

THE INFLUENCE OF MINERAL FERTILIZATION TYPE ON THE PROTEIN CONTENT OF RAPESEED SEEDS AND MEAL

Petrișoara ȘUVET, Lucian BOTOȘ, Simona NIȚĂ, Iinca Merima IMBREA,
Ștefan Laurențiu BĂTRÎNA, Adrian BORCEAN, Ioana Alina HÎNDA, Florin IMBREA

University of Life Sciences "King Mihai I" from Timișoara,
119 Calea Aradului Street, Timișoara, Romania

Corresponding author email: stefan.batrina@usvt.ro

Abstract

Rapeseed (Brassica napus) stands as one of the preeminent oil-bearing crops globally, attributable to its elevated seed oil concentration, robust hybrid production capabilities within the extant crop, and its versatile applications. The advent of erucic acid and glucosinolate-free hybrids has significantly broadened the utility of rapeseed-derived cakes and meal, characterized by a substantial protein content ranging from 38% to 41.9%. This study aimed to elucidate the impact of mineral fertilizer type on the protein content of both rapeseed and its resultant meal. Three distinct mineral fertilizers were tested in the experiment: E34 (10:24:0 + 0.1Zn + 0.1Br + 20 SO₃), DAP (18:46:0), and the 20:20:0 fertilizer type. The findings unequivocally underscored the discernible impact of fertilizer type on the protein content of rapeseed seeds. Notably, the protein content ranged from 19.80% in the 20:20:0 fertilization variant to 22.04% in the E34 fertilization variant. Similarly, the protein content of the resultant meal exhibited variance, oscillating between 38.07% in the 20:20:0 fertilization variant and 39.81% in the E34 fertilization variant. These outcomes accentuate the pivotal role of mineral fertilization in modulating the nutritional composition of rapeseed and its derivatives.

Key words: fertilization, protein, rapeseed.

INTRODUCTION

According to the US Department of Agriculture/Foreign Agricultural Service (USDA/FAS, 2018/19), rapeseed meal is the second largest global production of protein meals in the world, following soybean meal (Gherasimescu et al., 2023). The emergence of erucic acid-free and glucosinolate - free rapeseed hybrids has expanded the use of rapeseed for fodder in cattle feed, being an important source of proteins and amino acids that stimulate lactation, and more recently in human nutrition as a source of plant protein due to its special fatty acid composition (Bătrîna et al., 2021; Șuveț et al., 2021; Dziekanski et al., 2022; Sitnicki et al., 2024). Rapeseed-based feeding is the most important source of non-GMO proteins in Europe and is very important for dairy and meat producers in meeting the market demand for non-GMO products (Gofferje et al., 2015; Reichert et al., 2020; Schafer et al., 2018; Bătrîna et al., 2020). Another use of the cakes resulting from oil extraction is to produce natural fertilizers (Suvet et al., 2023).

Many studies (Sanchez-Vioque et al., 2001; Chang and Nickerson, 2014, 2015; Jang et al., 2011; Shin et al., 2011; Bandara et al., 2017; Zhang et al., 2018; Fetzer et al. 2019; He et al., 2019;) mention the use of rapeseed proteins for use in the production of adhesives, polymers, adhesives, and lubricants.

This study aimed to elucidate the impact of mineral fertilizer type on the protein content of both rapeseed and its resultant meal.

MATERIALS AND METHODS

The study examining the impact of various mineral fertilizers on the protein content of rapeseed seeds and meal was carried out on cambic chernozem soil. This research also explored how different fertilization levels interact with the specific climatic conditions experienced during 2021-2022 at the Educational Station of the University of Life Sciences 'Regele Mihai I' from Timișoara. The experiment was focused on the influence of three types of mineral fertilizers, namely: E34 (10:24:0 + 0.1Zn + 0.1Br +20 SO₃), DAP (18:46:0) and fertilizer type 20:20:0. The

fertilizers were applied fractionally: 200 kg for preparing the germination bed and 200 kg in spring. The used rapeseed hybrid was Astronom. The preceding plant was autumn wheat.

