

Variation of Total Amino Acid Content in Wheat Grains in Relation to Fertilization

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Abstract. The aim of this study was to evaluate the quality of wheat grains, in terms of total amino acid (TAA) content, in relation to fertilization. The field experiments were located at the University of Life Sciences "King Mihai I" from Timisoara, during 2018 – 2022. The 'Alex' wheat variety was cultivated. Nitrogen (N, ammonium nitrate) fertilization was done in five doses: 0, 50, 100, 150, and 200 kg ha⁻¹ a.s. (a.s. – active substance). The Super Fifty (SF) biofertilizer was applied foliarly, at each N level, in doses: 0, 1, 2, 3, 4, 5 L ha⁻¹. The total amino acid content (TAA, mg kg⁻¹) ranged from 962.09±21.66 mg kg⁻¹ (N0, SF0) to 1388.06±52.14 mg kg⁻¹ (N200, SF2). Comparative analysis of the data indicated statistically significant differences between the mean values (ANOVA Test), between the median values (Kruskal-Wallis Test), and between certain experimental variants with nitrogen associated with the foliar biofertilizer (N200 and N0, N200 and N50, N150 and N50, respectively N100 and N50). The direct and interaction effect of the two fertilizers on the TAA index was described by a polynomial model, with $R^2 = 0.556$, $p < 0.001$. From the values of the equation coefficients and the graphic models (3D, isoquants), divergent action of the N and SF factors on the formation and variation of TAA was observed. Multivariate analysis explained how low nitrogen doses (N0, N50) were associated with high concentrations of the SF biofertilizer (SF4, SF5) and medium-high N doses (N100, N150, N200) were associated with moderate concentrations of the SF biofertilizer (SF2, SF3) in the formation of total amino acid content values (TAA, mg kg⁻¹).

Keywords: amino acids; comparative analysis; fertilization; nutritional quality; quality indices; wheat

INTRODUCTION

Due to the energy stored in the grain, in the form of specific compounds, wheat is essential for global food safety and security (Shiferaw et al., 2013; Gahlot et al., 2020; Husenov et al., 2021; Emebiri, 2023; Khalid et al., 2023; Zhou et al., 2025; Tan et al., 2026). Wheat occupies a basic place in human nutrition, positioned in the top three crops worldwide, along with rice and corn, and represents an accessible source of protein and calories for human nutrition (Bibi și Ilyas, 2020). Wheat provides an important share of dietary calories, through its high content of dietary fiber, proteins, minerals, vitamins, amino acids and other active principles necessary in human nutrition (Khalid et al., 2023).

In the context of increasing human population and food needs, the demand for wheat is forecast to increase, along with the optimization of technological practices for sustainable production (Ma and Cai, 2024). The focus on increasing wheat yields has been associated with a decrease in protein content (Emebiri, 2023).

The mechanisms of wheat grain quality formation have been studied to support a sustainable wheat industry (Peng et al., 2022). Improving wheat quality has been studied by improving the genetic base, through sustainable technologies with balanced input supply (Khalid et al., 2023). The interest in plant protein resources was the basis for comparative studies between wheat genotypes regarding protein content, protein quality, mineral content and active principles in wheat grains (Szuba-Trznadel et al., 2024). Differential values of some quality indices (e.g. amino acids, gluten) in wheat grains have been recorded in relation to different wheat genotypes, with importance for the destination of production and the quality of finished products (Wang et al., 2025).

Variations in wheat grain and flour quality indices were recorded in relation to different wheat varieties and cultivation locations, in conditions of correlations of quality indices and statistical reliability of the data (Siddiqi et al., 2020). Quantitatively and qualitatively, proteins in wheat grains are genetically defined, and dependent on the interaction "genotype × environment × crop technology" (Peng et al., 2022). Variation in protein content in wheat grains has been associated with increased amino acid content in wholemeal flour through breeding processes, as a result of the impact of selection on key genes in wheat quality (Emebiri, 2023). The quality of wheat processing and finished products is largely dependent on the proteins stored in the grains and the quality of these proteins (Peng et al., 2022).

