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# Evaluating Soybean Meal Quality Using Near-Infrared Reflectance Spectroscopy

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# Evaluating Soybean Meal Quality Using Near-Infrared Reflectance Spectroscopy

## Abstract

The objective of this study was to establish a range of soybean meal quality to evaluate the correlations between official analytical methods and near-infrared reflectance spectroscopy (NIRS). Crushed soybean white flakes (Mark Hershey Farms, Lebanon, PA) exposed to mechanical oil extraction, but not heat processing, were used in this experiment. Ground samples (500 g) were put into cotton bags and autoclaved at 262°F for 0, 5, 10, 15, 30, 45, and 60 min at 29 PSI. This was done to simulate varying degrees of heat processing. A total of 2 samples per treatment were autoclaved in 3 separate blocks. The duplicate samples were divided and analyzed using NIRS and official analytical analysis (wet chemistry). Crude protein (CP), total lysine (Lys), Lys:CP, available Lys, available Lys:total Lys, protein solubility in potassium hydroxide (KOH), trypsin inhibitor activity (TIA), urease activity index (UAI), individual amino acids (AA), and total AA were analyzed to determine the degree of processing using official analytical methods. The correlation coefficient (R) and coefficient determination ($r^2$) between NIRS and official analytical methods were established for CP, total Lys, available/reactive Lys, Lys:CP and available/reactive Lys:total Lys. Data were analyzed using the SAS (v. 9.4, SAS Institute Inc., Cary, NC) GLIMMIX procedure and the CORR procedure to determine the degree of association of NIRS and official analytical analysis. When measured using official analytical methods, CP, total AA, Ala, Asp, Glu, Gly, Iso, Leu, and Val decreased (linear, $P < 0.05$), whereas available/reactive Lys:total Lys, Lys:CP, available Lys, KOH, trypsin inhibitor, urease, Lys, and Cys decreased (quadratic, $P < 0.05$) with increasing exposure time to the autoclave. There was a positive correlation between official analytical and NIRS results for CP, Lys:CP, available Lys:total Lys, total AA, Ala, Cys, Lys, and a negative correlation for Thr. A linear model was best fit ($P = 0.011$, $r^2 = 0.489$) to predict CP using NIRS. A quadratic model was best fit to use NIRS total Lys ($P = 0.011$, $r^2 = 0.969$), reactive Lys ($P = 0.001$, $r^2 = 0.988$), and their ratio ($P = 0.001$, $r^2 = 0.981$) to predict official analytical results. In conclusion, increasing soybean autoclave exposure time decreased soybean meal quality as measured by crude protein, total Lys, Lys:CP, available Lys, available Lys:total Lys, KOH solubility total AA, and additional AA. In addition, regression models were successful at using NIRS for Lys, reactive Lys, Lys:CP, and reactive Lys:total Lys to predict official analytical results.

## Keywords

soybeans, soybean meal, heat processing, NIR, lysine, available lysine

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# Evaluating Soybean Meal Quality Using Near-Infrared Reflectance Spectroscopy