In order to assess the protein content, rapeseed meal and seeds were first defatted, then proteins were extracted using a strong alkali solution, followed by hydrolysis to break down proteins into amino acids. These amino acids are derivatized using phenyl isothiocyanate to form volatile derivatives suitable for GC-MS analysis. The total protein content was estimated by quantifying these derivatives using calibration curves based on standard proteins.

RESULTS AND DISCUSSIONS

The study of seed protein content [8.5% moisture]

The data regarding the protein content in seeds, presented in Table 1 and Figure 1, indicate that it ranged from 19.80% in the fertilization variant a3 (20:20:0) to 22.04% in the fertilization variant a1 (E34). The average protein content recorded for the experiment was 20.63%. Compared to the experiment's average, the a1 fertilization variant registered an increase of 1.42%, a difference statistically marked as highly significant. The protein content in the case of a3 fertilization level was 0.83% below the experiment's average, recording a negative increase, statistically significant in a negative sense.

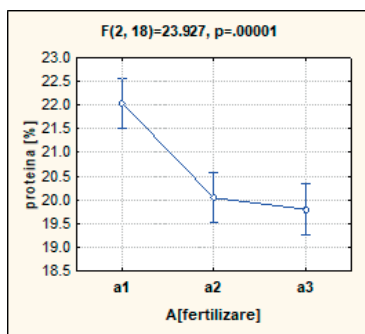


Figure 1. Variation of seed protein content in the 3 types of fertilization (factor A)

In conclusion, factor A (type of fertilization) has a significant action on protein synthesis; among the three levels of fertilization followed in the experiment, there are significant differences regarding the protein content.

Table 1. Protein content in the seed, depending on the type of fertilization

Factor A	Protein (%)	Diff.	Signif.
a1 - E34	22.04	1.42	***
a2 - DAP	20.04	-0.59	ns
a3 - 20:20:0	19.80	-0.83	0
Average	20.63	Mt	

DL 5% = 0.75 %; DL 1% = 1.02; DL 0.1% = 1.40

The protein fluctuations from one level of fertilization to another are significant.

The protein content decreases with the level of fertilization, showing a descending trend. Protein content varies between 22% and 19.8%. The differences between fertilization levels are highly significant ($p < 0.00$).

The protein content in seeds, based on the experimental year, is presented in Table 2 and Figure 2.

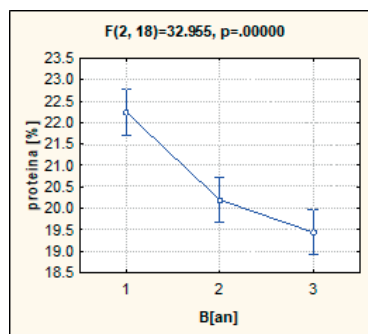


Figure 2. Yearly variation of protein content in seeds [factor B]

Table 2. Protein content in seed by experimental year

Factor B	Protein (%)	Diff.	Signif.
b1 - 2019	22.24	1.61	***
b2 - 2020	20.20	-0.43	ns
b3 - 2021	19.44	-1.18	**
Average	20.63	mt	

DL 5% = 0.75 %; DL 1% = 1.02; DL 0.1% = 1.40

Beyond the level of mineral fertilization, the protein content in seeds is also influenced by climatic conditions, namely temperature and humidity during the fruiting period. As a result, the protein content in the experimental cycle from 2019 to 2021 ranged from 19.44% in 2021 to 22.24% in 2019, a year characterized by very high temperatures and a lack of moisture. Therefore, compared to the experiment's average of 20.63%, in 2019 the protein content exceeded the control value by 1.61%, a difference statistically assured as highly significant. The protein content in 2020

registered a value roughly equal to the experiment's average.

In years with a precipitation deficit during the fruiting period, the protein content was higher, but seed production was lower. In conclusion, we can state that factor B (year) has a highly significant impact, the difference between production years being highly significant. The annual fluctuations in protein content are highly

significant (meaning they are statistically assured at the $\alpha=0.001\%$ level).

