Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 22, Issue 1, 2022
PRINT ISSN 2284-7995, E-ISSN 2285-3952

THE ACCUMULATION OF GLUTEN PROTEINS IN WINTER WHEAT UNDER THE INFLUENCE OF THE USED TECHNOLOGY

Alina Laura AGAPIE[1], Cristian BOSTAN[1,2], Nicolae Marinel HORABLAGA[1,2], Oana Maria BOLDURA[2], Adina HORABLAGA[2], Dorin RECHIȚEAN[1], Lorena-Diana POPA[3], Florin SALA[2]

[1]Agricultural Research and Development Station Lovrin, 200, Lovrin, 307250, Romania, Email: cristian.bostan.tm@gmail.com, alinamartinig@yahoo.com, hnm75@yahoo.com, dorin_rechitan@yahoo.com
[2]Banat's University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania" from Timisoara, 300645, 119, Calea Aradului, Timisoara, Romania, Emails: cristian.bostan.tm@gmail.com, hnm75@yahoo.com, ahorablaga@yahoo.com, florin_sala@usab-tm.ro
[3]Agricultural Research and Development Station Secuieni, Secuieni, Neamț, Romania.
Emails: dy.hemp420@gmail.com

Corresponding author: cristian.bostan.tm@gmail.com; hnm75@yahoo.com

Abstract

The bread quality of wheat is one of the most important aspects targeted by crop technologies, given the great diversity of foods obtained from this crop and given the fact that bread is one of the main foods in the daily diet of many people. This paper highlights the role of mineral fertilization with nitrogen on the accumulation of gluten proteins. The study was conducted at SCDA Lovrin, in a long-term experiment with fertilizers. The Lab-on-a-Chip (LoaC) technique was used to extract gliadin and glutenin, followed by polyacrylamide gel electrophoresis. Ammonium nitrate was used for fertilization, with the following graduation: N0, N30, N60, N90 and N120. Nitrogen fertilizers significantly influence the quality of wheat crop. Thus, the percentage of protein and wet gluten increase by up to 47% and 70%, respectively. The accumulation of glutenins and glutenins at the level of molecular weight, increases exponentially with the dose of fertilizer administered to the crop, the highest values being registered at doses N90 kg / ha and N120 kg / ha, values statistically assured very significant for the probability of transgression of 0.1%. Also, the gliadin / glutenin ratio, whose value is an indicator for the bread quality of the grains, records the best value (as close as possible to 1) in the variant fertilized with the maximum dose of nitrogen.

Key words: *gliadin, glutenin, gluten, protein, nitrogen, wheat quality*

INTRODUCTION

Given the multitude of products obtained from wheat flour, wheat is arguably one of the most important crops in the world, sown on significant areas. One of the main objectives of wheat breeding programs is to improve quality [4], [16]. Therefore, the creation of ecobiotypes with high protein content is considered.

The quality of the products that reach the consumer's table is given by the quality of the gluten from the wheat flour, that vascoelastic network obtained after mixing the flour with water. The vascoelastic properties of the dough obtained are decisive for the bread quality of the wheat [2], [18].

Wheat proteins can be classified as follows: structural proteins (non-gluten) and storage proteins (gluten).

Gluten proteins are represented by prolamine, so named because of its high content of amino acids, proline and glutamine [13]. In turn, prolamins include: sulfur-rich prolamins, sulfur-poor prolamins and high molecular weight gluten (HMW) subunits. Sulfur-rich prolamins are β- and γ-gliadins, B- and C-LMW glutenins. Sulfur-poor prolamins include ω-gliadin and D-LMW-glutenins [18], [14], [15]. In general, wheat flour proteins include 45% gliadin, 45% gliadin and 10% soluble proteins.