Variation in the technological quality of wheat grains (grinding quality, protein complex, enzymatic activity) was recorded in relation to technology elements (e.g. sowing date, fertilization rates) (Lachutta and Jankowski, 2024). Variable rates of phosphorus and iron fertilizers differentially influenced quality indices, such as fiber, protein and moisture content of wheat grains (Shahbazi and Nematollahi, 2019). Soil tillage methods

and variants and adequate fertilizer rates were considered important for optimizing wheat yields and quality (Ahmadi et al., 2024). The effect of controlled shading, under experimental conditions, has been recorded in the variation of amino acid content and protein accumulation in wheat (Ma et al., 2024). Based on case studies, the importance of the balance between wheat crop productivity, product quality and safety, associated with technological practices and environmental factors, has been communicated (Xu and He, 2024).

Ensuring the quality of wheat in relation to its final use, the quality of finished products, and the nutritional benefits for humans was evaluated in relation to climate change and its impact on the quality of the raw material – wheat grains (Han et al., 2025). The increase in thermal values, associated with the decrease in precipitation, influenced the phenology of plants with effects on the formation of yields and seed quality in wheat crops (Poggi et al., 2022). Variations in wheat grain quality components have been reported in relation to cultivated genotypes, type of stress and duration of action of stress factors (Zahra et al., 2023). Variations in the quality of wheat, flour and finished products were recorded, in relation to variations in climatic factors (Zhou et al., 2025). Higher temperatures led to rapid grain filling, with effects on the gliadin/glutenin ratio and grain quality (Poggi et al., 2022). Differentiated variations in protein and starch content in relation to thermal values over the day have been reported, as well as the alteration of key protein quality parameters when thermal thresholds are exceeded (Han et al., 2025). The stressful effect of atmospheric factors (e.g. O₃) on wheat crops was recorded, with a reduction in yield and the values of some quality indices (Cook et al., 2025).

Non-destructive methods are increasingly present and promoted in the quality assessment of wheat grains, to provide precise information and facilitate the development of models for the valorization of production in relation to the quality of finished products (Lullien-Pellerin, 2024).

The present study evaluated the direct and interaction effect of nitrogen (N) and the foliar biofertilizer Super Fifty (SF) on the total amino acid content (TAA) of wheat, 'Alex' variety.

MATERIAL AND METHODS

The study and the field experiment were conducted within the DER (Didactic and Experimental Resort) of the University of Life Sciences “King Mihai I” from Timisoara (ULS), between 2018 and 2022. The experiments were in non-irrigated conditions, on flat land, chernozem soil, Figure 1. The values of the climatic parameters, during the experimental period, are graphically shown in Figure 2.



Figure 1. Experimental location

The soil was prepared for sowing using conventional technology (plowing, harrowing). Each year, sowing was done in the first decade of October. The 'Alex' wheat variety represented the biological material.

Nitrogen fertilization (N, ammonium nitrate) was done in spring, in five levels (0, 50, 100, 150 and 200 kg ha⁻¹ N a.s. (a.s. – active substance). At the end of twinning - beginning of elongation, 29 - 30 BBCH Code (Meier, 2001) nitrogen was applied manually and uniformly on the experimental variants. The biofertilizer product Super Fifty (SF), in six concentrations (0, 1, 2, 3, 4, and 5 L ha⁻¹), was applied to each nitrogen level, at BBCH code stage 37 – 39 (Meier, 2001). This resulted in 30 variants, in repetitions, with a surface area of 18 m² per variant.

The total amino acid content (TAA, mg kg⁻¹) of wheat grains was evaluated. The determinations were

made on average grain samples from the experimental period, within the Research Platform of ULS Timisoara, using accredited laboratory methods.

