PROTEIN AND NITROGEN VARIABILITY IN PLANT BIOMASS: IMPLICATIONS FOR CHELATE BIOFERTILIZER FORMULATION USING THE KJELDAHL METHOD

[a]Avazyazov Muhammad Akbarovich, [b]Ashirov Mansur Allanazarovich,
[a]First-year master's student of the Faculty of Chemical Technologies of UrSU
[b]Ph.D. in chemistry, Senior researcher of Khorezm Ma'mun Academy

Abstract

Accurate protein quantification in plant biomass is essential for developing effective chelate biofertilizers, as amino acids serve as key chelating agents. This study employed the Kjeldahl method to determine the protein content in the leaves of tomato (Solanum lycopersicum) and alfalfa (Medicago sativa). The methodology involved the conversion of organic nitrogen to ammonium sulfate, followed by titration, to estimate total nitrogen content. Results indicated average protein contents of 9.198% for tomato and 28.45% for alfalfa, with minimal deviation in experimental precision. The study also highlighted the challenges posed by non-protein nitrogen and amino acid variability in protein quantification. These findings provide valuable data for optimizing chelate biofertilizer formulations, contributing to improved nutrient uptake and sustainable agricultural practices.

Keywords: Kjeldahl method, protein quantification, nitrogen content, plant biomass, non-protein nitrogen (NPN), amino acid variability, conversion factor, biofertilizers, sustainable agriculture, nutrient uptake

Introduction

The Kjeldahl method is commonly used to determine the nitrogen content in plant biomass, with the total nitrogen value being multiplied by a conversion factor to estimate the protein content. This method operates under the assumption that most nitrogen in plant biomass is present within the amino acids that form proteins. Early research established that the average nitrogen (N) content of proteins is approximately 16%, resulting in the widely used conversion formula N x 6.25 (100/16 = 6.25) to calculate protein content from nitrogen levels. However, using the standard conversion factor of 6.25 presents two key challenges. First, not all nitrogen in plant biomass is found in proteins; some is present in other nitrogen-containing compounds, such as amides, free amino acids, peptides, nucleic acids, nitrogenous lipids, ammonium salts, nucleotides, nitrates, creatine, choline, and secondary compounds, collectively known as non-protein nitrogen (NPN). Second, the nitrogen content of individual amino acids varies depending on their molecular weight and the number of nitrogen atoms, which can range from one to four, depending on the specific amino acid [2,3].

The accurate quantification of protein content in plant biomass is crucial for the development of chelate biofertilizers, as amino acids serve as effective chelating agents. In this study, the Kjeldahl method was employed to determine the protein content in the leaves of tomato (Solanum lycopersicum) and alfalfa (Medicago sativa). The Kjeldahl method, which involves the conversion of organic nitrogen to ammonium sulfate followed by titration, provides a reliable estimate of the total nitrogen content in plant tissues. By multiplying the total nitrogen content by a conversion factor, the protein content can be calculated. The results obtained from this study will contribute to the optimization of chelate biofertilizer formulations, ultimately enhancing plant nutrient uptake and growth. The findings underscore the significance of accurate protein quantification in plant biomass for the advancement of sustainable agricultural practices.

Materials and methods

Chemicals

Copper (II) sulfate (according to GOST 4165 obtained from Chemical invest, Tashkent), Potassium sulfate (according to GOST 4145 obtained from Chemical invest, Tashkent), Concentrated sulfuric acid (according to GOST 4204), Boric acid (according to GOST 9656), methyl red, methyl blue, ethyl alcohol (according to GOST 5962), Sulfuric acid (standard titer molar concentration c (H2S04) = 0.05 mol/dm^3).

Experiment

A 1 g sample of tomato and alfalfa green mass was placed into a clean, dry Kjeldahl flask. Mineralization was performed in a fume hood. An 8 g catalyst mixture ($CuSO_4$ and K_2SO_4 in a 1:10 mass ratio) was added to the flask, followed by the careful addition of 12 cm^3 of concentrated sulfuric acid. The contents were thoroughly mixed using gentle circular motions to ensure complete wetting of the sample.

The flask was positioned on a heater with its axis inclined at an angle between 30° and 45° to the vertical. A small glass funnel or sleeve was inserted into the neck of the flask to minimize acid volatilization during mineralization. Initially, moderate heating was applied to prevent violent foaming, with periodic rotation of the flask to ensure proper mixing.

Figure 1. Mineralization process

After cooling, the mineralizate was quantitatively transferred to a distillation flask. The Kjeldahl flask was rinsed three times with 20-30 cm^3 portions of distilled water. Prior to ammonia distillation, the mineralizate was diluted with 200 cm^3 of distilled water. A receiving flask was prepared with 30 cm^3 of boric acid solution (40 g/dm^3) and five drops of a mixed indicator (0.20 g methyl red and 0.10 g methylene blue dissolved in 100 cm^3 of 96% ethanol).