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## Summary

The objective of this study was to establish a range of soybean meal quality to evaluate the correlations between official analytical methods and near-infrared reflectance spectroscopy (NIRS). Crushed soybean white flakes (Mark Hershey Farms, Lebanon, PA) exposed to mechanical oil extraction, but not heat processing, were used in this experiment. Ground samples (500 g) were put into cotton bags and autoclaved at 262°F for 0, 5, 10, 15, 30, 45, and 60 min at 29 PSI. This was done to simulate varying degrees of heat processing. A total of 2 samples per treatment were autoclaved in 3 separate blocks. The duplicate samples were divided and analyzed using NIRS and official analytical analysis (wet chemistry). Crude protein (CP), total lysine (Lys), Lys:CP, available Lys, available Lys:total Lys, protein solubility in potassium hydroxide (KOH), trypsin inhibitor activity (TIA), urease activity index (UAI), individual amino acids (AA), and total AA were analyzed to determine the degree of processing using official analytical methods. The correlation coefficient (R) and coefficient determination ($r^2$) between NIRS and official analytical methods were established for CP, total Lys, available/reactive Lys, Lys:CP and available/reactive Lys:total Lys. Data were analyzed using the SAS (v. 9.4, SAS Institute Inc., Cary, NC) GLIMMIX procedure and the CORR procedure to determine the degree of association of NIRS and official analytical analysis. When measured using official analytical methods, CP, total AA, Ala, Asp, Glu, Gly, Iso, Leu, and Val decreased (linear, $P < 0.05$), whereas available/reactive Lys:total Lys, Lys:CP, available Lys, KOH, trypsin inhibitor, urease, Lys, and Cys decreased (quadratic, $P < 0.05$) with increasing exposure time to the autoclave. There was a positive correlation between official analytical and NIRS results for CP, Lys:CP, available Lys:total Lys, total AA, Ala, Cys, Lys, and a negative correlation for Thr. A linear model was best fit ($P = 0.011$, $r^2 = 0.489$) to predict CP using NIRS. A quadratic model was best fit to use NIRS total Lys ($P = 0.011$, $r^2 = 0.969$), reactive Lys ($P = 0.001$, $r^2 = 0.988$), and their ratio ($P = 0.001$, $r^2 = 0.981$) to predict official analytical results. In conclusion, increasing soybean autoclave exposure time decreased soybean meal quality as measured by crude protein, total Lys, Lys:CP, available Lys, available Lys:total Lys, KOH solubility total AA, and additional AA. In addition, regression models were successful at using NIRS for Lys, reactive Lys, Lys:CP, and reactive Lys:total Lys to predict official analytical results.

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## Introduction

Soybeans are the most abundant oilseed worldwide that provide by-products, including soybean meal and oil. After solvent extraction, soybean meal is further processed by heating to destroy antinutritional factors. However, there can be negative effects of over-processing, such as the Maillard browning reaction and amino acid (AA) digestibility loss. In the Maillard reaction, a free amino acid—most commonly Lys—will bind to a reducing sugar, therefore browning the soybean meal. Quality of soybean meal is of the utmost importance when considered for diet formulation in swine diets. Over-processing or under-processing soybean meal can impact the nutritional value, and ultimately animal performance. Near-infrared reflectance spectroscopy (NIRS) can serve as a tool for a nutritionist to save time and money, compared to official analytical analysis to determine soybean meal quality for formulation. Therefore, the objective of this study was to create a gradient of soybean meal quality to evaluate the correlations between official analytical methods and NIRS when measuring the protein quality of soybean meal.

## Procedures

A total of 900 kg of soybean white flakes were collected from a soybean crush plant (Mark Hershey Farms, Lebanon, PA). The soybean white flakes were defined as the soybean after oil extraction but prior to the heat processing step. Soybean white flakes were ground using a coffee grinder, and then two 1,000 g samples were autoclaved at 262.4°F for 0, 5, 10, 15, 30, 45, and 60 min. A total of 2 samples per treatment were autoclaved in 3 blocks to provide 3 replications per treatment and 6 observational units per treatment. Samples were split for official analytical methods and NIRS analysis. Treatments were randomized within block to ensure no effects of time or autoclave order. All treatments within block were run in the same week as the first sample of the block. Samples were collected from each treatment and analyzed for total AA, available Lys, UAI, TIA, and protein solubility KOH.

The autoclave initiation consisted of a 15 min warm-up to bring the chamber temperature and pressure to 262.4°F and 29 PSI, respectively. For the sterilizing stage, the chamber temperature and pressure remained as required (either 0, 15, 30, 45 or 60, and 29 PSI). The samples were cooled for 5 min at 230°F and 2 PSI before discharge from the chamber.