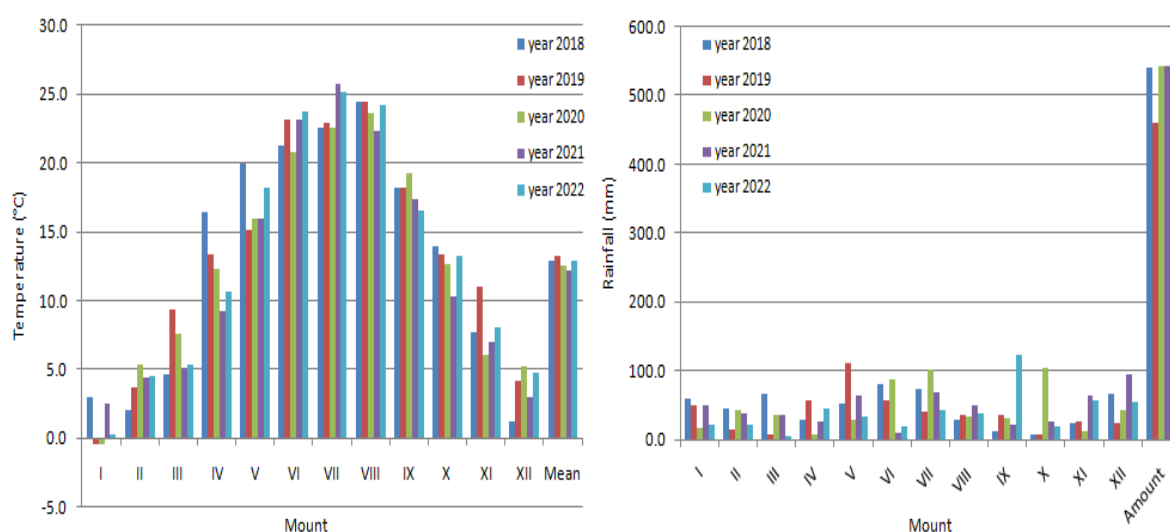


Figure 2. Climatic parameters

The general statistical analysis and characterization of the data was performed (descriptive statistical analysis). The variation of TAA in relation to fertilizer products was evaluated, as a direct and interaction effect (regression analysis). The comparative analysis of the variants was done, through specific tests (ANOVA, Kruskal-Wallis, Mann-Whitney Test). The correlation of the factors (N, SF) in the formation of TAA values was evaluated, and the level of similarity between the experimental variants given by N and SF was evaluated (multivariate analysis). The PAST software v.4.17c (Hammer et al., 2001), and the Mathematica software v.14.2 (2024) were used in relation to the purpose of the study.

RESULTS AND DISCUSSIONS

Total amino acid content (TAA, mg kg⁻¹) varied in the range (962.09, 1388.06). The normal distribution of the data series on nitrogen (N) levels in relation to Super Fifty (SF) was recorded, with $r = 0.982$ (N0), $r = 0.966$ (N50), $r = 0.955$ (N100), $r = 0.954$ (N150), and $r = 0.927$ (N200), Figure 3. The profile of total amino acid content (TAA) is presented in Figure 4. The ANOVA test (Two-Factor) indicated the presence of variance in the data set, and the statistical reliability of the data, according to Table 1.