The distillation flask was connected to an ammonia distillation apparatus. Subsequently, 42 cm^3 of sodium hydroxide solution (33% mass fraction) was carefully added to the flask containing the mineralizate through a dropping funnel. The funnel was rinsed two or three times with 10 cm^3 portions of distilled water, leaving a small amount of water as a seal. The solution in the distillation flask was then heated to ensure uniform boiling.

After the distillation was completed, the receiving flask was lowered, and the drain tube of the condenser was rinsed with distilled water into the receiving flask. Ammonia was titrated with 0.05 mol/dm^3 sulfuric acid solution from a burette until the indicator color changed from green to violet.

Concurrent with the sample testing, a control experiment was performed to evaluate the contamination levels of water and reagents with ammonia. During the distillation into boric acid, the volume of sulfuric acid used for titration in the control experiment was 0.15 cm^3, which is within the recommended limit of 0.5 cm^3. The mass fraction of nitrogen in the test sample (X_1, %) during the distillation of ammonia into boric acid was calculated using the following formula:

$$X_1 = \frac{(V_1 + V_0) * K * 0.0014 * 100}{m}$$

The mass fraction of crude protein in the test sample X_2, %, was calculated using the formula:

$$X_2 = 6.25 * X_1$$

Results

The precision of the experiments was assessed. The mean value of the experimental results for tomato samples was 9.198%. The absolute discrepancy was 0.44%, the relative divergence was 4.78%, the standard deviation was 0.22%, and the relative standard deviation was 2.39%.

Table 1. Experimental Data for Tomato Samples

Sample number (Tomato)	V_1	V_2	X_1(%)	X_2(%)
1	10.9	0.15	1.505	9.41
2	10.4	0.15	1.435	8.97
3	10.4	0.15	1.435	8.97
4	10.7	0.15	1.477	9.23
5	10.9	0.15	1.505	9.41

The average value of the experimental outcomes for alfalfa specimens was 28.45%. The absolute difference was 0.35%, the relative deviation was 1.23%, the standard deviation was 0.146%, and the relative standard deviation was 0.513%.

Table 2. Experimental Data for Alfalfa Samples.

Sample Number (Alfalfa)	V_1	V_2	X_1(%)	X_2(%)
1	32.9	0.15	4.585	28.66
2	32.7	0.15	4.557	28.48
3	32.5	0.15	4.529	28.31
4	32.5	0.15	4.529	28.31
5	32.7	0.15	4.557	28.48

Conclusion

The study successfully employed the Kjeldahl method to determine the protein content in tomato and alfalfa leaves, contributing valuable insights for optimizing chelate biofertilizer formulations. The findings demonstrated the significance of accurate protein quantification, with tomato samples exhibiting an average protein

content of 9.198% and alfalfa samples showing 28.45%. The precision of the experimental results was confirmed by low standard deviation and relative deviation values, highlighting the reliability of the method. The study underscores the importance of considering non-protein nitrogen and amino acid variability when calculating protein content using conversion factors. These results are pivotal for advancing sustainable agricultural practices by enhancing nutrient uptake and plant growth through more effective biofertilizers.

References

1. Standardinform. (2019). GOST 13496.4—2019 Fodder, mixed fodder, and raw mixed fodder. Methods for determining nitrogen and crude protein content. Moscow: Standardinform.
2. Maynard, A. B., & Loosli, J. K. (1969). Animal nutrition. McGraw-Hill.
3. Herrera, C. M. (1982). Defense of ripe fruit from pests: Its significance in relation to plant-disperser interactions. The American Naturalist, 120(2), 218–241.
4. Lourenço, S. O., Barbarino, E., Lanfer Marquez, U. M., & Aidar, E. (1998). Distribution of intracellular nitrogen in marine microalgae: Basis for the calculation of specific nitrogen-to-protein conversion factors. Journal of Phycology, 34(5), 798–811.
5. Conklin-Brittain, N. L., Dierenfeld, E. S., Wrangham, R. W., Norconk, M., & Silver, S. C. (1999). Chemical protein analysis: A comparison of Kjeldahl crude protein and total ninhydrin protein from wild, tropical vegetation. Journal of Chemical Ecology, 25(12), 2601–2622.
6. Fujihara, S., Kasuga, A., & Aoyagi, Y. (2001). Nitrogen-to-protein conversion factors for common vegetables in Japan. Journal of Food Science, 66(3), 412–415.
7. Fujihara, S., Kasuga, A., & Aoyagi, Y. (2001). Nitrogen-to-protein conversion factors for common vegetables in Japan. Journal of Food Science, 66(3), 412–415.