The bread-making properties of wheat are given by storage proteins, as follows: density and extensibility are given by the gliadin monomer, and hardness and flexibility by the

glutenin polymer [6], [7], [8], [21], [22].
In addition to genetics, the technological factor, especially crop fertilization, plays an important role in achieving quality production. The quality of the dough and the bread-making properties of the wheat are strongly influenced by the mentioned factors.
Nitrogen fertilizers greatly influence both crop productivity and quality.
To identify and characterize the main components involved in determining the baking qualities of wheat and flour, an impressive number of techniques have been used over time, using the most diverse principles [1], [9], [3].
The first techniques tried to separate the protein fractions from wheat and flour, based on their differences in solubility in a number of solvents. Subsequently, a functional analysis (solubility, foaming capacity and emulsification) of some glutenin fractions, obtained after its hydrolysis with fungal proteases [17], was successful.
Another group of techniques used in the study of these components were chromatographic (adsorption, distribution chromatography, ion exchange chromatography, exclusion chromatography), electrophoretic (starch gel electrophoresis, polyacrylamide, isoelectric focusing, isotachophoresis) and spectroecopic (based on IR and Electron Spin Resonance) [11], [5], [12].
In the present study we evaluated the influence of chemical nitrogen fertilizers on the accumulation of gliadin and glutenin, the distribution of protein subunits in molecular weight, the technique used being Lab on a Chip, followed by polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

The study was conducted at ARDS Lovrin, in a long-term experiment with fertilizers.
The soil on which the experimental device was placed is a typical chernozem, weakly glazed and weakly alkalized.
The annual average rainfall in the area is 520 mm. The average annual temperature is 10.8°C.
The variety that was used in this experiment is the Ciprian variety, created and approved by ARDS Lovrin.
Ammonium nitrate was used for nitrogen fertilization of the crop, with the following graduation: N0 (V_1), N30 (V_2), N60 (V_3), N90 (V_4) and N120 (V_5).
The Lab-on-a-Chip (LoaC) technique was used to extract gliadins and glutenins, a rapid technique frequently used to separate and quantify proteins. Wheat grains, obtained after harvesting the crop, were ground to obtain flour. For extraction, 30 g of flour treated with 300 μL 70% ethanol were used. 200 μL solution was used for gliadin extraction and 100 μL for glutenin extraction. Extraction of gluten-free spores took place after removal of globulins and albumin and were used.
After evaporation of the ethanol, 350 μL 2% SDS solution containing 5% β-mercaptoethanol were used for the extraction of gliadins, maintained for 5 min at 100^0C.For the extraction of glutenins, the same volume of solution was used to which 0.0625 M tris base and the same temperature conditions were added. The final solution for the extraction of gluten proteins contains 4 μL sample to which was added 2 μL Agilent sample buffer and 84 μL deionized water [10], [20], [19], [23].
The molecular weights of the proteins were determined in the range 12.5 - 230 kDa, using chip electrophoresis techniqueon Agilent 2100 Bioanalyzer with Protein 230 Plus Lab-on-a-Chip kit. After analysis, each subunit was manually integrated and their percentage was calculated from the time-corrected area. The results were statistically analyzed using analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

After harvesting the culture, a conclusive sample was taken from each experimental variant which was analyzed from a qualitative point of view. The following were determined: protein, wet gluten, starch, glassiness.
Then the quality of protein and gluten was evaluated. The aim was to accumulate gliadins, accumulate glutenins and distribute them by molecular weight.
The percentage of protein and gluten,

respectively, increase significantly under the influence of the doses of fertilizers administered to the crop, in proportion to the increase of the dose (Table 1).

Table 1. Variation of protein and wet gluten content under the action of mineral nitrogen fertilization

Experimental variant	Protein (%)	Difference and significance	Wet gluten (%)	Difference and significance
V_1	10.7	Mt	21.2	Mt
V_2	11.0	0.3	24.2	3.0
V_3	12.8	2.1*	26.2	5.0*
V_4	15.2	4.5***	34.0	12.8***
V_5	15.7	5.0***	36.1	14.9***

Protein: DL 5% - 1.66; DL 1% - 2.75; DL 0.1% - 3.16.
Wet gluten: DL 5% - 4.9; DL 1% - 6.2; DL 0.1% - 12.1.
Source: Original data.