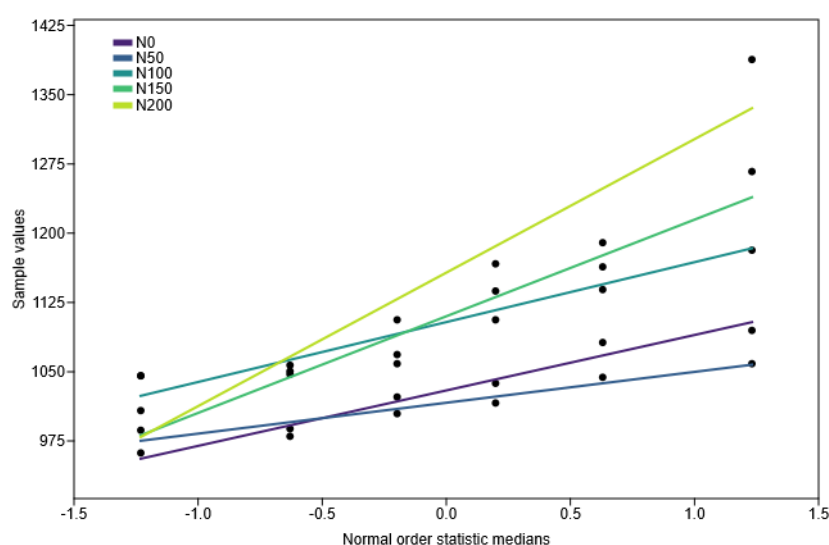
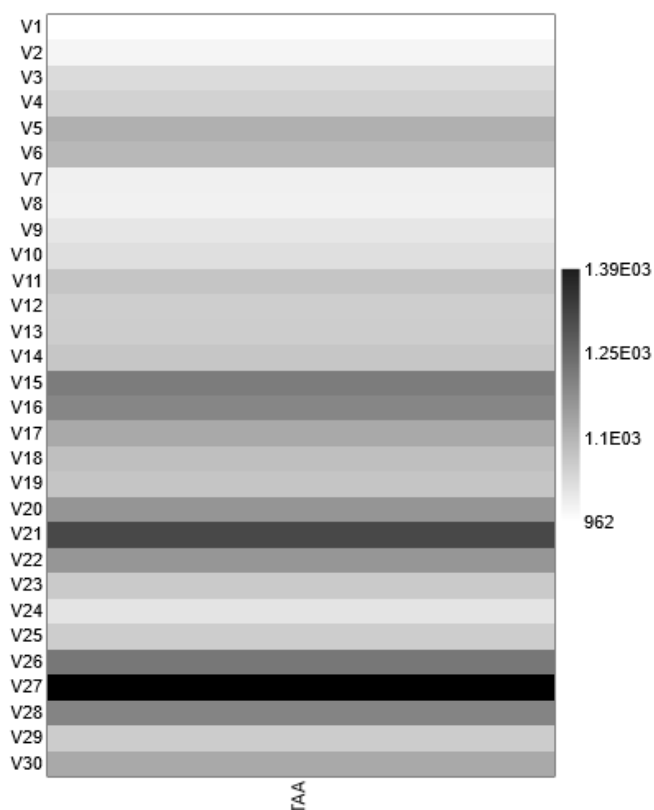
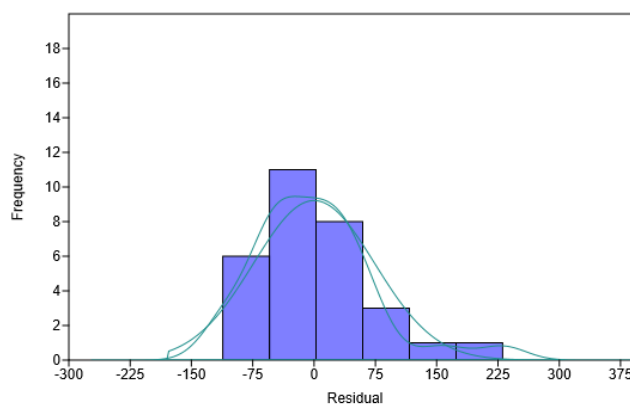


Figure 3. Normal distribution of TAA data series

Table 1. ANOVA test results

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	66051.28	5	13210.26	2.835991	0.042911	2.71089
Columns	83703.01	4	20925.75	4.492362	0.009426	2.866081
Error	93161.47	20	4658.073			
Total	242915.8	29				

Comparative analysis of the data indicated differences between the mean values, in conditions of statistical safety ($p = 0.027$) according to ANOVA (Test for equal means), with the residual values in Figure 5. The Kruskal-Wallis test indicated differences between the median values in conditions of statistical safety, $H(\chi^2) = 12.44$, H_c (tie corrected) = 12.45, $p = 0.0143$.

**Figure 4.** TAA profile in wheat grains, 'Alex' variety, according to experimental variants**Figure 5.** Graphical distribution of residual values

Based on the results of the ANOVA and Kruskal-Wallis Tests, which confirmed differences between the means and medians, the pairwise Mann-Whitney Test was applied, with the results in Table 2. The color-coded fields indicated significant differences between the experimental variants, based on the values of total amino acid content (TAA). According to the results, statistically significant differences were recorded between N200 and N0, N200 and N50, N150 and N50, and N100 and N50, respectively.