The protein content in seeds resulting from the interaction of the factors fertilization level x experimental year (A x B) and the significance of the content differences compared to the control are presented in Table 3 and Figure 3.

Table 3. Protein content in seed obtained from the interaction between A x B and the significance of the production differences compared to the control

Factor B (year)	Factor A (fertilization)								
	a1 - E34			a2 - DAP			a3 - 20:20:0		
	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.
b1 - 2019	23.74	3.11	***	21.71	1.08	ns	21.27	0.64	ns
b2 - 2020	21.78	1.15	ns	19.72	-0.91	ns	19.10	-1.53	0
b3 - 2021	20.62	-0.01	ns	18.68	-1.95	ns	19.03	-1.60	0
Average	20.63								

DL 5% = 1.295%; DL 1% = 1.774% DL 0.1% = 2.418%

Regardless of the experimental year, the protein content in seeds shows a descending trend relative to the level of fertilization (from E34 to 20:20:0), except for the year 2021, which has a descending trend from a1 to a2, and from a2 to a3, the trend is ascending.

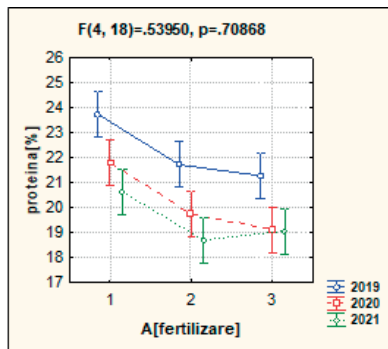


Figure 3. Variation in protein content (A x B interaction)

The contribution of factors A (fertilization), B (year), and the interaction AxB to the achievement of protein content in rapeseed is presented in Figure 4.

Factor A (fertilization) contributes to the achievement of production by 35.73%, factor B (year) contributes 49.22%, and the interaction A x B contributes 1.61%. Therefore, the greatest contribution comes from factor B (year), followed by factor A (fertilization) and the interaction A x B.

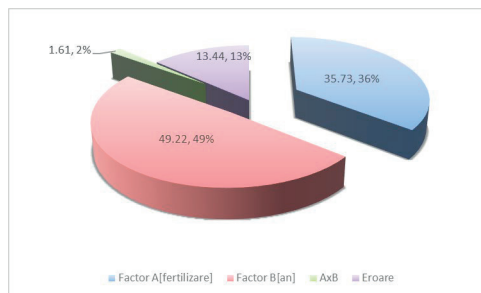


Figure 4. Contribution of factors A (fertilization), B (year) and A x B interaction

The protein content in meal [12% moisture]

The protein content in meal is presented in Table 4 and Figure 5. The obtained data indicate that it ranged from 38.07% in the fertilization variant a3 (20:20:0) to 39.81% in the fertilization variant a1 (E34).

Table 4. Protein content in meal, depending on the type of fertilization

Factor A	Protein (%)	Diff.	Signif.
a1 - E34	39.81	1.00	ns
a2 - DAP	38.55	-0.26	ns
a3 - 20:20:0	38.07	-0.74	ns
Average	38.81	Mt	

DL 5% = 1.20%; DL 1% = 1.65; DL 0.1% = 2.25

These results confirm the exceptional value of the meal obtained from oil extraction as fodder, considering that the Astronom hybrid is free from erucic acid and glucosinolates, and the resulting fodder contains approximately 38%

protein. Depending on the experimental year, the protein content in the meal (Table 5 and Figure 6) varied according to climatic conditions from 37.68% (in 2021) to 40.27% (in 2019).

