Development of a Ninhydrin Assay to Quantify Methionine Loss from Commercial Rumen Protected Amino Acid Products

T. Baillargeon*, A. Miske, M. Hernandez, N. Whitehouse

Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH 03824

Introduction

Ninhydrin (NH) reactions are widely used in biomedical, forensic, and nutritional research for characterizing amino acids (AAs). The reaction between α-AAs and NH creates a unique color called Ruhemann's purple which makes colorimetric quantification simple.

AAs are an important aspect of dairy cattle nutrition, and they are often limited by diet which makes supplementation valuable for producers. In North America, the first limiting AA in the dairy cow diet is Methionine (Met).

Rumen-protected amino acids (RPAAs) are typically coated with a pH-sensitive polymer, fats, or other compounds that help the AAs avoid degradation in the rumen. This allows for maximum absorption in the small intestine.

Methodology

1. DL-Met was weighed in 5mg increments starting at 0mg up to 50mg
2. DL-Met was dissolved in 3 mL DI water
3. 100 µL of a 1% NH solution was added to each sample
4. Samples were incubated for 30 minutes in a water bath heated to 49°C
5. Absorbance was measured at 570 nm on a spectrophotometer
6. Data was analyzed using JMP student edition 19

References

Chalupa, W., 1975. Rumen Bypass and Protection of Proteins and Amino Acids. Journal of Dairy Science 58, 1198–1218. https://doi.org/10.3168/jds.S0022-0302(75)84697-2

Friedman, M., 2004. Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. J Agric Food Chem 52, 385–406. https://doi.org/10.1021/jf030490p

Kung, L., Rode, L.M., 1996. Amino acid metabolism in ruminants. Animal Feed Science and Technology 59, 167–172. https://doi.org/10.1016/0377-8401(95)00897-7

Schwab, C.G., Broderick, G.A., 2017. A 100-Year Review: Protein and amino acid nutrition in dairy cows. Journal of Dairy Science 100, 10094–10112. https://doi.org/10.3168/jds.2017-13320

Stauß, A.C., Fuchs, C., Jansen, P., Repert, S., Alcock, K., Ludewig, S., Rozhon, W., 2024. The Ninhydrin Reaction Revisited: Optimisation and Application for Quantification of Free Amino Acids. Molecules 29, 3262. https://doi.org/10.3390/molecules29143262

Ninhydrin Images

The Ninhydrin Reaction

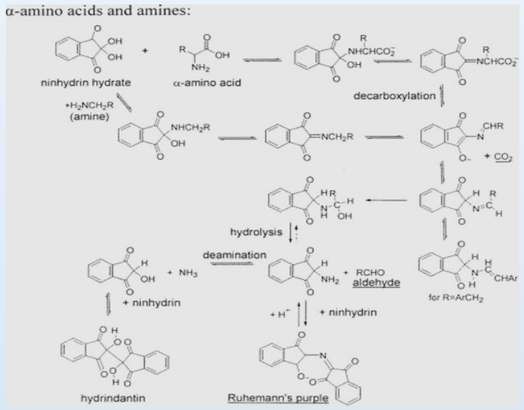


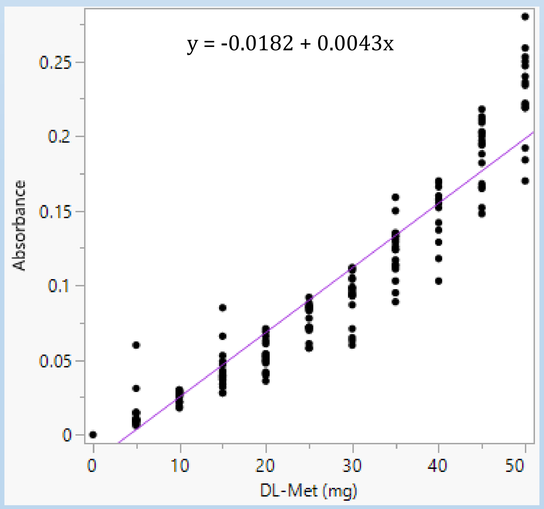
Figure 1: Mechanism of the reaction between α-AAs and ninhydrin hydrate to form Ruhemann's purple. (Friedman, 2004)

Visual of standard samples after the incubation of ninhydrin reaction

Figure 2: Tubes place in order of 0mg DL-Met on the left increasing by 5 mg increments up to 50 mg on the right

Graphs

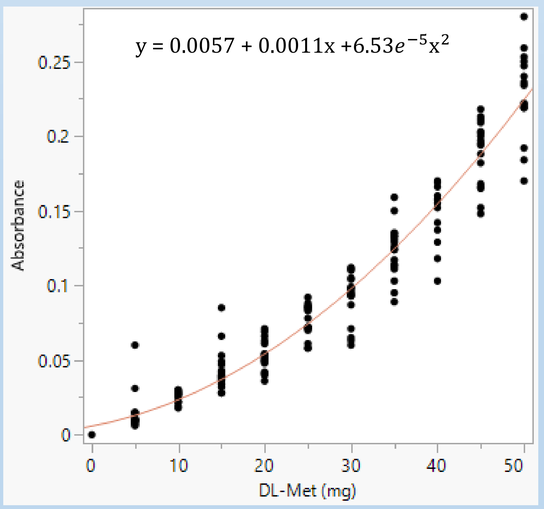
Linear Model Curve of Standard DL-Met Concentrations



Model	BIC	SSE	MSE	RMSE	R-square
Linear	-904.54	0.0867	0.00047	0.0216	0.908

Figure 3: Linear model of standard DL-Met concentrations. Equation represented as y=a+bx where a=intercept, b=slope, and x=concentration of DL-Met.

Quadratic Model Curve of Standard DL-Met Concentrations



Model	BIC	SSE	MSE	RMSE	R-square
Quadratic	-1001.08	0.0479	0.00026	0.0161	0.9493

Figure 4: Quadratic model of standard DL-Met concentrations. Equation represented as $y=a+bx+cx^2$ where a=intercept, b=slope, c=quadratic, and x=concentration of DL-Met.

Results

After outliers were removed an average of 17 runs were used to fit a curve model to the data.

A linear and quadratic model was fit to the data. The quadratic model fit the data more accurately as shown by the R-squared values (linear = 0.91, quadratic = 0.95).

Conclusions

The quadratic model of the data captures the curvature of an accelerated increase in absorbance for Ruhemann's purple.

This assay can be used to provide a clearer picture of product bioavailability to the animal on a commercial farm setting. This includes estimating loss of Met from rumen-protected products as they sit in feed and prior to reaching the abomasum.

Next Steps

Adapted the procedure to allow for release of unknown concentrations of DL-Met from commercial products and quantify them using colorimetric measurements in the quadratic equation.

Product A:

$0.13 = 0.0057 + 0.0011x + 6.53e^{-5}x^2$

DL-Met loss ~ **36.0 mg**

Product B:

$0.05 = 0.0057 + 0.0011x + 6.53e^{-5}x^2$

DL-Met loss ~ **18.9 mg**

Product C:

$0.01 = 0.0057 + 0.0011x + 6.53e^{-5}x^2$

DL-Met loss ~ **3.3 mg**

Acknowledgements

I would like to acknowledge the undergraduate students Ashley Miske and Madelynn Hernandez for the work on this assay as part of their capstones, The members of the Whitehouse lab that helped with the laboratory analysis and to Nancy Whitehouse for her expertise and advisement.