Table 2. Mann-Whitney pairwise results

	N0	N50	N100	N150	N200
N0		0.8102	0.0656	0.1282	0.0306
N50	0.8102		0.0131	0.0370	0.0131
N100	0.0656	0.0131		0.9362	0.5738
N150	0.1282	0.0370	0.9362		0.6889
N200	0.0306	0.0131	0.5738	0.6889	

Multivariate analysis (PCA) led to the diagram in Figure 6. The two main components explained 95.463% of the total variance. According to the PCA diagram, Figure 6, it was observed how low doses of nitrogen (N0, N50) were associated with high concentrations of the SF biofertilizer (SF4, SF5) in the formation of the values of the total amino acid content (TAA, mg kg⁻¹). Medium-high doses of N (N100, N150, N200) were associated with moderate concentrations of the biofertilizer SF (SF2, SF3) in the formation of total amino acid content values (TAA, mg kg⁻¹). At the same time, independent positioning of low SF concentrations (SF0, SF1) was observed, which were without notable influence in relation to TAA, under the study conditions.

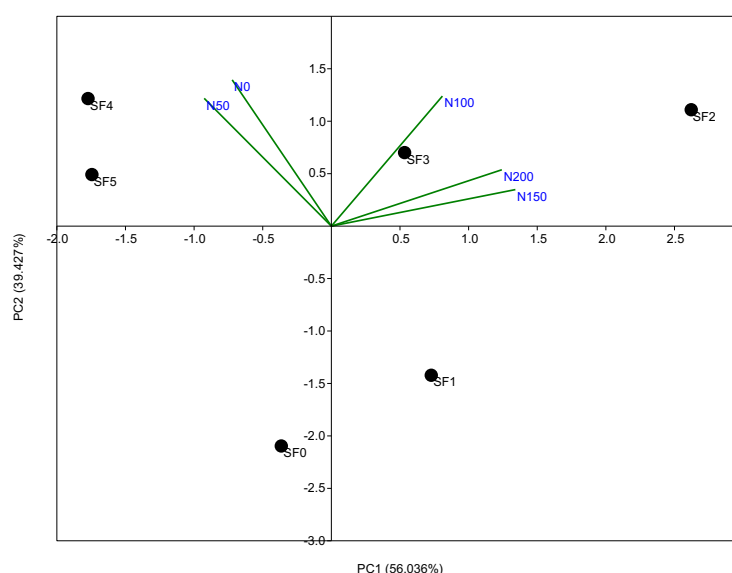


Figure 6. PCA diagram regarding the distribution of SF variants in relation to N (as biplot)

The multivariate analysis led to the loading values of the given nitrogen variants (N0 to N200) in relation to the Principal Components (Table 3) and of the given SF biofertilizer variants (SF0 to SF5), as factors in relation to the Principal Components (Table 4). The N150 variant presented the highest loading value in PC1 (0.5782), and the SF2 variant presented the highest score in PC1 (2.6218).

Table 3. Loading values of N variants in the Principal Components

	PC 1	PC 2	PC 3	PC 4	PC 5
N0	-0.3117	0.6015	0.2584	-0.0763	-0.6845
N50	-0.3997	0.5255	0.1101	0.3704	0.6441
N100	0.3489	0.5347	-0.6917	-0.3264	0.0862
N150	0.5782	0.1502	0.0164	0.7735	-0.2113
N200	0.5358	0.2317	0.6652	-0.3902	0.2542

Table 4. Score of SF variants in Principal Components

	PC 1	PC 2	PC 3	PC 4	PC 5
SF0	-0.3651	-2.0959	-0.3238	-0.0123	0.0682
SF1	0.7276	-1.4219	0.3847	0.1070	-0.0794
SF2	2.6218	1.1094	0.2125	0.0262	0.0537
SF3	0.5334	0.7011	-0.6304	-0.1556	-0.0581
SF4	-1.7731	1.2158	-0.1391	0.2613	0.0049
SF5	-1.7447	0.4914	0.4962	-0.2266	0.0106

The statistical analysis generated SDI values (Similarity and Distance indices), which showed the level of similarity between the variants, in relation to N (Table 5) and in relation to SF (Table 6). In relation to nitrogen (N), the highest level of similarity was recorded between N0 and N50 (SDI = 65.02), and in relation to Super Fifty (SF), the highest level of similarity was recorded between SF4 and SF5 (SDI = 84.27).