Very significant increases in the percentage of protein are registered at the application of doses of 90 kg/ha, respectively 120 kg/ha. Wet gluten varies in the range of 21.2% - 36.1%, with the best results obtained in the variants fertilized with high doses of nitrogen - 34%, respectively 36.1%, with up to 14.9% more than in the control variant, non-fertilized. Regarding the accumulation of gluten proteins (gliadin and glutenin) and this registers important changes under the influence of the dose used.

The accumulation of gliadin, presented in Table 2 and the distribution of gliadin subunits at the level of molecular weight, changes significantly when applying chemical fertilizers with nitrogen.

Table 2. Distribution of gliadins at the molecular weight

Molecular weight (kDa)	V_1- unfertilized	V_2 – fertilized with N_{30}	V_3 – fertilized with N_{60}	V_4 – fertilized with N_{90}	V_5 – fertilized with N_{120}
	The total concentration of gliadin extracted (ng/ μl)				
	3,355.2 ng/ μl	3,772.4 ng/ μl	3,850.7 ng/ μl	3,937.1 ng/ μl	4,522.1 ng/ μl
	Molecular weight distribution (ng/ μl)				
4.5	0.0	0.0	0.0	0.0	0.0
6.1 – 13.9	34.0	83.6	79.5	78.1	77.5
14.0 -16.2	67.7	74.5	76.6	78.1	87.8
16.3 – 37.8	85.7	95.6	86.2	76.1	136.7
37.9 – 46.9	2,534.8	2,858.0	2,496.5	2,964.0	3,384.7
47.0 – 57.6	434.5	494.2	632.1	569.4	636.8
57.7 – 90.5	90.5	81.7	55.6	81.6	74.9
ω-gliadin	96.1	121.6	293.1	149.3	124.7

Source: original data.

The total concentration of extracted gliadin, expressed in ng/μl, varies between 3,355.2 ng/μl - 4,522.1 ng/μl, the increasing trend being proportional to the dose of ammonium nitrate administered, a situation reported by other specialized studies (Table 3).

Analyzing the comparison in Table 3, we can say that the accumulation of gliadin under the influence of administered nitrogen doses indicates significant increases, statistically assured, in variants 3 and 4, and in the variant fertilized with maximum dose of nitrogen the increase of gliadin compared to non-fertilized variant is statistically assured very significant for the probability of transgression of 0.1%.

Table 3. Comparison table

Variant	Gliadin (ng/ μl)	%	Difference and significance
V_1	3,355.2	100	0.00
V_2	3,772.4	112.1	407.2
V_3	3,850.7	114.8	495.5*
V_4	3,937.1	117.3	581.9*
V_5	4,522.1	134.8	1,166.9***

DL 5% - 414.99; DL 1% - 603.62; DL 0.1% - 906.43
Source: Original data.

From the point of view of the molecular weight distribution, there is an increase of the values recorded in each of the analyzed ranges, an increase due to the increase of the amount of nitrogen administered to the culture.

The electrophoregrams obtained from gliadin extraction are presented in Figure 1.