### *Sample Analysis*

Official analytical methods were performed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri. Complete AA profiles were analyzed according to AOAC[3] Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006. Available lysine was analyzed using the fluorodinitrobenzene (FDNB) method, according to AOAC official method 975.44. Protein solubility using the KOH method was determined according to Parsons et al.[4] Trypsin inhibitor activity was measured according to AACC official method 22-40, 2006. Urease activity was measured according to AACC

[3] Association of Official Analytical Chemists. 2000. Official Methods of Analysis of the AOAC. 17th edition, Association of Official analytical Chemists.
[4] Parsons, C. M., K. Hashimoto; K. J. Wedekind, D. H. Baker, 1991. Soybean protein solubility in potassium hydroxide: an *in vitro* test of *in vivo* protein quality. J. Anim. Sci., 69 (7): 2918-2924.

International Method 22-90.01. Once treatments were analyzed, data were used to validate NIRS equations using a NIRS DS2500 (FOSS, Eden Prairie, MN). The estimates measured using NIRS were CP, total AA, and available lysine.

## Statistical Analysis

Data were analyzed as a randomized complete block design using the GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC) with soybean sample as the experimental unit, autoclave time as a fixed effect and period as a blocking factor. Orthogonal contrasts were used to evaluate means. The coefficients for the unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS. Least square means were calculated for each independent variable. Correlation analysis was performed to determine the degree of association between official analytical and NIRS results. Linear and/or quadratic regression was used to develop models for predicting official analytical total and available lysine, and Lys:CP using NIRS estimates. Results were considered significant if $P \leq 0.05$ with tendencies set at $0.05 < P \leq 0.10$.

# Results and Discussion

Increasing soybean autoclave exposure time darkened the color of the soybean meal and created evidence of the Maillard browning reaction (Figure 1). Crude protein, total AA, Ala, Asp, Glu, Gly, Iso, Leu, and Val decreased (linear, $P < 0.05$) when measured with official analytical methods with increasing autoclave exposure time (Table 1). Increasing autoclave exposure time decreased (quadratic, $P < 0.05$) Lys:CP, available Lys, available Lys:total Lys, KOH, trypsin inhibitor, urease, Lys, and Cys when measured using official analytical methods.

Crude protein, Lys:CP, total AA, and AA results of NIRS and official analytical analysis were used to determine the correlation coefficient ($r$) and coefficient of determination ($r^2$) for official analytical results and NIRS results (Table 2). There was a positive correlation ($P < 0.03$) between official analytical and NIRS results for CP ($r = 0.546$; $r^2 = 0.298$), Lys:CP ($r = 0.977$; $r^2 = 0.954$), available/reactive Lys:total Lys ($r = 0.935$, $r^2 = 0.981$) total AA ($r = 0.819$; $r^2 = 0.538$), Ala ($r = 0.491$; $r^2 = 0.241$), Cys ($r = 0.924$; $r^2 = 0.853$), Lys ($r = 0.975$; $r^2 = 0.950$), and a negative correlation for Thr ($r = -0.666$; $r^2 = 0.444$).

When total Lys (Figure 3), reactive Lys (Figure 4), Lys:CP (Figure 5), and reactive Lys:total Lys (Figure 6) were measured using NIRS they were considered a good predictor of official analytical results for total Lys ($r^2 = 0.969$), available Lys ($r^2 = 0.988$), Lys:CP ($r^2 = 0.954$), and available Lys:total Lys ($r^2 = 0.981$), respectively.

In conclusion, increasing soybean autoclave exposure time decreased soybean meal quality as measured by KOH solubility, Lys, available Lys, Lys:CP, and available Lys:total Lys. These results created a model to estimate the relationship between NIRS and official analytical results. Regression models were successful at using NIRS Lys, available Lys, their ratio, and Lys:CP. This demonstrated the ability of NIRS to be used as a tool to determine over-processing of soybean meal.

