13.4 | 11.7 | 13.9 | 12.0 | 13.9 | 11.5 | 13.3 | 11.5 |
| valine | 35 | 51.0 | 49.4 | 50.8 | 49.2 | 51.3 | 49.2 | 47.7 | 46.6 |
| LEAA ^c | | MET + CYS | MET + CYS | MET + CYS | MET + CYS | MET + CYS | MET + CYS | THR | THR |
| AAS ^d | | 1.04 | 0.97 | 1.05 | 0.99 | 1.04 | 1.00 | 1.09 | 1.05 |
| true digestibility (%) | | 97.88 | | 96.44 | | 97.50 | | 96.97 | |
| PDCAAS, untruncated | | 1.02 | 0.95 | 1.02 | 0.95 | 1.02 | 0.98 | 1.05 | 1.02 |
| PDCAAS, truncated | | 1.00 | 0.95 | 1.00 | 0.95 | 1.00 | 0.98 | 1.00 | 1.00 |

^aRecommended essential amino acid pattern for a pre-school child (2–5 years, mg/g protein); FAO/WHO, 1991. ^bAA values are expressed as the mean value of 2 replicates, in mg/g protein. ^cLimiting essential amino acid. ^dAmino acid score = amino acid content of test protein/ref AA pattern.

and amino acid recoveries, which are addressed in greater detail in a later discussion.

Table 2 compares the essential amino acid profiles to the FAO/WHO reference pattern for 2–5 year olds, as is specified in the PDCAAS methodology.⁸ Digestibility results and PDCAAS calculations are also included in Table 2. Again, discrepancies were noted between laboratories for PDCAAS results, which was not unexpected due to the differences already noted for protein, amino acids, and nitrogen recovery. This is addressed in greater detail in a later discussion. Digestibility values were 97.3% (mean value for ISP) and 97.0% (SPC), comparable to results reported in the literature of 98% and 95%, respectively, and similar to the digestibility of animal proteins.⁸ Figure 2 shows a comparison of the amino acid profile of ISP to reference patterns for all age groups of two and above,⁸ illustrating that ISP meets or exceeds the amino acid requirements for children aged two and above and adults throughout the life cycle.

On the basis of the observed discrepancies noted between laboratories for protein, moisture, amino acid profiles, amino acid recoveries, and calculated PDCAAS noted previously, our first

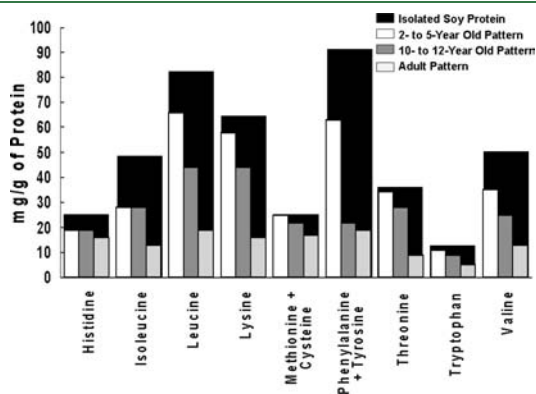


Figure 2. Comparison of the amino acid profile of isolated soy protein (ISP) to FAO/WHO reference patterns for children and adults.⁸ Values for ISP are mean values for three products (lab A).

Table 3. Comparison of Protein, Moisture, and Nitrogen Recovery Values for Isolated Soy Protein Set and Soy Protein Concentrate

| analysis | isolated soy protein set | | soy protein concentrate | |
|----------------------------------|--------------------------|--------------------|-------------------------|--------------------|
| | lab A | lab B | lab A | lab B |
| protein, as is (%) | 86.0 | 87.1 ^a | 74.6 | 74.7 |
| protein, dry basis (%) | 90.5 | 92.2 ^a | 78.4 | 78.9 |
| moisture (%) | 4.98 | 5.54 ^a | 4.80 | 5.33 ^b |
| amino acid nitrogen recovery (%) | 89.11 | 82.86 ^a | 89.07 | 83.54 ^b |

^a $P < 0.01$, lab B vs lab A for the ISP set. ^b $P < 0.01$, lab B vs lab A for SPC.

Table 4. PDCAAS Normalized Using Protein and Amino Acid Recovery Factors

| product | unadjusted PDCAAS | | protein-normalized PDCAAS | total AA recovery-normalized PDCAAS | AA hydrolysis recovery-normalized PDCAAS |
|-----------------------------|-------------------|--------------------|---------------------------|-------------------------------------|------------------------------------------|
| | lab A | lab B | lab B | lab B | lab B |
| isolated soy protein (mean) | 1.016 | 0.961 ^a | 0.978 ^a | 1.033 ^b | 1.015 |
| soy protein concentrate | 1.053 | 1.002 | na | 1.068 | 1.058 |

^a $P < 0.01$, compared to lab A unadjusted PDCAAS. ^b $P = 0.03$, compared to lab A unadjusted PDCAAS.

objective was to determine the cause of the differences. Our second objective was to determine if a method of normalization could be employed to correct for these differences.