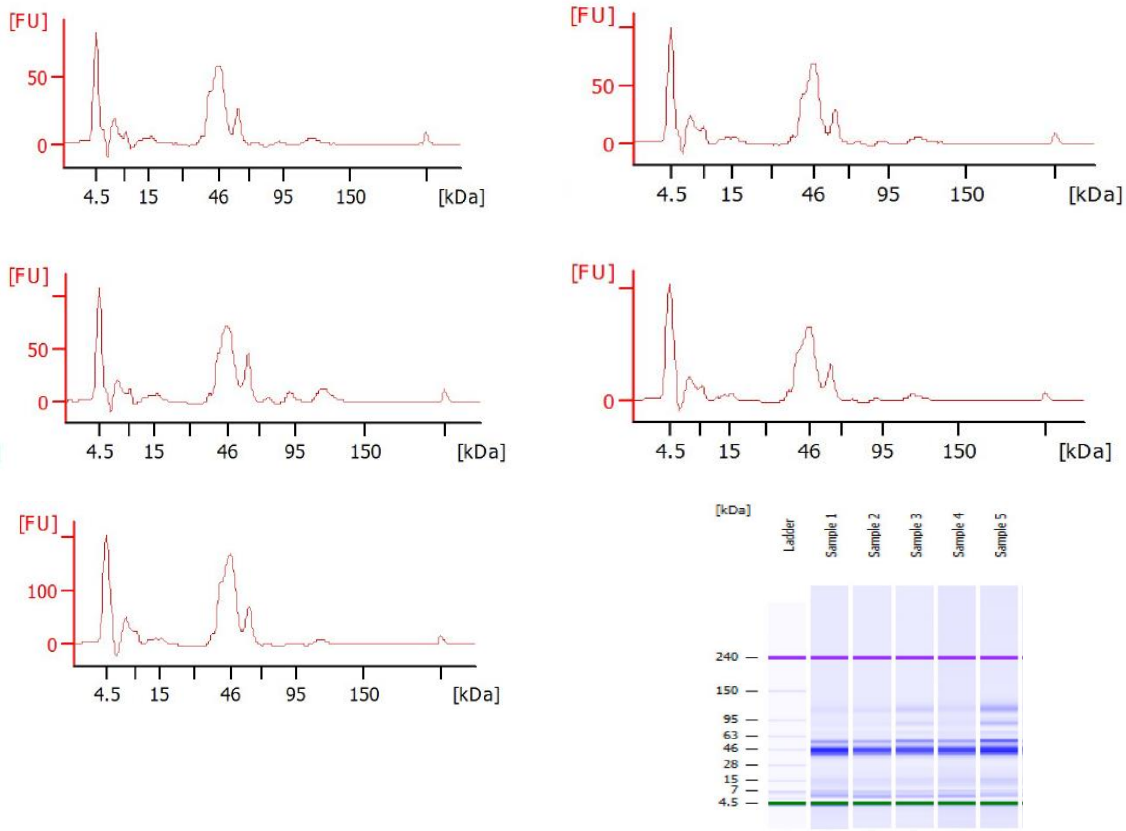


Fig. 1. The electrophoregrams of gliadin accumulation under the influence of the agrofund used
Source: Original figure.

Omega - gliadins, which are high molecular weight gliadin subunits, it accumulates in the range of 90-120 kDa. As the dose of fertilizer applied increases, the amount of omega gliadin also increases. In proportion to this the highest value accumulates when applying the nitrogen dose of 60 kg/ha - 293.1 ng/µl. Then there is a decrease of high values of fertilizer administered, up to 124.7 ng/µl, at the maximum dose of nitrogen administered.

Regarding the weight of ω - gliadin in the analyzed samples, it varies from 3.7% to 2.4%, with the highest value in the control variant, unfertilized. As the dose of nitrogen increases, so does the amount of ω - gliadin in the flour, but percentage, in the total amount determined, its share decreases by up to 1.3% (Figure 2, Figure 3).

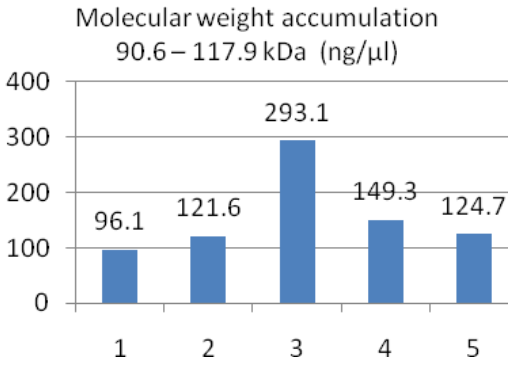


Fig. 2. Accumulation of ω - gliadin depending on the dose of nitrogen administered
Source: Original figure.

In order to highlight the correlation that is established between the amount of gliadin that accumulates in the control variant and in the variants fertilized with the four nitrogen graduations, a linear regression was used, presented in Figure 4.