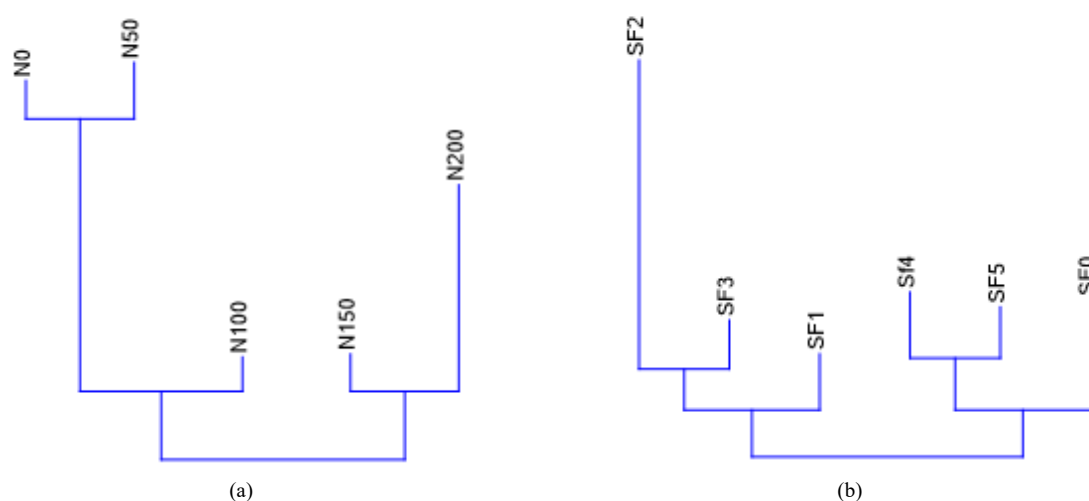
Table 5. SDI values for variants given by N

	N0	N50	N100	N150	N200
N0		65.02	233.20	334.01	451.72
N50	65.02		253.30	336.08	467.18
N100	233.20	253.30		146.98	255.21
N150	334.01	336.08	146.98		167.31
N200	451.72	467.18	255.21	167.31	

Table 6. SDI values for the given SF variants

	SF0	SF1	SF2	SF3	SF4	SF5
SF0		166.40	427.75	203.10	162.23	155.52
SF1	166.40		270.69	126.60	221.15	194.66
SF2	427.75	270.69		257.58	420.35	405.23
SF3	203.10	126.60	257.58		173.87	179.40
SF4	162.23	221.15	420.35	173.87		84.27
SF5	155.52	194.66	405.23	179.40	84.27	

Cluster analysis generated the association dendrograms of variants based on similarity, in relation to nitrogen, Figure 7 (a), Coph.corr. = 0.811, and in relation to the Super Fifty biofertilizer, Figure 7 (b), Coph.corr. = 0.900. In relation to nitrogen (N), the grouping of the first three nitrogen levels in one cluster was recorded, and the other two levels (N150,N200) in another cluster.

**Figure 7.** Cluster diagrams in relation to TAA values given by N (a) and in relation to TAA values given by SF (b)

In relation to Super Fifty (SF) the formation of two clusters was observed, with three variants each. One cluster included the variants SF1, SF2 and SF3, with the SF2 variant being positioned out of the way. The second cluster included the variants SF0, SF4 and SF5. The graphical distribution is in concordance with the SDI values, Table 6.

The variation of TAA in relation to SF at each N level was described by Equations (1) to Equation (7), presented in Table 7. The variable “ x ” was represented by the N level (kg ha^{-1}), according to the position in Table 7, and the variable “ y ” was represented by the SF levels (L ha^{-1}).