The sample set consisted of three ISPs and one SPC. The data in Tables 1 and 2 show that within a lab the ISPs were not significantly different from each other for protein, amino acid profile, or PDCAAS. However, since the product families (ISP vs SPC) are significantly different, it warranted independent evaluation. A comparison of protein, moisture, and amino acid recovery values for the ISP set and the SPC sample are shown in Table 3. For the ISP set, both protein as-is and moisture were statistically higher for lab B vs lab A (protein bias +1.05, $P < 0.01$; moisture bias +0.56, $P < 0.01$), which led us to suspect that systematic bias was a factor. Dry basis protein comparisons observed identical trends and even larger biases (protein bias lab B +1.65, $P < 0.01$). For SPC, there was no statistical difference between laboratories for either as-is protein ($P = 0.81$) or dry basis protein ($P = 0.10$) at the $P < 0.05$ level. PDCAAS values (see Table 4) for the ISP set were statistically lower for lab B vs lab A (0.96 vs 1.02, $P < 0.01$). No statistically significant difference was seen for the SPC sample between laboratories ($P = 0.09$).

The next aspect of between lab comparison that was investigated was amino acid nitrogen recovery. The amino acid hydrolysis methods are a compromise between amino acid release and destruction. Labile amino acids are partially or fully destroyed, while some amino acids are resistant to hydrolysis. The most labile amino acids, namely, cysteine, methionine, and tryptophan, are addressed using separate methodologies. Internally generated amino acid data on soy ingredients has resulted in very good estimates of average expected recoveries (93%). Official AOAC methods cited and used above have suggested that it can be appropriate to adjust recoveries to 95% assuming that a window of acceptable recovery (86–105%) is first achieved. There were observed clearly statistically significant differences in recoveries between lab A and lab B. These differences were independent of the product type and very consistent (standard deviation = 0.55). Since protein measurements are inherent to amino acid (AA) concentrations (g AA/100 g protein), we would expect protein differences to confound the recovery from different laboratories. For the ISPs, the average recoveries from lab A vs lab B were 89.11% and 82.86%, a difference of 6.25% ($P < 0.01$). For the SPC sample, the average recovery for lab A was 89.07% vs 83.54% for lab B or a difference of 5.53% ($P < 0.01$) (Table 3).

These findings suggest that the application of normalization factors to the protein and nitrogen recoveries could correct errant PDCAAS results. Since the recoveries from lab B were significantly lower than that from lab A, and lower than what would be expected, the decision was made to correct the recoveries to lab A. AOAC has cited 95% as acceptable recovery,¹⁶ and analysis conducted internally for soy ingredients has resulted in a long-term average value of 93% recovery. Although it could be justified to adjust recoveries to this higher range (93–95%), we chose to adjust only to the value obtained for lab A (89.11%).

Protein determinations and calculated nitrogen recovery values from amino acid hydrolysis methods are both technique dependent and include multiple sources of random and systematic errors. For the purpose of PDCAAS determination, protein values are used to convert amino acid values from an as-is product basis to a protein basis as follows:

$$\begin{aligned} \text{gAA}/100 \text{ g product as-is}/\text{protein as-is} \\ = \text{gAA}/100 \text{ g protein dry basis} \end{aligned}$$

Assuming a nitrogen (N) conversion factor of 6.25, this is sometimes described as gAA/16gN. Therefore, protein values will impact protein basis amino acid values, the resulting nitrogen recovery calculations, and PDCAAS values derived from these results. Amino acid values are also impacted by the hydrolysis conditions as discussed previously. Nitrogen recovery therefore is a function of both the protein determination and hydrolysis. Normalization factors can be applied to correct for protein and total nitrogen recovery (includes effects from both protein and digestion) or effects from only the hydrolysis.

For the ISP set, dry basis protein values from lab B were 1.65% higher than that from lab A. In addition, nitrogen recoveries from lab B were 6.25% lower. Since the work was completed at two different laboratories, analyzed at different times, the data must be expressed on a dry basis to account for moisture differences. Note that dry basis protein values are also a function of the oven moisture, which could also contribute to the between lab differences. Since this study was not designed to investigate oven moisture methods independent of protein, this potential source of error was included in the dry basis protein measurement. It is usually not possible, unless the errors are obvious, to know which protein values are more accurate; however, errors that result in artificially high protein values will result in nitrogen recoveries that are artificially low. Since the nitrogen recoveries from lab B were unexpectedly low, outside of normal tolerance as discussed previously, and protein determination values were also statistically higher, as well as being outside of normal expected tolerances for between lab comparisons, (>1% based on internal collaborative data), a protein normalization factor (NF_P) for nitrogen recovery was calculated for the ISP set. To test the hypothesis that protein was a significant contributor to the nitrogen recovery differences, a normalization factor for protein was calculated as follows:

$$\begin{aligned} NF_P = \text{mean dry basis protein for lab B}/\text{mean dry basis protein for lab A} \\ = 92.16/90.51 = 1.018 \end{aligned}$$

The factor of 1.018 applied to the nitrogen recoveries is mathematically equivalent to the application of the inverse ($1/1.018 = 0.9823$) to the protein values. We choose to apply these directly to the recoveries, however, to simplify the steps and reduce rounding errors. The application of the protein normalization factor only accounted for 25% of the total nitrogen recovery bias between the two laboratories. A significant bias for lab B still existed (bias for lab B = -4.7% , $P < 0.05$). Furthermore, it also did not rectify the discrepancy in PDCAAS values between laboratories (see Table 4). This suggested that there were other factors that accounted for the differences in PDCAAS values, and additional examination and possible correction were needed. A protein normalization factor was not applied to the SPC sample since protein results were not significantly different between laboratories.