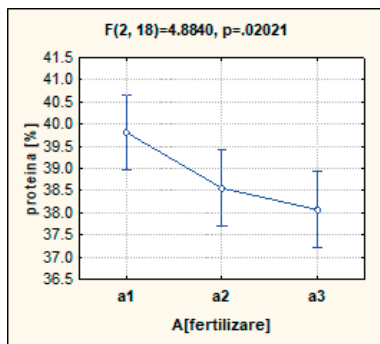


Figure 5. Protein content variation in meal across the three types of fertilization (factor A)

The protein content decreases with the level of fertilization; the two variables are inversely proportional. The evolution of protein content is descending. The protein content varies between 39.8% (a1), 38.5% (a2), and 38% (a3). The differences between the levels of fertilization are significant ($p = 0.02$, i.e., $p < 0.05$).

The protein content in meal depending on the experimental year is presented in Table 5 and Figure 6. From the data analysis, it is observed that it is directly correlated with the protein content in the seed.

Table 5. Protein content in meal by experimental year

[Factor B]			
Factor B	Protein (%)	Diff.	Signif.
b1 - 2019	40.27	1.46	***
b2 - 2020	38.48	-0.33	ns
b3 - 2021	37.68	-1.13	**
Average	38.81	mt	

DL 5% = 1.20 %; DL 1% = 1.65; DL 0.1% = 2.25

The highest protein content, around 40.3%, was obtained in b1 (year 2019) – class B, a content that significantly differs from the protein content obtained in b2 (year 2020) and b3 (year 2021).

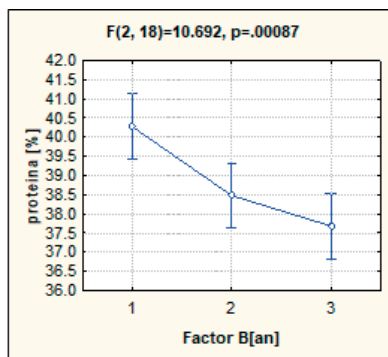


Figure 6. Yearly variation of protein content in seeds (factor B)

The protein content in the meal under the interaction of experimental factors, fertilization and year, is presented in Table 6 and Figure 7.

Table 6. Protein content in meal obtained from the interaction between A x B and the significance of the production differences compared to the control

Factor B (year)	Factor A (fertilization)								
	a1 - E34			a2 - DAP			a3 - 20:20:0		
	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.
b1 - 2019	41.69	2.88	**	40.62	1.81	ns	38.51	-0.30	ns
b2 - 2020	39.19	0.38	ns	37.87	-0.94	ns	38.37	-0.44	ns
b3 - 2021	38.54	-0.27	ns	37.17	-1.64	ns	37.34	-1.47	ns
Average							38.81 %		

DL 5% = 2.09%; DL 1% = 2.86% DL 0.1% = 3.90%

Compared to the control – the average of the experiment, only at a1 variant in the year 2019, was there a significantly distinct difference in the protein content in the meal, while for the other variants, the differences were not significant. Regardless of the production year, the highest protein content was obtained at the fertilization level a1, and the lowest protein content was achieved at the fertilization level a3. The protein contents obtained at a1, over the

three production years, varied between 41.7% and 38.5%, and those from a3 varied between 38.5% (b1), 38.4% (b2), and 37.3% (b3).

The contribution of factors A (fertilization), B (year), and the interaction A x B to achieving the protein percentage in the meal is presented in Figure 8. Factor A (fertilization) contributes to the production by 18.06%, factor B (year) contributes 39.54%, and the interaction AxB contributes 9.2%. Therefore, the greatest

contribution comes from factor B (year), followed by factor A (fertilization) and the interaction A x B.

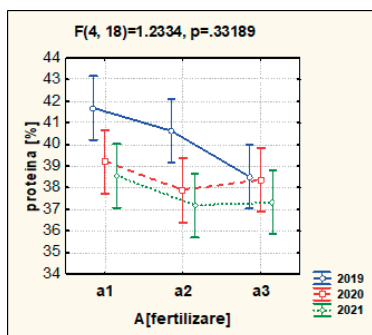


Figure 7. Meal protein content variation (A x B interaction)