Table 7. Equations and statistical safety parameters in describing the variation of the TAA index in wheat, 'Alex' variety

Quality Index	Nitrogen Level	Equation	Eq. No	R^2	F	p
TAA	N = 0	$\text{TAA} = -1.727x^2 + 35.97x + 955.7$	(1)	0.937	22.226	0.0158
	N = 50	$\text{TAA} = 0.5852x^2 + 11.54x + 982.2$	(2)	0.841	7.9537	0.0632
	N = 100	$\text{TAA} = -17.38x^2 + 93.86x + 1028$	(3)	0.738	4.2146	0.1344
	N = 150	$\text{TAA} = -26.3x^2 + 113x + 1069$	(4)	0.745	4.3736	0.1290
	N = 200	$\text{TAA} = -30.31x^2 + 141.7x + 1081$	(5)	0.4413	1.1850	0.4175

The direct and interdependent action of the variables N and SF in the formation and variation of the total amino acid content (TAA) values in wheat, 'Alex' variety, under the study conditions was evaluated. Regression analysis led to Equation (6), with $R^2 = 0.566$, $F = 6.2536$, $p < 0.001$, with graphical representation in Figure 8.

$$\text{TAA} = ax^2 + by^2 + cx + dy + exy + f \quad (6)$$

where: TAA – Total amino acids (mg/kg);

x – N (nitrogen level, kg ha^{-1} a.s.); y – SF (Super Fifty concentration, L ha^{-1}), variables of Equation (6);

b, c, d, e, f – coefficients of the Equation (6);

$a = 0.00114671$;

$b = -15.02546428$;

$c = 1.00516952$;

$d = 100.68532142$;

$e = -0.21485428$;

$f = 905.51069047$.

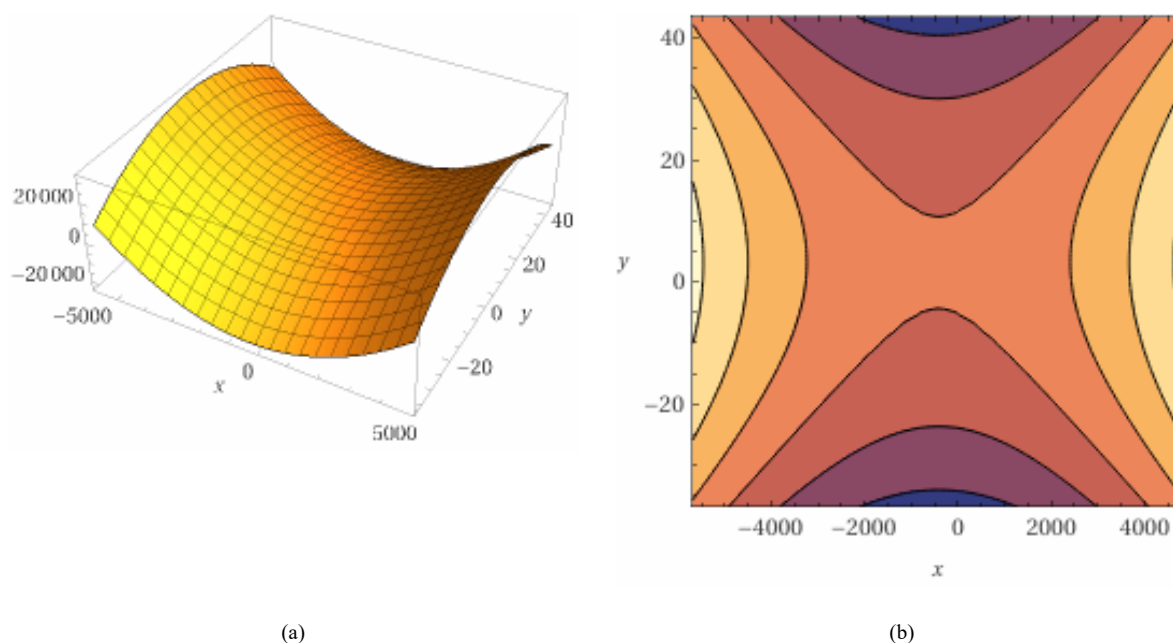


Figure 8. Graphical distribution of TAA values in relation to N (x – axis) and SF (y – axis) in wheat, 'Alex' variety; (a) – 3D representation, (b) – representation in isoquants format