**Table 1. Effect of autoclave heating time on soybean meal quality when measured using official analytical procedures[1]**

| | | | | | | | | | Probability, $P<$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item, % | 0 | 5 | 10 | 15 | 30 | 45 | 60 | SEM | Linear | Quadratic |
| CP[2] | 39.3 | 38.7 | 38.4 | 38.5 | 38.3 | 38.2 | 38.2 | 0.36 | 0.060 | 0.268 |
| Total lysine | 2.51 | 2.19 | 2.13 | 2.06 | 1.90 | 1.74 | 1.62 | 0.021 | 0.001 | 0.001 |
| Lys:CP | 6.2 | 5.7 | 5.5 | 5.4 | 4.8 | 4.6 | 4.3 | 0.01 | 0.001 | 0.001 |
| Available Lys | 2.44 | 1.97 | 1.80 | 1.70 | 1.40 | 1.22 | 1.05 | 0.024 | 0.001 | 0.001 |
| Available Lys:total Lys | 97.2 | 89.8 | 84.8 | 82.6 | 74.0 | 70.1 | 64.9 | 0.85 | 0.001 | 0.001 |
| KOH[2] | 89.9 | 59.2 | 38.1 | 31.8 | 24.2 | 21.1 | 16.8 | 6.48 | 0.001 | 0.001 |
| Trypsin inhibitor index, TIU/g | 3205.0 | 483.8 | 423.5 | 390.7 | 39.8 | 133.7 | 188.3 | 72.05 | 0.001 | 0.001 |
| Urease, $\Delta$pH | 0.7 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.06 | 0.001 | 0.001 |
| Total AA[2] | 28.85 | 27.46 | 27.45 | 27.28 | 27.29 | 26.71 | 26.27 | 0.264 | 0.001 | 0.092 |
| Other AA | | | | | | | | | | |
| Ala | 1.69 | 1.64 | 1.64 | 1.64 | 1.65 | 1.63 | 1.62 | 0.016 | 0.018 | 0.526 |
| Asp | 4.50 | 4.32 | 4.33 | 4.30 | 4.31 | 4.21 | 4.17 | 0.037 | 0.001 | 0.230 |
| Cys | 0.64 | 0.55 | 0.54 | 0.53 | 0.50 | 0.47 | 0.44 | 0.006 | 0.001 | 0.001 |
| Glu | 6.91 | 6.63 | 6.65 | 6.64 | 6.68 | 6.60 | 6.55 | 0.066 | 0.004 | 0.215 |
| Gly | 1.70 | 1.66 | 1.66 | 1.66 | 1.68 | 1.66 | 1.64 | 0.016 | 0.049 | 0.990 |
| Iso | 1.85 | 1.77 | 1.78 | 1.78 | 1.79 | 1.76 | 1.74 | 0.017 | 0.003 | 0.753 |
| Leu | 3.01 | 2.89 | 2.90 | 2.91 | 2.93 | 2.89 | 2.87 | 0.031 | 0.013 | 0.414 |
| Met | 0.57 | 0.56 | 0.56 | 0.56 | 0.57 | 0.56 | 0.57 | 0.010 | 0.115 | 0.211 |
| Pro | 1.97 | 1.87 | 1.86 | 1.79 | 1.86 | 1.80 | 1.69 | 0.108 | 0.070 | 0.999 |
| Thr | 1.49 | 1.44 | 1.45 | 1.46 | 1.47 | 1.45 | 1.46 | 0.018 | 0.417 | 0.410 |
| Val | 2.02 | 1.95 | 1.95 | 1.95 | 1.96 | 1.95 | 1.91 | 0.027 | 0.033 | 0.727 |

[1]Soybean white flakes after oil extraction but prior to heat processing, were ground using a blender, and 500 g samples were put into cotton bags to be autoclaved. Samples were autoclaved at 262°F for 0, 5, 10, 15, 30, 45, and 60 min at 29 PSI. A total of 2 samples per treatment were autoclaved in 3 blocks to provide 3 replications per treatment.

[2]CP = crude protein. AA = amino acids. KOH = protein solubility in potassium hydroxide.