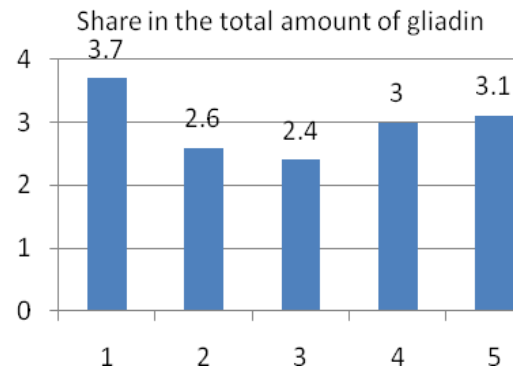


Fig. 3. The share of ω - gliadin in the total amount of protein
Source: Original figure.

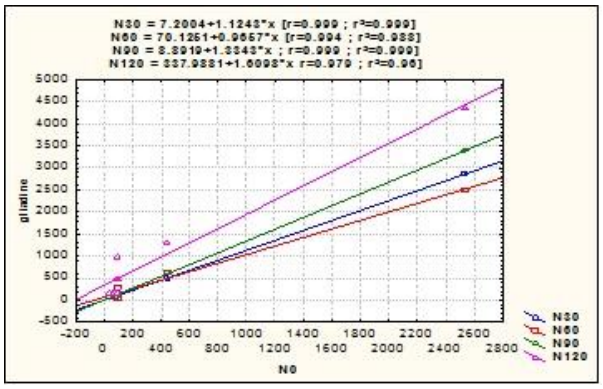


Fig. 4. The correlation between gliadin and nitrogen doses administered to the culture
Source: Original figure.

A very significant correlation is observed between the amount of gliadin belonging to the unfertilized control and the amount of gliadin accumulated at the N30, N60, N90 graduations.

The value of the correlation coefficients r = 0.999 (N30), r = 0.994 (N60), r = 0.999 (N90) indicates a very significant correlation between the amount of gliadin accumulated on the indicated agrofunds. At the maximum applied nitrogen dose - N120 kg/ha active substance, the correlation coefficient r = 0.979, indicates a distinctly significant linear correlation.

Of the two gluten proteins, gluten is the one that is associated with the bread quality of the dough, being responsible for their elasticity and viscosity.

Under the action of the agrofund administered to the crop, glutenin registers a slight decrease to low nitrogen values, followed by a significant increase when applying the doses of 90 kg/ha and 120 kg/ha, respectively.

Table 4 shows the distribution of glutenins in terms of molecular weight, under the influence of the five levels of fertilization used.

Table 4. Distribution of glutenins at the molecular weight

Molecular weight (kDa)	V_1- unfertilized	V_2 – fertilized with N_{30}	V_3 – fertilized with N_{60}	V_4 – fertilized with N_{90}	V_5 – fertilized with N_{120}
	The total concentration of glutenins extracted (ng/ μl)				
	1,396.1 ng/ μl	1,209.5 ng/ μl	1,136.6 ng/ μl	1,846.3 ng/ μl	2,348.9 ng/ μl
	Molecular weight distribution (ng/ μl)				
4.5	0.0	0.0	0.0	0.0	0.0
6.1 – 13.7	0.0	0.0	0.0	0.0	0.0
13.8 -44.2	1,051.4	1,092.3	788.1	893.1	911.5
44.3 – 57.6	273.4	117.1	265.7	417.5	506.6
57.8 – 112.7	14.5	0.0	34.1	174.1	389.6
112.8 – 173.3	41.8	0.0	25.2	176.9	355.4
173.4 – 222.7	18.0	0.0	23.5	113.1	185.9

Source: Original data.

Analyzing Table 5, which represents the table of comparisons between the five agrofunds studied, shows the influence of fertilization on glutenin accumulation. Thus, large amounts of nitrogen bring distinctly significant (N90) and very significant (N120) increases, by 32% and 68% respectively more than the control, unfertilized variant.