Improving wheat quality indices is a major objective for research (Tan et al., 2026). Biostimulant products applied to wheat crops contributed to increasing yield and mineral element content in grains (Popko et al., 2018). Direct and interaction effects of phosphorus and iron, at different application rates, were recorded in the variation of protein, fiber and moisture content of wheat grains (Shahbazi and Nematollahi, 2019). Fertilizer supplementation (e.g. phosphorus) contributed under the study conditions to increase the protein and gluten content, with importance for the quality of the finished products (Ahmadi et al., 2024). Fertilizer management practices (types and application rates) differentially influenced the yield and quality of wheat and microbiological properties of the soil (Sheoran et al., 2025). The effect of technological substitution variants of organic fertilization was recorded in the variation of wheat quality indices (Wang et al., 2025). Foliar treatments with different amino acids led to an increase in the yield, protein and gluten content, with positive effects on the finished products (Tan et al., 2026).

In the context of this study, variable nitrogen fertilization rates and foliar treatments with the Super Fifty biofertilizer, based on algae extracts, led to the variation of the total amino acid content of wheat grains and the differentiation of variants in conditions of statistical safety. Variants with positive effects on the total amino acid content were identified, and multivariate analysis identified the correlation of the treatments in relation to TAA values.

Various mathematical models have been developed to describe the variation of wheat quality indices (e.g. protein, fiber) in relation to fertilizers and application rates (Shahbazi and Nematollahi, 2019). Through modeling, the potential fertilizing effect of CO₂ was formulated, as a counterbalance to yield losses, and the modification of temporal patterns in relation to humidity and plant water requirements (Bouras et al., 2019). Regression modeling has described the variation in amino acid content in different wheat varieties depending on the year of release of the genotypes (Emebiri, 2023). Simulation models have been used to explain the variation in wheat quality in relation to atmospheric factors with a stress effect (e.g. O₃), as well as the interdependence between different quality indices in wheat grains (Cook et al., 2025). Modeling of wheat quality in relation to climatic factors has shown, in relation to the specifics of some areas, that factors associated with water had a more pronounced impact on wheat quality than those associated with temperature (Zhou et al., 2025).

The present study found models in the form of polynomial equations and graphical models (3D, isoquants) that described the variation of total amino acid content in relation to fertilizers (N, SF) and application rates. The results recorded have scientific and practical importance for optimizing nitrogen and foliar biofertilizer (SF) doses in wheat crop, in relation to the formation of total amino acid content.

CONCLUSIONS

Nitrogen (N) fertilization and the foliar biofertilizer Super Fifty (SF) based on algae extract, differentially influenced the total amino acid (TAA) content of wheat, 'Alex' variety, under the study conditions.

Statistical analysis, through the applied tests, found significant differences between the mean and median values in relation to the TAA values by experimental variants.

Multivariate analysis explained how high concentrations of the SF biofertilizer (SF4, SF5) were associated with low doses of nitrogen (N0, N50) in the formation of the values of the total amino acid content (TAA, mg kg⁻¹). Moderate concentrations of the SF biofertilizer (SF2, SF3) were associated with medium-high doses of N (N100, N150, N200) in the formation of the values of the total amino acid content (TAA, mg kg⁻¹). Low concentrations of the biofertilizer (SF0, SF1) were positioned independently, without notable influence in relation to TAA, under the study conditions.

Mathematical models in the form of polynomial equations and graphical models (3D, isoquants) described the variation of the total amino acid content (TAA, mg kg⁻¹) in relation to N and SF. The divergent action of the two factors (N, SF) in the formation of TAA values, recommends a differentiated approach for optimization. Under the conditions of the present study, the combination of N200 with SF2 led to the highest value of the total amino acid content in wheat grains.

The results recorded recommend the continuation and development of studies on other wheat genotypes, to optimize fertilization in relation to the total amino acid content, but also other grain quality indices for the improvement of crop technologies. It is also of interest to extend the study to other agricultural crops.

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