**Table 2. Near-infrared reflectance spectroscopy (NIRS) Pearson correlation coefficient (R) and coefficient of determination (r²) to analytical laboratory values[1]**

| Item | Pearson R | $r^2$ | Probability, $P <$ |
|---|---|---|---|
| CP[2] | 0.546 | 0.298 | 0.011 |
| Lys:CP | 0.977 | 0.954 | 0.001 |
| Reactive Lys:total Lys | 0.935 | 0.981 | 0.001 |
| Total AA[2] | 0.819 | 0.538 | 0.001 |
| Other AA | | | |
| Ala | 0.491 | 0.241 | 0.024 |
| Asp | 0.119 | 0.014 | 0.607 |
| Cys | 0.924 | 0.853 | 0.001 |
| Glu | -0.270 | 0.073 | 0.237 |
| Gly | 0.108 | 0.012 | 0.641 |
| Iso | -0.214 | 0.046 | 0.352 |
| Leu | -0.138 | 0.019 | 0.551 |
| Lys | 0.975 | 0.950 | 0.001 |
| Met | 0.021 | 0.000 | 0.928 |
| Pro | -0.021 | 0.000 | 0.929 |
| Thr | -0.666 | 0.444 | 0.001 |
| Val | -0.413 | 0.170 | 0.063 |

[1]Predictability of the variance between NIRS and official procedures for individual AA analysis for under-processed, adequately processed, and over-processed soybeans.
[2]CP = crude protein. AA = amino acids.

**Figure 1. Ground soy white flakes exposed to an autoclave at 262°F for 0, 5, 10, 15, 30, 45, and 60 min at 29 PSI, simulating the heat step in soybean meal processing to create a range of soybean meal quality.**
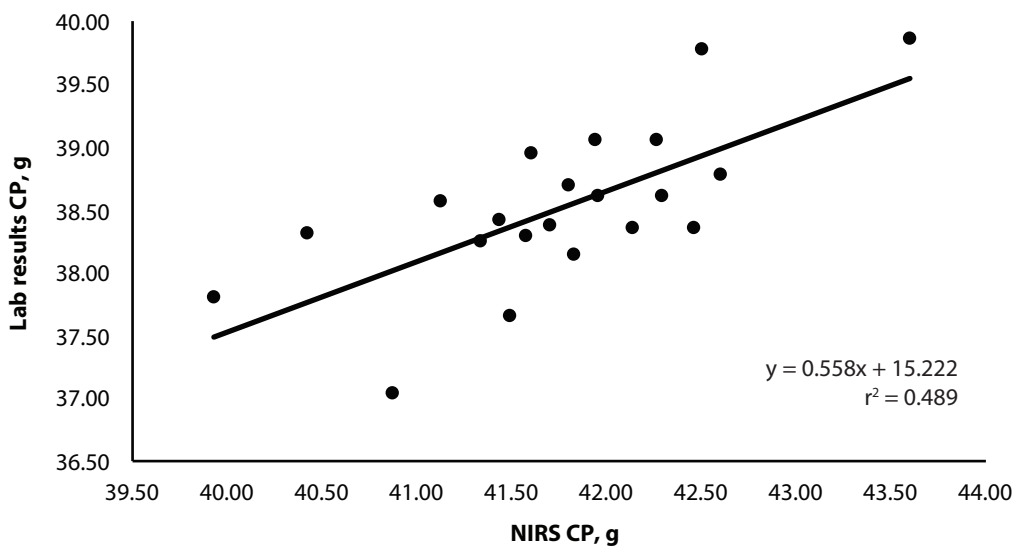