Table 5. The comparison table

Varianta	Glutenin (ng/ μl)	%	Difference and significance
V_1	1,396.1	100	0.00
V_2	1,209.5	86.6	-186.73
V_3	1,136.6	81.4	-259.60°
V_4	1,846.3	132.2	450.20**
V_5	2,348.9	168.2	952.80***

DL 5% - 215.00; DL 1% - 312.73; DL 0.1% - 469.09
Source: Original data.

Regarding the bread quality of the flour, the studies indicate a superior quality to the increased accumulation of high molecular weight (HMW) subunits of glutenin. HMW subunits of glutenin accumulate starting at 112.8 kDa. There is a progressive accumulation, with the highest values - 290 ng/μl and 541.3 ng/μl at the highest doses of nitrogen, very significant differences from the control variant, Figure 5.

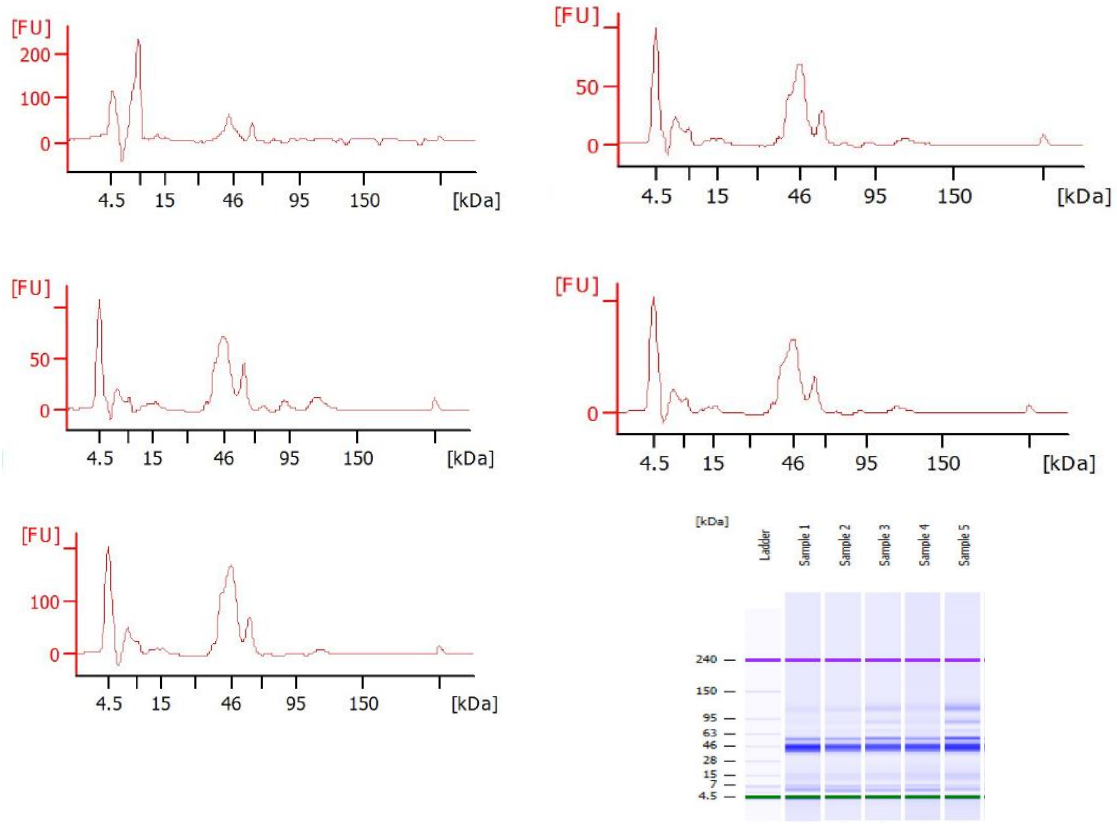
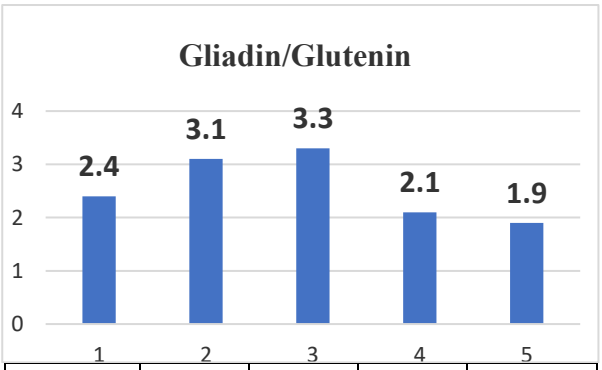