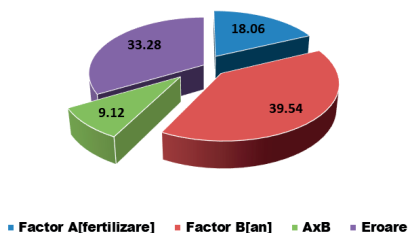


Figure 8. The contribution of factors A (fertilization), B (year), and the interaction A x B to the protein content in the meal

CONCLUSIONS

The protein content in seeds fluctuated between 19.80% for the fertilization variant with the 20:20:0 type of fertilizer and 22.04% for the E34 fertilization variant. The average protein content recorded for the experiment was 20.63%, a value close to the protein content in soybeans. These results underline the significance of the type of fertilizer on protein production, with significant differences in protein content among the three types of fertilization used in the experiment. The fluctuations in protein content from one fertilization level to another are significant.

Beyond the type of mineral fertilization, the protein content in seeds is also influenced by climatic conditions, namely temperature and humidity during the fruiting period. Accordingly, the protein content in the experimental cycle from 2019 to 2021 varied between 19.44%

in 2021 and 22.24% in 2019, a year characterized by very high temperatures and a lack of moisture. Thus, compared to the experiment's average of 20.63% in 2019, the protein content exceeded the control value by 1.61%, a difference statistically significant as highly significant. The protein content in 2020 recorded a value approximately equal to the experiment's average.

In the production of seed protein content, the type of fertilization contributed 35.73%, climatic conditions contributed 49.22%, and the interaction of fertilization type x climatic conditions contributed 1.61%.

The protein content in meal varied between 38.07% for the 20:20:0 fertilization variant and 39.81% for the E34 fertilization variant. Depending on the experimental year, the protein content in the meal fluctuated due to climatic conditions from 37.68% (in 2021) to 40.27% (in 2019).

The contribution of the fertilization type to the protein content in the meal was 18.06%, climatic conditions contributed 39.54%, and the interaction of fertilization type x climatic conditions contributed 9.2%.

These findings confirm the exceptional value of the meal resulting from oil extraction as fodder, considering the Astronom hybrid is free from erucic acid and glucosinolates, and the fodder obtained contains approximately 38% protein.

REFERENCES

- Bandara, N., Esparza, Y., Wu, J. (2017). Exfoliating nanomaterials in canola protein derived adhesive improves strength and water resistance. *RSC Advances*, 7, 6743–6752.
- Bătrina, Ș. L., Jurcoane, Ș., Popescu, I., Marin, F., Imbrea, I. M., Crista, F., Imbrea, F. (2020). *Camelina sativa*: A study on amino acid content. *Romanian Biotechnological Letters*, 25, 1136-1142.
- Bătrina, Ș., Jurcoane, Ș., Imbrea, I.M., Pop, G., Popescu, I.M. & Imbrea, F. (2021). Nutritive quality of camelina varieties with special focus on oil. *Scientific Papers. Series A. Agronomy*, 64 (1), 212-216.
- Chang, C. & Nickerson, M.T. (2014). Effect of plasticizer-type and genipin on the mechanical, optical, and water vapor barrier properties of canola protein isolate- based edible films. *European Food Research and Technology*, 238, 35–46.
- Chang, C. & Nickerson, M.T. (2015). Effect of protein and glycerol concentration on the mechanical, optical, and water vapor barrier properties of canola protein isolate- based edible films. *Food Science and Technology International*, 21, 33–44.