$$y = 0.558x + 15.222$$
$$r^2 = 0.489$$

**Figure 2. Linear regression analysis of near-infrared reflectance spectroscopy (NIRS) crude protein (CP) compared with official analytical results.**
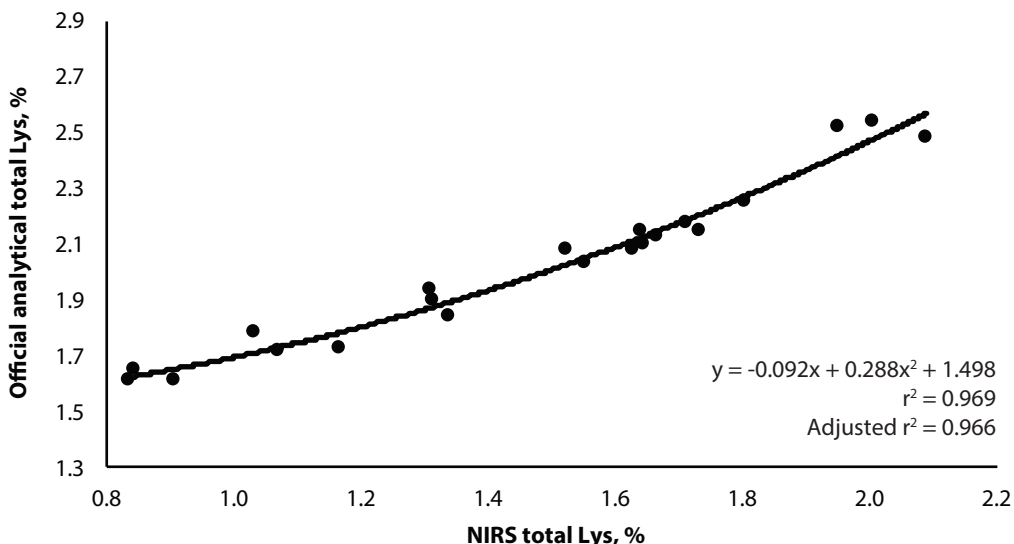
Figure 3. Quadratic regression analysis of near-infrared reflectance spectroscopy (NIRS) total lysine compared with official analytical results.
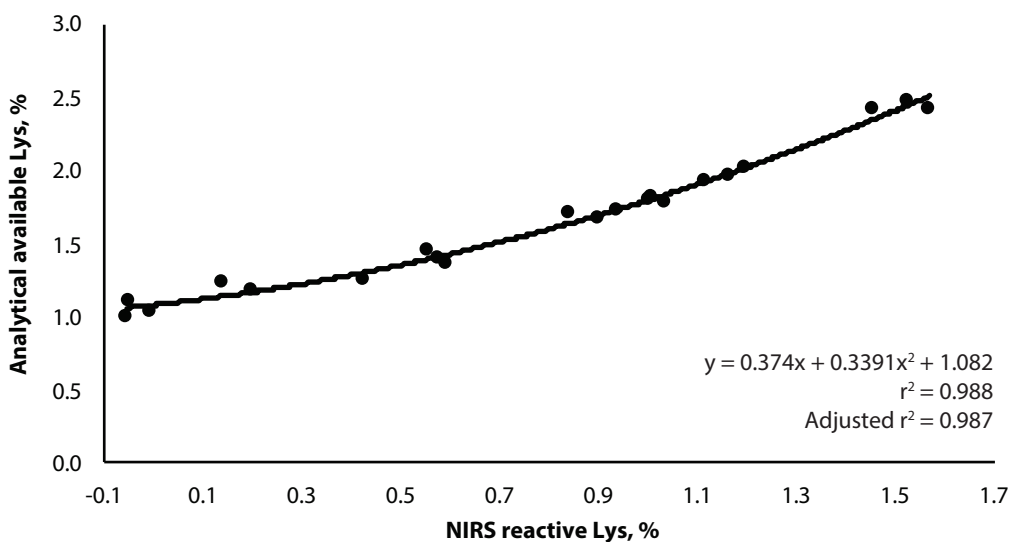