Fig. 5. The electrophoregrams of glutenin accumulation under the influence of the agrofund used
Source: original figures.



1,396.1	1,209.5	1,136.3	1,846.3	2,348.9
3,355.2	3,772.4	3,937.1	3,937.1	4,522.1

Fig. 6. The gliadin/glutenin ratio
Source: Original graph.

The gliadin/glutenin ratio is considered an indicator of grain quality.
The closer its value is to 1, the better the bread value of the flour. Under the influence of the fertilizer doses administered to the crop, this ratio has an interesting evolution, which emerges from Figure 6.
The closest value of 1 is registered in the variant fertilized with 120 kg/ha nitrogen 1.9, followed by the variant fertilized with 90 kg/ha nitrogen - 2.1. Compared to the unfertilized control, there are decreases of this quality indicator by up to 20%.

CONCLUSIONS

Nitrogen fertilizers significantly influence the quality of wheat crop. Thus, the percentage of protein and wet gluten increase by up to 47% and 70%, respectively.
At the same time, the quality of gluten, given the participation rate of gluten proteins is considerably influenced. The proportion of

gliadin in the flour increases in proportion to the dose of nitrogen applied to the wheat crop. ω-gliadin, also called anti-bread quality protein, accumulates in an amount that increases to the level of the N60 agrofund, after which there is a decrease in high values of the amount of nitrogen administered. The lowest value is recorded in the control version, unfertilized. However, the share of the total amount of protein decreases with increasing nitrogen dose, from 3.7% - value belonging to the unfertilized control to 2.4%, a value found in the version fertilized with 60 kg/ha nitrogen.

The accumulation of glutenins at the level of molecular weight, increases exponentially with the dose of fertilizer administered to the crop, the highest values being registered at the doses N90 kg/ha and N120 kg/ha.

Also, the gliadin/glutenin ratio, whose value is an indicator for the bread quality of the grains, registers the best value (as close as possible to 1) in the version fertilized with the maximum dose of nitrogen.

ACKNOWLEDGEMENTS

The authors thank the ARSD Lovrin management for facilitating this study.

REFERENCES

[1]Chan, K.X., Wirtz, M., Phua, S.Y., Estavillo, G.M., Pogson, B.J., 2013, Balancing metabolites in drought: The sulfur assimilation conundrum, Trends Plant Sci., 18:18-29.

[2]Finney, K.F., Barmore, M.D., 1948, Loaf volume and protein content of hard winter and spring wheat, Cereal Chem., 25:291-312.

[3]Goetz, H., Kuschel, M., Wulff, T., 2004, Comparison of selected analytical techniques for protein sizing, quantity and molecular weight determination, J. Biochem. Biophys. Methods, 60(3):281-293

[4]Guzman, C., Peña, R.J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., Rutkoski, J., Poiland, J., Battenfield S., 2016, Wheat quality improvement at CIMMYT and the use of genomic selection on it, Appl. Trans. Genom., 11:3-8

[5]Gygi, S.P., Corthals, G.L., Zhang, Y., Rochon, Y., Aebersold, R., 2000, Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology, Proc. Natl. Acad. Sci., 97:9390-5.

[6]Horvat, D., Kurtanjek, Z., Drezner, G., Simic, G., Magdic, D., 2009, Effect of HMM glutenin subunits on wheat quality attributes, Food Technol. Biotechnol., 47(3):253-259.