The next hypothesis for testing was simply the application of a normalization factor to account for the total nitrogen recovery

differences, which included protein, moisture, and hydrolysis effects. As stated earlier, the total amino acid recovery (N_R) is calculated as follows:

$$\% \text{ nitrogen recovery } (N_R) = \left(\sum_{AA=1-18} \text{gAA}/16\text{gN} \cdot N_{AA} \right) / 16$$

Specifically, the total normalization factor (NF_T) to correct for nitrogen recovery was calculated as follows for the ISP set:

$$\begin{aligned} NF_T = N_R \text{ mean of lab A}/N_R \text{ mean of lab B} \\ = 89.11/82.86 = 1.075 \end{aligned}$$

For the SPC sample, this was also calculated as follows:

$$\begin{aligned} NF_T = N_R \text{ mean of lab A}/N_R \text{ mean of lab B} \\ = 89.07/83.54 = 1.066 \end{aligned}$$

These normalization factors were then applied to the amino acid values from lab B to correct recoveries to match lab A, and PDCAAS values were then recalculated. For the ISP set, this resulted in a possible overcorrection as lab B was now statistically higher in PDCAAS (Table 4). Although statistically significant, lab B was only 0.017 higher in PDCAAS, which was of no practical significance.

For the SPC sample, the application of the total nitrogen recovery normalization factor increased the PDCAAS values in an incrementally similar fashion for lab B, but there were no statistically significant differences between lab A and B in all uncorrected or corrected cases for PDCAAS. The lack of statistical significance in part is due to the low sample number ($n = 2$).

Because of a possible overcorrection for the ISP set, the next hypothesis investigated a third nitrogen recovery normalization option. This option considered only the impact due to hydrolysis, independent of protein. This factor (NF_H) was determined as described below for the ISP set:

$$NF_H = NF_T/NF_P = 1.075/1.018 = 1.056$$

When this factor was applied to the ISP set from lab B, it resulted in no statistically significant difference in PDCAAS between labs A and B (lab B was 0.001 lower). Even though the difference in PDCAAS values for the SPC sample was not statistically significant before correction, the correction also resulted in numerically closer PDCAAS values for the SPC product as well (Table 4).

A thorough investigation of future data sets will require testing of all three hypotheses to obtain the root cause of the differences. The distribution of analytical errors as addressed is multifaceted and will be different for different data sets. The simplest and most practical approach, however, would be the correction based on total nitrogen recovery. This evaluation determined that the largest source of analytical error is the amino acid hydrolysis. This is also expected to be the case for future data sets. Fundamentally, there should be no reason why the NF_H normalization factor due to hydrolysis only is a better normalization factor than alignment of the data sets based on total nitrogen recovery since protein values are confounded with and impact recovery. In reality, amino acid recovery is a function of the recovery of three hydrolysis methods including alkaline hydrolysis for tryptophan and performic acid hydrolysis for the sulfur amino acids. This adds some additional uncertainty to the total nitrogen recovery correction that may in part explain why the total nitrogen recovery was not the statistical best fit with this particular data set.

We conclude that nitrogen recovery is a critical quality measure of amino acid analysis and will have a significant impact on

PDCAAS values. This can be mitigated with the application of amino acid nitrogen recovery factors. Application of these factors was able to explain the differences in PDCAAS values between laboratories, and we recommend that all PDCAAS determinations should take nitrogen recovery into account to correct for analytical method error. This is especially useful in cases where *a priori* knowledge of products and processes are known not to specifically target or destroy individual amino acids which would impact recovery. Even though literature values and official methodology often cite nitrogen recovery or amino acid data is provided that can be used to calculate recovery, it is also helpful to have a repeatable history of amino acid recovery values on like or similar products or ingredients from a validated laboratory for comparison. Thus, laboratories with extensive experience in amino acid testing for specific products could develop their own product-specific nitrogen recovery factors, based on their historic data. If a laboratory performs less frequent testing for a specific product or ingredient, they could employ a nitrogen recovery factor based on the values cited in the literature or official methods, e.g., 95% per AOAC method.¹⁶

Normalized data indicate that the four ingredients evaluated, three different ISPs and one SPC, have a truncated PDCAAS of 1.00, supporting the position of soy protein as a high quality protein, comparable to meat, egg, and dairy proteins.

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