Figure 4. Quadratic regression analysis of near-infrared reflectance spectroscopy (NIRS) reactive Lys compared with official analytical results.
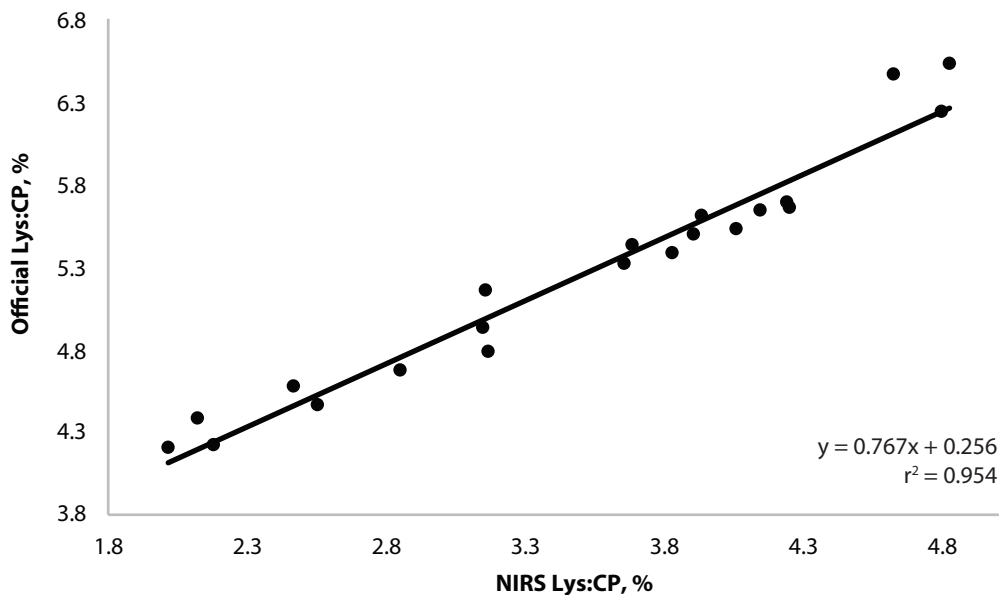
**Figure 5. Linear regression analysis of near-infrared reflectance spectroscopy (NIRS) Lys:CP compared with official analytical results. CP = crude protein.**
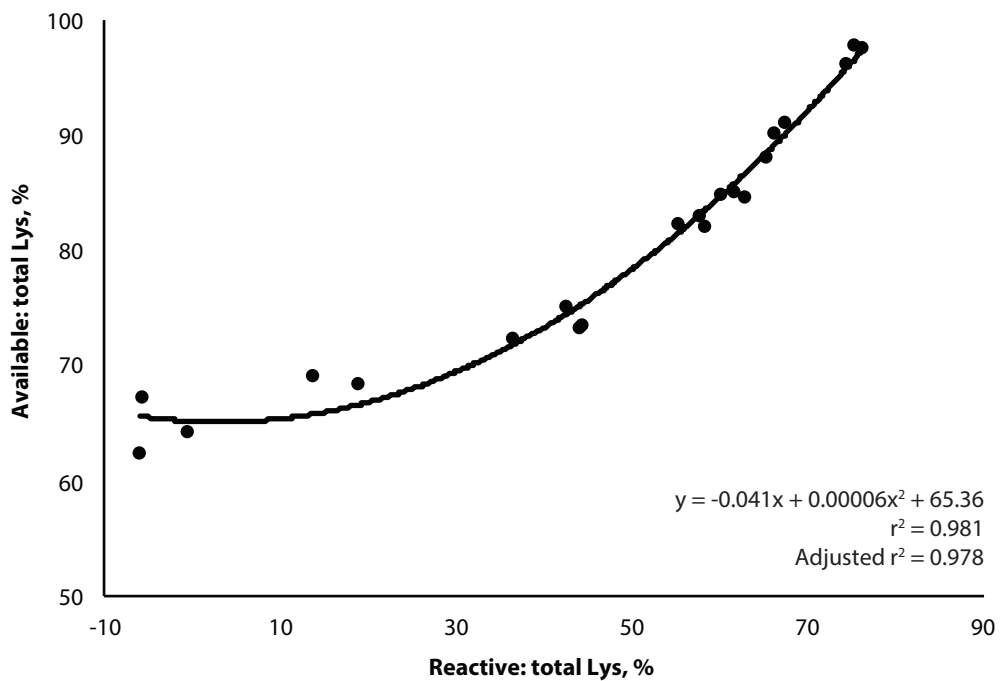


**Figure 6. Quadratic regression analysis of near-infrared reflectance spectroscopy (NIRS) reactive Lys:total Lys compared with official analytical results.**