[7]Horvat, D., Crossed, N., Signukic, D., Magdi, D., 2013, Characterization of bread wheat cultivars (*Triticum aestivum* L.) by glutenin proteins, Cereal Res. Commun., 41(1):133-140.

[8]Horvat, C., 2014, Storage proteins in wheat (*Triticum aestivum* L.) and the ecological impacts affecting their quality and quantity, with a focus on nitrogen supply, J. Agric. Environ. Sci., 1(2):57-76.

[9]Järvan, M., Lukme, L., Adamson, A., Akk, A., 2017, Responses of wheat yield, quality and bread-making properties on the sulphur fertilization, Acta Agric. Scan. B. Soil Plant Sci., 67(5):444-452.

[10]Kuschel, M., Neumann, T., Barthmaier, P., Kratzmeier, M., 2002, Use of lab-on-a-Chip technology for protein sizing and quantitation, J. Biomol. Tech., 13(3):172-178.

[11]Lilley, K.S., Razzaq, A., Dupree, P., 2002, Two-dimensional gel electrophoresis: recent advances in sample preparation, detection and quantitation, Curr. Opin. Chem. Biol., 6:46-50.

[12]Liu, H., Lin, D., Yates, J., 2002, Multidimensional separations for protein/peptide analysis in the post-genomic era. BioTechniques, 32:898-911.

[13]Malik, A.H., 2009, Nutrient uptake, transport and translocation in cereals: Influences of environment and farming conditions. Introductory paper at the Faculty of Landscape Planning, Horticulture and Agricultural Science, 1:46.

[14]Masci, S., Lafiandra, D., Porceddu, E., Lew, E.J.L., Tao, H.P., Kasarda, D.D., 1993, D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine, Cereal Chem., 70(5):581-585.

[15]Masci, S., Egorov, T.A., Ronchi, C., Kuzmicky, D.D., Lafiandra, D., 1999, Evidence for the presence of only one cysteine residue in the D-type low molecular weight of wheat glutenin, J. Cereal Sci., 29(1):17-25.

[16]Michel, S., Kummer, C., Gallee, M., Hellinger, J., Ametz, C., Akgöl, B., Epure, D., Löscheneberger, F., Buerstmayr, H., 2018, Improving the baking quality of bread wheat by genomic selection in early generations, Theor Appl Genet., 131**:**477-493.

[17]Popineau, Y, Huchet, B., Larre, C., Berot, S., 2002, Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration, J. Cereal Sci., 35:327-335.

[18]Shewry, P.R., Halford, N.G., 2002, Cereal seed storage proteins: Structures, properties and role in grain utilization, J. Exp. Bot., 53:947-958.

[19]Torbica, A.M., Zivancev, D.R., Nikolić, Z.T., Dordević, V.B., Nikolovski, B.G., 2010, Advantages of the Lab-on-a-chip method in the determination of the Kunitz Trypsin inhibitor in soybean varieties, J., Agric. Food Chem., 58(13):7980-7985.

[20]Uthayakumaran, S., Batey, I.L., Wrigley, C.W., 2005, On-the-spot identification of grain variety and wheat-quality type by Lab-on-a-chip capillary electrophoresis, J. Cereal Sci., 41(3):371-374.

[21]Uthayakumaran, S., Listiohadi, Y., Baratta, M.,

Batey, I.L., Wrigley, C.W., 2006, Rapid identification and quantitation of highmolecular-weight glutenin subunits, J. Cereal Sci., 44(1):34-39.
[22]Uthayakumaran, S., Zhao, F.J., Sivri, D., Roohani, M., Batey, I.L., Wrigley, C.W., 2007, Defect identification in wheat grain by micro-fluidic electrophoresis: Sulfur deficiency and bug damage, Cereal Chem., 84(4):301-303.
[23]Živančev, D., Horvat, D., Torbica, A., Belović, M., Šimić, G., Magdić, D., Đukić, N., 2015, Benefits and limitations of Lab-on-a-Chip method over reversed-phase high-performance liquid chromatography method in gluten proteins evaluation, J. Chem., 2015:430328.