- Dziekanski, P., Prus, P., Sołtyk, P., Wronska, M., Imbrea, F., Smuleac, L., Pascalau, R., Błaszczuk, K. (2022). Spatial Disproportions of the Green Economy and the Financial Situation of Polish Voivodeships in 2010–2020. *Sustainability*, *14*, 13824.
- Gherasimescu, L., Bătrina, Ș.L., Imbrea, I.M., Imbrea, F. (2023). The evolution of agricultural yields. A case study on Timiș County, *Scientific Papers. Series A. Agronomy*, *66(1)*, 685-689.
- Egües, I., González, A.M., Herseczki, Z., Marton, G. Labidi, J. (2010). Hemicelluloses obtaining from rapeseed cake residue generated in the biodiesel production process. *Journal of Industrial and Engineering Chemistry*, *16*, 293–298.
- Fetzer, A., Müller, K., Schmid, M., Eisner, P. (2020). Rapeseed proteins for technical applications: Processing, isolation, modification, and functional properties. *Industrial Crops & Products*, *158(15)*, 112986.
- Fetzer, A., Herfellner, T., Eisner, P. (2019). Rapeseed protein concentrates for non-food applications prepared from pre-pressed and cold-pressed press cake via acidic precipitation and ultrafiltration. *Industrial Crops and Products*, *132*, 396–406.
- Gofferje, G., Schmid, M., Stabler, A. (2015). Characterization of *Jatropha curcas* L. Protein cast films with respect to packaging relevant properties. *International Journal of Polymer Science*, *2015*, 9.
- He, R., Dai, C.X., Li, Y., Wang, Z.G., Li, Q., Zhang, C., Ju, X.R., Yuan, J. (2019). Effects of succinylation on the physicochemical properties and structural characteristics of edible rapeseed protein isolate films. *Journal of the American Oil Chemists' Society*, *96(10)*, 1103–1113.
- Jang, S.A., Lim, G.O., Bin Song, K. (2011). Preparation and mechanical properties of edible rapeseed protein films. *Journal of Food Science*, *76*, C218–C223.
- Reichert, C.L., Bugnicourt, E., Coltelli, M.B., Cinelli, P., Lazzeri, A., Canesi, I., Braca, F., Martínez, B.M., Alonso, R., Agostinis, L., Verstichel, S., Six, L., Mets, S.D., Go´mez, E. C., Ißbrücker, C., Geerinck, R., Nettleton, D.F., Campos, I., Sauter, E., Pieczyk, P., Schmid, M. (2020). Bio-based packaging: materials, modifications, industrial applications and sustainability. *Polymers*, *12*, 1558.
- Sanchez-Vioque, R., Bagger, C.L., Rabiller, C., Gueguen, J. (2001). Foaming properties of acylated rapeseed (*Brassica napus* L.) hydrolysates. *Journal of Colloid and Interface Science* *244*, 386–393.
- Schafer, D., Reinelt, M., Stabler, A., Schmid, M. (2018). Mechanical and barrier properties of potato protein isolate-based films. *Coatings* *8(2)*, 58.
- Shin, Y.J., Jang, S.A., Song, H.Y., Song, H.J., Bin Song, K. (2011). Effects of combined fumaric Acid-UV-C treatment and rapeseed protein-gelatin film packaging on the postharvest quality of 'Seolhyang' strawberries. *Food Science and Biotechnology*, *20*, 1161–1165.
- Sitnicki, M.W., Prykaziuk, N., Ludmila, H., Pimenowa, O., Imbrea, F., Smuleac, L., Pascalău, R. (2024). Regional perspective of using cyber insurance as a tool for protection of agriculture 4.0. *Agriculture*, *14*, 320.
- Șuveț P. F., Șmuleac L. I., Botoș L. F., Pașcalău R., Bătrina Ș.L., Borcean A., Imbrea F. (2021). Research on the influence of technological links on oil rapeseed production, *Scientific Papers. Series A. Agronomy*, *64(2)*, 321-324.
- Șuveț, P. F., Șmuleac, L. I., Botoș, L. F., Pașcalău, R., Bătrina, Ș.L., Imbrea, I.M., Borcean, A., Imbrea F. (2023). Agronomic response of rapeseed and oil yield on different mineral fertilizing scheme. *Scientific Papers. Series A. Agronomy*, *66(1)*, 570-577.
- Zhang, Y., Liu, Q., Rempel, C. (2018). Processing and characteristics of canola protein- based biodegradable packaging: a review. *Critical Reviews in Food Science and Nutrition* *58*, 475–485
- <https://apps.fas.usda.gov/PSDOnline/Circulars/2019/10/production.pdf> accessed on 22.04.2024.